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NEW PROCESSES FOR NUTRIENT RECOVERY FROM WASTES

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NEW PROCESSES FOR NUTRIENT RECOVERY FROM WASTES

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Calcium phosphate concentrate mined from animal waste.

Picture by Matias Vanotti, USDA.

Global demand for mineral fertilizers is continuously increasing, while large amounts of organic wastes are being disposed without use as a resource, resulting in soil, water and air pollution. Current trends of intensification, expansion and agglomeration of livestock production result in a net import of nutrients that lead to a surplus in some production areas. Therefore, new processes and technologies to recover and re-use nutrients from both solid and liquid wastes are desirable to close the loop on the nutrient cycle in modern human society and address future scarcity of non-renewable nutrients and fossil-based fertilizers. This Research Topic aims to present scientific progress regarding processes and technologies that allow recovery and re-use of nutrients from wastes, the selective recovery of mineral nutrients (ammonia and phosphates), the production of new organic fertilizers, and evaluation of their relative agronomic efficiency. The articles within provide a stronger recognition of the importance of nutrient recovery and upcycling in the new horizons of the circular economy.

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Editorial: New Processes for Nutrient Recovery From Wastes

Matias B. Vanotti^{1*}, Maria C. García-González², Beatriz Molinuevo-Salces² and Berta Riaño²

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Keywords: nutrient recovery, nutrient re-use, upcycling, waste, manure, organic fertilizers, value-added products, circular economy

Editorial on the Research Topic

New Processes for Nutrient Recovery From Wastes

Global demand for mineral fertilizers is continuously increasing, while large amounts of organic wastes are being disposed without use as a resource, resulting in soil, water, and air pollution. Current trends of intensification, expansion, and agglomeration of livestock production result in a net import of nutrients that lead to a surplus in some production areas (Vanotti et al., 2019). Therefore, new processes and technologies to recover and re-use nutrients from both solid and liquid wastes are desirable to close the loop on the nutrient cycle in modern human society and address future scarcity of non-renewable nutrients and fossil-based fertilizers. Development of technologies for nutrient-reuse was identified as one of the main challenges in waste management within a circular economy (Bernal, 2017). Another main challenge in this context is to provide needed information for efficient substitution of mineral fertilizer with nutrients from organic wastes (Bernal, 2017). This Research Topic aims to present scientific progress regarding processes and technologies that allow recovery and re-use of nutrients from wastes, the selective recovery of mineral nutrients (ammonia and phosphates), the production of new organic fertilizers, and evaluation of their relative agronomic efficiency. The Research Topic comprises 13 articles, including 11 Original Research articles and 2 Reviews.

Solid-liquid separation up-front in a treatment train allows recovery of the organic compounds that can be used for manufacture of compost materials, peat substitutes, quick-wash phosphorus, and biochars (Vanotti et al., 2019). Vanotti et al. presents a multi-stage treatment system in a swine facility that used high-rate solid-liquid separation as a first step (capture of 90% TSS, 69% organic N, and 84% organic P), followed by biological treatment of the ammonia in the separated liquid fraction, and efficient calcium phosphate recovery in the absence of ammonia and alkalinity buffers. The system was operated full-scale for 7 years. This allowed documentation of water quality improvements and the halt of sludge accumulation in the converted swine lagoons. Ro et al. contribute with a connected paper that shows an additional environmental benefit of the same system: the reduction on ammonia emissions. Using open-path tunable diode laser (TDL) absorption spectroscopy, the total farm-level NH₃ emissions were reduced below minimum detection level. Szogi et al. presents the use of a Quick Wash (QW) process to mine the phosphorus that has accumulated for years in the sludges at the bottom of anaerobic swine lagoons in the USA. The QW process uses acidification of the organic solids to release the phosphate, and precipitation of the phosphate as calcium phosphate. The recovered product was amorphous calcium phosphate with P₂O₅ grades (33–35%) higher than rock phosphate, but with the advantage that there is no need for additional chemical treatment for its use as plant fertilizer.

The circular economy approach requires the transformation of organic wastes through treatment and their reuse in agriculture. An important aspect for effective reuse of the new products is to know their relative agronomic efficiency and pollution risks compared to that of a mineral

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fertilizer. Santos et al. assessed the fertilizer value and the pollution risks of composts. The composts were prepared using mixtures of dewatered swine manure and cotton gin waste at ratios of 4:3 and 3:4. The compost with higher manure proportion was more efficient for N fertilization. However, both composts increased soil total N, soil available P, and plant biomass production and can be used as organic fertilizer to substitute mineral fertilizer, with the advantage of reduced nitrate leaching risk.

The production of biochars through pyrolysis or gasification processes could be an effective way of recycling the phosphorus (P) contained in organic materials. For both processes, the thermal conversion conditions and the type of biowaste are important factors that influence the subsequent physical and chemical properties of produced biochars. In fact, differences in their plant nutrient composition is the main concern with the use of biochar as a soil fertilizer amendment. In the present Research Topic, Novak et al. reported that pyrolyzed biochars produced from poultry litter feedstocks have significantly higher P and K contents than lignocellulosic-based biochars, and that various blends of feedstocks can be used to produce designer biochars that match specific crop nutrient needs. Li et al. studied the plant availability of P in five different gasification biochars and observed a strong influence of the type of feedstock on P availability. Specifically, gasification biochars derived from poultry manure, wheat straw and shea nut wastes presented higher P extractability relative to triple superphosphate (indicating the fertilizer value) than sewage sludge-based gasification biochars. In addition, Li et al. found that pyrolysis of poultry litter at 450°C or less significantly decreased the water-soluble proportion and lability of P in the biochar but did not affect the long-term P bioavailability. Under these conditions, they produced a P-enriched, slow release soil amendment that minimizes P runoff following field application and environmental risk of fertilization.

Gaseous ammonia (NH₃) emissions and a lack of N capture and reuse is a major concern in livestock farming today. In this Research Topic, three papers focus on technologies that could capture and recover the NH₃ emanating from manures. Oliveira Filho et al. and Molinuevo-Salces et al. studied a gas-permeable membrane (GPM) technology for reducing N content from liquid manures (digested and raw) and its recovery in a concentrated stable ammonium solution. The membrane manifolds are submerged and capture the NH₃ before it reaches the air. The studies used low-rate aeration to increase manure pH and promote NH₃ capture, contributing to NH₃ emissions

mitigation and sustainable livestock waste treatment. Oliveira Filho et al. found that the GPM technology was efficient for recovering N from swine manure and effluents obtained by manure co-digestion with vegetable wastes; the NH₄⁺ removal rate was >74%, and >95% of the removed N was recovered as a stable fertilizer salt solution. Molinuevo-Salces et al. showed that it is feasible to combine N-recovery by GPM technology and anaerobic digestion for the treatment of swine manure. The combined treatment resulted in a 96% ammonium recovery and a 69% total COD removal. A variety of valuable products was obtained, specifically sustainable energy in the form of methane and a stable ammonium sulfate solution for use in fertigation. Moore et al. used an innovative ARS air scrubber that captures NH₃ from exhausted air in animal feeding operations while simultaneously reducing emissions of dust and odor. Full-scale testing showed NH₃ capturing efficiencies over 90%. A variety of acid salts (alum, sodium or potassium bisulfate, ferric chloride, and ferric sulfate) were found to work as well as strong acids (hydrochloric, phosphoric, and sulfuric) for capturing NH₃.

Acien-Fernández et al. reviewed the relevance of microalgae-based processes for the recovery of nutrients from wastewaters. Under optimal conditions, up to 200 t per hectare and year of microalgae biomass can be produced containing 25 t of N and 2.5 t of P. Hernández et al. studied the recovery of N in the form of protein concentrates from this biomass that could be used for animal feed in aquaculture. The maximum protein recovery was 55% of the initial biomass protein. The left-over materials after protein extraction had value for methane (CH₄) production through anaerobic digestion, yielding 180 mL CH₄ g VS⁻¹.

Finally, Magrí used a bibliometric approach to review global trends in the area of knowledge of nutrient (N and P) management from digestates. The recovery of nutrients from digestates has undergone accelerated development in recent years. Physicochemical methods usually target the production of high quality, nutrient-rich concentrates that can be placed on the market.

The 13 articles composing this Research Topic provide a stronger recognition of the importance of nutrient recovery and upcycling in the new horizons of the circular economy.

AUTHOR CONTRIBUTIONS

MV and MG-G contributed to the conception and coordination of this Research Topic. All authors contributed to the writing of the present editorial article.

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High-Rate Solid-Liquid Separation Coupled With Nitrogen and Phosphorus Treatment of Swine Manure: Effect on Water Quality

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This study determined the water quality improvements in swine lagoons by an innovative swine manure treatment system operating at full-scale during five pig production cycles. The system performed high-rate solid-liquid separation, biological ammonia treatment and phosphorus treatment. The treatment system met the environmental performance standards for swine waste management systems in new or expanding operations in North Carolina. The system substantially reduced odor by 99.9%; pathogens by 99.99%, nutrients (phosphorus and nitrogen) by > 90%, and heavy metals by 99%. As the treated effluent and/or rainwater renovated the liquid in the anaerobic lagoons, they became aerobic ($E_h > 300$ millivolts). By the end of the second year, the $\text{NH}_4\text{-N}$ concentration in the lagoons liquid declined from the initial 370–485 mg L^{-1} to lower than 15. After conversion, the sludge accumulation in the former lagoons was halted. This was a significant outcome because one converted lagoon served twice the number of animals than before implementation of the innovative manure treatment system, which is similar to a situation of herd expansion. These findings showed that environmentally superior waste management technologies can have substantial positive impacts on water quality in intensive swine production.

Keywords: water quality, solid-liquid separation, flocculants, nutrient recovery, swine lagoons, pig manure, nitrification, recovered calcium phosphate

INTRODUCTION

Typically, waste from confined swine production operations in the southeastern U.S. is stored and treated in large, open anaerobic lagoons prior to application on cropland (Barker, 1996a; Westerman et al., 2010). After year 2000, there was great public interest in developing new swine manure treatment systems in the region to address multiple environmental and health concerns associated with the anaerobic lagoon system. These concerns included emissions of ammonia (Aneja et al., 2008), pathogens (Sobsey et al., 2001; Vanotti et al., 2005), odors (Schiffman and Williams, 2005; Loughrin et al., 2006), and deterioration of water quality (Mallin, 2000). Consequently, demonstrations of new treatment systems were conducted on-farm to demonstrate feasibility of environmental superior waste management technologies (EST) that could address

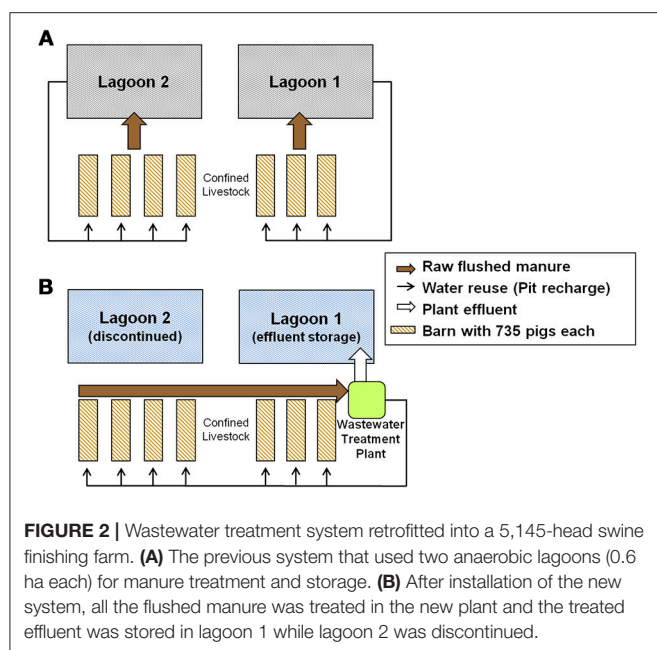
five environmental standards: “1. Eliminate the discharge of animal waste to surface waters and groundwater through direct discharge, seepage or runoff; 2. Substantially eliminate atmospheric emissions of ammonia; 3. Substantially eliminate the emission of odor that is detectable beyond the boundaries of the parcel or tract of land on which the swine farm is located; 4. Substantially eliminates the release of disease-transmitting vectors and airborne pathogens; and 5. Substantially eliminates nutrient and heavy metal contamination of soil and groundwater” (Williams, 2009). Nutrients of concern were nitrogen (N) and phosphorus (P), and heavy metals of concern were copper (Cu) and zinc (Zn). As a result of this process, new legislation in North Carolina was enacted enforcing the environmental performance standards of EST for the construction of new swine farms or expansion of existing swine farms (NC Legislature, 2007; 15A NCAC 02T, 2010; Sommer et al., 2013).

Typically, the separation efficiencies of mechanical solid-liquid separators are less than 68% (Chastain et al., 2001) and typically less than 34% (Riaño and García-González, 2014). Organic nutrients (N and P) are contained mostly in fine manure particles < pore size 0.3 mm (Vanotti et al., 2002) that are not separated with normal screening (Riaño and García-González, 2014). However, new advances in both equipment and flocculant polymer applications developed for high-rate separation treatment have improved removal efficiency of solids and plant nutrients (N and P) (Vanotti and Hunt, 1999; Hjorth et al., 2010; Chastain, 2013). More and more often, new treatment systems for manure encompass three or four process units in tandem to meet various environmental standards and nutrient recovery targets. The swine waste management system described in this work is a manure treatment system developed to meet the EST environmental standards referenced above (Vanotti

et al., 2010). The system consisted of high-rate solid-liquid separation followed by ammonia treatment and phosphorus recovery. A detailed description of this system as well as system drawing, first year performance of the treatment plant, and economic considerations, are found in Vanotti et al. (2009). While treatment performance of the system *per se* can be correctly assessed at steady state over relatively short periods during cold and warm weather conditions, its effect on water quality needs longer periods of monitoring due the large volume and slow hydraulic retention time of existing lagoons. In a previous study, we were able to evaluate side by side the water quality of a swine lagoon (total volume 24,145 m³) being cleaned with the treated effluent from a multiple-stage treatment system (4,360-head swine unit) compared with an identical control anaerobic swine lagoon receiving raw effluent from another 4,360-head swine unit (Vanotti and Szogi, 2008). Results of that evaluation showed the converted lagoon was transformed into an aerobic reservoir (dissolved oxygen, DO, 6.95 mg L⁻¹) within a year, and by the second year, the following concentration reductions in the lagoon liquid were realized: 73% of total suspended solids (TSS), 77% of biochemical oxygen demand (BOD), and 92% of ammonium (NH₄-N) (Vanotti and Szogi, 2008). In the present study, the multi-stage treatment system (performing the same environmental functions as before) was retrofitted into a 5,145-swine farm that for the previous 11–12 years used two anaerobic lagoons (16,552 and 13,120 m³) to treat the manure (Figure 1). With the implementation of the new system, one lagoon was discontinued, and the other lagoon was used as effluent storage and received the effluent from all the barns after treatment in the new plant (Figure 2). This lagoon served twice the animals as before, similar to a situation of herd expansion. Thus, it presented unique conditions that have not been experimented before or could be predicted without experimental data on water quality.



FIGURE 1 | Aerial picture of waste treatment system and barns. It treated all the manure flushed from seven barns with 735 pigs each. Existing lagoons are shown in the foreground.



The objectives of this study were to: (1) Report the overall treatment efficiency consisting of high-rate solid-liquid separation followed by ammonia and phosphorus treatment evaluated intensively at steady state over a 2 years period and five swine production cycles. (2) Report the corresponding water quality improvements in the converted lagoons. (3) Report the changes in the sludge depth of the lagoons during a 6 years period of the new system operating at full-scale.

MATERIALS AND METHODS

Farm Description

The full-scale manure treatment system was installed on a swine farm near Clinton, Sampson Co., North Carolina and evaluated intensively with regards to water quality during 2 years under steady-state conditions that included five complete swine production cycles. The evaluation monitored the treatment plant and lagoons. Changes in the sludge depth in the lagoons were not clear in the first 2 years. For this reason, measurements of sludge depth were collected and reported for an additional 4 years of operation of the new treatment system.

The farm had 12.9 ha (32 acres) with a permitted capacity of 5,145-head feeder-to-finish swine placed in seven barns (735 heads/barn). The traditional anaerobic lagoon system (Barker, 1996a; NRCS, 2004), which is typical in North Carolina, was used for about 11 years before the new treatment system started operation in Dec. 2006. Production records during the three growing cycles before the start of the new plant showed the farm produced an average of 584,000 kg total live weight (487,000 kg net gain production) per growing cycle (5,296 pigs/cycle). The manure was collected under the barns using slatted floors and a pit-recharge system (Barker, 1996b). The liquid manure contained in the pits was emptied weekly by gravity into

the anaerobic lagoons. There were two anaerobic lagoons for treatment and storage of the manure flushed from the barns (Figure 2A). Lagoon 2 received the flushes from four barns (1–4); it had a surface of 0.62 ha, a depth of 3.66 m and a design volume of 16,552 m³. This volume included (1) a minimum treatment volume of 11,240 m³ based on Steady State Live Weight (SSLW) (2,940 head × 61.24 kg/head = 180,045 kg) and anaerobic treatment volume guidelines of 6.243 m³/100 kg SSLW (1 ft³/lb SSLW), and (2) a temporary storage volume of 5,016 m³ based on waste volume generated stored for 180 days (0.00849 m³/100 kg SSLW/d or 0.00136 ft³/lb SSLW/d), a positive balance of rain – evaporation (17.78 cm) and an additional 17.78 cm storage for a “25-year, 24 h” storm event. Lagoon 1 received the flushed raw manure from three barns (5–7); it had a surface of 0.60 ha, a depth of 2.74 m and a design volume of 13,120 m³. This volume included (1) a minimum treatment volume of 8,433 m³ based on SSLW (2,205 heads × 61.24 kg/head = 135,034 kg) and same anaerobic treatment volume guidelines, and (2) a temporary storage volume of 4,312 m³ also based on waste volume generated stored for 180 days, rain – evaporation of 17.78 cm, and a “25-year, 24-h” storm storage of 17.78 cm. After treatment in the lagoons, the liquid was sprayed onto the farms’s fields growing small grains and forages with a permitted capacity to utilize a total 5,390 kg of plant available N per year (average N application rate of 417.8 kg N/(ha.year). The lagoon supernatant liquid was recycled into the subfloor pits to facilitate waste flushing (Figure 2A).

With the new treatment system, the flow of raw wastewater into the lagoons was discontinued; instead, all the raw wastewater was sent to the treatment plant (Figure 2B). The barn pits were flushed once a week as before, but the flushed manure (barns 1–7) was diverted into a homogenization tank that mixed the manure before the solid-liquid separation step. A portion of the water after ammonia treatment was used to recharge the barn pits for the flushing (Figure 3). Water in excess of that needed for barn pit recharge was treated in the phosphorus treatment + disinfection module and stored in lagoon 1 for use in crop irrigation. As mentioned before, the new treatment system was evaluated intensively during five growing cycles of pigs (Figure 4). Within production cycles, the total pig weight in the seven barns varied greatly, from a low monthly average of 71.8 Mg to a high of 519.3 Mg (Figure 4). The average live animal weight (LAW) in the seven barns during the 5-cycle evaluation period was 335.8 Mg. This value is also referred to as steady-state live weight (SSLW, dotted line Figure 4). During the 5-cycle evaluation period, the farm sold an average of 624,345 ± 21,867 kg total live weight (516,239 ± 13,791 kg net gain produced) in each of the five growing cycles (5,265 ± 132 pigs/cycle).

The water quality of the two lagoons was monitored intensively during a 3 years period: the year before the project started when both lagoons performed anaerobic treatment, and the following 2 years when lagoon 1 received effluent from the alternative treatment system and lagoon 2 received only rain water. To help comparison of results of water quality monitoring, the same 36 months scale was used in the figures in this paper. After the intensive 3 years evaluation by the ARS

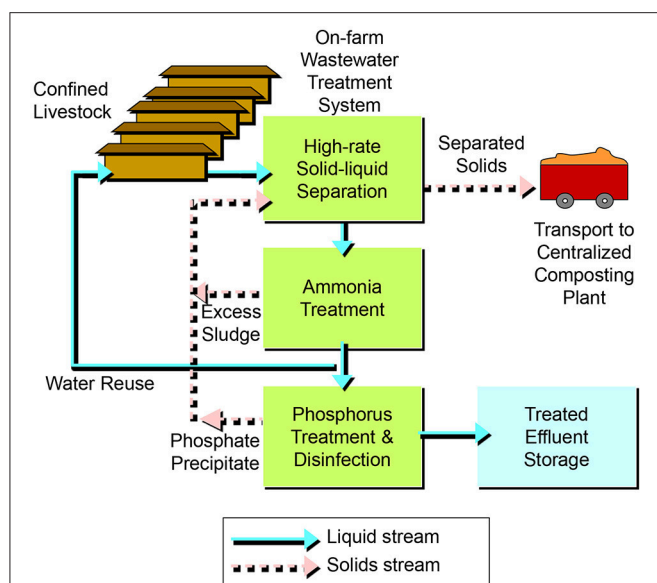


FIGURE 3 | Schematic diagram of wastewater treatment system using high-rate solid-liquid separation, ammonia treatment and phosphorus treatment.

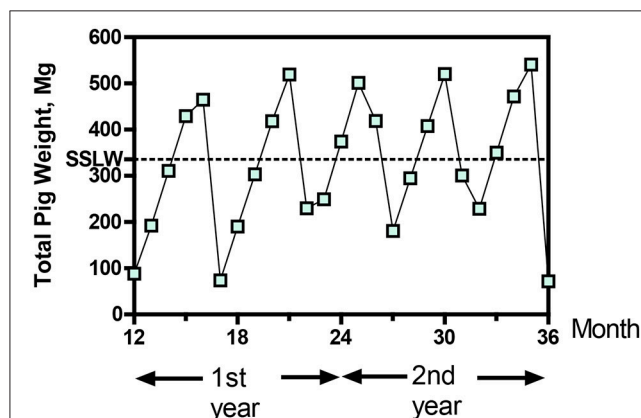


FIGURE 4 | Average monthly total live weight of the pigs during five production cycles in seven barns during demonstration of new wastewater treatment process. Dotted line is the steady state live weight (SSLW).

team (2006–2008), the treatment system kept operating full-scale without changes in swine inventory for an additional 4 years. As part of the permit No AW1820164 for using the innovative animal waste management system, the operator had to report to the State Permitting Authority the following parameters: daily volume of separated solids, the quarterly total volumes of the system wastewater influent and effluent, and quarterly chemical analyses of Total N, $\text{NH}_4\text{-N}$, $\text{NO}_3\text{+NO}_2\text{-N}$, BOD_5 , Total P, TS, Cu, Zn, pH, and fecal coliforms in the separated solids (except BOD_5), the influent into the homogenization tank and the plant effluent from the phosphorus settling tank. After 3 years of successful compliance, the frequency of sampling and chemical analyses was reduced to two times per year to demonstrate compliance in both

summer and winter seasons. During this extended period, the analyses were also done at ARS laboratory.

Wastewater Treatment System Description

The multistage system (Vanotti et al., 2010) consisted of three steps or process units in tandem: high-rate solid-liquid separation, biological ammonia treatment, and phosphorus treatment/disinfection (Figure 3). For a completed description and the schematic drawing of this system, see Vanotti et al. (2009). Before the first step, subfloor wastewater was emptied weekly by gravity into a receiving pit and pumped by a 946 L min^{-1} pump into a 379 m^3 capacity homogenization tank. The manure was kept well mixed using a 3.5 kW , $12.1 \text{ m}^3 \text{ min}^{-1}$ submersible mixer. The homogenized liquid manure was conveyed into the first step in the system—the liquid/solid separation process—at a process flow of $9.1 \text{ m}^3 \text{ h}^{-1}$. The process used polymer flocculation to enhance the separation of fine suspended particles (Vanotti and Hunt, 1999; Garcia et al., 2007). Solids were separated by a rotary press separator (Fournier Industries Inc., Quebec, Canada) with a dual 1.2 m rotary press, two polymer preparation tanks, a polymer metering pump, manure feed pump and in-line flocculator. The polymer was dry cationic, linear polyacrylamide (PAM) with 35% mole charge (SNF Floerger, Riceboro, GA). The prepared polymer solution contained $2.14 \text{ g PAM L}^{-1}$ (0.2%) and was mixed with the liquid manure at a rate of 6%. This resulted in a final polymer dosage of 128 mg L^{-1} . Separated manure solids were transported offsite to a solids processing facility and composed with cotton gin waste to produce value-added organic fertilizers, soil amendments and plant growth media (Vanotti et al., 2006). The separated wastewater was stored in another 379 m^3 tank and then pumped continuously into the second step of the system – the biological ammonia treatment process. This process used nitrification-denitrification (NDN) with a Modified Ludzack-Ettinger (MLE) configuration (Tchobanoglous et al., 2003). There were three tanks in the second step: the first tank was an anoxic tank (277 m^3) for denitrification (DN), the second tank was an aeration tank (227 m^3) for nitrification, and the third tank was a settling tank for clarification. Nitrification converted $\text{NH}_4\text{-N}$ into $\text{NO}_2\text{-N}$ and $\text{NO}_3\text{-N}$. The nitrified wastewater was continually recycled into the DN tank using the pre-denitrification configuration (MLE). In the DN tank, suspended denitrifying bacteria used soluble manure carbon in the separated manure to transform NO_2 and NO_3 into N_2 gas. The nitrification process used high-performance nitrifying bacterial sludge (HPNS), which was developed for high-ammonia wastewater and cold temperatures (Vanotti et al., 2013). To start the nitrification process, the aeration tank was inoculated with one liter of HPNS. Then a multiplication step was conducted in the same tank during 40 days to achieve full-scale nitrification activity prior to starting the complete system (Vanotti et al., 2009). Air was supplied continuously with a 10 HP rotary lobe blower and 98 fine-air diffusers. The DN tank contained a 1.7 kW , $9.8 \text{ m}^3 \text{ min}^{-1}$ submersible mixer. A settling tank (14.3 m^3) with conical bottom clarified the effluent after nitrification. The settled sludge was returned into the DN tank. The rates of sludge and nitrified liquid recycling into the DN tank were 0.5 and 3.5 times the

inflow rate, respectively. The clarified effluent was stored in a 277 m³ tank and used to refill the barn pits (**Figure 2B**). The average mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS) in the nitrification tank during evaluation were $2,450 \pm 1,680$ mg L⁻¹ and $1,980 \pm 1,440$ mg L⁻¹, respectively. Target MLSS concentrations were $> 2,000$ and $< 4,000$ mg L⁻¹. Once a week, the operator used a settling test (15 min, 1 L graduated cylinder) to estimate the MLSS in both denitrification and nitrification tanks based on an empirical relationship obtained at the site: [settled solids vol. (mL L⁻¹) = $-66.7 + 0.1132$ MLSS (mg L⁻¹); $r^2 = 0.759$] so that the settled solids volume in the 15 min test stayed between 160 and 390 mL L⁻¹ (corresponding to the 2,000–4,000 mg MLSS L⁻¹ target). This information was used by the operator to divert more or less sludge from the settling tank into the solids separator up-front to meet the MLSS target range. Considering a specific nitrification activity of 20.76 mg N/g MLVSS/h and a nitrification tank volume of 227 m³, the nitrification capacity of the unit was 223 kg N/day. In the third step of the system—the phosphorus treatment/disinfection process - the soluble P was recovered as a calcium phosphate solid (Vanotti et al., 2003), and pathogens were substantially reduced by the alkaline environment (Vanotti et al., 2005). The effluent was treated with hydrated lime slurry [12% Ca(OH)₂] in a 0.3 m³ reaction chamber. The pH of the process was maintained at 9.5 by a pH probe and pH controller linked to the lime injection pump. The average lime consumption rate was 1.18 kg m⁻³. The P precipitate (calcium phosphate) was separated in a settling tank with conical bottom (8.8 m³) and further dewatered using the solid/liquid separation unit in the first unit of the system (Garcia et al., 2007). Phosphorus and manure solids as well as excess NDN sludge were combined in one solids stream for off-farm transport (**Figure 3**).

The average wastewater flows through the new treatment system (2 years averages) were the following: 36.3 m³ d⁻¹ of raw manure were flushed from the barns and treated (plant influent); 6.2 m³ d⁻¹ after N treatment were recycled to refill the barn's pit recharge system; 31.6 m³ d⁻¹ after P treatment (plant effluent) were stored in lagoon 1 for use in crop irrigation. On average, the flushed manure volume from the barns contained 17.1% recycled

effluent from the treatment system and 82.9% of newly generated manure, urine, and water wasted by pigs. The newly generated wastewater stream (flushed manure—water reuse) averaged 30.1 m³ day⁻¹ or 40.8 L per 455 kg live animal weight (LAW) per day. For comparison, the industry average in feeder to finish operations using pit-recharge systems is 45.8 L per 455 kg LAW per day (1.62 ft³ per 1,000 lbs. LAW per day) (Chastain et al., 1999).

Water Sampling and Monitoring

For the treatment system, composite liquid samples were collected twice per week during a 2 years period from four locations: (i) the homogenization tank containing raw flushed manure (plant influent), (ii) after solid-liquid separation treatment, (iii) after N treatment, and (iv) after P treatment (plant effluent). Samples were composited of four sub-samples taken over 3.5 days periods using refrigerated automated samplers (Sigma 900max, American Sigma, Inc., Medina, NY). Wastewater flows throughout the system were measured with five liquid-level ultrasonic probes and data logger (SR50 Sonic Ranging Sensor and CR800 data logger, Campbell Scientific Inc., Logan, UT). The ultrasonic probes measured liquid levels in the homogenization tank, separated water tank, clean water tank, and settling tank. The measurements of liquid height and area of the tanks were used to calculate actual volume dynamics and flows. The data logger also monitored air and water temperatures, precipitation, DO, ORP, and process pH. Average monthly maximum, average and minimum air temperatures and total monthly rain are shown in **Figure 5**. Average monthly minimum and maximum of daily air temperatures ranged from -8.4 to 38.8°C , average monthly air temperature was 16.5°C , and it ranged from 5.5 to 27.2°C , and precipitation averaged 1,048 mm per year.

Lagoon liquid samples were collected monthly during a 3 years period to monitor water quality characteristics at least 1 year before and 2 years after the treatment system was implemented. Sub-samples were collected from the lagoon supernatant within a 0.30 m depth using a 500 mL polyethylene dipper with a 3.6 m handle. From each lagoon, two composite

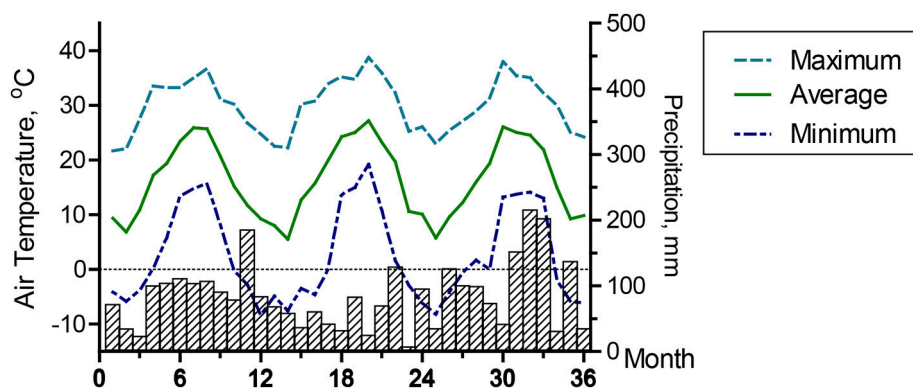


FIGURE 5 | Air temperature and precipitation during the 3-yr water quality monitoring period. Data are monthly maximum, average, and minimum of daily air temperatures, and monthly precipitation.

samples were obtained by mixing in a bucket eight sub-samples collected around the lagoon.

Collected samples were: (1) transported on ice to the ARS Coastal Plains Research Center in Florence, SC, for water quality analyses, or (2) overnight shipped with cold packs to the ARS Sustainable Agricultural Systems Laboratory and Environmental Microbial Safety Laboratory in Beltsville, MD, for microbiological analyses, and to the ARS Animal Waste Management Research Unit in Bowling Green, KY, for odor analyses.

The sludge depth in the lagoons was monitored yearly during 9 years: 3 years before and 6 years after the new system was implemented. The distance from the liquid surface level to the top of the sludge layer was measured with a sonar and the distance from the liquid surface to the lagoon bottom (soil) was measured with a pole. From 2004 to 2006, the sonar measurements were made from a boat at 8 or 10 sampling points per lagoon. Afterwards, the sonar measurements were done with a remote control boat that collected $1,150 \pm 170$ points per lagoon. Sludges were sampled five times to measure chemical characteristics using Sludge Judge probes 4.5 m long \times 3.2 cm outside diameter (OD). Volume of sludge was calculated based on height using the formula volume of a trapezoid and design dimensions of the lagoons.

Analytical Methods

Water quality analyses were performed according to Standard Methods for the Examination of Water and Wastewater (APHA, 1998). Total solids (TS), total suspended solids (TSS), and volatile suspended solids (VSS) used Standard Method 2540 B, D, and E, respectively. Chemical analyses consisted of chemical oxygen demand (COD) using Method 5220 D, 5 days biochemical oxygen demand (BOD₅) using Method 5210 B, ammonia (NH₄-N) using Method 4500-NH₃ G, nitrate plus nitrite (NO₃ + NO₂-N) using Method 4500-NO₃ F, pH using Method 4500-H⁺ B, electrical conductivity (EC) using Method 2510 B, alkalinity using Method 2320 B and endpoint pH of 4.5, soluble P (SP or PO₄) using Method 4500-P F after filtration through a 0.45- μ m membrane filter. Total P (TP) and total Kjeldahl N (TKN) were determined using acid digestion (Gallaher et al., 1976) and colorimetric phosphorus and nitrogen methods adapted to acid digests (Technicon Instruments Corp, 1977). Organic P was the difference between total P and PO₄ analyses. Organic N was the difference between Kjeldahl N and NH₄-N analyses, and Total N was the sum of TKN and nitrate + nitrite. The potassium (K), calcium (Ca), magnesium (Mg), sodium (Na), copper (Cu), and zinc (Zn), were determined using nitric acid/peroxide block digestion (Peters, 2003) and inductively coupled plasma (ICP) analysis (Method 3125 A). Oxidation-reduction potentials (ORP) were measured at the time of sampling using a Ag/AgCl reference electrode and corrected to standard hydrogen electrode (Eh) values (Standard Method 2580 B). Reduction in odor was characterized as described by Loughrin et al. (2009) that measured in the liquid the concentration of five odor compounds characteristic of swine manure (phenol, *p*-cresol, *p*-ethylphenol, indole, and skatole) using extraction with Twister stir bars (Gerstel, Baltimore, MD) coated with polydimethylsiloxane followed by thermal desorption and gas

chromatography-mass spectrometry. Total aromatic malodors were the sum of the five odor compounds. Microbiological analyses of liquid samples were done using standard protocols for pathogens and indicator microbes for the examination of wastewater (Vanotti et al., 2005).

Statistical Analysis

Data management, descriptive statistics (PROC MEANS), regression (PROC REG), and mean comparison for repeated measurements (PROC MIXED) analyses were performed with SAS (SAS Institute, 2008).

RESULTS AND DISCUSSION

Water Quality Improvements by Treatment System

The wastewater treatment performance of the plant are presented in **Table 1**; the various columns show changes in water quality indicators as the liquid manure passed through the three treatment steps as well as the overall system efficiency. The intensive evaluation period encompassed five cycles of pig production; this allowed assessment of the performance of the system through varying environmental conditions and waste loadings. High treatment efficiencies were obtained consistently despite air temperatures varying from -8.4 to 38.8°C (**Figure 5**) and large fluctuations in the strength of the manure. Taken on average through the evaluation period, flushed manure had high strength (TS $3.0 \pm 1.2\%$) according to the manure strength scale of Garcia-González and Vanotti (2015). The variation in concentrations because of changes in pig weight during production cycles was big: volatile solids, for example, averaged $17,800 \text{ mg L}^{-1}$, but ranged from $2,850 \text{ mg L}^{-1}$ up to about $41,800 \text{ mg L}^{-1}$ while BOD₅ averaged $7,360 \text{ mg L}^{-1}$ and ranged from 730 to over $25,000 \text{ mg L}^{-1}$ (**Table 1**). Other quality parameters such as TKN (average $2,050 \text{ mg L}^{-1}$, range 810 – $4,220 \text{ mg L}^{-1}$) and NH₄-N (average $1,290 \text{ mg L}^{-1}$, range 310 – $3,445 \text{ mg L}^{-1}$) were also distinctive of a high-strength swine wastewater. The variations in wastewater volumes were also big because of the pig production cycles: average monthly volume of flushed wastewater was $1,095 \text{ m}^3$, but ranged from 396 up to $2,233 \text{ m}^3$. Similarly, the clean treated effluent volumes averaged 963 m^3 per month, and ranged from 290 to $1,666 \text{ m}^3$. In terms of mass loadings, the total nitrogen load into the treatment system (flushed manure) during the five pig cycles averaged 80.6 kg N d^{-1} ($29,510 \text{ kg N yr}^{-1}$) and the monthly averages ranged from 20.9 to $211.5 \text{ kg N d}^{-1}$. The average NH₄-N load was 51.9 kg d^{-1} (range 13 – 150 kg d^{-1}), and the average TP load was 21.4 kg d^{-1} . The on-farm system removed 67.75% of TS, 90.2% of VS, 97.2% of TSS, 98.4% of VSS, 96.3% of COD, 99.4% of BOD₅, 95.7% of TKN, 96.5% of NH₄-N, 93.3% of TP, 98.8% of Zn, 98.8% of Cu, 56.0% of EC, and 77.5% of alkalinity (**Table 1**).

High-Rate Solid-Liquid Separation

The first step of the system was a high-rate solid-liquid separation via polymer flocculants (Chastain, 2013). The separation up-front allowed recovery of the organic materials in the manure, which can be utilized for the manufacture of composts, biochars,

TABLE 1 | Wastewater treatment plant performance by treatment step and overall system efficiency^a.

Water quality parameter	Raw flushed swine manure (system influent)	Treatment step			System efficiency
		After solid-liquid separation treatment	After ammonia treatment	After phosphorus treatment (system effluent)	
		mg L ⁻¹ ^b			%
TSS	11,754 ± 6,417	1,254 ± 1,015	227 ± 199	325 ± 215	97.2
VSS	8,926 ± 5,103	891 ± 756	154 ± 129	142 ± 105	98.4
TS	30,065 ± 12,475	14,244 ± 5,104	9,824 ± 2,312	10,008 ± 2,495	67.7
VS	17,799 ± 8,725	5,322 ± 2,893	1,818 ± 827	1,738 ± 1,046	90.2
COD	22,204 ± 14,363	8,196 ± 5,286	1,058 ± 541	821 ± 405	96.3
Soluble COD	7,338 ± 6,012	6,073 ± 4,098	862 ± 393	684 ± 308	90.6
BOD ₅	7,364 ± 6,313	3,185 ± 2,692	62 ± 88	41 ± 61	99.4
TKN	2,054 ± 778	1,466 ± 600	138 ± 166	87 ± 130	95.7
NH ₄ -N	1,290 ± 615	1,213 ± 451	124 ± 171	45 ± 92	96.5
NO ₂ + NO ₃ -N	1.4 ± 4.6	0.2 ± 1.5	221 ± 179	162 ± 144	–
Organic N	739 ± 447	230 ± 290	33 ± 38	36 ± 51	95.1
Total N	2,055	1,466	359	249	87.9
TP	492 ± 272	151 ± 79	83 ± 30	33 ± 23	93.3
Soluble P	94 ± 63	82 ± 42	76 ± 29	19 ± 17	79.8
Organic P	380 ± 259	62 ± 63	11 ± 12	12 ± 14	96.8
K	1,648 ± 562	1,551 ± 475	1,420 ± 371	1,443 ± 370	12.4
Ca	417 ± 196	106 ± 58	39 ± 18	90 ± 95	78.4
Mg	219 ± 110	44 ± 30	16 ± 7	12 ± 5	94.5
Zn	25.4 ± 12.6	2.9 ± 2.8	0.4 ± 0.4	0.3 ± 0.3	98.8
Cu	16.8 ± 11.1	2.0 ± 2.4	0.2 ± 0.1	0.2 ± 0.1	98.8
Fe	39.9 ± 21.3	4.81 ± 4.55	0.49 ± 0.40	0.39 ± 0.35	99.0
S	128 ± 60	49 ± 19	34 ± 8	31 ± 8	75.8
Na	512 ± 215	487 ± 188	434 ± 152	441 ± 157	13.9
ORP, mV	–64 ± 72	6 ± 135	202 ± 177	ND ^c	–
Alkalinity, mg CaCO ₃ L ⁻¹	7,027 ± 2,175	5,469 ± 1,505	1,422 ± 1,013	1,580 ± 835	77.5
pH	7.80 ± 0.35	7.78 ± 0.23	7.98 ± 0.50	9.72 ± 0.69	–
EC, mS cm ⁻¹	14.97 ± 4.36	14.09 ± 4.08	7.25 ± 1.91	6.58 ± 1.57	56.0

^aData are means ± standard deviations for 122 sampling dates (2 years of continuous operation).

BOD₅, 5 days biochemical oxygen demand; COD, chemical oxygen demand; EC, electrical conductivity; ORP, oxidation reduction potential; TKN, total Kjeldahl nitrogen; TP, total phosphorus; TS, total solids; TSS, total suspended solids; VSS, volatile suspended solids.

^bExcept for ORP (mV), EC (mS cm⁻¹), and pH. ORP values are standard hydrogen electrode (Eh); measurements were done weekly in grab samples (n = 56).

^cND, Not Determined.

and other value-added products. It also allowed treatment of the liquid through biological nitrogen steps and phosphorus recovery/disinfection in an economical way to meet specific environmental standards. Compared to the flushed manure, the separation process concentrated the suspended solids > 25 times. It produced a relatively dry manure cake with 24.9% solids. The capture of the fine suspended solids through flocculation resulted in large decreases in TSS (90%) and COD (63%) concentrations (Table 1). TKN and total P were reduced by approximately 30% and 70%, respectively. Organic N and P were reduced 69 and 84%, respectively. In contrast, the soluble ammoniacal nitrogen (NH₄-N) and soluble P were unaffected by the solids separation process. The high-rate solid-liquid separation was also effective reducing heavy metals Cu and Zn concentrations; this was one of

the five environmental treatment objective of EST. Initial Cu and Zn concentrations (16.8 and 25.4 mg L⁻¹) were both reduced > 88% just with the high-rate solid-liquid separation.

Biological Ammonia Treatment

The NDN step using the MLE process configuration treated NH₄-N effectively. Nitrification was accomplished using high performance nitrifying sludge (HPNS) adapted to high-ammonia and low temperatures (Vanotti et al., 2013). The pre-denitrification configuration of the MLE process allowed suspended denitrifying bacteria to consume most of the COD and BOD₅ remaining in the wastewater after solid-liquid separation. The average ratio COD/TN of the manure liquid after solid-liquid separation was 5.6 and appeared a good

balance for N removal in this system without external carbon addition. On average, the NDN step reduced COD by 87% and BOD₅ by 98% relative to their concentration after solid-liquid separation (Table 1). The average ammonia (NH₄-N) removal efficiency was high (average = 90%) in spite of large variations of influent NH₄-N concentrations (310–3,445 mg L⁻¹) and monthly NH₄-N loading rates (14.7–117.3 kg NH₄-N d⁻¹; average load = 47.7 kg d⁻¹). Average TKN removal efficiency was 91%. Influent TKN concentration varied from 810 to 4,220 mg L⁻¹, and the N mass loading rates varied monthly from 16.8 to 166.1 kg TKN d⁻¹ (average TKN load = 58.7 kg d⁻¹). The process responded well to cold temperatures experienced during evaluation. Monthly average water temperatures during cold weather (Dec–Feb) were 9.1–11.3°C, and corresponding daily minimum average water temperatures were 8.0–9.1°C. The N removal performance in this study was consistent with the performance obtained by Riaño and García-González in a full-scale, on-farm treatment plant in Castilla y León region, Spain, that also used a combination of high-rate solid-liquid separation with flocculants and nitrification-denitrification to treat raw swine manure: nitrification-denitrification step alone removed 84.5% of COD, 95.9% of TKN and 98.0% of NH₄-N, while the combined system removed 97% of COD, 97% of TKN and 89% of TP. In France, a combination of solid-liquid separation using centrifuges and biological NDN treatment have been successfully established at large scale in approximately 300 units treating about 15% of the swine effluents produced in Brittany region to remove N surplus and also the P excess (Bernet and Béline, 2009).

The N removal unit produced a cleaner and oxidized effluent with 124 mg L⁻¹ of NH₄-N, 221 mg L⁻¹ of NO₃-N + NO₂-N, 227 mg L⁻¹ of TSS, 62 mg L⁻¹ of BOD₅, and ORP 202 mV (Table 1). Part of the N treated effluent was recycled on the farm to refill the pits under the barns and facilitate flushing (under the previous anaerobic lagoon system, the anaerobic lagoon liquid supernatant was used for the flushing). This recycling of clean water with low ammonia into the barns improved the

environment in the barns that benefited health and productivity of the animals. Production records for the five growth cycles before and the five cycles after conversion of waste management technology showed improvements in several animal productivity and health indicators. With the new manure treatment system, the animal mortality was decreased 47%, daily weight gain was increased 6.1%, and feed conversion was improved 5.1%. As a result, the farmer sold 28,100 kg more hogs (a 5.8% increase) per growth cycle using the new system compared to the previous anaerobic lagoon management.

Phosphorus Recovery Treatment

The clarified effluent from the biological N removal step was treated with hydrated lime that precipitated the phosphorus at process pH of 9.5. Removal efficiencies of the soluble phosphate with the treatment system averaged 80% for wastewater containing an average of 94 ± 63 mg PO₄-P L⁻¹ (Table 1). The overall treatment system (three steps) recovered 93.3% of the TP, with two steps contributing significantly: the high-rate solid-liquid separation (first step) removed the organic P efficiently (from 380 to 62 mg P L⁻¹), and phosphorus module (third step) removed the soluble P efficiently (from 76 to 19 mg PO₄-P L⁻¹).

Odor and Pathogen Reductions

The substantial elimination of malodorous compounds was an important environmental standard to meet. A complete odor evaluation in this system have been reported by Loughrin et al. (2009). Five characteristic aromatic malodor compounds (phenol, p-cresol, p-ethylphenol, p-propylphenol, indole, and skatole) were measured in the liquid at the successive stages of the treatment system (Table 2). Results obtained showed a 99.9% reduction of total odors (the sum of concentration of the five malodor compounds) in the treated effluent compared to the untreated swine manure. The solid-liquid separation step was not efficient to separate the malodorous compounds in the flushed manure and 89% of these compounds remained in the liquid fraction. However, they were effectively destroyed during the

TABLE 2 | Removal of odor compounds and pathogen indicator microorganisms by on-farm wastewater treatment system using high-rate solids separation coupled with ammonia and phosphorus treatment.

	Raw flushed swine manure (system influent)	After solid-liquid separation treatment	After ammonia treatment	After phosphorus treatment (system effluent)	Removal efficiency with system
Odor Compounds^a	ng mL⁻¹				%
Total	71,269 ± 14,733	63,642 ± 12,366	40 ± 17	44 ± 11	99.9
Skatole	2,943 ± 496	2,540 ± 420	0 ± 0	0 ± 0	100.0
Pathogen/pathogen indicators^b	log₁₀ cfu mL⁻¹				%
Total fecal coliforms (Mac+ 44.5)	4.11 ± 0.19	3.47 ± 0.16	0.84 ± 0.23	0.17 ± 0.18	99.99
Enterococci (mEnt)	5.11 ± 0.13	3.62 ± 0.18	1.53 ± 0.34	1.14 ± 0.35	99.99
Salmonella (XLT4)	1.79 ± 0.11	1.14 ± 0.30	0.00 ± 0.00	0.00 ± 0.00	100.00

^aData are means ± standard error of 15 monthly determinations that included cold and warm weather months. Total odor compounds are the sum of concentrations of five malodorous compounds contained in the liquid (phenol, p-cresol, p-ethylphenol, indole, and skatole) that are characteristic of swine manure.

^bData are means ± standard error of log₁₀ colony forming units (cfu) per mL for duplicate samples of six determinations that included cold and warm weather conditions.

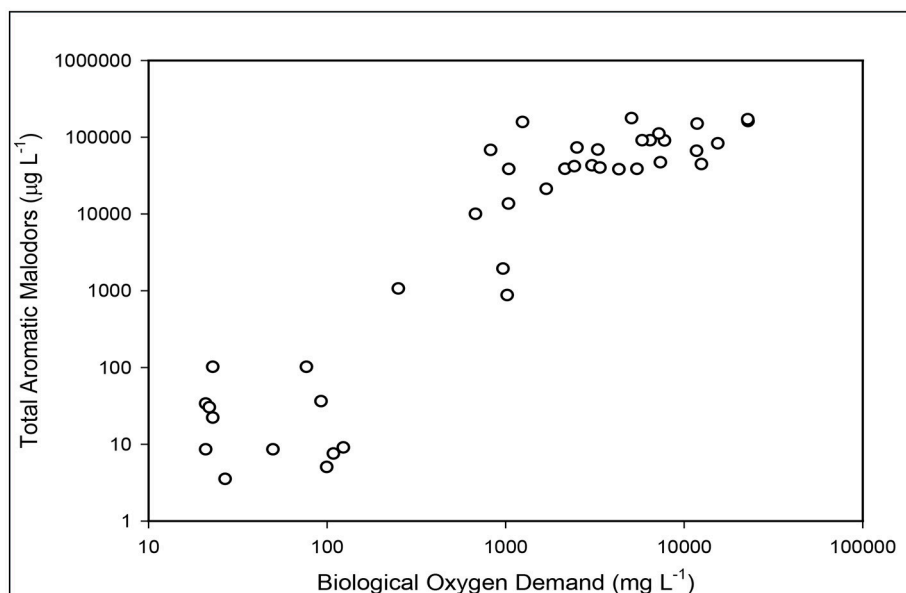


FIGURE 6 | Relationship between total odor compounds in the liquid and BOD₅ concentration as the liquid is being treated in the new plant.

subsequent biological ammonia treatment step. One important finding was that the concentration of total odor compounds in the liquid was related to BOD₅ concentration (**Figure 6**). This relationship was used later by the State Permitting Authority to determine the level of odor acceptable using this innovative animal waste management system, as a replacement of measuring odor intensity levels at the property level, which was more complicated to measure. It was established that, to demonstrate odor compliance, the BOD₅ concentration in the effluent samples shall not exceed 150 mg L⁻¹.

The substantial elimination of pathogens was another important environmental standard to meet. The multistep treatment system was efficient reducing pathogens in the liquid swine manure (**Table 2**). Results showed a steady reduction of microbial indicators and pathogens by each step in the treatment system. The largest reduction was obtained in the biological ammonia removal step (2.63 and 2.09 log₁₀ reductions for total fecal coliforms and enterococci, respectively). The phosphorus treatment with its high pH provided a level of disinfection needed to meet the EST criteria of 4-log pathogen indicator reduction (99.99%). *Salmonella*, which was present in the raw manure at 1.79 log₁₀ cfu/mL, was eliminated by the second step in treatment system.

Water Quality Improvements in Lagoons

Initial Lagoon Conditions

Table 3 and **Figure 7** show the water quality changes in the two study lagoons during the 36 months monitoring period. **Table 3** show yearly changes of all the water quality parameters measured, and **Figure 7** show monthly changes of selected parameters. This monitoring period includes a common year before the project started (0–12 months) when both lagoons received raw

manure directly from the barns (anaerobic lagoon management, **Figure 3A**) and the subsequent 2 years (12–36 months) when lagoon 1 received all the effluent from the new treatment plant, while lagoon 2 stopped receiving wastewater (raw or treated) (new manure management, **Figure 3B**). During initial conditions (0–12 months), the liquid characteristics in the two lagoons were similar as determined by water quality indicators shown in **Table 3**. The average TKN and NH₄-N concentrations (539–671 mg L⁻¹ and 371–485 mg L⁻¹, respectively) were consistent with range values of 340–650 mg TKN L⁻¹ and 280–570 mg NH₄-N L⁻¹ reported for liquid in 10 swine lagoons in North Carolina (Bicudo et al., 1999). Under traditional management, the monthly average TKN concentrations varied significantly within a year, from a low of about 325 to a high 829 mg L⁻¹ in lagoon 1 and 487–819 in lagoon 2 (**Figure 7**). The NH₄-N, which comprised 71% of the TKN, followed the same cyclic variation within a year. These N concentration cycles in the traditional lagoon followed seasonal temperature variations (**Figure 5**) with the lowest NH₄-N concentrations at the end of summer and highest at the end of winter. This is consistent with the previous study that monitored NH₄-N in traditional lagoon during 3 years (Vanotti and Szogi, 2008).

Lagoon Liquid Cleanup

In month 12 of the 3 years water quality monitoring period, manure flushes to both lagoons were halted and the conventional anaerobic lagoon treatment was discontinued. At that point, lagoon 1 received all the treated effluent generated by the new wastewater treatment plant. It went from receiving raw waste from 3 barns (permitted for 2,205-head feeder-to-finish swine) to receiving treated waste from 7 barns (5,145-heads). Lagoon 2 did not receive any effluent (treated or untreated), only rainwater,

TABLE 3 | Lagoon liquid analyses of two swine lagoons before and after implementation of new treatment system using high-rate solids separation coupled with ammonia and phosphorus treatment^a.

Water quality parameter ^b	Sampling period (3 years)							
	Year before the project started (traditional lagoon management)		1st year of new treatment operation		2nd year of new treatment operation		Lagoon effect	Time (year) effect
	Lagoon 1	Lagoon 2	Lagoon 1	Lagoon 2	Lagoon 1	Lagoon 2		
mg L ^{−1b}							Prob > t	
TSS	532 ± 189	435 ± 183	417 ± 150	424 ± 109	207 ± 94	290 ± 64	0.9412	<0.0001
VSS	417 ± 138	321 ± 117	320 ± 111	307 ± 86	140 ± 78	221 ± 77	0.7159	<0.0001
TS	11,709 ± 846	12,164 ± 2,750	9,728 ± 1,419	10,520 ± 1,346	8,174 ± 712	8,332 ± 1,335	0.2859	<0.0001
VS	2,968 ± 1,181	2,747 ± 1,050	1,960 ± 762	2,118 ± 742	1,420 ± 307	1,625 ± 326	0.8013	<0.0001
COD	2,298 ± 799	2,126 ± 301	1,548 ± 443	1,794 ± 206	907 ± 373	1,113 ± 404	0.3796	<0.0001
Soluble COD	1,390 ± 192	1,628 ± 311	1,068 ± 296	1,255 ± 102	668 ± 264	761 ± 233	0.0032	<0.0001
BOD ₅	190 ± 150	219 ± 135	165 ± 130	195 ± 118	81 ± 80	71 ± 44	0.5518	<0.0001
TKN	539 ± 163	671 ± 108	291 ± 89	359 ± 103	140 ± 96	144 ± 76	0.0088	<0.0001
NH ₄ -N	371 ± 167	485 ± 128	181 ± 75	251 ± 117	65 ± 66	60 ± 53	0.0215	<0.0001
NO ₂ + NO ₃ -N	0.0 ± 0.0	0.0 ± 0.0	1.1 ± 1.9	0.1 ± 0.4	11.5 ± 23.5	0.0 ± 0.0	0.0550	0.0697
TP	76 ± 15	83 ± 15	65 ± 15	73 ± 23	77 ± 14	86 ± 14	0.0556	0.0168
Soluble P	50 ± 6	54 ± 7	47 ± 10	52 ± 14	64 ± 13	67 ± 15	0.1244	<0.0001
K	1,391 ± 132	1,537 ± 132	1,327 ± 150	1,436 ± 192	1,388 ± 147	1,332 ± 177	0.1089	0.1099
Ca	46.6 ± 15.3	53.4 ± 19.0	51.1 ± 13.8	57.5 ± 15.5	32.6 ± 6.4	35.3 ± 6.5	0.1090	<0.0001
Mg	13.1 ± 2.01	13.4 ± 3.0	16.1 ± 5.3	17.7 ± 6.4	23.7 ± 6.4	28.7 ± 11.2	0.1291	<0.0001
Zn	0.71 ± 0.42	1.04 ± 0.58	0.69 ± 0.50	1.21 ± 0.56	0.37 ± 0.14	0.61 ± 0.14	0.0009	0.0011
Cu	0.49 ± 0.20	0.67 ± 0.32	0.17 ± 0.06	0.49 ± 0.12	0.09 ± 0.04	0.22 ± 0.08	<0.0001	<0.0001
Fe	2.29 ± 0.48	2.71 ± 0.40	1.43 ± 0.52	1.86 ± 0.34	0.95 ± 0.43	1.35 ± 0.34	<0.0001	<0.0001
S	38.6 ± 9.0	33.6 ± 14.1	46.5 ± 6.4	42.8 ± 15.1	27.5 ± 10.2	39.1 ± 13.1	0.7181	0.0021
Na	391 ± 45	442 ± 58	389 ± 54	447 ± 87	424 ± 70	398 ± 67	0.0822	0.9379
ORP, mV	60 ± 127	−4.8 ± 102	181 ± 168	179 ± 132	287 ± 126	287 ± 119	0.4901	<0.0001
Alkalinity, mg CaCO ₃ L ^{−1}	3,438 ± 1273	3,621 ± 470	2,360 ± 253	2,858 ± 356	1,817 ± 318	1,863 ± 371	0.0830	<0.0001
pH	8.19 ± 0.15	8.11 ± 0.20	8.20 ± 0.32	8.09 ± 0.29	8.28 ± 0.18	8.22 ± 0.19	0.1542	0.2469
EC, mS cm ^{−1}	8.19 ± 1.38	9.37 ± 0.86	6.74 ± 0.40	7.58 ± 0.38	6.02 ± 0.68	5.66 ± 0.90	0.0046	<0.0001

^aData are means ± standard deviations of monthly samples. During the previous year, both lagoons were managed as typical anaerobic swine lagoons receiving raw swine manure. Afterwards, lagoon 1 received all the swine effluent after being treated in the new plant, and lagoon 2 did not receive any swine influent (treated or untreated). Monthly changes for selected parameters are shown in **Figure 7**.

^bUnits in mg L⁻¹ except for ORP, EC, and pH. ORP values are standard hydrogen electrode (Eh).

and its situation resembles that of an inactive lagoon after depopulation of pigs (Sheffield, 2000). Excess water over storage capacity of the lagoons was applied onto crops and forages on the farm. Rainfall averaged 1,026 mm per year and contributed 7,500 m³ of rain water annually to each lagoon (drainage area of each lagoon was 0.634 ha) or 13,000 m³ of rainwater per lagoon in the 2 years period. Actual lagoon volumes were 9,565 ± 750 m³ (lagoon 1) and 13,057 ± 1,180 m³ (lagoon 2). Thus, rain alone renovated 70 and 50% of the total lagoon volumes per year (lagoon 1 and 2, respectively). Lagoon 1 received also the clean plant effluent, 11,552 m³ per year (31.6 m³ d⁻¹) with a renovation capacity of 1.2 lagoon volumes per year (HRT = 0.8 years).

Statistical tests showed significant reduction with time on most water quality parameters measured in the lagoons ($P < 0.001$), an effect that was consistent across lagoons (**Table 3**). The

exceptions were K, Na, TP, and pH, which were not significantly changed with time. In three instances when statistical differences ($P < 0.01$) in water quality parameters between lagoons occurred (Cu, Zn, and Fe, **Table 3**), the concentration decrease was quicker in lagoon 1 that received the treated water (**Table 3** and **Figure 7**). By the second year of new treatment operation, the following average reductions in water constituents were realized in lagoon 1 (**Table 3**): 61% of TSS, 66% of VSS, 57% of BOD₅, 74% of TKN, 82% of NH₄-N, 48% of Zn, 82% of Cu, 47% alkalinity, and 26% of EC. Corresponding reductions in lagoon 2 (inactive) were: 33% of TSS, 31% of VSS, 68% of BOD₅, 79% of TKN, 88% of NH₄-N, 41% of Zn, 67% of Cu, 49% alkalinity, and 40% of EC. Therefore, when multistep EST treatment technology is implemented in a swine operation with anaerobic lagoons, an additional environmental benefit is obtained: the progressive cleanup of the lagoon liquid without having to stop production.

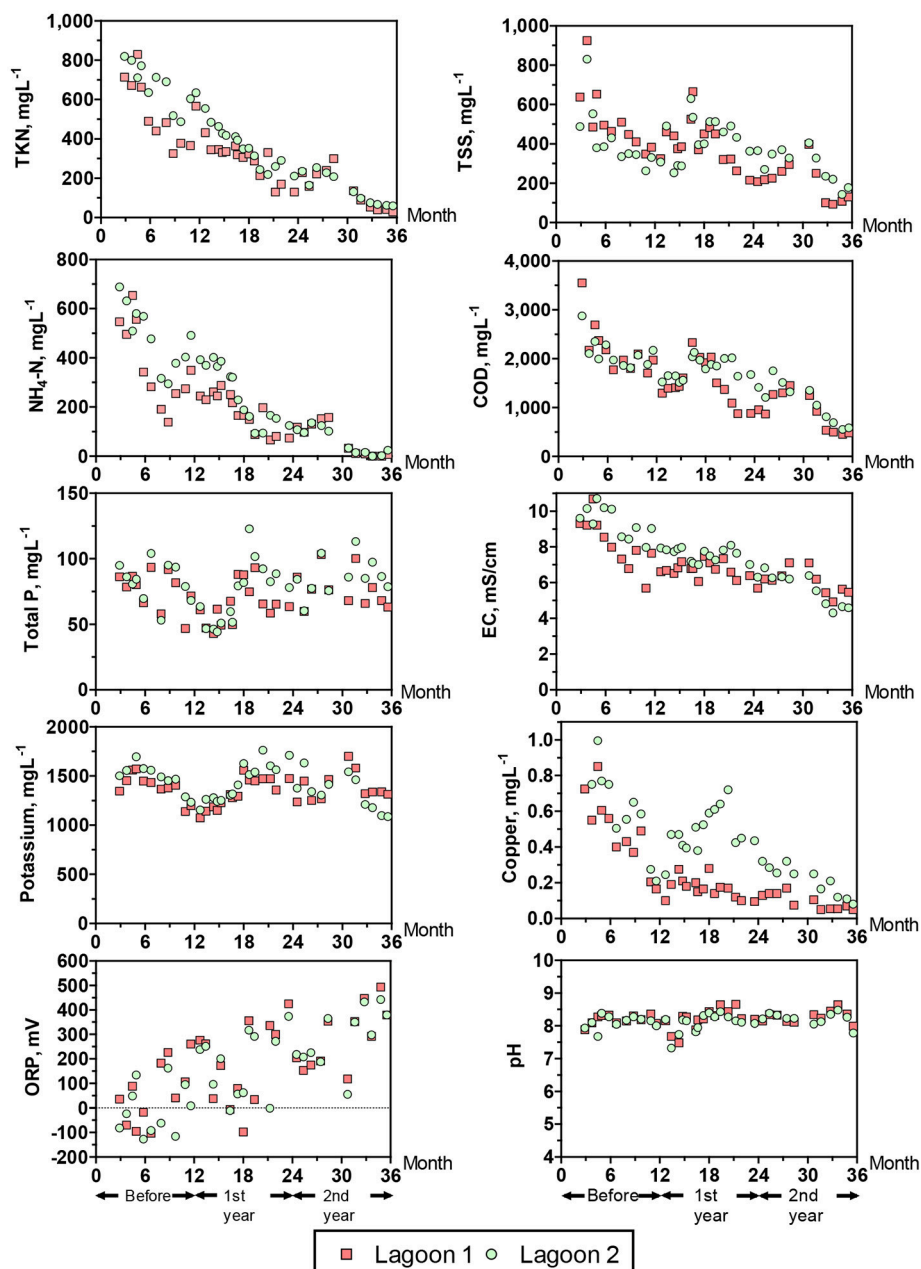


FIGURE 7 | Water quality changes in the two study lagoons during a 36 months period. 0–12 months = lagoons being managed as typical anaerobic swine lagoons receiving raw swine manure. Thereafter, lagoon 1 received all the swine effluent after treatment in the new plant, and lagoon 2 did not receive any swine influent (treated or untreated). Yearly averages are shown in **Table 3**.

Even though lagoon 1 served the production of more than twice the number of animals than it did before with the traditional lagoon system (average LAW increased from 144 to 336 Mg), remarkably, the overall cleaning performance of the new plant effluent on lagoon 1 liquid was similar to the cleaning performance by rainwater alone under lagoon inactivation and abandonment of production (lagoon 2). Indeed, the results of this study were used by the State Permitting Authority to issue Permit

No AWI820164 using the innovative animal waste management system that would allow the expansion of total swine animal capacity in this farm from 5,145 to 11,015 feeder-to-finish using the same acreage (12.9 ha).

The NH₄-N concentration in the lagoons before the project started were 371 ± 167 mg L⁻¹ in lagoon 1 and 485 ± 128 mg L⁻¹ in lagoon 2 (**Table 3**). During the last 6 months the NH₄-N concentrations were very low: 10.0 ± 11.9 mg L⁻¹ in lagoon 1 and

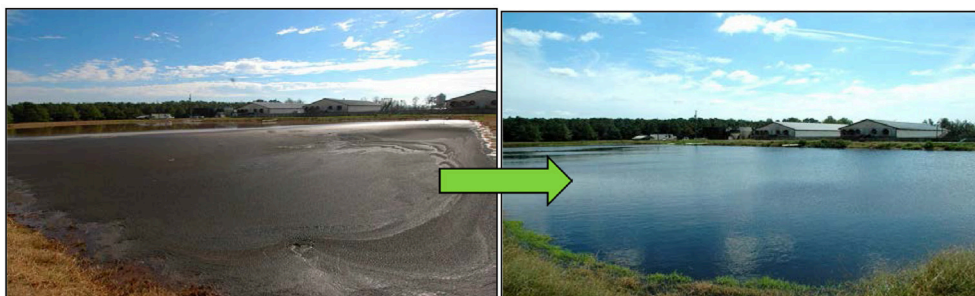


FIGURE 8 | Swine lagoon conversion into aerobic pond. Picture on the left shows Lagoon 1 under traditional management before start of the project, and picture on the right shows the same lagoon after the wastewater treatment plant (background) was in operation for about 10 months.

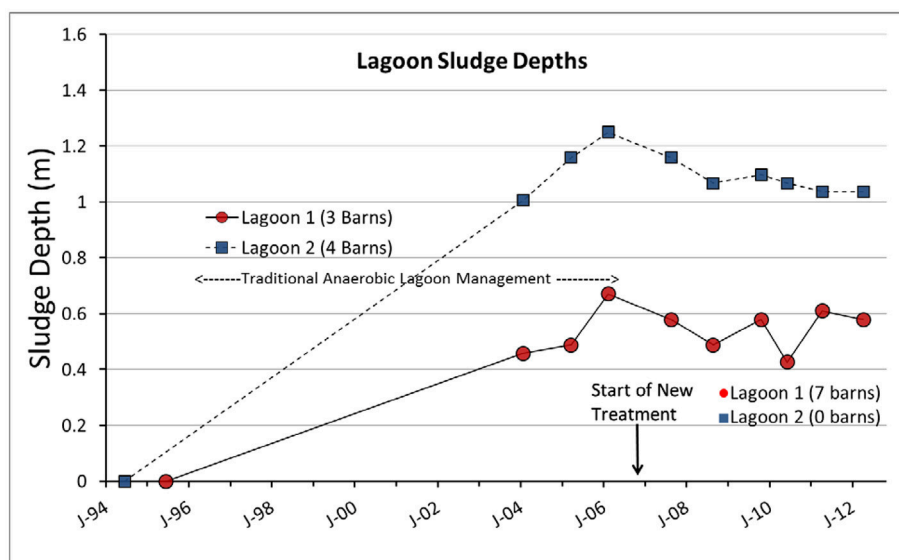


FIGURE 9 | Sludge depth dynamics of the two swine lagoons. The new treatment plant was installed after 11–12 years of conventional anaerobic lagoon treatment.

$14.9 \pm 12.5 \text{ mg L}^{-1}$ in lagoon 2 (months 30–36, **Figure 7**); they approached average concentration of $4 \text{ mg NH}_4\text{-N L}^{-1}$ reported for 30 lagoons in swine operations in North Carolina that were depopulated and inactive for 6 ± 4 years (Sheffield, 2000). In a companion paper, Ro et al. (2018) measured the ammonia emissions from this project including lagoons using open-path tunable diode laser and found that the ammonia emissions were reduced to below detectable levels. Another important parameter is the effect on EC (water salinity) that is an important water quality guideline on crop productivity, for example FAO (1994) guidelines indicate that the yield potential of irrigated cotton is reduced from 100 to 90% to 75 and 50% with irrigation water EC of 5.1, 6.4, 8.4 and 12 mS cm^{-1} , respectively. In the study, the average EC of the lagoon liquid before the project started was $8.19\text{--}9.37 \text{ mS cm}^{-1}$ in lagoon 1 and 2, respectively. The EC was lowered to 5.33 ± 0.37 and $4.51 \pm 0.19 \text{ mS cm}^{-1}$ during the last quarter (**Figure 7**), which is optimal for cotton irrigation. As clean plant effluent and/or rain water replaced the liquid in the two lagoons, they become aerobic. From the point of

view of microbial metabolism, a redox potential (ORP) > 300 millivolts is associated with aerobic, oxidized conditions (Reddy et al., 2000). The transition from anaerobic to aerobic, oxidized conditions took about 1.5 years. Average ORP were -4.8 to 60 millivolts during traditional management before the project started (**Table 3**); they increased steadily with time to reach consistent levels > 300 millivolts in the second half of second year (months 30–36, **Figure 7**). In addition to these chemical indicators of aerobic conditions, in 10 months of the new manure management the lagoon 1 changed color from brown to blue (**Figure 8**).

Changes in Sludge Accumulation in the Lagoons

Before the conversion and under traditional anaerobic lagoon management, the sludge in lagoon 1 accumulated to a depth 0.67 m (or $2,620 \text{ m}^3$) in 11 years of continuous swine production (serving 2,205-head feeder-to-finish) and sludge

TABLE 4 | Chemical composition of the lagoon sludges^a.

Component	Sludge lagoon 1	Sludge lagoon 2
	g L ^{-1b}	
TS	208.3 ± 111.4	177.3 ± 60.9
VS	140.3 ± 102.4	105.8 ± 36.9
TSS	88.9 ± 25.0	91.9 ± 12.0
COD	105.9 ± 39.0	105.1 ± 20.4
Soluble COD	1.5 ± 0.7	1.7 ± 0.8
BOD ₅	4.44 ± 2.98	5.36 ± 3.10
TKN	6.23 ± 2.43	6.41 ± 2.45
NH ₄ -N	0.51 ± 0.17	0.60 ± 0.15
NO ₂ + NO ₃ -N	0 ± 0	0 ± 0
TP	6.59 ± 0.92	6.58 ± 1.10
Soluble P	0.26 ± 0.14	0.18 ± 0.04
K	1.65 ± 0.19	1.53 ± 0.15
Ca	3.50 ± 1.37	4.13 ± 1.18
Mg	3.18 ± 1.82	3.35 ± 1.71
Zn	0.37 ± 0.22	0.45 ± 0.14
Cu	0.40 ± 0.13	0.42 ± 0.12
Fe	0.72 ± 0.41	0.85 ± 0.22
S	1.34 ± 0.23	1.34 ± 0.20
Na	0.45 ± 0.05	0.42 ± 0.03
Alkalinity	26.2 ± 10.7	25.28 ± 7.05
pH	7.89 ± 0.27	7.80 ± 0.21

^aData are means ± standard deviations for 5 sampling dates (months 0–24).

^bUnits in g L⁻¹ except for pH.

in lagoon 2 accumulated to a depth of 1.25 m (volume 4,440 m³) in 12 years of continuous swine production (serving 2,940-head feeder-to-finish) (Figure 9). Therefore, the average rate of sludge accumulation in the two lagoons was 0.1170 ± 0.0127 m³ sludge/feeder-to-finish head/year. It was consistent with the sludge generation standard for NC anaerobic swine lagoons of 0.1249 m³ sludge/feeder-to-finish head/year (33 gal/animal of 135 lb/year) (AG-604, 2000).

After conversion, the sludge accumulation on both lagoons was halted (Figure 9). During the 6 years of new treatment, the sludge depth in lagoon 1 (that received all the plant effluent) did not increase; it was stabilized at a depth of about 0.55 ± 0.07 m (volume = 2,100 ± 290 m³). Similarly, lagoon 2 (discontinued lagoon) did not accumulate more sludge after discontinuation; the sludge depth remained about constant at 1.08 ± 0.05 m (volume = 3,750 ± 180 m³).

Table 4 shows the composition of the sludges in the two lagoons determined five times at the beginning of the study (months 0–24). The sludges were of mineral nature, thick, black, with tar like smell, with similar chemical composition in the two lagoons (Table 4). A salient characteristic is the large amount of P contained in the lagoon sludges. Considering sludge volume and P concentration, there were 17.2 and 29.2 metric tons of P (39.5 and 66.9 metric tons P₂O₅) in lagoon 1 and 2, respectively. Therefore, new technologies that could harvest the P contained

in lagoon sludges could have a great impact on global P cycling. One such technology is the Quick Wash process presented in this special issue (Szogi et al., 2018). It recovered 80% of the P from swine lagoon sludges.

CONCLUSION

More and more often, new treatment systems for manure combine three or four process units to meet various environmental standards and recovery targets. In North Carolina, USA, construction of new swine farms or expansion of existing swine farms are required new waste management systems that can replace anaerobic lagoon treatment for the waste and meet new environmental standards of ammonia and odor emissions, pathogens release, and the substantial elimination of soil and groundwater contamination by nutrients (phosphorus and nitrogen) and heavy metals. A treatment system that met these multiple standards was implemented at full-scale in a swine farm and operated for 6 years. It combined high-rate solid-liquid separation with N and P removal processes. The treatment plant removed from the manure: 97% of TSS, 90% of VS, 99% of BOD₅, 96% of TKN and NH₄-N, 93% TP, 99% of Zn and Cu, 99.9% odors and 99.99% pathogens. This study determined the water quality improvements in lagoons by an innovative swine manure treatment system operating at full-scale during five pig production cycles. After conversion, the sludge accumulation in the lagoons was halted. As plant effluent or rainwater replaced the liquid in the old lagoons, they became aerobic (Eh > 300 millivolts). In 2 years, the NH₄-N concentration in the lagoons liquid was reduced from the 370 to 485 to lower than 15 mg L⁻¹. While clean water is more valuable for both environmental quality and crop production, it is significant that the treatment process transformed the lagoon's water from a constituent-laden legacy condition to relatively cleaner water. Moreover, the transformation was accomplished while doubling the number of animals.

AUTHOR CONTRIBUTIONS

The author MV has designed and conducted the full-scale project, performed data summarization, and written the manuscript. KR measured ammonia emissions. AS assisted with water quality work. JL did odor quantification. PM did the pathogen assessment.

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High-Rate Solid-Liquid Separation Coupled With Nitrogen and Phosphorous Treatment of Swine Manure: Effect on Ammonia Emission

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A new treatment system was developed to meet multiple environmental performance standards including to substantially reduce ammonia emissions. It was tested full-scale for 2-years in a 5,145-head finishing swine farm with two anaerobic lagoons. The system combined high-rate solid-liquid separation with nitrogen and phosphorus removal processes. Both vertical radial plum mapping (VRPM) and floating static chamber techniques were used to measure NH₃ emission fluxes from anaerobic storage lagoons and the total farm-level NH₃ emission rates. The VRPM used an open-path tunable diode laser absorption spectroscopy (TDL) and the flux chamber used a photoacoustic gas analyzer to accurately measure NH₃ concentration. After the treatment system started, one of the two lagoons became inactive without receiving anymore flushed manure. The ammonia emission flux from the other lagoon with the treated effluent decreased from 43.9 to 6.8 kg-N ha⁻¹ d⁻¹ 1.5 years after implementation of the new treatment system. The NH₃ emission flux from the inactive lagoon also decreased similarly because the already stored old manure of the lagoon prior to inactivation was diluted with rainfalls and lost some NH₃ via volatilization. The total farm-level NH₃ emission rates decreased from 1.72 g s⁻¹ to below detection level of the VRPM technique. Using the minimum detection level of the TDL with $R^2 > 90\%$ (i.e., 8.1 8.1 μL L⁻¹-m), the total farm-level NH₃ emission rates in the second year were less than 0.04–0.15 g s⁻¹. These results suggested that the impact of the new treatment system on NH₃ emission reduction was equivalent to closing conventional swine lagoons while actively growing 5,145 pigs with minimal ammonia emissions from the farm.

Keywords: total farm ammonia emission rate, swine lagoon, manure treatment, vertical radial plume mapping method, lagoon emission flux

INTRODUCTION

Ammonia (NH₃) is an important fugitive gas mostly emitted from livestock operations in the United States (Doorn et al., 2002; Ro et al., 2017). Ammonia, a precursor of nitrate due to microbial nitrification/denitrification, causes acidification of both soil and surface water, and eutrophication in water bodies. It is also a principal source of atmospheric aerosols. Anaerobic

lagoons are being widely utilized in the southeastern U.S. for storage and treatment of manure from confined swine production operations. The anaerobic treatment reduces the organic load of liquid manures but releases inorganic nitrogen as ammonia. Thus, ammonia emission from the lagoons is of environmental and health concerns in geographic areas with very intense confined swine production (Barker, 1996; Aneja et al., 2000; McCubbin et al., 2002; Szogi et al., 2006; Blunden and Aneja, 2008; Westerman et al., 2010). Addressing the environmental and health issues caused by ammonia emissions and potential contamination of water bodies with swine manure effluents Vanotti et al. (2010), developed an on-farm wastewater treatment system and demonstrated its high efficacy to meet multiple environmental performance standards (Vanotti et al., 2009) on a 5,145-head swine finishing farm. The multi-stage treatment system used solid separation, nitrification-denitrification, and phosphorous removal/disinfection processes. The new treatment system replaced the conventional anaerobic treatment lagoons of a swine farm and improved lagoon water quality dramatically. The treatment system removed 97% of total suspended solids (TSS), 99% of biochemical oxygen demand (BOD), 93% of total phosphorous (TP), 96% of total Kjeldahl nitrogen (TKN), and ammoniacal nitrogen ($\text{NH}_4\text{-N}$) from the manure (Vanotti et al., 2018).

Although the new treatment system significantly improved the lagoon water quality (Vanotti et al., 2018) and reduced odors (Loughrin et al., 2009), its impact on ammonia (NH_3) emission had not been reported. The improved lagoon water quality would directly impact the NH_3 emissions from the lagoon and the animal houses using the cleaner plant-treated water for flushing manure (Szogi et al., 2006; Ro et al., 2008). Therefore, it is of great interest to assess the NH_3 emission reduction from the whole swine farm utilizing the new second-generation manure treatment system.

Micrometeorological techniques such as the integrated horizontal flux (IHF) and the backward Lagrangian stochastic (bLS) dispersion techniques can be used to measure the total ammonia emission from a swine farm. Although the bLS technique has shown its high accuracy in measuring gas emissions from point and distributed emission sources (Ro et al., 2009, 2011, 2013, 2014), one of the concerns for the use of the bLS technique in measuring emissions from a real farm with a variety of structures (buildings, stand pipes, etc.) is its underlying assumption of idealized wind flow over flat and homogenous terrain. The wind disturbance caused by farm building structures can be minimized by measuring downwind concentrations further downwind distance from the farm such as 10 times the height of the buildings (Flesch et al., 2005). However, measuring downwind concentration at longer distances from an emission source may not be feasible for certain conditions due to limited open space or dilution of emitted gas concentration below the detection limit of an analytical technique.

In contrast, the IHF technique, a mass-balance based method, is not very sensitive to changes in wind turbulence, which can be used to measure an average gas emission rate of a large area (Wilson et al., 1983). The U.S. Environmental Protection Agency published the vertical radial plum mapping (VRPM) technique

based on the IHF principles as Other Test Method 10 (OTM–10) in measuring fugitive gas emission rates from closed landfills (EPA, 2005). The VRPM technique estimates the horizontal flux of gas passing downwind of the emission source based on measured wind speed profiles and path integrated concentrations (PICs) (Ro et al., 2009, 2011; Viguria et al., 2015). The VRPM utilizes a bivariate Gaussian smooth basis function minimization (SBFM) to reconstruct a crosswind-smoothed mass-equivalent concentration map in a vertical plane from the downwind PIC data. Once all parameters for the bivariate Gaussian function are measured for a specific run, the VRPM procedure calculates the mass-equivalent concentration values for every square elementary unit ($4 \times 4 \text{ m}$) in a vertical plane. Then, the VRPM procedure computes and integrates the elementary unit flux over the entire vertical plane with corresponding wind speed data. Arcadis Inc. (Research Triangle Park, NC) developed the computer-based VRPM software which automatically calculates the emission rates based on the downwind PIC data, wind speed and direction information using the VRPM algorithm.

The objective of this study was to measure the ammonia emissions from the 5,145-head swine finishing farm with the new on-farm manure treatment system during a 2-year evaluation using the VRPM technique.

MATERIALS AND METHODS

Farm Description

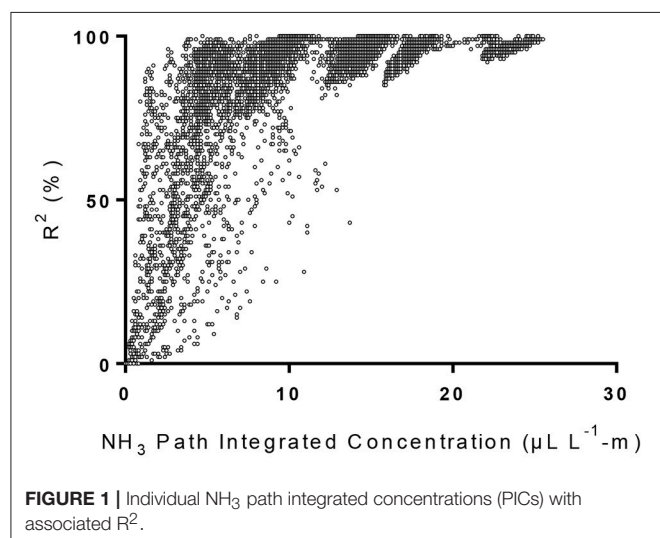
The total NH_3 emissions from a finishing swine farm in North Carolina with a full-scale manure treatment system were measured from December 2006 to September 2008. The farm had seven swine houses with a permitted capacity of 5,145 head feeder to finish (735 head/barn). Two traditional anaerobic lagoons were used for about 11 years before the new manure treatment plant started operation in December 2006. With the new treatment system, the flushing of raw wastewater into the lagoons was discontinued. The treatment system consisted of three process units in series: polymer-enhanced solid-liquid separation, biological N removal, and alkaline phosphorus extraction and disinfection. More detailed description of the new manure treatment system and the farm operation can be found elsewhere (Vanotti et al., 2018).

Open-Path Tunable Diode Laser Absorption Spectroscopy

An open-path tunable diode laser absorption spectrometer (TDL; GasFinder2.0 for NH_3 , Boreal Laser Inc., Edmonton, AB, Canada) was used to measure path-integrated concentrations (PICs) of NH_3 . The TDL mounted on an automatic positioning device (APD; Model 20 Servo, Sagebrush Technology, Inc., Albuquerque, NM) emits a collimated beam (1,512 nm) aimed at distant three mirrors (retroreflectors) from which it is reflected to the TDL's detector. The TDL was designed for a sampling rate of about 1 Hz and had continuous internal calibration updates every 40 samples using an internal reference cell. The signal from the measurement path is compared with the signal from the internal reference cell and calculate the average gas concentration in the path as $\mu\text{L L}^{-1}\text{-m}$ (i.e., $\mu\text{L L}^{-1}$ multiplied by path length

in m). The TDL also calculates the coefficient of determination (R^2) for each measurement to indicate the similarity between the waveform of the sample gas to that of the reference cell gas. A perfect match of the two waveforms would give R^2 of 1.0, and total mismatch would give R^2 of 0.0. Although the company recommends $5 \mu\text{L L}^{-1}\text{-m}$ as the minimum detection limit (MDL) for NH_3 , we decided to determine the MDL for our TDL that would yield $R^2 > 90\%$. Determining the reliable value of MDL was important because the new manure treatment system dramatically improved the lagoon water quality and the NH_3 emission was so low that the path-integrated NH_3 concentrations measured with our TDL became at or below the MDL during the second year of the manure treatment operation in 2008.

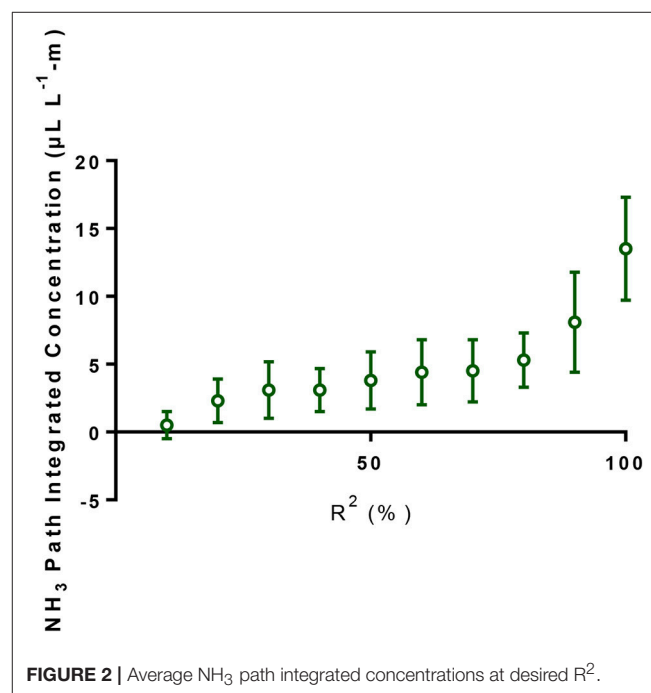
The MDL of our TDL was determined by measuring PICs through a 4.5-m PVC pipe (5.1 cm diameter) in which 3 L min^{-1} of a mixture of pure N_2 and calibrated NH_3 gas (5.0 ppm, National Welders Supply Co., Inc., NC) flew through the PVC pipe. The two ends of the PVC pipe were sealed with transparent plastic film. Initially the PVC pipe was filled with ultra-pure nitrogen gas (National Welders Supply Co., Inc.). Using a gas divider (SGD-710C, Horiba STEC, Sunnyvale, CA), gas mixtures of 10% $\text{NH}_3 + 90\% \text{ N}_2$ ($0.5 \mu\text{L L}^{-1}\text{-m NH}_3$), 30% $\text{NH}_3 + 70\% \text{ N}_2$ ($1.5 \mu\text{L L}^{-1}\text{-m NH}_3$), 50% $\text{NH}_3 + 50\% \text{ N}_2$ ($2.5 \mu\text{L L}^{-1}\text{-m NH}_3$), 70% $\text{NH}_3 + 30\% \text{ N}_2$ ($3.5 \mu\text{L L}^{-1}\text{-m}$), and 100% NH_3 ($5.0 \mu\text{L L}^{-1}\text{-m}$) were introduced into the PVC pipe. The measured PICs and the corresponding R^2 -values were plotted as shown in **Figure 1**. We also plotted the average NH_3 PICs at different R^2 -values (**Figure 2**). The average PIC was $5.3 \mu\text{L L}^{-1}\text{-m}$ at R^2 of 80% (**Figure 2**), which was similar to the NH_3 MDL ($5.32 \mu\text{L L}^{-1}\text{-m}$) reported by the U.S. Environmental Protection Agency's (EPA's) Environmental Technology Verification (ETV) statement (Myers et al., 2000). The MDL with R^2 of 90% was $8.1 \mu\text{L L}^{-1}\text{-m}$. We used the MDL of $8.1 \mu\text{L L}^{-1}\text{-m}$, when we observed the NH_3 PICs were below MDL during the second year of the new manure treatment system in 2008, to calculate the MDL for the VRPM NH_3 emission rate determination.



Total NH_3 Emission Rate Measurements Using the VRPM Technique

Total NH_3 emission rates from the swine farm (animal houses, lagoons, and the wastewater treatment system) were measured using the VRPM technique. The VRPM technique utilized the TDL mounted on the APD to measure downwind NH_3 PICs with 3 distant retroreflectors, one positioned at ground-level and the other two mounted on a weather station mast (10 or 20 m height). The upwind NH_3 concentrations were measured with a photoacoustic gas analyzer (INOVA, California Analytical, Orange, CA). The ranges of path lengths and the heights of the three retroreflectors are shown in **Table 1**. The path length between the TDL and the retroreflectors ranged from 129 to 266 m. The VRPM system was positioned downwind from the farm on a vertical plane, approximately perpendicular to the mean wind direction. The wind directions were mostly NNW or NNE. **Figure 3** shows the VRPM setup with the vertical plane approximately perpendicular to the NNE wind. The APD sequentially directed the infrared collimated beam of the TDL to each retroreflector. At each retroreflector, the TDL collected about 12–15 downwind PIC datasets before moving to the next position. Two cup anemometers (CS800-L Climatronics Wind Speed and Direction Sensor, Campbell Scientific, Logan, UT) mounted on the weather station mast at 2 and 10 m heights continuously measured wind speed and direction information during the emission monitoring campaigns.

The post-data filtering criteria recommended by the USEPA OTM-10 were used to remove error-prone data: concordance factor (CCF) > 0.8 (except when estimating the below minimum detection level emission rates with all PICs assuming to have the MDL of $8.1 \mu\text{L L}^{-1}\text{-m}$) and the mean wind direction between -10° and $+25^\circ$ from perpendicular to the vertical



optical plane. After filtering data with the above criteria, the relative accuracies (calculated emission rate/actual emission rate) of the VRPM technique were 1.23 ± 0.17 (i.e., 23% over the actual emission rate) and 0.97 ± 0.44 (i.e., 3% less than the actual emission rate) for multi land emission sources and lagoon emission, respectively (Ro et al., 2011; Viguria et al., 2015).

Lagoon Emission

In addition to the VRPM technique for measuring whole farm NH₃ emission rates, the lagoon emissions were further verified using floating chamber techniques to compare the emission fluxes before and after the new treatment system reaching its steady state condition. The lagoon NH₃ emission fluxes were measured on June 18, 2007 while the treatment system was still under a startup period and on July 24, 2008 when the new treatment system was operating steady state.

Floating Static Chamber System

The static flux chamber was made of polyvinyl chloride (PVC) pipe (15.2 cm diameter and 29.8 cm length) one end sealed with

an endcap. It was placed at the center of a 50.8 cm square foam sheet (thickness of 5.1 cm) to float on the lagoon water surface. The system was designed to submerge 11 cm of the PVC pipe under water to achieve a complete seal of the chamber headspace. The headspace gas was pulled from the chamber through Teflon tubing to the photoacoustic gas analyzer positioned at the bank of the lagoon and the analyzed gas was recirculated back to the chamber, thereby achieving well mixed condition inside the chamber. The floating flux chamber was launched into the lagoon at about 10 m away from the bank. The lagoon NH₃ emission was measured from 6 (3 points in each lagoon in 2007) to 10 (4 points in Lagoon 1 and 6 points in Lagoon 2 in 2008) different locations of the lagoons. After the treatment system started, Lagoon 2 became inactive without receiving anymore flushed manure from the pig houses. Instead, all manure flushed from the houses was treated first by the new treatment system and the treated effluent was stored in Lagoon 1 before use in crop irrigation. The increase in headspace NH₃ concentration was measured every 0.5–1 min with the photoacoustic gas analyzer for 10 min. The time-series headspace NH₃ concentration data were then used to calculate emission fluxes.

The NH₃ emission flux was calculated by fitting the time-series headspace NH₃ concentration data to a theoretical equation derived from performing a mass balance around the chamber. Assuming that the chamber is completely mixed and sealed, NH₃ mass balance around the chamber gives

$$\frac{dC}{dt} = k(C_s - C)d \quad (1)$$

TABLE 1 | Retroreflector positions.

Retroreflectors	Height above ground (m)	Pathlength (m)
Retroreflector 1 (M1)	1.0	199.2–266.2
Retroreflector 2 (M2)	5.0 or 10.0	128.6–228.5
Retroreflector 3 (M3)	10.0 or 18.4	128.6–228.5

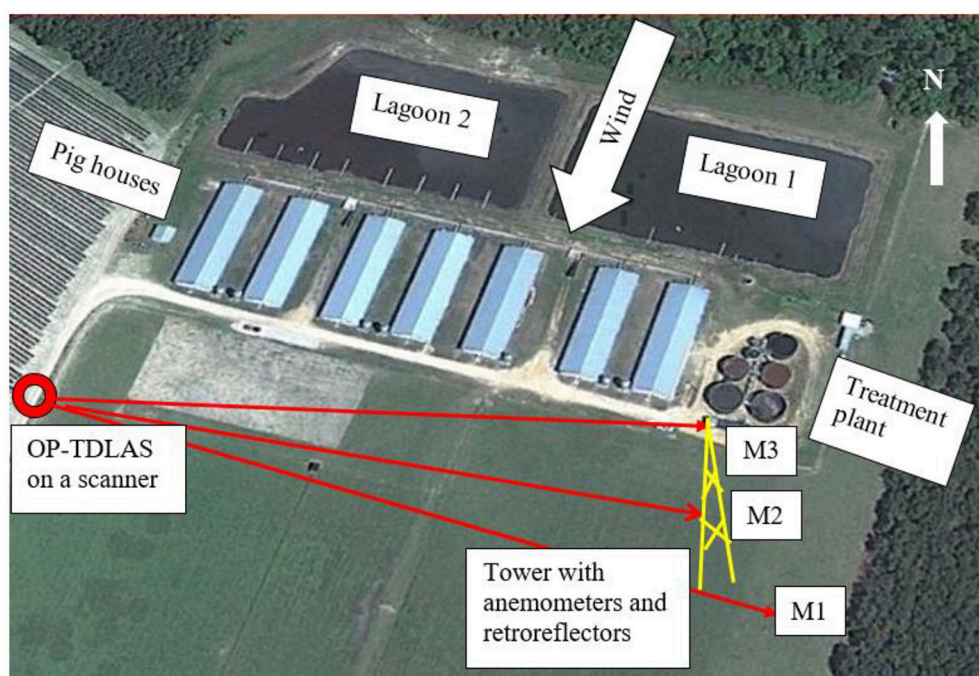


FIGURE 3 | VRPM beam geometry for NNE wind (M1, retroreflector 1 m above ground; M2, retroreflector at 5–10 m above ground; M3, retroreflector at 10–18.4 m above ground).

Where

- C = bulk headspace NH₃ concentration (mg m⁻³),
 C_S = NH₃ concentration at the water surface (mg m⁻³),
 d = mixing height of the flux chamber (m²)
 $= A_d/V$
 A_d = water surface area (m²),
 V = headspace volume (m³),
 k = mass transfer coefficient (m min⁻¹),
 t = time (min),

Integrating Equation (1) gives

$$C = C_S - (C_S - C_0) \exp\left(\frac{-kd}{t}\right) \quad (2)$$

Where C_0 = initial headspace concentration (mg m⁻³).

Instead of assuming initial concentration of ambient concentration, all three parameters (i.e., C_S , C_0 , and k) were estimated simultaneously via non-linear regression analysis of the time-series headspace NH₃ concentration data using GraphPad Prism 7.03 (GraphPad Software, Inc., La Jolla, CA). This simultaneous regression of multi-parameters were recommended by the American Society of Civil Engineers (ASCE) in estimating oxygen transfer efficiency with the similar equation (ASCE, 1992). These three parameters estimated from fitting the time-series NH₃ concentration data to Equation (2), were then used to calculate the instantaneous flux (J) as time approached zero:

$$\left. \frac{dC}{dt} \right|_{t \rightarrow 0} \left(\frac{V}{A_d} \right) = J = k(C_S - C_0) \quad (3)$$

Statistical Analysis

The central tendency and precision of measurements were presented with arithmetic averages and standard deviations (given as \pm values). All statistical parameters, analyses of variance (ANOVA) tests, and multi-regression analyses were obtained/performed using GraphPad Prism 7.03.

RESULTS AND DISCUSSION

Ammonia Emission Flux From Lagoons Using Floating Static Flux Chamber Technique

The increases in static flux chamber headspace NH₃ concentration with time at 6 (6/18/2007) and 10 (7/24/2008) different locations of the two lagoons and average NH₃ fluxes from each lagoon are shown in **Figure 4** and **Table 2**. Ammonia fluxes from the two lagoons ranged from 14.4 to 78.8 kg-N ha⁻¹ d⁻¹ with an average flux of 32.8 ± 24.2 kg-N ha⁻¹ d⁻¹ in 2007 (**Table 2**). These NH₃ emission fluxes were comparable to that from conventional swine lagoons without any manure treatment during summer months as reported in the literature (Arogo et al., 2003). It also indicated that the new treatment system had not yet been effective in reducing NH₃ emission from the lagoon 1 receiving treated effluent. Therefore, we decided to discontinue the VRPM monitoring of whole farm NH₃ emission rates until

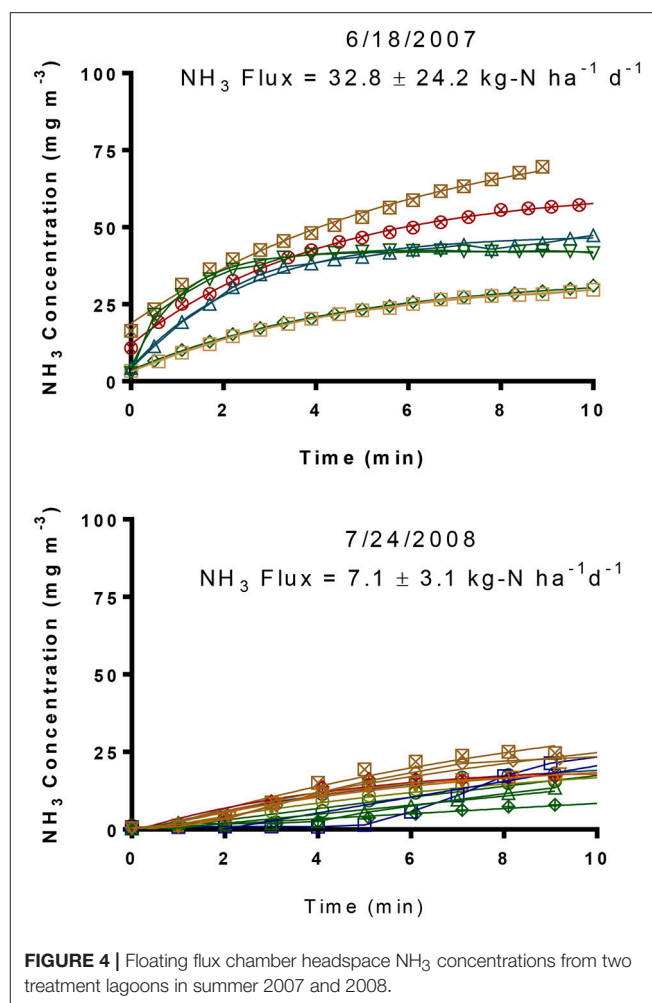


FIGURE 4 | Floating flux chamber headspace NH₃ concentrations from two treatment lagoons in summer 2007 and 2008.

TABLE 2 | NH₃ emission fluxes from the two lagoons.

	Emission Flux in June 2007 (kg-N ha ⁻¹ d ⁻¹)	Emission Flux in July 2008 (kg-N ha ⁻¹ d ⁻¹)
Lagoon 1	43.9 ± 32.4 ^{a†}	6.8 ± 2.8 ^b
Lagoon 2	21.7 ± 6.5 ^a	7.3 ± 3.6 ^b
Average for both lagoons	32.8 ± 24.2	7.1 ± 3.1

[†] Emission flux values followed by the same superscript letter (a or b) were not significantly different at $P < 0.05$ (ANOVA).

the new treatment system reached steady state and impacted NH₃ emission from the farm.

Figure 4 (bottom) shows that the flux-chamber time-series headspace ammonia concentrations increased to less than 25 mg L⁻¹ after the chamber was deployed. In contrast, the NH₃ concentrations increased to 75 mg L⁻¹ 10 min after deployment, indicating much higher emission fluxes in 2007 than in 2008. The NH₃ emission fluxes from the two lagoons ranged from 1.9 to 11.0 kg-N ha⁻¹ d⁻¹ with an average flux of 7.1 kg-N ha⁻¹ d⁻¹. The average NH₃ emission fluxes were 6.8 ± 2.8 kg-N ha⁻¹ d⁻¹ from Lagoon 1 and 7.3 ± 3.6 kg-N ha⁻¹ d⁻¹ from

the inactive Lagoon 2. The significant decrease in NH₃ emission fluxes resulted from improving lagoon water quality by the new treatment system for Lagoon 1. The NH₃ emission flux from Lagoon 2 also decreased because the already stored old manure of the Lagoon 2 prior to inactivation was diluted with rainfalls and lost some NH₃ via volatilization. The decrease in NH₃ emission flux was further validated by the fact that the total ammoniacal N (TAN) of the lagoon liquid in June 2007 was 197 mg L⁻¹ [or 20.0 mg L⁻¹ NH₃ (aq)-N] while that in July 2008 was only 32.7 mg L⁻¹ {32.5 and 33.0 mg L⁻¹ TAN [or 5.1 and 2.8 mg L⁻¹ NH₃ (aq)-N] for Lagoons 1 and 2, respectively} (Vanotti et al., 2018). These results suggested that the impact of the new treatment system on NH₃ emission reduction was equivalent to closing conventional swine lagoons while actively growing 5,145 pigs.

Whole Farm Ammonia Emission Reduction With the VRPM Technique

The NH₃ emission rates, measured with the VRPM technique, were the whole-farm emission rate that included three main NH₃ sources: (1) the NH₃ emissions from seven animal houses, (2) the new wastewater treatment system, and (3) the two lagoons (Figure 3). These NH₃ emissions rates were measured from 12/7/2006 to 9/17/2008 (Table 3 and Figure 5). The start date (12/7/2006) was a day before the start date of the new wastewater treatment system. The NH₃ emission rate on 12/7/2006 was 0.80 g/s. However, 3 months after starting the new treatment system, the NH₃ emission rate increased to 2.64 g/s, because the air temperature along with the wind speed were much higher. In addition, it was too short period of time to observe the impact of the new treatment system on lagoon water quality (the lagoon renovation capacity with treated effluent was 1.2 lagoon volumes per year or hydraulic residence time, HRT = 0.8 years). After observing the NH₃ emission flux from the lagoons was comparable to that from conventional swine lagoons without any treatment (Figure 4 top), NH₃ emission rate was not measured for the rest of 2007 to give more time for the new manure treatment system fully impacting the total farm emission. In 2008, NH₃ emission rates were monitored in relatively cold (4/4/2008), mild (9/16–9/17/2008), and hot days (7/24/2008 and

8/20/2008). Regardless the wide variation of air temperatures and wind speeds, in the second year, downwind NH₃ PICs were all below the MDL (i.e., 8.1 μL L⁻¹-m) of TDL. Below-detection-limit emission rates of these days were estimated by assuming all PICs had the MDL concentration of 8.1 μL L⁻¹-m (Table 3). These below-detection emission rate ranges from 0.04 to 0.15 g/s, more than an order of magnitude lower the initial emission rates. These data clearly demonstrated that the new treatment system substantially reduced the NH₃ emission from the swine farm.

The NH₃ emissions rates obtained prior and during initial operation of the new wastewater treatment in this full-scale study were compared to emissions rates from other full-scale studies with conventional and converted lagoons in North Carolina. Initially with lower air and lagoon water temperatures (December 2006), the estimated live weight (LW) based emission rate of 0.80 g s⁻¹ [1.19 kg N wk⁻¹ (1,000 kg LW)⁻¹] was similar to the winter combined house-lagoon NH₃ emission rate of 1.06 kg N wk⁻¹ (1,000 kg LW)⁻¹ from a conventional swine farm with similar number of pigs for a 5,784-head finisher farm using anaerobic lagoon technology (Aneja et al., 2008a). The emission rate obtained immediately after beginning the new treatment system in February 2007 was 2.64 g s⁻¹ [3.92 kg N wk⁻¹ (1,000 kg LW)⁻¹], due to very high wind speed at that day (8.33 m/s compared to 2.67 m/s in 12/7/2006) while the water temperatures were similar. The emission rate was almost linearly increased

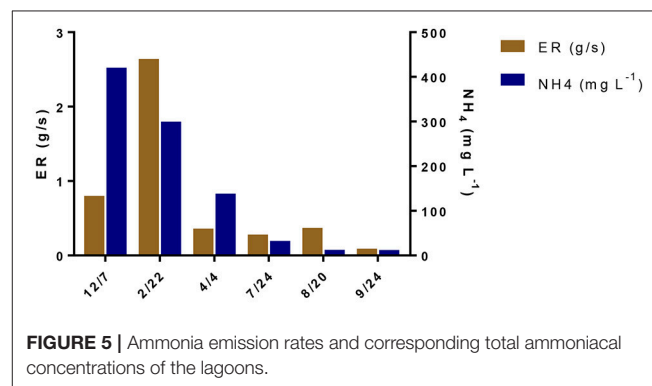


FIGURE 5 | Ammonia emission rates and corresponding total ammoniacal concentrations of the lagoons.

TABLE 3 | Summary of temperatures, wind speed, and NH₃ emission rates from the swine farm.

Sampling period [†]	Mean air temperature [‡] (°C)	Wind speed (m s ⁻¹)	Average NH ₃ concentration [§] (μL L ⁻¹)	NH ₃ emission rate (g s ⁻¹) [#]
12/7/2006 12:15–14:55	15.3 ± 0.6	2.67 ± 0.92	0.29 ± 0.05	0.80 ± 0.16
2/22/2007 15:45–16:30	22.4 ± 0.05	8.33 ± 2.20	0.27 ± 0.03	2.64 ± 0.32
4/4/2008 10:15–12:30	16.9 ± 2.7	3.46 ± 1.03	<MDL [¶]	< 0.15
7/24/2008 11:50–18:20	29.3 ± 1.1	1.59 ± 0.75	<MDL	< 0.12
8/20/2008 11:10–15:30	30.9 ± 1.0	2.38 ± 1.06	<MDL	< 0.13
9/16/2008 14:10–16:15	22.5 ± 0.6	3.52 ± 1.23	<MDL	< 0.05
9/17/2008 9:30–10:45	19.2 ± 0.6	3.01 ± 1.12	<MDL	< 0.04

[†] New wastewater treatment system started operation December 9, 2006.

[‡] Mean air temperatures measured at 2 m above ground on site during measurement period.

[§] NH₃ concentration.

[¶] <MDL, below minimum detection limit (<8.1 μL L⁻¹-m or about 0.08 μL L⁻¹ for a 100-m path length).

[#] Calculated assuming 8.1 μL L⁻¹-m for all PICs.

with wind speed. Note that during this initial startup period, the effects of the new treatment system on lagoon water quality were minimal.

After 1.5 years of operation the total emissions (combining animal houses, treatment plant, and lagoons) of the treatment plant were $<0.15 \text{ g s}^{-1}$ [$0.22 \text{ kg N wk}^{-1}$ ($1,000 \text{ kg LW})^{-1}$] for April 2008 (Table 3). Astonishingly, even for hot days in July and August 2008, the downwind NH₃ concentrations were below its detection limit. Comparing to swine lagoon NH₃ emission rates with similar water temperature of 30.2°C [2.4 kg N wk^{-1} ($1,000 \text{ kg LW})^{-1}$, (Szogi et al., 2006)], this reduction in total NH₃ emission was indeed remarkable.

Compared to the average of the first two emissions for the periods of December 2006 and February 2007 (1.72 g s^{-1}), total NH₃ emissions from the April 2008 to September 2008 periods were reduced by 94%. In comparison, the new treatment plant removed an average of 96.5% of the soluble NH₄-N contained in the raw flushed manure with most of the removal (84%) occurring at the biological N treatment step (Vanotti et al., 2018). Concentrations of NH₄-N in the lagoons were also reduced significantly during the emission evaluation period from 371 to 485 mg L^{-1} in 2006 to lower than 65 mg L^{-1} in 2008 (Vanotti et al., 2018).

Low NH₃ emission rates similar to those recorded in 2008 were also found in earlier studies (Aneja et al., 2008b) of a first generation version of the same wastewater treatment system (Vanotti et al., 2007) that was retrofitted in a 4,360-finishers production unit and removed 98.7% of the soluble NH₄ from liquid waste. According to Aneja et al. (2008b), the NH₃ emissions for this first-generation wastewater treatment plant were $0.02 \text{ kg N wk}^{-1}$ ($1,000 \text{ kg LW})^{-1}$ in the warm season and $0.0004 \text{ kg N wk}^{-1}$ ($1,000 \text{ kg LW})^{-1}$ in the cold season. These low NH₃ emissions represented reductions of 94.4% for the warm season and 99.0% for the cool season with respected to a conventional lagoon system (Aneja et al., 2008a). On a separate study of the same first-generation wastewater treatment system, Szogi et al. (2006) found that total annual NH₃ emission from the converted lagoon was $1,210 \text{ kg N y}^{-1}$ and equivalent to $0.10 \text{ kg N wk}^{-1}$ ($1,000 \text{ kg LW})^{-1}$. In contrast the NH₃ emissions from the conventional lagoon totaled $12,540 \text{ kg N y}^{-1}$ [$1.22 \text{ kg N wk}^{-1}$ ($1,000 \text{ kg LW})^{-1}$]. Compared with the conventional lagoon, annual NH₃ emissions from the converted lagoon were reduced 90% (Szogi et al., 2006).

The NH₃ concentration in the air inside the houses was also reduced as a result of recycling cleaner water for pit recharge under the animal houses (Szogi and Vanotti, 2008). Compared with the previous conventional lagoon management, the new treatment system lowered NH₃ concentrations in the house

exhaust air by an average of 75.1%. Even though the wastewater treatment had instant effect on reducing NH₃ in the manure, and the NH₃ in the recycled water used for flushing the barns, the beneficial effects on emissions at the farm level were not seen immediately; the dirty liquid in the former lagoons needed time to be cleaned up. Actual lagoon 1 volume was $9,565 \pm 750 \text{ m}^3$ and received $11,552 \text{ m}^3$ per year of the clean plant effluent (Vanotti et al., 2018). In this study, one year of continuous operation of the wastewater treatment plant was necessary to observe marked differences in NH₃ emissions at the farm level.

SUMMARY

The NH₃ emissions from a 5,145-head finishing swine farm before and after the new manure treatment system implementation were measured using both micrometeorological VRPM and static chamber techniques. The NH₃ emission flux from the lagoons decreased from 32.8 to $7.1 \text{ kg-N ha}^{-1} \text{ d}^{-1}$. The reduction in the total farm NH₃ emission was even more dramatic: All downwind NH₃ concentrations 1.5 year after starting up of the new treatment system were all below MDL. The dramatic decrease in total ammoniacal nitrogen in the lagoon validated this exceedingly low NH₃ emission from the farm.

AUTHOR CONTRIBUTIONS

KR designed and conducted the emission experiments, collected and post-processed data, and wrote the draft. MV, AS, JL, and PM conducted water quality work, data summarization and processing. All authors participated in writing of the manuscript.

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Economic Recovery of Calcium Phosphates From Swine Lagoon Sludge Using Quick Wash Process and Geotextile Filtration

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To maintain the waste treatment capacity of anaerobic swine lagoons, excessive accumulation of sludge in the bottom of the lagoon is traditionally removed by dredging and pumping followed by dewatering prior to land application. A widespread and low-cost method of dewatering this lagoon sludge in U.S. farms is the use of geotextile bag filtration. Because of typically high phosphorus (P) contents in lagoon sludge, the dewatered sludge is eventually hauled to distant fields to reduce the environmental risk of excess P in land nearby the lagoon. The inclusion of a P recovery approach, called Quick Wash (QW), along with the geotextile bag technology could eliminate or reduce hauling costs of dewatered sludge and produce a valuable P product for use as fertilizer. The QW process uses a novel combination of acid, base, and organic polyelectrolytes to selectively extract and recover P from manure solids. The objective of this study was to evaluate the potential advantage and technical feasibility of combining the QW process and geotextile dewatering in a system to extract and recover P from lagoon sludge. Laboratory tests results showed that the amount of recoverable P from lagoon sludge depends on the pH level obtained by acidification. The highest release of P in solution, 83% of total initial P, was obtained when the lagoon sludge was acidified to pH 3. The amount of P recovered as a precipitate with lime addition at pH 10 was about 79% of the initial total P in the sludge. A mass flow balance confirmed that about 20% of the total P remained in the dewatered sludge solids. The effluent contained extremely low soluble P concentrations. The calcium phosphate product was identified as amorphous calcium phosphate (ACP) with very low heavy metal content. The recovered ACP had P grades (33.2–35.5% P_2O_5), higher than rock phosphate, with the advantage that there is no need for additional chemical processing for its use as fertilizer. Results of this study support the technical feasibility of using the QW process in combination with geotextile bag filtration for the economic recovery of P from anaerobic swine lagoons.

Keywords: phosphate, phosphorus, nutrient recovery, anaerobic lagoon, dredging, sludge, solid-liquid separation, pig manure

INTRODUCTION

With a total inventory of 8.9 million heads, North Carolina is the second largest swine (*Sus scrofa* L.) producer in the United States (NASS, 2018). This intensive swine production generates large amounts of manure which is typically treated in anaerobic lagoons (Barker, 1996). One challenging aspect of managing anaerobic swine lagoons is the accumulation of sludge in the bottom of the lagoon. Eventually, the excessive accumulation of sludge reduces the liquid storage volume of the lagoon and the ability of the lagoon to treat waste (Hamilton, 2010). As part of the lagoon management, excess lagoon sludge is removed by pumping after mechanical agitation or dredging. Once the sludge is removed from the lagoon, it is land applied at agronomic rates according to its nutrient content (Schmidt, 2013). However, nutrient management plans may limit P application to crop or pastureland near the lagoon because lagoon sludge usually has a high phosphorus (P) content. Indeed, more than 90% of the total mass of P in lagoons is found concentrated in the solid fraction of the sludge (Bicudo et al., 1999; Szogi and Vanotti, 2016). Therefore, to reduce the risks of excess soil P losses via leaching and runoff into water resources, the dewatering of lagoon sludge is a critical step to facilitate long distance transportation of sludge and offset local soil P imbalances. In North Carolina's swine farms, a low-cost and widespread method of dewatering lagoon sludge is the use of geotextile bag filtration with injection of a flocculant polymer (Spearman, 2017). The polymer is added to increase retention of the small particulate solids by the dewatering bag. The dewatered sludge collected in the geotextile bags is eventually hauled to distant locations for land application. The inclusion of a P recovery approach along with the geotextile bag technology could: (1) eliminate or reduce hauling costs of dewatered sludge, and (2) produce a valuable concentrated P product for use as fertilizer. Since P is a finite mining resource being depleted due to its global demand for agricultural production (Gilbert, 2009; Karunanithi et al., 2015), P recovery is essential to close the P loop in production agriculture and reduce the P pollution risks due to soil leaching and runoff P losses into aquatic environments (Keyzer, 2010; Szogi et al., 2015b; Jurgilevich et al., 2016).

A number of technologies for P recovery are already operational at full-scale in industrial, municipal, and agricultural treatment systems (Karunanithi et al., 2015; Katagi et al., 2016). Most technologies have the purpose to recover phosphate at various points in the treatment process from liquid phase, sludge, or sludge ash (Cieřlik and Konieczka, 2017). Technologies for phosphate recovery from the liquid phase consist of feeding P-rich wastewater into a precipitation or crystallization tank, in which calcium (Ca) or magnesium (Mg) salts are added to recover phosphate as Ca phosphate or struvite (Desmidt et al., 2015). Technologies for chemical recovery of P from municipal sludge, biosolids, and incinerator ashes were reported to extract P by acid leaching or washing with recovery of P as a Ca or Mg precipitate (Takahashi et al., 2001; Franz, 2008; Cieřlik and Konieczka, 2017). For animal waste, combined acidification and precipitation processes were reported for recovery of P from incinerated poultry litter (Kaikake et al., 2009). A novel

chemical P recovery process using a combination of acid, base, and organic polyelectrolytes called "Quick Wash" (QW) was used to selectively extract and recover P from both raw poultry litter and fresh swine manure solids (Szogi et al., 2008, 2015a). The QW is a patented treatment process (U.S. Patent 8,673,046; first filed Feb. 5, 2008) developed for rapid acid wet extraction of P from solid manure and P recovery in a solid concentrated form (Szogi et al., 2014).

The inclusion of the QW process in a lagoon sludge management plan offers an opportunity to recover P as a valuable fertilizer byproduct. The objective of this study was to evaluate the potential advantage and technical feasibility of combining the QW process with a geotextile dewatering technology to extract and recover P from lagoon sludge. In order to provide process data to operate a field scale system, laboratory experiments were performed to determine: (1) acid rates for optimal release of soluble P from swine lagoon sludge; (2) mesh size for effective dewatering of acidified sludge with geotextile bags; and (3) P recovery efficiency. In addition, the study included elemental composition of the products (washed sludge solids and recovered phosphate) and a mass flow balance.

MATERIALS AND METHODS

Process Description

Figure 1 show first the individual technologies: basic QW process (**Figure 1A**) and geotextile bag dewatering (**Figure 1B**), and their combination in one single system, hereafter called "QW retrofit" (**Figure 1C**). The basic QW process includes three steps (Szogi et al., 2014): (1) selective P extraction, (2) P recovery, and (3) P recovery enhancement (**Figure 1A**). In the first step, manure solids or slurry are mixed with an acidic solution to form a washed solid residue and a liquid P extract. In step 2, P is precipitated under alkaline conditions by adding calcium (Ca) or magnesium (Mg) to the liquid extract, and in the final step (step 3) the addition of an organic anionic polymer, polyacrylamide (PAM), enhances the formation and recovery of a P-rich precipitate. In the geotextile bag filtration system alone (**Figure 1B**), polymer (cationic PAM) is injected to enhance filtration and dewatering of solids retained in the geotextile bag, producing an effluent low in solids. In the QW retrofit (**Figure 1C**), the sludge is first acidified and then cationic PAM is injected to flocculate and retain solids in the geotextile bag (QW step 1). The acidic liquid flowing out of the geotextile bag is then collected in a settling tank to recover the P precipitate after reaction with hydrated lime and anionic PAM (QW steps 2 and 3). The P is recovered as a precipitate from the bottom of the settling tank and further dewatered. The effluent from the settling tank, low in solids and P, is then sent to the lagoon or eventually recycled as dilution water.

Phosphorus Extraction (Experiment 1)

The P extraction was conducted by filling 20 mL of lagoon sludge into 50-mL centrifuge tubes. Sulfuric acid (4.1 M) was added to the tubes at 10 rates ranging from 20 to 200 mmol L⁻¹ (equivalent to a concentrated acid rate of 2.1–20.8 g H₂SO₄ L⁻¹, 18M, 1.84 g cm⁻³) plus one tube with no addition of acid

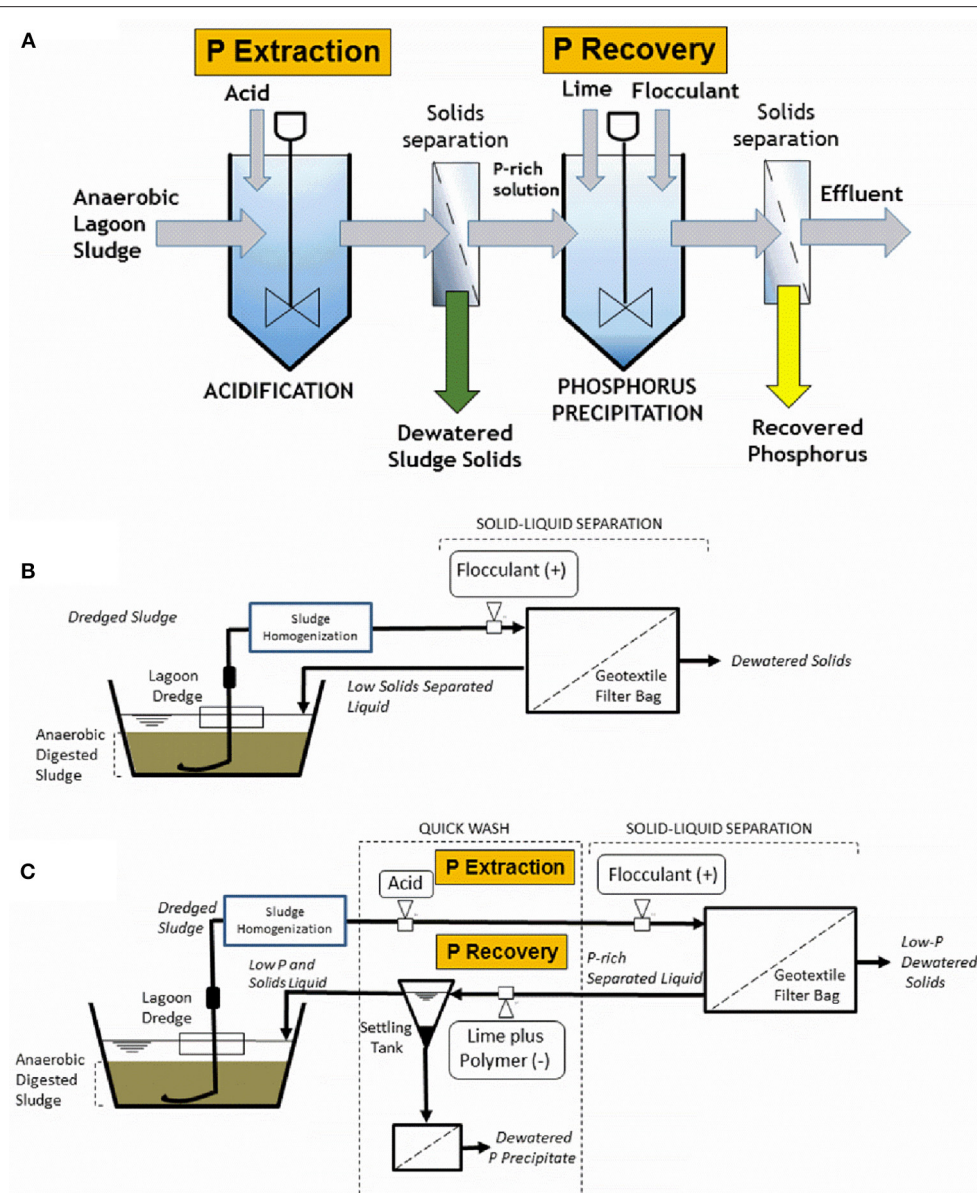


FIGURE 1 | Schematic diagrams of the basic Quick Wash process (A), the geotextile bag filtration for dewatering of sludge (B), and the combination of the Quick Wash process and the geotextile bag filtration into one system for P recovery from sludge (C).

(control). The tubes with the mixture of sludge and acid were capped and shaken on a reciprocating shaker (135 oscillations min^{-1}) for 20 min at ambient temperature (23°C). Subsequently, the tubes were uncapped and pH was measured using a pH meter (Denver Instruments, Arvada, CO). After measuring the pH, the acid-sludge mixtures were filtered through 0.2- μm filters. The collected clear filtrate was used for analysis of soluble inorganic P. The extraction experiment was carried out in duplicate.

Dewatering of Washed Solids (Experiment 2)

A filtration test was used to determine the effect of acidification and mesh size of geotextile bag on sludge dewatering. A

preliminary flocculation study was performed to determine the PAM rate (WERF, 1993). The polymer was a cationic PAM (0.05% solution) added at a single rate of 133 mg PAM L^{-1} which was within the optimum rate of 100–200 mg L^{-1} to flocculate swine manure slurries (Vanotti and Hunt, 1999). Prior to the filtration test, a set of 150-mL samples were previously acidified to pH 5.0, 4.0, and 3.0 with H_2SO_4 and conditioned with cationic PAM. Duplicate gravity filtration tests were carried out through 250-, 400-, and 600- μm mesh monofilament nylon filters (NMO, Krystil Klear Filtration, IN). The selected mesh size of the filters were in the range of opening sizes in geotextiles tubes used for dewatering manure and sludges (Szogi et al., 2006; Cantrell et al., 2008; Worley et al., 2008). Total suspended solids (TSS) were

determined in the filtrates, and their corresponding moisture content. The solid-liquid separation removal performance for every filter mesh was determined by the difference between the TSS concentration in the filtrate and the TSS in the original sample before acidification and PAM applications and filtration.

Phosphorus Precipitation and Recovery (Experiment 3)

Based on the results of the acidification and dewatering experiments, a 1.0-L sample of sludge was stirred for 20 min using an over-head mixer while adding H_2SO_4 to reach pH 3.0 and then conditioned with cationic PAM. The acidified sludge was gravity filtered through a 400- μm mesh filter. The separated washed solids retained in the filter were rinsed with distilled water and freeze dried prior to elemental analysis. The filtrate (acidified extract) was used in P precipitation experiments. The same filtration procedures were used to obtain acidified extracts at pH 4.0 and 5.0. From each acid extract, a set of 35-mL aliquots were placed in 50-mL centrifuge tubes and hydrated lime [10% w/v $\text{Ca}(\text{OH})_2$] was added to pH endpoints of 8.0, 9.0, and 10.0 units. To facilitate the dewatering and recovery of P, an anionic

PAM (0.05% solution) was added at a single rate of about 0.4 mg L^{-1} to thicken the precipitated material (Szogi et al., 2015a). Subsequently, the samples were centrifuged at 2,000 g for 10 min. The supernatant was poured from the centrifuge tubes into plastic vials and immediately analyzed. The P precipitates were washed with known volumes of de-ionized distilled water, centrifuged again at 2,000 g for 10 min, and decanted. The recovered P precipitates were freeze-dried prior to analysis.

Chemical Analysis

Sludge analyses and effluents from the QW process were performed according to Standard Methods for Examination of Water and Wastewater (APHA, 1998). The pH was determined electrometrically (Method 4500-H+ B). Alkalinity was determined by acid titration to the bromocresol green endpoint (pH 4.5) and expressed as $\text{mg CaCO}_3 \text{ L}^{-1}$ (Method 2320 B). TSS were determined according to Method 2540E. Total Kjeldahl nitrogen (TKN) was determined using acid digestion and the automated ascorbic acid and phenate methods adapted to digested extracts (Technicon Instruments Corp, 1977). Concentrations of total phosphorus (TP), and metals were determined in liquid and solids using nitric acid/peroxide block digestion (Peters, 2003) and inductively coupled plasma analysis (Method 3125 A). Ammonium-nitrogen ($\text{NH}_4\text{-N}$), nitrate-nitrogen ($\text{NO}_3\text{-N}$) and soluble P ($\text{PO}_4\text{-P}$) were determined by chemically suppressed ion chromatography using ASTM Standards D6919-09 and D4327-11 (ASTM International, 2009, 2011) after filtration through a 0.20- μm membrane filter.

Both washed sludge solids and P recovered precipitates were analyzed for carbon (C) and N by dry combustion (Elementar, Mt. Laurel, NJ). Minerals in the P recovered precipitates were identified by X-ray powder diffraction using an Olympus Terra (Waltham, MA) field-portable X-ray diffractometer (XRD) system operating at 30 kV and 0.3 mA with a Cu radiation tube. Data were collected from 5 to $55^\circ 2\theta$ in steps of $0.05^\circ 2\theta$ for qualitative XRD analysis including standards of

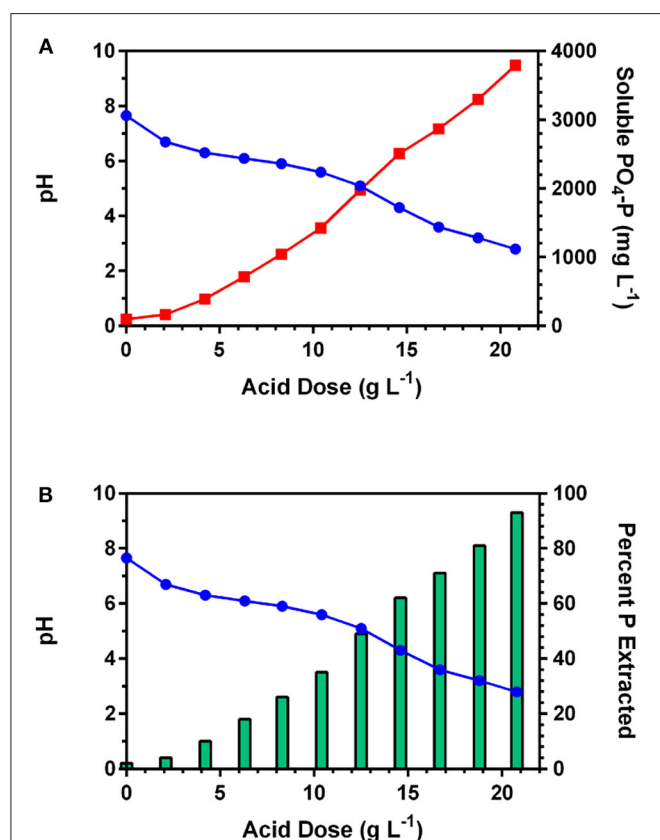


FIGURE 2 | Phosphorus extraction from anaerobic lagoon sludge at increasing concentration levels of sulfuric acid: (A) effect of decreasing pH (blue line) on soluble P concentration (red line); and (B) percent soluble P with respect to initial total P in the anaerobic lagoon sludge (bars).

TABLE 1 | Effect of mesh size and acidification of anaerobic lagoon sludge conditioned with cationic polymer on filtrate quality, solid/liquid separation efficiency and moisture of solids (Experiment 2).

Mesh size	Acidification	TSS filtrate	Solid/liquid separation efficiency ^a	Moisture separated solids
μm	pH	mg L^{-1}	%	%
250	5.0	250 ± 3	99.6	86
	4.0	570 ± 28	99.0	89
	3.0	328 ± 4	99.4	88
400	5.0	495 ± 13	99.1	88
	4.0	985 ± 78	98.3	88
	3.0	533 ± 75	99.1	87
600	5.0	813 ± 9	98.6	86
	4.0	$1,485 \pm 21$	97.4	88
	3.0	670 ± 42	98.8	88

^aEfficiency % = $[(\text{Initial TSS} - \text{Filtrate TSS}) / \text{Initial TSS}]$; Initial TSS = $57,450 \text{ mg L}^{-1}$.

amorphous calcium phosphate (ACP) and calcium carbonate (CaCO_3) supplied by Sigma-Aldrich (St. Louis, MO). In addition, carbonates in the recovered P precipitates were determined according to (Horváth et al., 2005).

Extraction and recovery efficiencies of P were determined using mass balances that included the sludge volume and P concentrations before and after treatment as well as the mass and concentration of P in precipitated materials. Data results were statistically analyzed by mean and standard deviation using PROC MEANS procedure in SAS (Statistical Analysis System, Cary, NC). Analysis of variance for the effect of acid extraction treatment on composition of washed sludge solids, lime addition treatments to acid extract on recovered P material and effluents was conducted using the General Linear Model (GLM) in SAS. Difference between means of any two treatments was considered significant when it was larger than the least square difference (LSD) with a probability of $t < 0.05$.

Origin and Characteristics of the Lagoon Sludge

The sludge was collected during the course of dredging an anaerobic swine lagoon used for storing and treating manure in a swine finishing farm located in North Carolina, USA. A sample of the dredged sludge was pumped into a 20-L plastic container, transported to the USDA-ARS laboratory in Florence, and stored at 4°C until use. The sludge was homogenized using a mechanical stirrer before taking samples for the experiments. On average, the sludge contained pH 7.65, alkalinity 11,290 mg L⁻¹, TSS 57.5 g L⁻¹, TKN 2,513 mg L⁻¹, $\text{NH}_4\text{-N}$ 323 mg L⁻¹, $\text{NO}_3\text{-N}$ 0 mg L⁻¹, K 606 mg L⁻¹, TP 4,070 mg L⁻¹, $\text{PO}_4\text{-P}$ 82 mg L⁻¹, Ca 5,553 mg L⁻¹, Mg 1,278 mg L⁻¹, Al 216 mg L⁻¹, Fe 511 mg L⁻¹, Cu 24 mg L⁻¹, and Zn 179 mg L⁻¹.

RESULTS

Phosphorus Extraction

Previous research on applying mineral acid to poultry litter and fresh swine manure has shown that over 60% of the initial TP was released into solution between pH 5.0 and 3.0 (Szogi et al., 2008, 2015a). At pH lower than 3.0, the acid extract becomes corrosive, and unnecessary oxidation of organic compounds can occur (Szogi et al., 2015a). In this study, the acid requirement to decrease the pH of the sludge and increase release of P from the solid fraction into a soluble fraction is shown in Figure 2A. Prior to acid addition, at pH 7.7, the soluble P concentration was 99 mg $\text{PO}_4\text{-P L}^{-1}$ representing only about 2% of the initial 4,070 TP mg L⁻¹ in the sludge. With addition of the acid, the pH declined with a significant increase of soluble $\text{PO}_4\text{-P}$ concentration in the acid extract. Since the concentration of soluble $\text{PO}_4\text{-P}$ strongly depends on the acid dose, Figure 2B shows the percent of $\text{PO}_4\text{-P}$

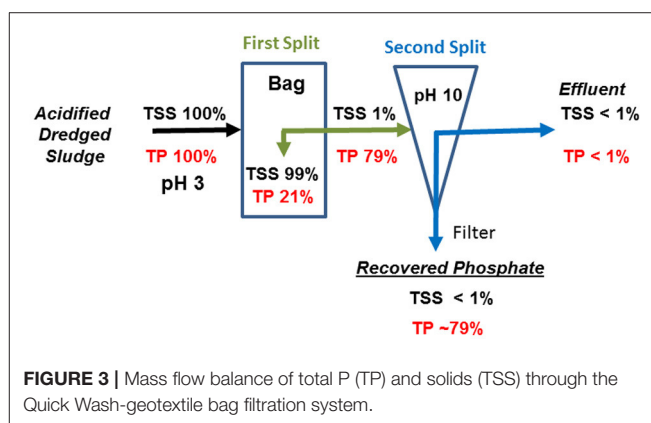


TABLE 2 | Phosphorus (P) mass flow of the Quick Wash process (Experiment 3).

P extraction (Step 1)			P recovery (Steps 2 and 3)					Effluent ^e	
pH	Acidified sludge		pH	Applied lime ^b	Removed from acid extract	Recovered mass	P content in precipitate	Return to lagoon	
	mg L ⁻¹	% ^a		g Ca(OH)_2 L ⁻¹	mg L ⁻¹	% ^c	% P_2O_5^d	mg L ⁻¹	%
5.0	2,022 ± 26	50	8.0	4.8	1,996 ± 80	49	36.0 ± 1.4	23 ± 0	<1
			9.0	5.3	1,928 ± 34	47	34.1 ± 0.8	5 ± 1	<1
			10.0	7.0	1,801 ± 36	44	30.7 ± 0.4	3 ± 0	<1
4.0	2,746 ± 75	67	8.0	7.3	2,733 ± 1	67	32.7 ± 2.4	20 ± 1	<1
			9.0	9.6	2,795 ± 121	69	35.3 ± 1.9	4 ± 1	<1
			10.0	11.4	2,776 ± 10	68	34.8 ± 0.2	2 ± 0	<1
3.0	3,398 ± 8	83	8.0	8.6	2,947 ± 16	72	35.0 ± 0.2	22 ± 4	<1
			9.0	9.4	2,872 ± 1	71	33.2 ± 0.3	3 ± 0	<1
			10.0	11.6	3,219 ± 321	79	35.5 ± 3.8	2 ± 1	<1

^a% P extracted = TP in the acid extract relative to initial TP content in the untreated sludge (4,070 mg L⁻¹).

^bApplication of lime plus anionic polymer for enhanced P precipitation.

^c% of the mass of P removed and from the acid extracts and recovered in the precipitate with respect to the initial TP content in the untreated sludge (4,070 mg L⁻¹).

^d% P_2O_5 = %P × 2.29 where %P is the dry content of P in the precipitated material (g TP per 100 g of precipitate).

^e% of the mass of P in the treated effluent with respect to the initial TP content in the untreated sludge (4,070 mg L⁻¹).

extracted with respect to the initial TP at increasing acid dose (H_2SO_4). At pH 2.8, the acid extract had a concentration of $3,793 \text{ mg L}^{-1}$ of $\text{PO}_4\text{-P}$ (Figure 2A) or 93% of the TP that was in the original sludge (Figure 2B).

Dewatering of Acidified Sludge

Dewatering technologies use solid-liquid separation methods to separate the liquid and solid fractions of sludge by gravity, mechanical, and chemical processes. Usually, dewatering efficiencies of mechanical or gravity separators are <60% for removal of solids. Whereas chemical applications of flocculants have been shown to enhance the dewatering efficiency to more than 90% solids removal for liquid manure (Vanotti and Hunt, 1999; Garcia et al., 2009). Furthermore, Coccolo et al. (2016) reported that acidification of pig manure slurry to pH 5.3 followed by cationic polymer addition promoted a faster dewatering of solids than a non-acidified control. In our study, we observed that the addition of acid and cationic PAM to lagoon sludge quickly produced firm, large flocs with rapid settling, and a clear supernatant liquid. The effects of mesh size and pH on TSS concentration in the filtrate and filtration efficiency of sludge conditioned with PAM are presented in Table 1. At the same acidification treatment (pH 5.0, 4.0, or 3.0), the filter with $250 \mu\text{m}$ mesh size produced filtrates with the lowest TSS concentrations when compared to filtrates obtained with 400 and $600 \mu\text{m}$ mesh size. Nevertheless, all filtration tests had a high efficiency of >98% retention of solids, and the separated solids had a narrow range moisture content of 86–89%. From these results we concluded that commercially available geotextile filtration bags with a $400\text{-}\mu\text{m}$ openings could be used effectively for solid-liquid separation

of acidified sludge conditioned with cationic PAM. The results reported for P recovery in the next section were obtained using the filtrates of the acidified sludge passed through the selected $400\text{-}\mu\text{m}$ mesh filter.

P Recovery and Mass Flow Balance

In the QW process, the recovery of P can be done either by addition of either Mg or Ca salts to form a phosphate precipitate under alkaline pH conditions (Szogi et al., 2014). Hao et al. (2013), indicated that efforts for recovery of P from waste streams should be aimed at forms of phosphate compounds acceptable by the fertilizer industry such as Ca phosphates. The potential use of recovered Ca phosphates from waste streams by the fertilizer industry led us to develop the QW process for recovery of Ca phosphates from various waste streams: poultry litter, manure solids (Szogi et al., 2008, 2015a), and lagoon sludge solids presented here. The laboratory results on P recovery and mass flow balances for the QW retrofit are presented in Table 2. The mass results are shown separately for the first step and second step of the QW process. The extraction of P (Step 1) was based on the acid extracts obtained by filtration through a $400\text{-}\mu\text{m}$ mesh of acidified sludge conditioned with cationic PAM. The P recovery (Steps 2 and 3) includes the results of the combined additions of lime and anionic PAM to enhance P precipitation from the acid extracts (Figures 1A,C).

In Table 2, the acid P extraction (Step 1) shows that with decreasing extraction pH from 5.0 to 3.0 there was an increase in soluble P from 2,022 to $3,398 \text{ mg L}^{-1}$, respectively. On a mass basis, from 50, 67, and 83% of the initial TP mass was in solution in the acid extracts at pH 5.0, 4.0, and 3.0, respectively. Following, the P recovery (steps 2 and 3) section of Table 2 shows the pH increase in three steps (pH 8.0, 9.0, and 10.0) by addition of hydrated lime to each of the three acid P extracts, resulting in nine doses of applied lime. Hydrated lime addition was effective to remove large amounts P from each acid extract by precipitating P. Overall, the amount of recovered P in the precipitates was strongly predisposed by the concentration of soluble P in the acid extracts. For instance, the highest concentration of soluble P was obtained at pH 3.0; subsequent addition of hydrate lime to a target pH of 10.0 removed $3,219 \text{ mg P L}^{-1}$ from the extract allowing the recovery of 79% of the initial mass of TP in the sludge. The removal of P from all acid extracts resulted in low TP concentrations in the effluents and represented <1% of the initial mass of TP in the sludge. Figure 3 summarizes the mass flows of solids and P for all three steps of the QW retrofit system when lagoon sludge was acidified at pH 3.0 and P recovered at pH 10.0. While 99% of the mass of TSS was retained in the filter as washed solids, the P mass split in two fractions: (1) 21% of P was retained in the filter with the washed solids (first split); and (2) 79% of the P was recovered as a precipitate. Overall, <1% of the mass of P was in the treated effluent.

Washed Solids

The original composition of the solid fraction of the sludge was significantly influenced by the acidification step of the QW process (Table 3). A significant increase in C, N, and S with a significant decrease in P concentration occurred in all acid

TABLE 3 | Chemical composition of anaerobic swine lagoon sludge solids before chemical extraction of phosphorus (no-acid), and after acid extraction treatment (washed solids) on a dry basis.

Component	Before acid treatment	After acid treatment (washed solids)		
	7.7	5.0	4.0	3.0
pH				
C (%)	16.5c	22.5ba	23.8a	21.3b
N (%)	2.4c	3.8a	3.5b	3.7a
S (g kg^{-1})	10.4d	12.9c	29.5a	22.8b
P (g kg^{-1})	70.8a	45.3b	29.3c	15.8d
N:P ratio ^a	0.3	0.8	1.2	2.3
K (g kg^{-1})	10.5a	1.5c	2.3b	1.2c
Ca (g kg^{-1})	96.7a	72.0b	68.8b	75.4b
Mg (g kg^{-1})	22.2a	5.3b	2.2c	2.1c
Na (g kg^{-1})	8.3a	1.1b	0.5b	0.7b
Cd (mg kg^{-1})	0.7c	1.9b	2.3a	1.8b
Cu (mg kg^{-1})	423c	433c	474b	451a
Ni (mg kg^{-1})	22.8c	26.2b	29.9a	25.1b
Pb (mg kg^{-1})	19.2a	16.8ab	13.2b	12.9b
Zn (mg kg^{-1})	3,121b	3,230b	4,921a	3,279b

Data are mean of duplicate samples; data in the same row followed by the same letter are not significantly different ($P < 0.05$).

^aN:P ratio = $\text{g N kg}^{-1} / \text{g P kg}^{-1}$.

washed solid residues when compared to the untreated sludge solids. The difference in P content were all highly significant between the untreated sludge solids and each of the wash solids after acid treatment. At pH 3, the P concentration in the wash solid was 4.5-fold smaller (15.8 g kg^{-1}) than the untreated sludge (70.8 g kg^{-1}). This result is consistent with the mass balance in **Figure 3** that about 79% of the P was removed from sludge solids allowing the subsequent recovery and off-farm export of P. The selective acid dissolution of P with respect N is represented by the increase in the original N:P ratio of the sludge before treatment from 0.33 to 2.3 after acidification at pH 3. Since P in the sludge solids was mostly in inorganic form, the release of soluble P under acidic conditions was accompanied by significant dissolution of K, Ca, Mg, and Na. It is important to notice the acidification step increased the concentration of heavy metals Cd, Cu, Ni, Pb, and Zn in the washed solids. Heavy metals remained in the washed solids most likely because effective dissolution of these elements requires stronger acidic conditions at pH levels lower than 2.0 (Scott and Horlings, 1975). However, the heavy metals concentrations in the acid washed solids were low enough to meet the U.S. EPA regulatory ceiling concentration limits of 85 mg Cd kg^{-1} , $4,300 \text{ mg Cu kg}^{-1}$, $420 \text{ mg Ni kg}^{-1}$, $840 \text{ mg Pb kg}^{-1}$, and $7,500 \text{ mg Zn kg}^{-1}$ for land application of biosolids (Metcalf Eddy Inc., 2003).

Recovered Phosphates

The chemical composition of selected P precipitates are presented in **Table 4**. They were obtained at pH 8.0, 9.0, and 10.0 after hydrated lime addition to acidified extracts (pH 3.0). On a dry matter basis, differences in elemental composition of the

precipitates were not significant across the three hydrated lime treatments with the exception of N and K concentrations. The total C content in the precipitates (1.4–1.5%) was low. It was partitioned into inorganic C (0.5–0.9%) and organic C (0.6–0.9%) forms. The organic C content was estimated by the difference in released CO_2 from total combusted C and carbonate measurements (2.3–4.3% as CO_3^{2-}). Only 2.3–5.7% of the initial N on a mass basis was recovered such that N concentrations (0.2–0.4 N%) in the P precipitates were low as compared to the initial lagoon sludge (2.4% N, **Table 3**). The recovered K, S, and Na were minor components in the precipitates since they remained mostly in solution after lime addition. Both P and Ca were the major components of the phosphate precipitates, whereas Mg was a third component with concentrations much lower than Ca (**Table 4**). The recovered Ca phosphates had mean P contents within the range of 33.2–35.5% P_2O_5 . On average, they were higher than rock phosphate from U.S. mines in North Carolina (29.8% P_2O_5) and Central Florida (32.5% P_2O_5) (FAO, 2004). The only two heavy metals detected in the recovered phosphate precipitates were Ni and Zn (**Table 4**), with concentrations of both heavy metals below 13 mg Ni kg^{-1} and 74 mg Zn kg^{-1} found in commercial P fertilizers (USEPA, 1999).

TABLE 4 | Chemical composition of the recovered phosphorus precipitates obtained from anaerobic swine lagoon sludge acid extract (pH 3) after lime addition to precipitate phosphorus at pH to 8.0, 9.0, and 10.0.

Component	Hydrated lime addition treatment pH		
	8.0	9.0	10.0
C (%)	1.4a	1.6a	1.5a
(% as Inorganic C)	0.5	0.8	0.9
N (%)	0.4a	0.3b	0.2b
P (g kg^{-1})	153a	145a	155a
(% as P_2O_5)	35.0	33.2	35.5
K (g kg^{-1})	1.9a	1.5b	1.4b
S (g kg^{-1})	19.0a	18.0a	22.0a
Ca (g kg^{-1})	253a	256a	269a
Mg (g kg^{-1})	41.4a	36.3a	38.0a
Na (g kg^{-1})	1.1a	1.1a	1.1a
Cd (mg kg^{-1})	<DL ^a	<DL	<DL
Cu (mg kg^{-1})	<DL	<DL	<DL
Ni (mg kg^{-1})	7.7a	8.0a	7.0a
Pb (mg kg^{-1})	<DL	<DL	<DL
Zn (mg kg^{-1})	20.4a	17.4a	23.5a

Data are mean of duplicate samples; data in the same row followed by the same letter are not significantly different ($P < 0.05$).

^a<DL = below detection limit of the method $<1.0 \text{ mg kg}^{-1}$ (Cd, Cu, Pb).

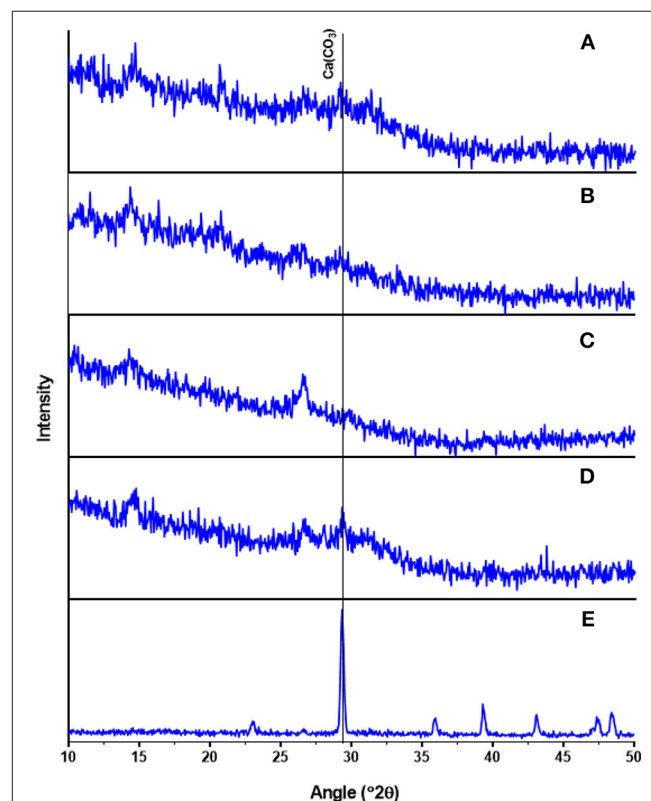


FIGURE 4 | Mineralogical characterization (x-ray diffraction) of precipitates obtained after lime addition to liquid extract of swine lagoon sludge (pH 3): (A) pH 8 precipitate; (B) pH 9 precipitate; (C) pH 10 precipitate; (D) amorphous calcium phosphate (ACP) standard; (E) calcium carbonate (CaCO_3) standard.

Several Ca-Mg phosphate minerals such as hydroxylapatite and struvite can co-exist in crystalline form contributing to phosphate recovered from waste streams (Zhang et al., 2010; Muster et al., 2013). However, our XRD analysis revealed that the presence of small peaks in all recovered P precipitate samples did not correlate with any known crystalline phosphate species (Figures 4A–C). For comparison, XRD analysis of standards of ACP and CaCO₃ as calcite were included in Figures 4D,E. Since the content of carbonates in the precipitates was in the low range of 2.3–4.3%, the intense characteristic peak ($2\theta = 30^\circ$) of calcite is not shown clearly in the XRD spectra of the recovered P precipitates. Magnesium had low concentration values but its consistent presence in the elemental composition of recovered P precipitates, hints that Mg was a component of the ACP as reported by Fernandes et al. (2012). However, Mg could be in the form of struvite which requires presence of three soluble ions (Mg²⁺, NH₄⁺, and PO₄³⁻). The precipitation of struvite (MgNH₄PO₄·6H₂O) is favored by raising the pH above 8.0 and Mg:P molar ratios between 1:1 and 1:3 (Muster et al., 2013). Although the three soluble ions required for struvite were in the acid extract at pH 3 (Table 5), the Mg:P molar ratio of 0.6:1 was not the most favorable for struvite precipitation. In addition, the presence of Ca in solution was reported to inhibit the crystal growth of struvite, leading to an amorphous material rather than crystalline struvite (Le Corre et al., 2005). In our study, the pH of the acid extract was raised with hydrated lime resulting in supersaturation of Ca at pH 8.0–10.0, and most probably inhibiting the crystal growth of struvite. On the other hand, small amounts of Mg were reported to be essential for the non-crystalline ACP to become thermodynamically stable at room temperature (Posner et al., 1984). Due to its amorphous nature, ACP does not have a fixed molecular formula. Therefore, ACP precipitates are distinguished only by their wide range of Ca/P molecular ratios (1.0–2.2); lower Ca/P ratios indicate higher contents of phosphate (Dorozhkin, 2010). From Table 4, the Ca/P ratios were estimated from the total Ca concentration by subtracting the Ca in the carbonates. Under this assumption, the Ca/P ratio of the P precipitates obtained at pH 8.0, 9.0, and 10.0 were 1.23, 1.28, and 1.25, respectively. These ratios are consistent

with the lower range of Ca/P ratio for ACP and the high grade phosphate (33.2–35.5% P₂O₅) of the recovered P precipitates (Table 4). From the XRD spectra and chemical analysis results we concluded that the recovered phosphates were similar in composition to ACP.

Effluent

Very low TP concentrations (2–21 mg L⁻¹) with respect to the initial acid extract (3,356 mg L⁻¹) were found in the final effluent (Table 5). As a result of lime addition to the acid extract and subsequent formation of P precipitates, the P removal efficiency was about 100% with <0.1% P mass remaining in the final effluent. Although, NH₄-N concentrations remained high in the final effluent, they declined with respect to the initial acid extract (1,049 mg L⁻¹) at the increasing alkaline pH (971–940 mg L⁻¹). On a mass basis, the NH₄-N in the effluent was 83–86% of the initial NH₄-N mass in the acid extract at pH 3. The decline of NH₄-N in the effluent was likely due to NH₃ losses by volatilization under the alkaline pH of the effluent. In spite of the alkaline pH, K, and Na remained in solution in sufficient high concentrations such that over 79% of the total initial mass of K and Na turn out in the effluents. Similarly,

TABLE 6 | Costs of chemicals for recovering P from 1,500 cubic meters of sludge using the Quick Wash—geotextile filtration system.

Item	Unit cost	Rate	Volume	Total
	US\$ kg ⁻¹	kg m ⁻³	m ³	US\$
Sulfuric acid	0.32	16.7 ^a	1,500	8,016
Cationic polymer	3.00	0.133	1,500	599
Anionic polymer	4.00	0.032	759 ^b	97
Lime	0.20	11.6	7593	1,761
Total chemical cost				10,473

^aRate of sulfuric acid to attain a pH of about 3.5 (Figure 2).

^bEstimated from the solid/liquid volume fraction of 0.506 obtained from our sludge filtration study.

^cRate of lime to attain a pH of 10.0 (Table 2).

TABLE 5 | Chemical composition of the liquid acid extract at pH 3, the effluent obtained after lime addition to the acid extract at pH of 8, 9, and 10 and settling, and mass percentage of each constituent remaining in the effluent with respect to its mass in the acid extract.

pH	Acid extract	Effluent after lime addition					
	(mg L ⁻¹)	(mg L ⁻¹)				(% mass) ^a	
	3.0	8.0	9.0	10.0	8.0	9.0	10.0
P	3,356 ± 36	21 ± 5	3 ± 0	2 ± 0	<0.1	<0.1	<0.1
NH ₄ -N	1,049 ± 9	971 ± 4	972 ± 1	940 ± 1	86	85	83
K	712 ± 4	630 ± 2	621 ± 4	613 ± 1	82	80	80
S	3,425 ± 28	3,329 ± 18	3,167 ± 5	3,061 ± 13	90	84	83
Ca	1,426 ± 18	498 ± 0.0	586 ± 3	1,236 ± 10	32	38	81
Mg	1,630 ± 18	879 ± 9	826 ± 1	824 ± 2	50	46	47
Na	374 ± 8	336 ± 7	323 ± 1	328 ± 6	83	79	82

^a% mass = (concentration × volume acid extract/ concentration × volume effluent) × 100.

83–90% of S mass remained in solution in the final effluents since it was added with the H_2SO_4 to acidify the sludge. On the other hand, the concentration and mass of Ca remaining in the final effluent increased from 32 to 97% with increasing alkaline pH due to addition of hydrated lime whereas the mass of Mg was about half (46–50%) of what was initially in the acid extract. The very low TP concentrations suggests that these effluents can be recycled into the QW retrofit for use as dilution water during acid mixing with the dredged sludge. At the same time, this recycling of the effluent could help to reduce NH_3 emissions. As an alternative to minimize gaseous NH_3 losses according to Vanotti and Szogi (2015), a gas-permeable membrane system can be included in the P recovery step (settling tank, **Figure 1C**) of the QW retrofit to capture and recover $\text{NH}_4\text{-N}$ from the effluents in a concentrated form.

Economic Considerations

The cost to recover P from lagoon sludge using the QW retrofit was calculated on the basis of processing one m^3 of sludge. Geotextile filtration bags are available commercially in 9- to 25-m circumference and practically any length, but typically 30.5-m (Worley et al., 2003). We assumed that the QW retrofit would use a 14-m circumference by 30.5-m length geotextile filtration bag with 425- μm mesh and a capacity to treat 1,500 m^3 of sludge. This is a volume of sludge estimated to be generated by 3,000 pigs in 5 years according to a sludge generation standard for North Carolina's swine lagoons of 0.125 m^3 per animal of 57 kg per year (33 gallons per animal of 125 lbs per year) reported by Sheffield et al. (2000). Treatment parameters used in calculating the processing cost are based on our laboratory study with the following conditions:

- Dredged sludge contains 4.1 g P L^{-1} or 9.4 kg $\text{P}_2\text{O}_5 \text{ m}^{-3}$.
- The solid/liquid volume fraction is 0.506, obtained from data of our sludge filtration study.
- The P recovery efficiency is 70%.
- The geotextile filtration bag cannot be reused once it is opened to remove the dewatered solids.
- The dewatered solids were applied on-farm.

The costs of chemicals are presented in **Table 6** with a total cost of US\$10,473 to treat 1,500 m^3 of dredged sludge. Additional materials and labor costs include the geotextile filtration bag at US\$ 2,500, the cost of site preparation (US\$ 340), and 30 h of labor at US\$ 30 h^{-1} (US\$ 900). The resulting cost for treating 1,500 m^3 of lagoon sludge would be US\$14,213, which represents a cost of US\$ 9.48 m^{-3} of processed lagoon sludge.

The amount of P potentially recovered in the precipitates is about 6.57 kg $\text{P}_2\text{O}_5 \text{ m}^{-3}$ of processed sludge with a fertilizer value of US\$ 6.17 (US\$ 0.94 $\text{kg}^{-1} \text{P}_2\text{O}_5$). In addition, the combined

fertilizer value of both N and P remaining in the washed solids is about US\$ 3.89 m^{-3} of processed sludge. A complete economic evaluation for deployment of this technology to recover P from lagoons per year still requires taking into account the annualized energy and capital or rental cost of the equipment (dredge, pumps, injection equipment, and tanks) and potential additional income from nutrient trading programs (Shortle, 2013).

CONCLUSIONS

This laboratory study showed that the amount of recoverable P from lagoon sludge depended on the acidic pH reached after application of sulfuric acid. The highest release of P in solution, 83% of total P, was obtained when the lagoon sludge was acidified to pH 3.0. The P released in the acidic extract was recovered as a calcium phosphate product using hydrated lime. The amount of P recovered at pH 10 was about 80% of the initial total P. The extremely low amounts of P in the effluent (2–3 mg L^{-1}) and a mass flow balance confirmed that about 20% of the total initial P remained in the dewatered sludge solids. The calcium phosphate product was identified as ACP. The recovered ACP had higher P grades (33.2–35.5% P_2O_5) than rock phosphate and the advantages of low heavy metal content and no need for additional chemical process for use as fertilizers. Results of this study support the technical feasibility of using the QW process in combination with geotextile bag filtration for economic recovery of P from anaerobic swine lagoons. Further tests at pilot scale are needed to determine: (1) solid-liquid separation efficiency of geotextile bags for treatment of acidified sludge; (2) confirm laboratory P recovery efficiencies; and (3) automation of the process. In addition, field trials will be needed to demonstrate the agronomic value of both washed solids and recovered phosphate products.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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Composts Produced From Pig Slurry Solids: Nutrient Efficiency and N-Leaching Risks in Amended Soils

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Composts were prepared from the solid fraction of pig slurry (SPS) by mixing with cotton gin waste (CGW) in two different proportions. The aim was to assess the fertilizer value of the two composts and to evaluate potential pollution risks due to the heavy metal (Cu and Zn) concentrations in the composts and the nitrate leaching following their soil application. For this, a pot trial using Italian ryegrass (*Lolium multiflorum* Lam.) as a test plant was run in a glasshouse. Two composts, A and B, prepared with SPS:CGW ratios of 4:3 and 3:4 (v:v), respectively (OM 63 and 57%, TN 36.1 and 32.7%, and TP 15.3 and 10.8 g kg⁻¹ in A and B, respectively), were applied to a sandy-loam soil at two doses based on the N supplied (D1: 360 mg TN pot⁻¹, equivalent to 52 kg N ha⁻¹, and D2: 720 mg TN pot⁻¹). They were compared with a soluble mineral fertilizer (3.62 g pot⁻¹ of N:P:K 10:10:10; providing 360 mg N pot⁻¹); unfertilized control soil was also tested. The potential risk of nitrate-N leaching in the soil after two simulated intense rainfall events was evaluated. The experiment was carried out in double-layer pots, which allowed the collection of leachates. Both composts were able to increase plant biomass production and soil TN and available P with respect to the control; available Cu and Zn in the soil did not increase significantly with respect to the control soil and mineral fertilizer treatment, although N deficiency limited plant growth at harvests 2 and 3. The compost prepared with a higher proportion of SPS (A) was more efficient for N fertilization (relative agronomic efficiency: 38.1–47.6%; nitrogen use efficiency: 34.1–41.9% of TN), due to its greater inorganic-N concentration. To obtain high P and K efficiencies, application rates based on compost TN are recommended, which also prevent Cu and Zn soil enrichment. Nitrate leaching was very low in all treatments (<0.5% of TN applied). Therefore, both composts can be used as organic fertilizers in the partial substitution of mineral fertilizers, without a significant nitrate leaching risk.

Keywords: nitrate, organic fertilizer, plant nutrient uptake, ryegrass, phosphorus

INTRODUCTION

The current Circular Economy approach requires the treatment of wastes so that their organic matter (OM) and nutrients can be recovered and used in agriculture. Materials derived from organic residues, such as compost, can be considered as sources of plant nutrients, for partial substitution of synthetic fertilizers (Flotats et al., 2011; Brockmann et al., 2014), in accordance with the key

principles of the Circular Economy (Bernal, 2017). Compost application to soil allows the recycling of nutrients in the soil-plant system (Tiquia et al., 2002; Bernal et al., 2009a; Flotats et al., 2011), increasing biomass production and soil fertility by improving the physicochemical and biological properties of the soil (Keener et al., 2000; Loecke et al., 2004; Cordovil et al., 2007; Bedada et al., 2014) as well as nutrient availability (Tejada and Gonzalez, 2006; Biau et al., 2012). Simultaneously, composting provides an efficient and cost-effective way of recycling these materials (Arcadis, EUNOMIA Research & Consulting, 2010; Burgos et al., 2006).

Composting has proved to be a feasible technology to treat the solid fraction of pig slurry (SPS) in areas with a high pig density, where there is excess slurry production with respect to the agricultural land available for its spreading. The stable final product is free of pathogens and rich in humified OM and nutrients (Vanotti et al., 2006; Brockmann et al., 2014; Santos et al., 2016; Sáez et al., 2017). Compared with other organic wastes (e.g., animal slurry, digestates, or untreated plant residues), qualitatively high-class composts show clear benefits as soil amendments/fertilizers: reliable sanitization, a high proportion of stable and humic-like substances, and high concentrations of nutrients (Bernal et al., 2009a, 2017; Moral et al., 2009), the latter allowing a reduction in mineral fertilization (Brockmann et al., 2014).

However, one of the main limitations to compost utilization as organic fertilizer is the fact that the exact amount of N available to plants is not clearly known (Bernal, 2017), which can induce an under- or over-application of N if the compost application rate is based on the total or mineral N content, respectively. Indeed, total N comprises both inorganic forms (mainly nitrate in mature compost; Bernal et al., 2009a), readily available to plants, and organic forms, which need to be mineralized in order to be available to plants (Bernal et al., 1998; Cordovil et al., 2006). So, when compost is applied on a total N basis, the fertilizer efficiency may be lower than that of mineral fertilizers. Furthermore, the rate of organic-N mineralization should be such that available N is produced when required by the crop (Cordovil et al., 2006); otherwise, it can be accumulated in the soil or lost to the environment (He et al., 2000).

Several parameters and criteria have been used for determining the efficiency of the nutrients present in fertilizers (Fixen et al., 2014). Nutrient use efficiency is an important concept in the evaluation of crop production systems and is commonly measured using several indicators (Gunnarsson et al., 2010; Fixen et al., 2014), such as agronomic efficiency and apparent recovery fraction (ARF) or efficiency. Modifications of the most common criteria also exist, to compare the efficiency of organic fertilizers with that of readily available mineral fertilizers. For instance, the relative agronomic efficiency (RAE) compares the agronomic efficiency of an organic fertilizer with that of a mineral fertilizer and also allows the extrapolation of the results to different total nutrient application rates (Brod et al., 2015). Much attention has been paid to N efficiency, due to the complicated N cycles in the soil, water, and atmosphere. The EU Nitrogen Expert Panel (2015) proposed an easy-to-use indicator for “nitrogen use efficiency” (NUE), applicable to agriculture and food production-consumption systems, based on the mass balance principle. The EU

Nitrogen Expert Panel (2015) considered a range of NUE values, indicating >100, soil N mining; 90–100, risk of soil N mining; 70–90, balanced fertilization; 50–70, risk of N losses; <50, a high risk of N losses due to inefficient resource use.

Another limitation associated with the use of composted manures, and in particular SPS, is the presence of high concentrations of Cu and Zn (Sáez et al., 2017). Both elements are supplied to piglets in great quantities, to promote growth (Cu), and prevent diseases (Zn). However, almost 90% is excreted in the feces (Legros et al., 2010) and is later concentrated in the SPS and in the derived compost (Sáez et al., 2017). Thus, the systematic application of composted pig manure as organic fertilizer could promote the accumulation of these elements in the soil (Gräber et al., 2005).

The over-application of mineral and organic fertilizers to crops can lead to an excess of nutrients and their consequent loss, initially through leaching, to the surrounding environment (Cavanagh et al., 2011). In Mediterranean climates, the amount of rainfall frequently is not sufficient to wash the nitrate down into aquifers, and it accumulates in the vadose zone (Jorge-Mardomingo et al., 2015). However, heavy storms in short time periods do occur, which can constitute a risk regarding nitrate pollution by leaching. The use of fertilizers in nitrate-vulnerable zones is regulated by EU legislation (Directive 91/676/EEC; EEC, 1991) that limits the application to 170 kg N ha⁻¹ year⁻¹, to minimize the impacts of nitrate pollution. The potential contamination of groundwater by nitrate from N fertilization depends mainly on the stability of the amendment used and therefore on the mineralization rate of the organic N and on the water input into the soil system, upon which the transport of nitrate to aquifers depends (Jorge-Mardomingo et al., 2015). Also, some physical properties of the composts, such as water holding capacity (WHC), can affect the water fluxes in the soil and thus the N losses (Corrêa et al., 2005). In this sense, the use of stabilized composts could reduce the potential nitrate leaching.

We hypothesized that mature compost derived from the SPS could be considered a fertilizing material due to the concentration of inorganic N readily available to plants and the organic-N forms, which will mineralize later in the soil, and that the nutrient efficiency will be similar in composts from the same origin although prepared with different SPS:cotton gin waste (CGW) proportions. Also, we considered that mature compost may have a low risk of nitrate pollution by leaching, relative to mineral fertilizer, and the application of pig slurry compost at a rate adequate for nutrient supply will prevent Cu and Zn toxicity to plants and their soil accumulation.

Therefore, the main objective of this study was to assess the fertilizer value of two composts produced from the SPS and CGW and to evaluate potential pollution risks due to the heavy metal (Cu and Zn) concentrations in the composts and the nitrate leaching following their soil application.

MATERIALS AND METHODS

Experimental Design

A 4-month pot trial with Italian ryegrass (*Lolium multiflorum* Lam.) was carried out in a greenhouse. The soil was collected

from the 0- to 15-cm soil layer of an agricultural field at UTAD (Universidade de Trás-os-Montes e Alto Douro), Vila Real, Portugal, air-dried, and sieved to 4 mm for the pot experiment and to 2 mm for analysis. The soil is non-calcareous ($\text{CaCO}_3 < 0.5\%$), with a sandy-loam texture, a WHC of 32%, and very low OM, total nitrogen (TN), and extractable P (Olsen) concentrations (Table 1). Two composts (A and B) were prepared from the SPS and CGW, at proportions of 4:3 and 3:4 (by volume), respectively, by the static composting system (Rutgers) in a pilot plant (Santos et al., 2016; Table 1). The composts were applied to the soil at two doses and compared with a soluble N:P:K (10:10:10) commercial fertilizer (N as NH_4NO_3).

Six treatments were tested as follows:

- AD1 and BD1: compost A and compost B at dose 1 (D1), using 10 and 11 g pot^{-1} of composts A and B, respectively — providing 360 mg TN pot^{-1} (equivalent to 1.44 and 1.59 t ha^{-1} of composts A and B, respectively or 52 kg N ha^{-1}), 350–272 mg P_2O_5 pot^{-1} and 402–439 mg K_2O pot^{-1} , respectively;
- AD2 and BD2: compost A and compost B at dose 2 (D2), adding double the amount of D1 (720 mg TN pot^{-1});
- F: mineral fertilizer (N:P:K 10:10:10) at a rate of 3.62 g pot^{-1} [360 mg N pot^{-1} (as in D1 of compost)], equivalent to 52 kg N ha^{-1} and 360 mg pot^{-1} of P_2O_5 and K_2O ; and
- C: control treatment without any fertilization.

A glasshouse experiment was run in 7-L double-layer polyethylene pots (System-Kick Brauckmann) which allow leachate collection from the bottom of the external part of the pot, through a polyethylene tube. The soil (9 kg of dry soil) was placed in each pot at a density similar to that in the field (1.3 g cm^3). For the compost treatments, the composts were mixed with the soil at the beginning of the experiment, and for the F treatment, the mineral fertilizer was divided into two applications (1.8 g pot^{-1} each time): before sowing and after the first harvest. All treatments were replicated four times (the total number of pots = 24). The moisture content of the soils was adjusted to 70% of the WHC with distilled water at the beginning of the experiment; then, 0.5 g of ryegrass seeds was added per pot (equivalent to 717 g m^{-2}). The soil moisture content was corrected weekly by weighing each pot and

adding water to reach 70% of the WHC. Any leachate produced after watering was collected from the bottom of the external part of the pot, quantified, and frozen for further analysis. The pots were placed in mobile racks which allowed frequent changes in position (every 4 days) inside the glasshouse (without temperature control). During the experiment, the plant material was harvested three times—30, 60, and 120 days after emergence—by cutting the aerial parts 2 cm above the soil surface. The fresh and dry weights were determined before and after drying at 60°C, respectively.

The potential nitrate leaching risk in the soil was assessed after the first and second harvests. For this, the moisture content of the soil was first adjusted to 70% of WHC; then, a heavy rain event (equivalent to 25 mm) was simulated by adding 1.75 L of distilled water to the top of the pot (equivalent to 60% of WHC). The leachates produced were quantified and frozen for further analysis.

At the end of the experiment, after the third harvest, the soil was separated from the main roots, homogenized, and sampled for analysis. The roots were cleaned—first by removing most of the soil particles with tap water, then two times with abundant distilled water, and, finally, by ultrasound (5 min at 750 W)—to eliminate any remaining soil particles adhering to the roots. Plant material (aerial part and roots) was dried at 60°C for 48 h and milled for analysis.

Analytical Methods

The moisture content of the plant material was determined by drying the sample at 60°C for 48 h in an oven with forced aeration. Then, chemical analyses were performed on the dry and ground samples: for compost and plants, total organic carbon (TOC) and TN by automatic microanalysis (EuroVector EuroEA 3000 elemental analyzer); after $\text{H}_2\text{O}_2/\text{HNO}_3$ microwave-assisted digestion, total P by a colorimetric method, K by atomic emission spectrophotometry (iCE 3000 series, Thermo Scientific), and Cu and Zn by atomic absorption spectroscopy (AAS) (iCE 3000 series, Thermo Scientific). The soil samples were analyzed for moisture content, by oven-drying the samples at 105°C for 12 h; WHC, gravimetrically, after allowing water-saturated soil to drain for 24 h; total CaCO_3 by the calcimeter method; pH, in a saturated soil paste; electrical conductivity (EC), in a 1:5 water extract; TOC and TN, by automatic microanalysis (Navarro et al., 1993); OM concentration, calculated by multiplying TOC by 1.72 (Nelson and Sommers, 1982); $\text{NH}_4^+ - \text{N}$, by a colorimetric method based on Berthelot's reaction (Sommer et al., 1992), after 2 M KCl extraction (1:20, w/v); $\text{NO}_3^- - \text{N}$, with a selective electrode (EPA 9210A) in a 1:20 water extract; available P, by a colorimetric method, after 0.5 M NaHCO_3 extraction (1:10, w/v) (Olsen-P; Watanabe and Olsen, 1965); available-K, by EAS, after 1 M $\text{CH}_3\text{COONH}_4$ extraction (1:10, w/v) (Kundsen et al., 1982); water-soluble and exchangeable Cu and Zn, by AAS, after 0.1 M CaCl_2 extraction (1:10, w/v) (McGrath and Cegarra, 1992). The leachates were analyzed for water-soluble nitrogen (N_w), using an automatic analyzer for liquid samples (TOC-V CSN Analyzer, Shimadzu), and nitrate, by ionic chromatography (850 Professional IC, Metrohm, Herisau, Switzerland). All the analyses were performed at least in duplicate, with analytical errors of <2%.

TABLE 1 | Characteristics of the soil and composts (dry weight basis).

	Soil	Compost A	Compost B
pH	7.4 ± 0.1	6.8 ± 0.02	6.9 ± 0.03
EC (dS m^{-1})	0.10 ± 0.01	10.8 ± 0.17	10.1 ± 0.25
OM (%)	0.69 ± 0.01	63 ± 1.4	57 ± 0.6
TOC (g kg^{-1})	5.0 ± 0.1	303 ± 10	288 ± 8
TN (g kg^{-1})	0.4 ± 0.01	36.1 ± 0.5	32.7 ± 1.1
$\text{NH}_4^+ - \text{N}$ (mg kg^{-1})	34.8 ± 0.7	689 ± 8.1	466 ± 6.2
$\text{NO}_3^- - \text{N}$ (mg kg^{-1})	44.7 ± 0.8	8932 ± 528	5740 ± 294
P (g kg^{-1})	0.06 ± 0.01	15.2 ± 0.1	10.8 ± 0.4
K (g kg^{-1})	0.07 ± 0.01	22.1 ± 0.2	21.9 ± 0.3
Cu (mg kg^{-1})	3.2 ± 0.06	326 ± 10	262 ± 28
Zn (mg kg^{-1})	0.7 ± 0.01	947 ± 15	719 ± 67

Concentrations of P, K, Cu, and Zn: total for compost and available for soil. Soil CaCO_3 concentration of <0.5%.

Calculations and Statistical Analysis

The plant N-utilization efficiency was calculated on the basis of the ARF, as suggested by Gunnarsson et al. (2010), and was reported as cumulative according to the following equation:

$$ARF = \sum_{t=1}^3 \frac{(N \text{ uptake treatment}) - (N \text{ uptake control})}{TN_{\text{added}}} \times 100$$

in which N uptake treatment is the total N uptake (mg pot^{-1}) of a fertilizer treatment (AD1, AD2, BD1, BD2, and F) at time n (n = harvest 1, 2, and 3); N uptake control is the total N uptake (mg pot^{-1}) of the unfertilized control at time n (n = harvest 1, 2, and 3); TN_{added} is the total N added to each pot (mg pot^{-1}).

The RAE of N, P, and K in the compost treatments was calculated from the nutrient uptake in the aboveground biomass of the compost treatment in comparison with the mineral fertilizer, taking into account the input in the double-dose treatments (Brod et al., 2015):

$$RAE = 100 \times (\text{Uptake treatment} / \text{Applied treatment}) / (\text{Uptake mineral fert.} / \text{Applied mineral fert.}).$$

The NUE of the compost and of the mineral fertilizer was calculated by expressing as a percentage the ratio of the N_{output} (N uptake by plants) and N_{input} (N applied in the treatment) of the soil (EU Nitrogen Expert Panel, 2015).

$$NUE = 100 \times N_{\text{output}} / N_{\text{input}}$$

For statistical analysis, IBM SPSS Statistics 21.Ink software was used. The standard error of the means was calculated for the chemical and physicochemical characteristics of the soils and the plant biomass and nutrients; a one-way analysis of variance (ANOVA) was performed, and differences between means were determined using Tukey's test. A two-way ANOVA was used to determine significance in the plant parameters according to the harvest and treatment and to the type of compost and application rate. The normality and homogeneity of the variances were checked using the Shapiro–Wilk and Levene tests, respectively, before ANOVA.

RESULTS AND DISCUSSION

Biomass Production of *L. multiflorum*

The shoot biomass production of *L. multiflorum* (dry weight) increased in the compost treatments and with the compost application rate, in comparison with the control plants, at all harvests, without significant differences between the two composts (Figure 1). The root biomass production was highest in F and only treatment AD2 gave results statistically different from the control (Figure 1). The compost treatments at D1 gave shoot biomass values similar to that of F in the first harvest, with significantly greater values at D2, due to the greater supply of inorganic N (300 and 260 mg pot^{-1} in composts A and B, respectively) in comparison with D1 (150 and 130 mg pot^{-1} in composts A and B, respectively) and with the first application of F (180 mg pot^{-1}). The pig manure composts had high inorganic N concentrations

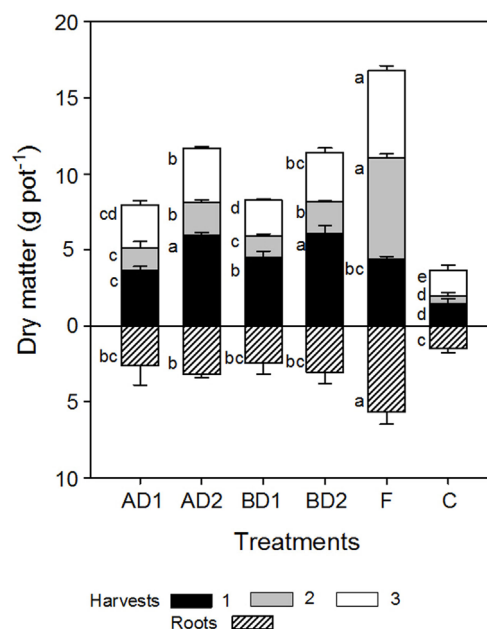


FIGURE 1 | Shoot biomass production of *Lolium multiflorum* at each harvest and root biomass at the end of the experiment (dry matter) in the different treatments: AD1, compost A at dose 1; AD2, compost A at dose 2; BD1, compost B at dose 1; BD2, compost B at dose 2; F, mineral fertilizer; C, non-fertilized control. For each harvest, values (bars) with the same letter do not differ significantly according to the Tukey test ($P < 0.05$).

(Table 1), supplying adequate N for correct plant growth after soil application. Thus, this readily available N seems to be the main factor responsible for the augmented plant growth (Eghball and Power, 1999), demonstrated by the positive correlation ($r = 0.98$; $p < 0.001$) between the aerial biomass production (DM) and the TN supplied by the amendments. The dependence of DM yields of Italian ryegrass on the N supply was suggested by Douglas et al. (2003), who used different organic wastes in a field experiment. Treatment F led to the greatest plant DM production at harvests 2 and 3, as the mineral fertilizer was divided into two applications, at the beginning of the experiment and after the first harvest, providing available N for the subsequent plant growth.

Inorganic N (NH_4^+ and NO_3^-) in the composts, readily available to plants, was responsible for the effects seen at the first harvest, but organic N needed to be mineralized for plant uptake prior to the following harvests. The decreased yields at harvests 2 and 3 for the compost treatments may indicate scarce mineralization of the organic N in both composts, as observed in previous studies (Bernal et al., 1998; Moral et al., 2009), due to the microbial stability of the OM, with a low amount of degradable TOC (Bernal et al., 1998, 2009a). Indeed, the N mineralization of mature compost is usually slow and may not have been sufficient to provide enough inorganic N to the crop for the following harvests. Cordovil et al. (2006) reported a good relationship between the N supplied to ryegrass by different organic amendments and their potentially mineralizable N. Different studies revealed differences in the mineralizable N in compost; for instance, Moral et al. (2009) reported N-mineralization values ranging from 0 to

34% of TN in composts prepared from manures, but only from 1 to 4.7% of TN in those prepared from pig slurry.

Also, fresh organic wastes and immature or unstable compost can cause N immobilization in the soil after their application, mainly for high C/N ratios, reducing plant-available N and leading to low yields (Bernal et al., 1998; Douglas et al., 2003; García-Gómez et al., 2003). Increases in the biomass production of maize (12–15%) with the use of composted pig manure, in comparison with the fresh manure, were found by Loecke et al. (2004) during a 2-year experiment. Cordovil et al. (2007) also found a greater wheat production with composted pig manure than with pig slurry, and Bedada et al. (2014) observed an increase in crop production of 11%, in comparison with the control treatment, when compost prepared with cattle manure and agricultural wastes was applied. Thus, compost maturity guarantees the N mineralization after soil application, avoiding soil microbial N immobilization.

Nutrient Uptake and Accumulation by *L. multiflorum*

Compost application significantly increased the concentrations of the macronutrients N and K in the plants, compared with the control, at each harvest, without significant differences relative to

treatment F (Table 2). Only treatment BD1, at the first harvest, reduced the N concentration in the plants with respect to F, due to the low inorganic-N concentration in compost B (Table 1). All compost treatments led to greater P concentrations in the plants than did treatment F at harvests 2 and 3, even at D1. This was probably due to a concentration effect associated with the low yields obtained at those harvests for the compost treatments (Ylivainio et al., 2008). So, both composts were able to substitute only partially for mineral fertilizer.

Besides the significant, positive responses of the N, P, and K concentrations in ryegrass to compost application, a significant decline in N with the harvesting cycle was observed, which may be due to the consumption of the easily available N and scarce N mineralization discussed previously, or to nutrient leaching after each “rain event” (discussed below). Despite the slightly higher N concentration in plants from treatment D2, relative to D1, in the first harvest, the values for the compost-based treatments did not differ significantly ($P < 0.001$), and only TN in BD2 was higher than the control values. Considering the optimal shoot N, P, and K concentrations in Italian ryegrass (30–42 g N kg⁻¹ DM, 3.5–5 g P kg⁻¹ DM, and 25–35 g K kg⁻¹ DM; Bergmann, 1986), N and P might have limited the growth, particularly at harvests 2 and 3. The P concentration in the aboveground biomass was

TABLE 2 | Plant nutrient concentrations in the aerial parts of *L. multiflorum* at the three harvests, according to the different treatments applied to the soil: AD1, compost A at dose 1; AD2, compost A at dose 2; BD1, compost B at dose 1; BD2, compost B at dose 2; F, mineral fertilizer; C, non-fertilized control.

Treatment	N (g kg ⁻¹)	P (g kg ⁻¹)	K (g kg ⁻¹)	Cu (mg kg ⁻¹)	Zn (mg kg ⁻¹)
Harvest 1					
AD1	28.4 ± 2.72 ab	0.96 ± 0.05	37.7 ± 2.4 a	9.6 ± 0.53 ab	19 ± 2.5 ab
AD2	32.0 ± 1.50 ab	0.99 ± 0.07	38.2 ± 1.0 a	9.9 ± 0.88 a	23 ± 3.5 a
BD1	23.4 ± 0.59 b	0.89 ± 0.05	34.7 ± 0.9 ab	8.1 ± 0.38 bc	19 ± 4.9 ab
BD2	35.4 ± 8.80 a	0.93 ± 0.12	38.0 ± 0.9 a	10.0 ± 1.1 a	19 ± 1.6 ab
F	32.8 ± 1.78 a	0.88 ± 0.15	34.7 ± 3.0 ab	10.4 ± 0.4 a	17 ± 2.7 ab
C	23.6 ± 2.29 b	0.82 ± 0.06	33.3 ± 0.3 b	7.4 ± 0.35 c	15 ± 0.9 b
ANOVA	**	n.s.	**	***	*
Harvest 2					
AD1	17.7 ± 1.21 ab	1.22 ± 0.05 a	28.3 ± 1.3 ab	5.7 ± 0.26	15 ± 1.9
AD2	14.7 ± 6.02 b	1.28 ± 0.09 a	28.9 ± 1.2 ab	5.8 ± 1.88	17 ± 4.0
BD1	17.1 ± 1.77 ab	1.23 ± 0.08 a	26.2 ± 0.7 b	5.0 ± 0.09	15 ± 1.7
BD2	17.0 ± 0.93 ab	1.28 ± 0.07 a	28.0 ± 0.8 ab	4.3 ± 0.43	17 ± 4.0
F	21.5 ± 1.24 a	0.98 ± 0.14 b	31.0 ± 4.0 a	6.7 ± 2.40	15 ± 2.1
C	17.5 ± 1.09 ab	1.13 ± 0.08 ab	26.8 ± 1.3 ab	4.5 ± 0.64	16 ± 1.0
ANOVA	*	**	*	n.s.	n.s.
Harvest 3					
AD1	6.3 ± 0.35	0.77 ± 0.04 a	20.6 ± 1.7 ab	2.3 ± 0.16 a	16 ± 2.8
AD2	6.9 ± 0.29	0.86 ± 0.03 a	21.4 ± 1.3 ab	2.3 ± 0.29 a	17 ± 2.9
BD1	7.0 ± 0.46	0.95 ± 0.21 a	19.9 ± 0.1 b	2.0 ± 0.23 a	16 ± 1.2
BD2	7.1 ± 0.48	0.96 ± 0.07 a	22.4 ± 0.4 a	2.3 ± 0.49 a	21 ± 3.7
F	6.5 ± 1.10	0.53 ± 0.04 b	19.0 ± 0.7 b	1.2 ± 0.24 b	19 ± 9.3
C	6.5 ± 0.47	0.76 ± 0.08 ab	19.4 ± 1.5 b	1.4 ± 0.19 b	15 ± 0.8
ANOVA	n.s.	***	**	***	n.s.
ANOVA					
Harvest × Treatment	***	***	***	***	n.s.

Mean ± SD (n = 4).

n.s.: not significant.

*, **, ***Significant at $P < 0.05$, 0.01 , and 0.001 , respectively. For each parameter, values followed by the same letter do not differ significantly according to the Tukey test ($P < 0.05$).

within the lower range found by Ylivainio et al. (2008), who suggested that a low P concentration can be due to a dilution effect caused by the enhanced growth of *L. multiflorum*—as shown at harvest 2, when the P concentration was lowest for plants of treatment F, which also gave the highest yield. The N:P ratio in the aerial parts of the plants can indicate the nutrient that is restricting plant growth (Güsewell, 2004). The N:P ratio of >20 found at the first harvest, for all treatments, suggests that plant growth was initially restricted by P availability, while, at harvest 3, the N:P ratio of <10 indicates that N limited plant growth at the end of the experiment for the compost treatments (Güsewell, 2004). At the second harvest, for all treatments, the N:P ratio was adequate. These results indicate that the plant-available N from compost and F was adequate immediately after soil addition, but the precipitation or fixation of P can occur shortly after addition to the soil (e.g., due to insoluble Ca-phosphate formation), being released later on during the growing session. Organic acids in the soil, produced from root exudates, may compete for the same binding sites as P, depressing the P sorption strength of the soil (Ylivainio et al., 2008). The exudation of organic anions by the roots has been found to occur in the rhizosphere, associated with NO_3^- -N nutrition in preference to NH_4^+ -N (Marschner, 1995; Hinsinger et al., 2003); also, localized release of H^+ by some portions of the roots has been reported to occur as a response to P deficiency, favoring P solubility in the soil (Hinsinger et al., 2003). Jones (1998) reported two mechanisms of P release in the soil rhizosphere: direct ligand exchange, whereby citrate directly replaces P on ligand exchange surfaces, and complexation with metal ions in the solid phase, which constitutes the exchange matrix holding P (such as Ca^{2+} in rock phosphate). According to Jorgensen et al. (2010), composting of the separated solids from animal slurry does not alter the P availability but P precipitation as Ca or Mg phosphate may occur during composting due to the pH increase. Ylivainio et al. (2008) indicated that the high proportion of inorganic P in manure compost makes it a convenient P fertilizer for *L. multiflorum* due to its high root-to-shoot ratio of P (as shown in the present experiment; Tables 2 and 3); these authors referred to it as a P-efficient species with relatively low external P requirements.

The nutrient uptake by the aerial parts of *L. multiflorum* during the pot trial is shown in Figure 2. Compost application,

particularly at the high rate (D2), significantly increased the N, P, and K accumulation by the shoots, when compared with the control (Figure 2). The uptake of N, P, and K was greatest in treatment F (324, 13.5, and 467 mg pot⁻¹, respectively), with a considerable difference from BD2 (270, 11.4, and 362 mg pot⁻¹, respectively). Composts A and B did not show any significant differences at the same application rate, but nutrient uptake increased with the compost application rate. An increase in N uptake with the compost application rate was also found by Cordovil et al. (2006), but differences were not found when organic and inorganic fertilizations were combined, suggesting that the mineral N supplied could have stimulated not only N uptake but also that of other nutrients. Also, Ylivainio et al. (2008) reported a higher P uptake by *L. multiflorum* when composted manure was applied at a double rate and even greater uptake at a quadruple dose, at all harvests.

High concentrations of Cu and Zn are usually found in pig slurry compost (Sáez et al., 2017). The Spanish legislation for fertilizing products (Ministerio de la Presidencia, 2013) classifies composts according to the total Cu and Zn concentrations. The composts in the present experiment belonged to class C, with Cu between 300 and 400 mg kg⁻¹ and Zn between 500 and 1000 mg kg⁻¹, limiting the use to 5 t ha⁻¹ year⁻¹ on agricultural soil. The concentrations of Zn and Cu in the plants were analyzed in order to determine their accumulation due to the use of pig slurry composts. At the first harvest, the shoot Cu and Zn concentrations were generally higher in the compost treatments than in control plants, but without statistically significant differences from treatment F. The concentrations of Zn measured in the aerial parts of the plants can be considered normal for plant nutrition (27–150 mg kg⁻¹; Kabata-Pendias, 2001) and are within the range found for grasses (12–80 mg kg⁻¹; Kabata-Pendias, 2001). Although the shoot Cu concentration remained higher in the compost treatments than in the control, the values are within the normal range for plants (5–30 mg kg⁻¹; Kabata-Pendias, 2001) and the range for grasses (1.3–33.1 mg kg⁻¹; Kabata-Pendias, 2001). The Cu concentration was decreased at the following harvests; this may be due to the ability of Cu to form chelates with the stable and humified OM (Bernal et al., 2009b). Therefore, both composts used here, A and B, did not present any risk regarding metal toxicity to plants.

TABLE 3 | Concentrations of nutrients in the roots of *L. multiflorum* at the end of the experiment.

Treatment	N (g kg ⁻¹)	P (g kg ⁻¹)	K (g kg ⁻¹)	Cu (mg kg ⁻¹)	Zn (mg kg ⁻¹)
AD1	6.54 ± 1.01	0.38 ± 0.03	9.4 ± 0.70 b	38.1 ± 2.4 b	33.5 ± 2.3 bc
AD2	6.59 ± 0.61	0.45 ± 0.07	11.6 ± 0.58 ab	43.4 ± 7.6 ab	34.6 ± 3.6 abc
BD1	6.26 ± 0.61	0.38 ± 0.03	9.4 ± 0.74 b	47.7 ± 5.4 ab	38.4 ± 4.4 ab
BD2	5.76 ± 0.36	0.41 ± 0.06	12.7 ± 0.11ab	51.8 ± 5.6 a	41.1 ± 2.1 a
F	5.87 ± 0.68	0.44 ± 0.03	14.8 ± 0.85 a	46.0 ± 8.4 ab	29.6 ± 2.3 c
C	7.34 ± 1.29	0.38 ± 0.03	9.9 ± 0.64 b	51.7 ± 2.1 a	32.8 ± 2.5bc
ANOVA	n.s.	n.s.	**	*	**

Mean ± SD (n = 4).

n.s.: not significant.

*, **, ***Significant at $P < 0.05$, 0.01, and 0.001, respectively. For each parameter, values followed by the same letter do not differ significantly according to the Tukey test ($P < 0.05$).

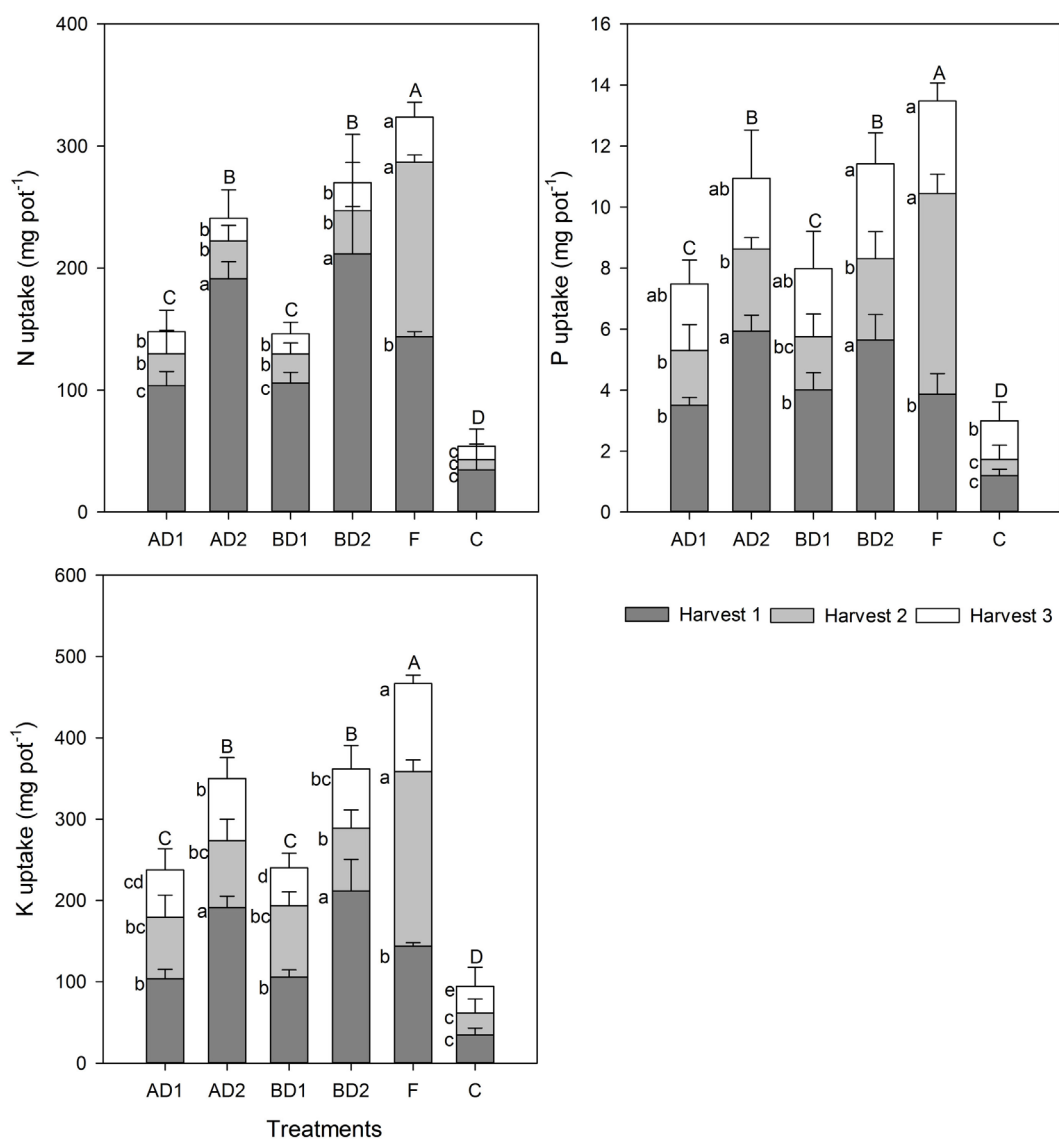


FIGURE 2 | Nutrient uptake in the aerial parts of *Lolium multiflorum* at each harvest during the pot trial, according to the different treatments applied to the soil: AD1, compost A at dose 1; AD2, compost A at dose 2; BD1, compost B at dose 1; BD2, compost B at dose 2; F, mineral fertilizer; C, non-fertilized control. For each harvest, values (bars) with the same letter do not differ significantly according to the Tukey test ($P < 0.05$). Capital letters refer to the accumulated values (sum of harvests 1 + 2 + 3) and lower-case letters refer to each harvest.

Fertilizing Efficiency

The nutrient fertilizing efficiency of the compost was estimated as the ARF, calculated according to Eq. 1. With respect to the control, the N-ARF of the composts ranged from 24 to 29%, without significant differences between the compost types or doses (Table 4). Similar values (28%) were found by Thomsen and Kjellerup (1997) for manure without litter (cattle feces), being 8–14% for manure with litter (cattle feces + urine + straw), related to the inorganic-N concentration; lower values were found in winter, associated with N leaching. The mineral fertilizer treatment (F) reached the greatest N-ARF value, because all the N was in inorganic forms (NH_4NO_3), whereas in composts A

and B, 26.7 and 19.0%, respectively, of the TN was in inorganic forms (Table 1). This indicates that the inorganic N present in compost A was assimilated by the plants, but partial mineralization of the organic N of compost B may have occurred, rendering it available to plants. Cordovil et al. (2006) compared the potentially mineralizable N of several organic materials (determined in incubation experiments) with the plant N uptake in a pot experiment. Their results showed that the plant N uptake from pig manure compost exceeded the potentially mineralizable N (33.3 and 25%, respectively), similar to the results found here, while compost from municipal solid waste led to very similar values of N uptake and potentially mineralizable N (19%). Also,

TABLE 4 | Evaluation of the fertilizer efficiency by the Apparent Recovery Fraction (ARF) and Relative Agronomic Efficiency (RAE) for N, P, and K, and the Nitrogen Use Efficiency (NUE).

Treatment	N-ARF (%)	N-RAE (%)	NUE (%)	P-ARF (%)	P-RAE (%)	K-ARF (%)	K-RAE (%)
AD1	26.6 ± 5.01b	47.6 ± 4.75	41.9 ± 5.01b	2.9 ± 0.51bc	56.1 ± 6.95b	64.5 ± 11.7b	66.3 ± 6.99a
AD2	26.5 ± 3.30b	37.8 ± 4.20	34.1 ± 3.30b	2.6 ± 0.52c	40.0 ± 7.56b	57.6 ± 5.79b	47.9 ± 4.96b
BD1	23.9 ± 2.45b	42.6 ± 3.42	37.9 ± 2.45b	4.2 ± 1.02b	74.6 ± 10.8a	60.1 ± 7.38b	60.3 ± 4.34a
BD2	28.0 ± 5.14b	38.1 ± 5.84	35.0 ± 5.14b	3.5 ± 0.43bc	52.7 ± 5.73b	55.0 ± 5.97b	45.0 ± 4.36b
F	74.5 ± 3.34a	n.a.	89.4 ± 3.34a	6.6 ± 0.37a	n.a.	123 ± 3.43a	n.a.
ANOVA treatment [†]	***	*	***	***	***	***	***
Dose [‡]	n.s.	n.s.	n.s.	***	**	n.s.	n.s.
Compost [‡]	n.s.	**	*	n.s.	***	n.s.	***
DxC [‡]	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

n.a.: not applicable.

[†]One-way.

[‡]Two-way ANOVA.

Mean ± SD (n = 4).

n.s.: not significant.

*, **, ***Significant at $P < 0.05$, 0.01 , and 0.001 , respectively. For each parameter, values followed by the same letter do not differ significantly according to the Tukey test ($P < 0.05$).

Douglas et al. (2003) found a very low N-ARF of green compost, in spite of its high TN concentration (4.3–5.8 g kg⁻¹).

Compared with the efficiency of the mineral fertilizer, the values of RAE (Table 4) confirm that the mineralization of organic N in the mature composts occurred in the soil during the crop development, accounting for 10–20% of the TN in compost A and an average of 22% in compost B. According to Bernal et al. (1998), the potentially mineralizable N increases with composting time, representing about 9% of the TN in mature compost derived from sewage sludge. Although the NUE is considered applicable to agriculture and food production–consumption systems, this parameter also can be applied to small agronomic experiments (EU Nitrogen Expert Panel, 2015). Considering the inputs and outputs in the present system (pots), the maximum NUE occurred with F, its value decreasing with the compost application rate (Table 4). The NUE values may also indicate that N mineralization occurred in the system, representing 8–18% of TN, with greater values for compost B.

The ARF of P was low in all treatments, the highest value being in treatment F (6.6%; Table 4). This implies that this nutrient was applied in excess of crop requirements and accumulated in the soil. In the work of Ylivainio et al. (2008), the ARF of P for ryegrass in the first growing year was 20–26% for manure compost applied at different rates, with similar values in the treatments with mineral fertilizer (21.7–26.8%). Grigatti et al. (2017) found, in a 21-week pot experiment, P-ARF values for green waste compost of 10–40%, depending on the characteristics (P fractionation) of the compost. They used a P-application rate (15 mg kg⁻¹) similar to that in the present experiment; the ARF was related to the H₂O + NaHCO₃-extractable P, which was mainly inorganic P. The solubility of the P from compost may depend on the degree of maturation. During composting of animal manures, Eneji et al. (2003) found that water-soluble P decreased during composting, shifting toward the more resistant HCl-soluble P (associated with Ca and Mg), which was the dominant phase at the end of composting. The high degree of maturation of the compost used in the present experiment (Santos et al., 2016) may have reduced the availability of P. The RAE of P in compost B (74.6 and 52.7%,

respectively, for doses 1 and 2) was greater than in compost A (56.1 and 40.0%, respectively, in doses 1 and 2), due to the lower concentration of P in compost B, giving a greater efficiency of P uptake by the plants. Brod et al. (2015) determined the P-RAE of different organic waste materials for ryegrass: the greatest values occurred for dairy cattle manure, with a greater efficiency than the mineral fertilizer (Ca(H₂PO₄)₂), followed by liquid digestate and chicken manure (72–78%), with lower values for fish sludge and solid digestate (47–70%), for one or up to four harvests in a soil of pH 6.9. These values were well predicted using the NaHCO₃-extractable P (Grigatti et al., 2017).

A very different behavior was shown by K, with 100% of the K added to the soil in F assimilated by the plants while the ARF reached very high values for composts A and B, without differences according to the compost type and dose (Table 4). This is due to the highly available (mainly soluble) forms of K present in the composts; the fraction not taken up by plants can be retained in the soil as exchangeable and soluble forms, but can also be lost by leaching. The high EC of the leachates of the compost treatments (up to 630 µS cm⁻¹ in the first rain event and up to 263 µS cm⁻¹ in the second), with respect to the control (116 and 127 µS cm⁻¹, rain event 1 and 2, respectively) and treatment F (219 and 170 µS cm⁻¹, rain event 1 and 2, respectively), may indicate the leaching of soluble salts, including K.

Nitrogen Leaching and Residual Soil Nutrients Availability

Losses of N during the experiment could have occurred by leaching or denitrification (Hoffman and Van Cleemput, 2004; Burgos et al., 2006; Demuras et al., 2016). For leaching to occur, N must be in a water-soluble, mobile form, and the rainfall must be abundant enough to transport N through the soil. Nitrate is the N form most susceptible to leaching, since ammonium N can be retained in the soil cation exchange capacity. In this sense, water-soluble TN (N_w) present in the leachate from the first rain event occurred as nitrate (data not shown); then, only N_w was measured in the following leachates.

Considering the threshold of 50 mg l^{-1} for NO_3^- concentration in ground and surface water set by the European Legislation (European Union, 1998), all the leachate samples were far away from that value (maximum NO_3^- concentration 5.28 mg l^{-1}). All the fertilizer treatments had higher N losses than the control during the experiment (1.7- to 2.1-times greater), without significant differences between treatments or rain events (**Figure 3**). The amount of N leached during the rain events did not differ statistically among the fertilizer treatments, and similar values also occurred during the irrigation in the soils treated with compost B (**Figure 3**). For TN, the total proportion lost (during irrigation and rain events) was lowest when each compost was applied at a high rate (**Figure 3**). The WHC of the composts may have had a strong influence, reducing N leaching (Fulekar, 2010), as compost can improve soil properties to achieve more efficient water utilization. In fact, compost B, prepared with a greater amount of CGW than compost A, produced a slightly lower volume of leachate during the rain events than compost A, especially at D2 ($2,100 \pm 395$ and $1,695 \pm 380 \text{ ml pot}^{-1}$ in AD2 and BD2, respectively; $2,180 \pm 212 \text{ ml pot}^{-1}$ in F). In terms of the doses applied, no significant differences were found when comparing the amounts of N leached (**Figure 3**). A higher NO_3^- -N application might imply a greater N leaching risk, due to the excess of NO_3^- with respect to the plant requirements. However, the lack of a significant change in the loss of N by leaching (or NO_3^- accumulation in the soil, **Table 5**) indicates that the NO_3^- -N provided by the compost was used by the plants (as the ARE, RAE, and NUE also indicate). Burgos et al. (2006) pointed out that N losses by leaching tend to be higher when poorly stabilized organic materials rich in N are applied, but also stated that NO_3^- losses by leaching from compost-amended soils are usually very low. Demuras et al. (2016) concluded from a field experiment that the replacement of organic with mineral N-fertilization systems was not effective at mitigating NO_3^- leaching, although the residual N remained in the soil for further mineralization. In fact, they did not find any reduction in N leaching during winter, when mineral fertilizer was used instead of organic N.

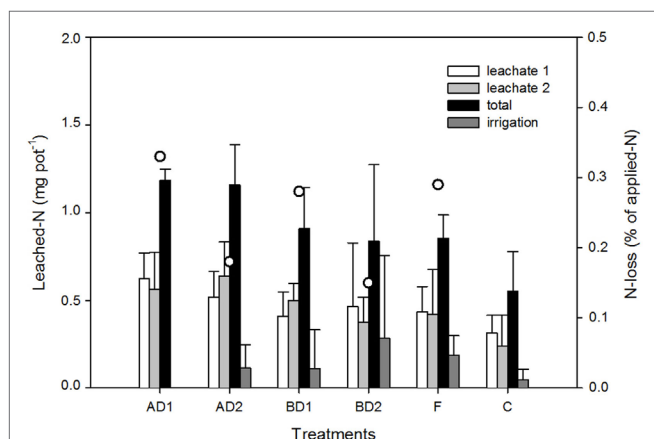


FIGURE 3 | Nitrogen leached during each rain event simulated during the pot trial. No significant differences were found among the treatments. Dots indicate total N leached in each treatment as a percentage of TN added.

The chemical properties of the soils at the end of the experiment are shown in **Table 5**. The TOC in the soil showed small and non-significant differences between the treatments with compost and the control. Evanylo et al. (2008) found small differences in the TOC in soils amended with compost at low doses, indicating that a relevant proportion of the OM from the compost may have been mineralized during the experiment. All the treatments were able to increase TN in the soil, at the end of the experiment, with respect to the control soil, without differences among the fertilizing treatments. Increases in TN in the soil after application of composts produced from livestock manure or from municipal solid wastes have been found in pot studies (Soumaré et al., 2003; Evanylo et al., 2008; Karami et al., 2011)—indicating that the resistant fraction of the organic N in mature composts accumulated in the soil, being incorporated into the humified OM.

The lack of significant differences in the soil NH_4^+ -N concentration among the treatments indicates its complete nitrification, in similar proportions in all treatments. The lowest concentrations of NO_3^- -N were observed in the soils amended with compost A, in spite of its high NO_3^- -N concentration (**Table 1**), but the RAE and NUE results indicate lower N mineralization than in compost B. No statistically significant differences occurred for the rest of the treatments, suggesting that the inorganic N supplied by the amendments may have been used by the plants or lost from the soil. The low N losses by leaching during the two rain events (**Figure 3**) indicate that the N applied in excess accumulated in the soil, as losses by denitrification may have been irrelevant at a soil moisture content of $<70\%$ WHC. According to Estavillo et al. (1996), N losses by denitrification from mineral fertilizers can be 9–17% during spring/autumn, reaching 2% from mature composts (Cambardella et al., 2003). In fact, the TN accumulation in the soil with the compost and mineral fertilizer treatments occurred as organic N (**Table 5**).

The concentrations of available P and K were lowest for treatment AD1 and greatest in treatment F (**Table 5**), which may be due to the excess P supplied by the treatments, giving a low ARE, as discussed previously, and the highly soluble nature of the K supplied by all treatments. However, a greater proportion of the excess P provided by the mineral fertilizer may have remained in the soil in the available fraction, in comparison with that provided by the compost, because P in well-matured compost can be found in less available forms (Eneji et al., 2003; Grigatti et al., 2017).

The elements Cu and Zn are essential plant micronutrients, but their accumulation in the soil can cause toxicity to plants (Kabata-Pendias, 2001). The use of compost from pig manure can lead to soil accumulation, this being one of the main environmental risks associated with compost use. In fact, limits for the Cu and Zn concentrations in compost for agricultural use have been set in several countries and in the EU for soil protection (Bernal et al., 2017). In the present experiment, the composts did not significantly increase the available Cu and Zn concentrations in the soil, with respect to the control (**Table 5**), and similar values were obtained for Zn in treatment F. These results, together with the plant concentrations found, indicate that these elements in the compost had a beneficial effect, as plant micronutrients. Considering the total concentrations of Cu and Zn in the compost and the application rate, the increases in their total levels

TABLE 5 | Total organic carbon (TOC), total N (TN), inorganic-N forms, available P and K, and the soluble and exchangeable fraction of Cu and Zn (CaCl₂-extractable) of the soils at the end of the experiment.

Treatments	TOC	TN	NH ₄ ⁺ -N	NO ₃ ⁻ -N	P _{avail}	K _{avail}	Cu	Zn
	(g kg ⁻¹)	(g kg ⁻¹)	(mg kg ⁻¹)	(mg kg ⁻¹)	(mg kg ⁻¹)	(mg kg ⁻¹)	(mg kg ⁻¹)	(mg kg ⁻¹)
AD1	4.0 ± 0.14	0.43 ± 0.02 a	17.5 ± 1.86	9.58 ± 0.53 b	63.2 ± 2.96 c	48.4 ± 2.49 e	2.97 ± 0.10 b	0.65 ± 0.01 c
AD2	4.8 ± 0.38	0.45 ± 0.02 a	13.7 ± 3.58	9.71 ± 1.18 b	81.5 ± 4.77 b	64.9 ± 1.84 c	3.32 ± 0.15 a	1.07 ± 0.18 b
BD1	4.8 ± 0.21	0.44 ± 0.03 a	17.6 ± 2.19	11.0 ± 0.80 ab	98.6 ± 5.46 a	74.0 ± 1.27 b	3.48 ± 0.11 a	1.53 ± 0.16 a
BD2	4.3 ± 0.22	0.42 ± 0.03 a	16.8 ± 1.04	11.3 ± 0.32 ab	82.1 ± 6.97 b	64.7 ± 1.33 c	3.40 ± 0.14 a	1.17 ± 0.13 b
F	4.9 ± 0.36	0.44 ± 0.04 a	15.9 ± 0.72	12.1 ± 1.29 a	97.3 ± 2.19 a	79.0 ± 3.65 a	3.42 ± 0.18 a	1.54 ± 0.03 a
C	3.8 ± 0.16	0.37 ± 0.01 b	18.3 ± 3.41	11.6 ± 0.34 ab	63.2 ± 3.48 c	56.0 ± 1.44 d	3.40 ± 0.16 a	0.76 ± 0.15 c
ANOVA	n.s.	**	n.s.	**	***	***	**	***

Mean ± SD (n = 4).

n.s.: not significant.

*, **, ***Significant at P < 0.05, 0.01, and 0.001, respectively. For each parameter, values followed by the same letter do not differ significantly according to the Tukey test (P < 0.05).

in the soil were low (Cu < 0.4 and Zn < 1.0 mg kg⁻¹ soil), with a scarce risk of soil accumulation at the compost rates applied. However, their pollution risk due to accumulation resulting from repeated applications over a long period needs to be tested in field experiments.

CONCLUSION

Both composts prepared from the SPS can be considered organic fertilizer products, since they efficiently increased the biomass yield of *L. multiflorum*, providing nutrients for plant growth; N was provided in readily available forms (inorganic N) and was slowly released by partial mineralization of the organic N during the crop growth. Differences in the timing of the supply of N and P to plants could have been responsible for plant growth restriction due to limited P or N supply in the early or later stages of the growing period, respectively, suggesting that the combined application of compost with mineral fertilizer during the growing season may ensure adequate plant growth prior to the subsequent harvests.

The RAE (47.6%) of N was highest for compost A (prepared with the greatest SPS:CGW ratio), due to the concentration of inorganic N directly available to plants. According to the average RAE values, 41% of the N from pig slurry compost can partially replace N from mineral fertilizer. The fertilizer efficiency of P was low for all treatments, but the P-RAE percentages reached 56 and 75% in composts A and B, respectively, at a low application rate.

Taking into consideration the limitation of the present experiment run in pots and under controlled conditions, the microbial stability of mature compost and an adequate C/N ratio for soil application seem to control the N supply to crops as a slow release fertilizer. Under these experimental conditions, the use of both composts as soil fertilizers did not show any threat in terms of nitrate leaching, even after a heavy rain event and with a double

dose of compost. As a consequence, it can be concluded that the fertilizing value of both composts can partially substitute for mineral fertilizer.

An experiment under real field conditions may be needed to confirm the present results. To supply the nutrients required for efficient crop production at each particular stage of the growing session, the combination of mineral and organic fertilizers, using composts as a base fertilization before sowing and complementing with mineral fertilizer during the growing season, might be worth testing under real conditions. Thus, the results obtained here for NUE, ARE, and RAE can be used to calculate the compost application rate and mineral fertilizer requirement under a combined fertilization plan, with a partial mineral fertilizer substitution by compost to avoid over-fertilization.

AUTHOR CONTRIBUTIONS

The author AS has performed the experimental work, statistical analysis, and written the first version of the manuscript; MB has designed the experiment and supervised the experimental work, corrected the paper, and written the final version; DF supervised the experimental work of AS and revised the paper; and RM revised the final version of the paper.

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Concentration and Release of Phosphorus and Potassium From Lignocellulosic- and Manure-Based Biochars for Fertilizer Reuse

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Biochars pyrolyzed from plant residues and animal manure feedstocks may contain disproportionate amounts of phosphorus (P) and potassium (K). Unequal nutrient characteristics can impact the biochars ability to properly supply as well as improve soil P and K fertility levels. A soil incubation study was performed to test the hypotheses that biochar produced from poultry litter will release more water soluble dissolved P (DP) and K (DK) concentrations and would also increase soil plant available P and K concentrations as compared to lignocellulosic-based biochars. Biochar was pyrolyzed at 500°C from hardwood waste products (HW), pine chips (PC; *Pinus taeda*), poultry litter (PL; *Gallus domesticus*), and an 80:20 pine chip/poultry litter blend, which were then added at 20 g kg⁻¹ to a sandy Norfolk E soil (Typic Kandiuult). Un-amended (no biochar) Norfolk E soil served as a control. During the incubation, all treatments were leached four times with deionized water and the leachate analyzed for DP and DK; their concentration and mass released as a function of total amounts present were then calculated. At the conclusion of the study, soils were extracted using Mehlich-1 reagent for determination of plant-available P and K contents. Leachates from soil amended with 100% PL biochar and the 80:20 blend had significantly more DP and DK mass (59 and 1,018 mg, respectively) released compared to PC and hardwood biochars (0.07 and 23 mg). Significant amounts of DP were released from PL biochar with additional water leaching, but DK release results were mixed. Soil Mehlich-1 P and K contents were significantly increased using PL biochar compared to lignocellulosic-based biochars. Blending PC with PL feedstocks at 80:20 weight ratio reduced Mehlich-1 soil P concentrations to 35 mg kg⁻¹, which was more aligned with soil test P levels (30–50 mg kg⁻¹) recommended for a corn (*Zea mays*) production in southeastern USA Coastal Plain sandy soils. These results reveal that 100% PL biochar offers a higher potential to provide more P and K to soils than lignocellulosic based biochars, and that feedstock blends can be used to create designer biochars that align soil test fertilizer values with plant nutrient requirements.

Keywords: biochar, fertilizer, manure, phosphorus, plant, potassium

INTRODUCTION

Pyrolysis of organic feedstocks produces thermal energy, bio-oil, and a solid-product called biochar (Antal and Gronli, 2003). Many types of organic feedstocks are suitable materials to produce biochars, including animal manures, wood waste compounds, green-wastes, and other plant-based materials (Bridgewater, 2003; Laird et al., 2009; Sohi et al., 2009; Boateng et al., 2015). The structural components of biochar have been well-characterized and are reported to consist of aromatic and non-aromatic organic compounds (Antal and Gronli, 2003; Jindo et al., 2014), and an ash fraction containing an assemblage of inorganic elements (e.g., Ca, Mg, P, K etc.; Novak et al., 2009; Cantrell et al., 2012; Ippolito et al., 2015). Consequently, they are acknowledged as viable soil amendments to increase C sequestration (Day et al., 2004; Brassard et al., 2016; Smith, 2016; Lamb et al., 2018), crop yields (Jeffery et al., 2011), and fertility characteristics (Novak et al., 2009; Spokas et al., 2012; Bargmann et al., 2013).

A concern with the use of biochar as a soil fertility amendment, however, are vast differences in their plant nutrient composition (Cantrell et al., 2012; Novak et al., 2013; Domingues et al., 2017). For example, biochars produced from hardwoods (HW) and softwoods generally have lower concentrations of total P (TP) and total K (TK) as compared to animal-manure based biochars, such as poultry litter (PL, Cantrell et al., 2012; Novak et al., 2013). In these reports, PL biochar pyrolyzed at 700°C had 42.8 and 86.6 g kg⁻¹ of TP and TK contents, respectively, as compared to 0.28 and 2.7 g kg⁻¹ contained in pine chip (PC) biochar. The greater TP and TK concentrations in PL biochar was attributed to un-assimilated nutrients in the excreted animal manure (Novak and Busscher, 2012).

Besides examining TP and TK contents among biochar types, plant available P and K concentrations have also been reported (Ippolito et al., 2015). In this reference, the authors reported that available P contents in biochars produced from hardwoods and softwoods ranged from 25 to 200 mg kg⁻¹ respectively, and 240–1,400 mg kg⁻¹ for animal manure biochars. Additionally, available K was much higher in biochar produced from animal manures (>13,000 mg kg⁻¹) compared to lignocellulosic-based biochars (10–1,620 mg kg⁻¹). Thus, the capability of biochar to recycle P and K back into soil is partially dependent on feedstock selection.

For biochar to make a viable contribution to the soil P and K nutrient pools, these nutrients need to be released into soil solution for plant uptake, and should bolster extractable soil P and K concentrations in ranges suitable for crop production. In this regard, several studies have investigated P release dynamics from biochar itself (Angst and Sohi, 2013; Morales et al., 2013; Domingues et al., 2017) and from biochar mixed with soil (Morales et al., 2013; Parvage et al., 2013; Wang et al., 2015; Zhai et al., 2015; Li et al., 2017). In these studies, variable amounts of water soluble P were released from biochars or from soil + biochars mixtures.

Plant available P concentrations after applying biochar to soils have been reported. Zhai et al. (2015) reported an increase in Olsen extractable P from 3 to 46 and 13 to 137 mg kg⁻¹ after

a straw-derived biochar was applied (at 8% w w⁻¹) into two soils from China. Li et al. (2017) recently reported a recovery of between 21 and 29% of the applied P content using resin (plant available) placed into three Danish soils after mixing with PL biochar.

There are a few recent reports of K released from biochars and from biochar-treated soils. Angst and Sohi (2013) sequentially extracted a wood-based biochar using deionized water, reporting an initial large concentration release followed by a decline (cumulative extraction of 3,500–4,000 mg kg⁻¹) after 6 sequential extractions. Widowati and Asnah (2014) applied 30 t ha⁻¹ of biochar produced from plant material wastes to soil. They reported an increase of 69–89% in (NH₄OAc extractable) K which contributed to a 14% increase in maize production relative to a KCl control. Limwikran et al. (2018) assessed K releases from Thailand soils after incubation with 9 biochar types that were produced from shells, food-processing wastes and wood wastes. They reported dramatic differences in water-soluble K (8–64%) amounts released into soil following biochar applications. Unfortunately, these researchers did not include manure-based biochars to compare K release dynamics against the lignocellulosic-based biochars.

While the above reports are promising, the overall release dynamics of DP and DK from lignocellulosic- and manure-based biochars alone and after biochars are mixed with soil is still under question (Angst and Sohi, 2013; Gul and Whalen, 2016; Zhang et al., 2016). This lack of clear information confounds biochar selection, production, and application conditions for their predictable use as a soil fertility amendment and further raises doubt if the added biochar can improve crop yields in all soils (Jeffery et al., 2011; Spokas et al., 2012; Bierderman and Harpole, 2013; Laird et al., 2017). Moreover, the initial feedstock concentration used to produce the biochar does not provide a reliable measure of a biochar's nutrient value in crop production because DP and DK releases are also depended on soil biotic and abiotic processes that influence nutrient solubilization, precipitation, and other sequestration reaction (Joseph et al., 2010; Angst and Sohi, 2013).

We assert that a more direct assessment of a biochar's potential to increase P and K releases into the soil nutrient pool would be to expose biochar to soil for a long period of time (>100 d), periodically leach the system with water, and assess their concentrations in the leachates. In turn, P and K associated with the biochars are exposed to mineral weathering reactions at the surface and in pore spaces potentially resulting in their dissolution and release. Moreover, the additional incubation time would influence P and K release equilibrium reactions because of exposure to other microbial (e.g., nutrient mineralization, assimilation, etc.) as well as other chemical mechanisms (i.e., precipitation, binding by organic ligands, etc.) that can counter their bioavailability. Finally, the soil + biochar mixture can be extracted to assess if the biochar has raised soil nutrient concentrations to approach agronomic plant sufficiency levels (Jones, 2003).

Review of the literature exposes the dearth in reports concerning a comparison of DP and DK releases between biochar types (manure- vs. lignocellulosic-based feedstocks) and then

quantifying the potential changes in plant extractable P and K concentrations. For this study, our hypotheses were that poultry litter-based biochar after incubation in an acidic, sandy-textured soil would release more DP and DK into water leachates, which concomitantly would also increase soil plant available P and K concentrations relative to soil treated with lignocellulosic-based biochars. Therefore, our objectives were to: (i) determine the quantity of DK and DP released in water leachates from several biochar types after incubation in a highly weathered, sandy-textured soil; and (ii) extract the biochar treated and untreated soils for plant available P and K to determine their impact on supplying nutrients for typical corn production in Southeastern USA Coastal Plain soils.

MATERIALS AND METHODS

Site Description and Soil Characterization

Soil from a Norfolk E horizon was collected from a farm field located in the Middle Coastal Plain region in South Carolina, USA. The USDA Taxonomic classification for the Norfolk soil series is a Fine-loamy, kaolinitic, thermic Typic Kandiodult. Background soil chemical characteristics are presented in **Table 1**. Soil collection details, site location, past crop and tillage management, along with chemical and physical characteristics are given elsewhere (Novak et al., 2016). Briefly, the parent material of the Norfolk soil series is extremely weathered marine sediment following several millions of years of pedogenesis (Daniels et al., 1999). Particle size determination on this E horizon revealed that it is a sandy loam texture consisting of 71% sand, 24% silt and 5% clay. The site where the soil was collected has a mean annual precipitation of 1200-mm and a mean annual air temperature of 17.4°C. This extreme weathering environment has resulted in low soil organic carbon contents ($<1 \text{ g kg}^{-1}$), loss of base cations resulting in an acidic soil pH (5.4), and low fertility characteristics (Novak et al., 2016).

Biochar Preparation and Characterization

Biochar pellets were produced from 100% PC (*Pinus taeda*) and 100% ground PL (*Gallus domestics*) using a 6.4-mm dye

plate, and then sieved to acquire $> 2\text{-mm}$ sized material. The pellets were then pyrolyzed at 500°C as described in Novak et al. (2016). The pyrolyzed pellets were re-sieved, using a 2-mm sieve, to ensure that any pellets broken down during pyrolysis were removed. Because the 100% PL biochar contained a high TP content (**Table 1**), a blended pellet was made consisting of an 80:20 w w $^{-1}$ ratio of PC:PL feedstock. This blending ratio was found to better align the expected Mehlich P released into soil after biochar application with the recommended agronomic Mehlich extractable P contents for row crops in southeastern USA coastal plain soils ($30\text{--}50 \text{ mg kg}^{-1}$, Novak et al., 2014). Pellet production for the HW biochar was poor because of the predominance of “dust-like size” material (75% by weight of material $< 0.25 \text{ mm}$ in diameter) that did not bind together when pressed. Thus, it was used “as received” in this experiment.

The biochars ash, C, N, O, H, and S contents were measured on an oven-dried basis by Hazen Research, Inc. (Golden, Colorado) following ASTM D 3172 and 3176 standard methods (ASTM American Society for testing and materials, 2006). The TP and TK contents of each biochar were determined using the USEPA 3052 digestion method (US EPA, 1996), and then quantified with inductively coupled plasma optical emission spectroscopy (ICP-OES; Novak et al., 2009).

Scanning electron microscopy (SEM) images of two biochar samples (the blend and HW) were collected to show differences in surface morphology. The images were taken using a SEM (JEOL 6500; Tokyo, Japan) at the University of Minnesota-Surface Characterization Laboratory (**Figures 1A,B**). Meanwhile, SEM images from a 100% PL biochar pellet (Cantrell et al., 2014; Sigua et al., 2016), and for a 100% PC biochar pellet (Novak et al., 2015) were reported previously. Furthermore, P and K associated with both the 100% PL and 100% PC biochars were confirmed using energy dispersive spectroscopy analysis (see EDS scans in Novak et al., 2015; Sigua et al., 2016). After characterization, all biochars were stored in vacuum-sealed plastic bags to prevent exposure to atmospheric conditions until used in the soil column leaching experiment.

Soil Column Leaching for DP and DK Releases

The treatments ($n = 5$) for the DP and DK release study consisted of 2-mm sieved Norfolk E horizon soil mixed with: (a) no biochar (control); (b) 100% PC biochar pellets; (c) 100% PL biochar pellets; (d) the 80:20 PC:PL blend biochar pellet, and (e) mixed with the HW biochar. Twenty g kg^{-1} (w w $^{-1}$) of biochar were added to the Norfolk E soil and sufficient deionized water was added to bring their soil moisture contents to 10% (w w $^{-1}$) on a soil-dry basis. These treatments were mixed into soil and the placed into plastic PVC columns (16 cm height x 10 cm diameter (**Figures 1C,D**). Plastic mesh screening was placed on the bottom of each column to retain soil. Each column was then mildly tapped to obtain a bulk density of about 1.5 g cm^{-1} . The % pore space for each column was determined using Equation 1

TABLE 1 | Selected chemical properties of Norfolk E soil and biochars (% on a dry weight basis; PC, pine chip; PL, poultry litter; HW, hardwood; TP, total phosphorus; and TK, total potassium).

Properties	Norfolk E	PC:PL 80:20	100% PL	100% PC	HW
C (%)	–	83.6	48	88.8	72.5
N (%)	–	1.3	4	0.5	0.3
O (%)	–	3.0	5	5	15.3
H (%)	–	2.7	1.5	3.1	2.8
S (%)	–	0.2	0.8	< 0.01	0.0
Ash (%)	–	9.2	41	2.6	8.9
TP (mg kg^{-1})	50 [†]	6,275	3,1573	592	300
TK (mg kg^{-1})	231 [†]	14,434	69,380	3,014	6,500

[†]Published previously in Novak et al. (2016).

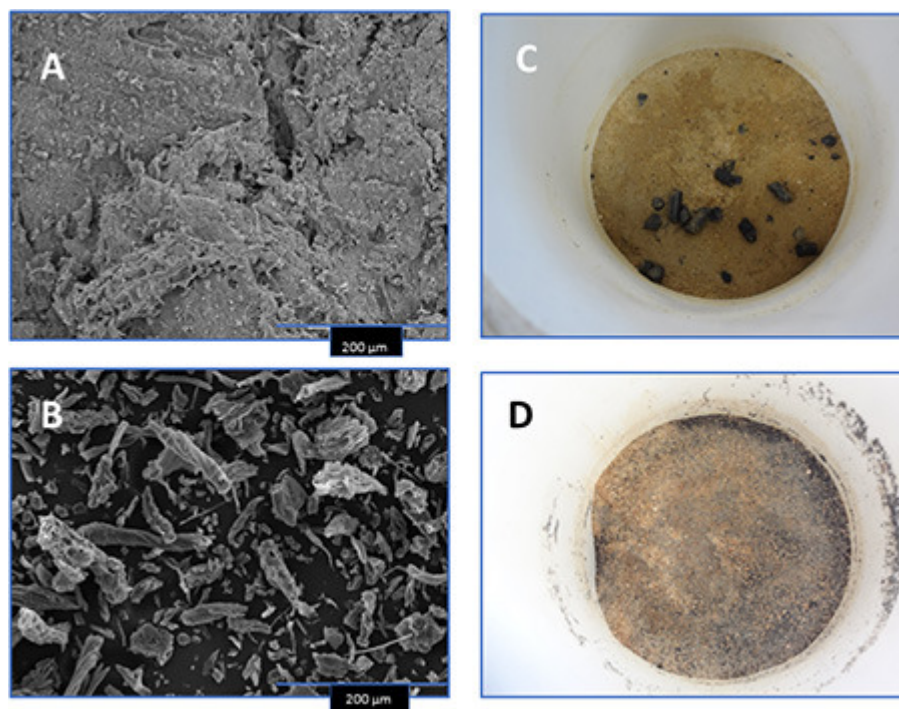


FIGURE 1 | Scanning electron microscopic images of select biochars [80:20 pine chip:poultry litter blend (A) and hardwood (B)], and photos depicting their placement into Norfolk E soil and settlement for water leaching (C,D).

(Troeh and Thompson, 2005)

$$[1]\% \text{pore space} = (1 - \text{bulk density in g/cm}^{-3} \div \text{particle density in g/cm}^{-3}) \times 100.$$

The total soil volume in each column was then calculated and multiplied by the % pore space to determine each column's pore volume (PV). The pore volume was used to fix the volume of leaching water needed to ensure that pores were flushed thoroughly. A value of 1.3 PV (≈ 285 mL) was assumed to adequately solubilize P and K salts, while also dislodging any entrapped DP and DK from surfaces, crevices, and pore spaces. In comparison, 1.3 PV of leaching water represented a rainfall event of approximately 40-mm.

The columns were arranged in a randomized design consisting of $n = 4$ replicates per treatment on a laboratory bench and were incubated for a total of 150 d. Their soil moisture contents were maintained at 10% which is the upper moisture content range for this soil at field capacity (Novak et al., 2009). Thus, the total column weights were monitored and sufficient deionized water was added when needed. On incubation days 32, 67, 95, and 128, the columns were placed on a wooden rack and leached with 1.3-PV of deionized water. Column leachate was collected until free drainage had ceased and their total volume measured. Afterwards, the bottle was shaken, and a 100-mL aliquot was removed for initial filtration using 0.7 μm GF/G WhatmanTM, (Buckinghamshire, UK) filter paper to remove sediments. Next, a 5-mL aliquot from the 0.7 μm filtered sample was re-filtered using 0.45 μm nylon syringe filter (Environmental Express,

Charleston, SC, USA) and their DP and DK concentrations quantified using ICP-OES as described in Novak et al. (2016).

After the final leaching event on day 128, the columns were air-dried for a few weeks. On day 150, the soil was physically removed by gently tapping the column. Next, subsamples of each soil were oven-dried (105C), and samples extracted using the double-acid Mehlich 1 reagent ($\text{HCl} + \text{H}_2\text{SO}_4$; Jones, 2001), and plant available P and K concentrations quantified using ICP-OES. The plant available P and K concentrations were then calculated on a mg kg^{-1} soil basis.

Statistics

The mass weights for DP and DK per leaching event was calculated by multiplying the total leachate volume with their respective concentrations. Next, the corrected cumulative mass of DP and DK was summed for each biochar treatment after correcting for DP and DK in soil alone and significant mean differences between treatments and by individual leaching events were determined using a 2-way ANOVA. In this 2-way ANOVA, the statistical factors were leaching number (L), biochar treatment, and their interaction. To distinguish the magnitude of DP and DK mass losses from sources, the % DP released was calculated on a TP content in the Norfolk E soil + biochar system, and then based on the TP content on a biochar alone basis. A 1-way ANOVA was used to compare the mean cumulative DP and DK released on both bases. A 1-way ANOVA was also used to compare plant extractable M1 P and M1 K contents between the Norfolk E soil treated with the different biochars. All statistical analyses were determined using Sigma Stat v. 11 (SSPS Corp., Chicago, IL, USA) at a $P < 0.05$ level of significance.

RESULTS

Chemical Characteristics of Biochars and Soil

The two SEM images demonstrate differences in the size of biochar material and surface roughness of the 80:20 PC:PL blend and the HW biochar (**Figures 1A,B**). The blended biochar appears to have polygonal-shaped material on surfaces, while the HW biochar is smaller in diameter and lacking polygonal-shaped material. After the pelleted and dust size biochars were mixed into the Norfolk E soil they were then incubated in the soil columns (**Figures 1C,D**).

As shown in **Table 1**, the untreated Norfolk E soil has TP and TK contents of 50 and 231 mg/kg, respectively. In contrast, all biochars had greater TP and TK contents relative to the Norfolk E soil. In fact, the highest TP and TK contents occurred in the 100% PL biochar followed by the PC:PL 80:20 blend. The TP and TK contents in the 100% PC and hardwood biochar were much lower relative to the other two biochars. The quantity of C, H, O, N, and S varied greatly among these four biochars. The highest ash contents occurred in the 100% PL biochar (41%; **Table 1**) with the remaining biochar having values < 10%.

P and K Mass Release Dissolved P

Relatively little DP was solubilized from the control soil (no biochar added) with each leaching event (L1 to L4; **Table 2**); only a cumulative mean mass of 0.06 mg DP was released. In contrast, significant amounts of DP were released per leaching event from the soil treated with 100% PL biochar; this treatment produced the highest cumulative DP released (59.32 mg). For each leaching event, this treatment also had the most significant DP mass releases among the four treatments. Blending the PL with PC feedstock resulted in almost an 80% reduction in DP released relative to samples from the 100% PL treatment. It is interesting that the cumulative DP loss (8.55 mg) from the 80:20 PC:PL blend is close to the theoretical 11.86 mg (20% of 59.32 mg) released from the 100% PL biochar. Meanwhile, DP masses released from soil treated with 100% PC and the HW biochar were similar to the control. Overall, the 2-way ANOVA for DP released revealed highly significant ($P < 0.01$; **Table 2**) impacts of leaching event, biochar treatment, and their interaction as well.

Dissolved K

The control soil, 100% PC and the HW biochar treated soils all released similar DK masses between the 4 leaching events (**Table 2**). Among the treatments, the greatest DK releases occurred from soil treated with 100% PL resulting in the greatest cumulative total release (1,018 mg). In this treatment, the greatest DK mass was released after L1 (723 mg) followed by a significant decline in mass values between L2 and L4. The next highest cumulative DK release occurred from soil treated with the 80:20 PC:PL blend. Here, there was an initial rapid release (L1, 80 mg), subsequently followed by statistically similar mass releases thereafter (L2 to L4). In the PL:PC blended biochar, there was a significant reduction in the mean DK mass released when leaching events were compared to the 100% PL

TABLE 2 | Mean dissolved phosphorus (DP) and potassium (DK) masses released after four water leaching events (L) from Norfolk E soil treated with 20 g kg⁻¹ biochar ($n = 4$; PC, pine chip; PL, poultry litter; and HW, hardwood).

Biochar treatment	DP (mg)				
	L1 [†]	L2	L3	L4	Σ
0 biochar (control)	0.00 a, A	0.06 a, A	0.00 a, A	0.00 a, A	0.06
80:20 PC:PL blend	1.22 a, A	2.29 b, B	2.89 b, B	2.15 b, B	8.55
100% PL	4.65 b, A	19.89 c, B	19.00 c, B	15.78 c, C	59.32
100% PC	0.05 a, A	0.02 a, A	0.00 a, A	0.00 a, A	0.07
HW	0.15 a, A	0.03 a, A	0.00 a, A	0.00 a, A	0.18
Factor	P				
Leaching no.	<0.001				
Biochar trt.	< 0.01				
Leaching no. X biochar trt	<0.001				

Biochar treatment	DK (mg)				
	L1	L2	L3	L4	Σ
0 biochar (control)	2.7 a, A	2.6 a, A	2.0 a, A	1.2 a, A	8.5
80:20 PC:PL blend	80 b, A	28 b, B	19 b, B	16 b, B	143
100% PL	723 c, A	142 c, B	88 c, C	65 c, D	1018
100% PC	5.9 a, A	5.1 a, A	3.5 a, A	2.8 a, A	17.3
HW	9.0 a, A	5.3 a, A	4.7 a, A	4.0 a, A	23
Factor					
Leaching no.	<0.001				
Biochar trt.	<0.001				
Leaching no. X biochar trt	<0.001				

[†] Lower case letters indicate significant difference between means within a column, while upper case letters indicate significant differences in means compared between columns using a 2-way ANOVA at a $P < 0.05$ level of significance.

biochar. In fact, blending of PC into the PL resulted in a 7-fold reduction in cumulative DK released. For DK released, leaching number, biochar treatment, and their interaction were also highly significant ($P < 0.001$).

Estimated DP and DK Losses on a System and Biochar Alone Basis

Calculating cumulative DP and DK mass balance releases on a system (soil + biochar) and biochar alone basis provides testimony for the large differences in their P and K recycling capabilities (**Table 3**). On a soil + biochar basis, there were no significant differences in DP released between the control soil and when the soil was treated with 100% PC or HW biochar. In these three treatments, there was approximately 0.0001% of the TP mass released as DP. Whereas, mixing in 100% PL biochar into the Norfolk E soil resulted in a significant increase to 0.11% of the total P released as DP.

On a biochar alone basis, a significantly higher percentage of DP was released from the 100% PL biochar and the 80:20 PC:PL blend (**Table 3**; 8.3 and 6.1%, respectively) compared to the other three treatments. Cumulative DP mass releases on a biochar alone basis were <1% of the TP content from the 100% PC and HW biochar treated soil.

TABLE 3 | Means of cumulative masses of dissolved phosphorus (DP) and potassium (DK) released in leachates calculated on a percentage of Total P (TP) and Total K (TK) in a soil + biochar system and on a biochar alone basis ($n = 4$; standard deviation in parentheses; PC, pine chip; PL, poultry litter; and HW, hardwood).

Cumulative DP mass released on a TP basis in		
Norfolk E soil +	Soil + biochar (%) [†]	Biochar alone (%)
0 biochar (control)	0.0001 (0.0001) a	0.0 a
PC:PL 80:20	0.02 (0.003) b	6.1 (1.1) b
100% PL	0.11 (0.009) c	8.3 (0.7) c
100% PC	0.0001 (0.0001) a	0.4 (0.6) a
HW	0.0001 (0.0001) a	0.7 (0.9) a
Cumulative DK mass released on a TK basis in		
Norfolk E soil +	Soil + biochar (%)	Biochar alone (%)
0 biochar (control)	0.003 (0.0003) a	0.0 a
PC:PL 80:20	0.06 (0.004) b	43.9 (3.1) b
100% PL	0.39 (0.03) c	65.5 (5.6) c
100% PC	0.01 (0.001) a	12.6 (1.4) d
HW	0.0000 (0.0000) a	0.7 (0.9) a

[†] Lower case letters indicate significant differences between means within columns as determined using a 1-way ANOVA at a $P < 0.05$ level of significance.

On a soil + biochar system basis, a significant mass of DK was released from soil treated with 100% PL (0.39% of the TK pool; **Table 3**). After adding in the 80:20 PC:PL blend, there was a significant reduction in % DK on a soil + biochar basis. In comparison, there were no significant differences in % DK released on a soil + biochar basis between soil treated the control, with 100% PC, and with HW biochar.

On a biochar alone basis, the 100% PL released 65% of DK from the TK pool (**Table 3**). Blending the PL with PC caused a significant reduction in cumulative % DK released, but still almost 44% by weight of DK was released from the TK pool. A lower % of DK was released from the 100% PC biochar, but the cumulative percentage released was significantly different than the control and the HW biochar.

Plant Extractable P and K in Biochar-Treated Soils

The control Norfolk E soil has Mehlich 1 P and K concentrations that were < 4 and 19 mg kg^{-1} , respectively (**Table 4**). However, supplementing the Norfolk E soil with 20 g kg^{-1} of 100% PL and 80:20 PC:PL blended biochars caused significant increases in the M1 P and M1 K concentrations. Adding 100% PC and hardwood biochar to soil at the same application rate produced no significant improvement in M1 P concentrations. In fact, the mean M1 P concentrations in these two treatments were similar to the mean value in the control soil. Treating soil with 100% PC biochar did not significantly increase the M1 K concentration, however, treatment with the hardwood biochar did cause a significant M1 K concentration increase. Adding the hardwood biochar to the Norfolk E soil at 20 g kg^{-1} resulted in a tripling of the M1 K concentration relative to the control.

TABLE 4 | Mean Mehlich 1 (M1) extractable phosphorus (P) and potassium (K) contents measured at end of study in Norfolk E soil after treatment with 2% biochar ($n = 4$; standard deviation in parentheses; PC, pine chip; PL, poultry litter; and HW, hardwood).

Norfolk E soil +	M1 P [†]	M1 K
	mg kg ⁻¹	
0 biochar (control)	3.84 (0.56) a	18.75 (5.09) a
80:20 PC:PL blend	35.37 (11.16) b	83.68 (12.20) b
100% PL	68.58 (12.11) c	162.56 (17.17) c
100% PC	5.08 (0.79) a	28.79 (2.30) a
HW	4.09 (0.48) a	59.35 (5.73) d

[†] Lower case letters indicate significant differences between means within columns as determined using a 1-way ANOVA at a $P < 0.05$ level of significance.

DISCUSSION

Composition of the Biochars

The biochars employed in this study corroborate other reports concerning the vast P and K compositional differences between manure- and lignocellulosic-based biochars (Spokas et al., 2012; Ippolito et al., 2015). Here we report that a manure-based biochar (100% PL) had higher TP and TK contents than lignocellulosic-based biochars. In terms of using these biochars to recycle P and K, the 100% PL biochar contains between 10 and 23 times the TK content and 53–105 times the TP content of the lignocellulosic-based biochars (**Table 1**). Their TP and TK content differences clearly show the recycling gains and losses of using either a manure-based biochar against lignocellulosic-based biochar-types in a nutrient recycling program.

From an agronomic perspective, blending the 100% PL feedstock with 100% PC material, a ratio of 80:20 illustrates the utility of producing designer biochars that have lower total TP and TK contents. In fact, as shown in **Table 1**, this blended biochar had its TP and TK contents reduced by 1/5 relative to the 100% PL feedstock. Blending feedstocks offers the utility of designing a biochar that can theoretically supply different amounts of nutrients to soils (Novak et al., 2014). This approach was subsequently reported to have a positive impact on wheat yields grown on poor quality soils (Sigua et al., 2016). Moreover, blending feedstocks with different moisture contents is reported to reduce thermal energy requirements for dewatering feedstocks during the pyrolysis process (Ro et al., 2010).

Release of Dissolved P and K

As reported by Angst and Sohi (2013), just because a feedstock contains a high content of a critical plant nutrients doesn't guarantee that these nutrients will be available to plants. The authors reported that nutrient releases from biochars may be limited due to binding with insoluble salts, inaccessibility of nutrients to water, microbial processes, and pore hindrances from physical microstructural properties (i.e., pore size, pore connectivity, etc.). As a test to these conditions, we incubated different biochar types in soil with anticipation that P and K release dynamics may be impacted by exposure to weathering

reactions, and after leaching with water, would impact their chemical form and physical location. These conditions should create the potential to better assess each biochars DP and DK release capability.

The biggest take away from the results characteristic is the magnitude of DP and DK released from 100% PL biochar. Moreover, soil treated with 100% PL biochar continued to release DP and DK with successive water leaching. Although the amount of release significantly declined by L4, there was still significant DP and DK mass releases relative to the other four treatments. This can be explained considering that the P and K in the 100% PL biochar was associated with salts that differed in their water solubility (K_{sp}). This is consistent with reports using EDS characterization of the polygonal-shaped material on 100% PL biochar that showed it possessed soluble salts composed of KNO_3 , KCl, and $\text{Ca}_3(\text{PO}_4)_2$ (Novak et al., 2015; Sigua et al., 2016). Both KNO_3 and KCl are highly water-soluble salts, so K ion would be readily released into the water leachate. In contrast, release of P from $\text{Ca}_3(\text{PO}_4)_2$ would be much slower since it has a lower water solubility constant ($K_{sp} = 1 \times 10^{-25} \text{ mol L}^{-1}$; Handbook of Chemistry Physics, 1981). Water solubility differences between materials holding P and K would account for the noted mass release differences per leaching event (Table 2). This is a credible explanation considering that the 100% PL feedstock used to produce the 80:20 PC:PL blend is similar to the PL characterized in the Novak et al. (2015) and Sigua et al. (2016) studies.

The presence of water soluble salts would explain the ability of 100% PL and the 80:20 PC:PL blend to release such significantly greater amounts of DP and DK on a biochar alone basis (Table 3). Minimal releases of DP and DK occurred from soil treated with the lignocellulosic-based biochars probably because of their relatively lower TP and TK contents (Table 1) and that the P and K are likely bound in structural forms that are not accessible to solubilization reaction with water.

Improvements in Soil Nutrient Levels Using These Biochars

A convenient method to determine the impact of biochar on soil fertility levels is to extract the biochar treated soil using an extractant solution specific for plant macro- or micro-nutrients held in soils. For Southeastern USA coastal plain soils, the common extractants for P and K are either Mehlich 1 or Mehlich 3 reagent (Sims, 1989). The double acid Mehlich 1 reagent was selected for this experiment because Mehlich 3's chemical formulation is more specific for plant micro-nutrient extraction (Mylavarapu et al., 2002). After soil is extracted and the P and K quantified, the next step involves comparing the extractable P and K concentrations to indices that have been calibrated against actual crop yields in field agronomic trials. These indices define whether Mehlich 1 extractable nutrient levels are satisfactory or unsatisfactory for optimal crop yields for soils in that geographic region. For corn grown in sandy soils in the Southeastern USA, medium and high index values established for Mehlich 1 extractable P are 16 to 30 and 31 to 60 mg kg^{-1} , respectively (Mitchell and Mylavarapu, 2014). From the same reference, the

medium and high critical indices for Mehlich 1 K are 36–78 and 79–117 mg kg^{-1} , respectively.

Extracting the control soil for plant available M1 P and K concentrations (Table 4, 2.84 and 18.75 mg kg^{-1} , respectively) showed that their values are far below the medium indices. This is a typical subsoil fertility characteristic of extensively weathered sandy-textured Ultisols in the Southeastern USA coastal plain region (Davis et al., 1996; Mitchell and Huluka, 2016). A soil fertility goal for row crop production in these sandy soils is to increase M1 P and M1 K contents to be in the medium to high range using either biochar or commercial fertilizer. Here additions of 20 g kg^{-1} (equivalent to 40 t ha^{-1}) of the lignocellulosic-based biochars failed to significantly increase M1 P concentrations, which attests to their poor ability to supply plant available P to the Norfolk E soil. While the HW biochar increased M1 K levels to be in the medium index level, the 100% PC biochar failed to meet this fertility goal. It can be argued that these are poor biochar types for improving plant nutrient fertility levels, but would better serve at increasing soil C sequestration (Novak et al., 2009). In contrast, the 100% PL and the blend were shown to have a significant impact with raising the M1 P and M1 K fertility levels. In fact, applying these two biochar types elevated the M1 K and M1 P concentrations to rank in the high index category.

In this study, we tested the hypotheses that biochar produced from PL will release more DP and DK amounts, which would subsequently increase soil plant available P and K concentrations as compared to lignocellulosic-based biochars. Our results support these hypotheses because biochars were shown to have different abilities to release DP and DK amounts which consequently impacted their ability to improve plant available P and K contents. While no plants were grown in this experiment, using 100% PL biochar served as a better soil fertility amendment to increase plant available P and K as compared to lignocellulosic biochar types. If blanket applied across a field, biochars at this high application rate (20 g kg^{-1} basis or 40 t ha^{-1}) would be too expensive for practical financial management. However, if biochars are locally applied near plant stems (i.e., side dressing, banding, etc.) the more prudent biochar selection would be a 100% manure-based or a manure + lignocellulosic-based biochar to supplement plant available P and K soil levels. This also assumes that commercial fertilizer would serve as the main source of P and K for crop production.

AUTHOR CONTRIBUTIONS

JN, MJ, and KS designed, performed the experiments, and composed the manuscript.

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Plant Availability of Phosphorus in Five Gasification Biochars

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The innovation and expansion in the bioenergy sector produce increased amounts of solid residues, e.g., ashes and biochars, which may derive from more diverse origins of biomass. Recycling of nutrients like phosphorus (P) in such residues to agricultural soils contributes to sustainability in both energy and agriculture systems. In this study, the P availability was tested in five gasification biochars (GBs) produced via a novel low-temperature (<750°C) gasification technology. The feedstocks used were wheat straw (STR), shea nut shells (NUT), poultry manure (POUL), and two types of sewage sludge mixed with wheat straw (SSA and SSB). A 16-week laboratory incubation study of the materials was conducted with three contrasting soils and resin-extractable P (available P) and pH were monitored. Another mini-plot experiment was done to examine the effects of GBs on spring barley on a loamy sand soil. Neither barley yield nor P uptake showed significant increase after application of the GBs or a mineral P fertiliser, indicating non-limiting P status in this soil and non-adverse effect on the crop growth. During the incubation, all GBs increased soil pH markedly, especially in the STR- and NUT-amended soils and in acid soils. Of the P applied in STR, NUT, and POUL 21–29% was recovered as resin-extractable P in the two acid soils after incubation, while in the alkaline soil the recovery from STR (49%) almost matched that from triple superphosphate (52%). Recoveries from SSA and SSB were similarly low (<14%). A significant positive relationship was identified between the resin-extractable P and the resulting pH in soils amended with some GBs with low P contents. These results revealed varying P availability of low-temperature GBs, which depends on the feedstock type and pH level in the soil, and it also showed a varying ability of GBs to substitute mineral P fertilisers.

Keywords: gasification biochar, low temperature circulating fluidised bed, phosphorus availability, spring barley, soil pH

INTRODUCTION

Combustion and gasification are the two primary forms of using biomass for power and heat generation. Appropriate reuse of the residue after gasification, a solid mixture of ash and char termed gasification biochar (GB), is crucial for sustainability of the energy system (Pan and Eberhardt, 2011; Ahrenfeldt et al., 2013) and a circular economy. Many elements are concentrated in the GB, especially essential non-renewable and irreplaceable nutrients for crop production such as phosphorus

(P), which need to be properly recycled (Mozaffari et al., 2002; Kuligowski et al., 2010; Müller-Stöver et al., 2012). Direct application of GB to agricultural soils is a low-cost strategy of nutrient recycling, but only if the nutrients in the GBs are available to and get utilised by the plants. The plant availability of P in GBs applied to agricultural soils remains uncertain.

Most studies on soil application of biochars have been conducted with biochars originating from pyrolysis (PB), i.e., operated in the absence of O₂ at low temperatures (e.g., 200–700°C, Spokas et al., 2012; Crane-Droesch et al., 2013). To achieve a higher energy efficiency, gasification is operated in the presence of low O₂ concentrations at temperatures of ca. 700–1,000°C. For both PB and GB, the operational temperature and the type of feedstock are important factors in determining their subsequent physical and chemical properties (Pan and Eberhardt, 2011; Trippe et al., 2015). With greater demand for biomass in the energy sector, it is desirable to use a broad variety of feedstocks. However, biomasses with high contents of low melting point ashes, such as straw, manure, and sewage sludge, often cause problems related to ash melt during combustion or gasification at high temperatures, e.g., >800°C (Thomsen et al., 2015). Such problems can be largely avoided with the low-temperature circulating fluidised bed (LT-CFB) gasifier, where the maximum process temperature does not exceed 750°C (Ahrenfeldt et al., 2013; Hansen et al., 2015).

There is a risk of self-ignition of some freshly produced GB residues if they come into contact with atmospheric oxygen because of the potentially large amount of unburned C and the high temperature. Adding water is a simple strategy to avoid this, which additionally reduces dust issues during the post-gasification processing of the biochars. During such wetting and subsequent drying processes before field application, soluble compounds may dissolve and reprecipitate as new compounds. It is unknown how this can affect the availability of P afterwards.

Only a few studies have examined the P availability of GBs from different origins. Pan and Eberhardt (2011) evaluated the P availability in ashes from gasification and combustion of wood chips using sequential chemical extraction. They showed that the majority of the P in gasification ash was in the potentially bio-available fraction. Some studies found that GBs from wheat straw and alfalfa stems were able to increase soil Olsen P levels, which was not the case for GBs from other types of feedstocks such as citrus peels (Mozaffari et al., 2002; Müller-Stöver et al., 2012). Another study on biochars from pig and poultry manure gasification reported that the initial P availability for spring barley was lower than that after the application of a commercial P fertiliser, but that in the longer term the P availability was similar for GB and the commercial fertiliser (Kuligowski et al., 2010).

In a meta-analysis of 40 studies with PBs, potential effects of biochar on crop yield appeared to be dependent on soil properties (Crane-Droesch et al., 2013). Soil properties also influence the P availability to crops in biochar-amended soils (Zhao et al., 2015). The complex soil processes that control P availability include adsorption–desorption of phosphate on the surfaces of iron and aluminium oxides, organic matter, and other clay minerals and/or precipitation as various calcium phosphates, and soil pH plays a crucial role for all these processes (Haynes, 1982; Holford, 1997; Weng et al., 2011). Adsorption–desorption of P and thus the

availability of P has previously been investigated in PB-amended soils (e.g., Morales et al., 2013; Xu et al., 2014). Variable, even conflicting, responses of P availability were reported depending on biochar and soil types, which were mainly attributed to the alterations caused by biochar addition to the aforementioned complex soil processes. There is little information about the P availability as affected by addition of GB to different soil types.

The objective of this work was to evaluate the P availability in five GBs produced by LT-CFB gasification of wheat straw, shea nut residues, poultry manure, and two types of sewage sludge—wheat straw mixtures. The study comprised one 16-week laboratory incubation experiment with three soil types with contrasting pH and clay contents, and one mini-plot field experiment on a loamy sand soil with spring barley as the test plant. The hypotheses were that: the P availability in GBs (1) varies among different types of feedstock and soil to which they are applied; (2) is lower than that of mineral P fertilisers but will contribute to crop P uptake and yield; and (3) is affected by drying and wetting of the material during storage.

MATERIALS AND METHODS

Gasification Biochars

The GBs originated from various types of biomass and were produced *via* LT-CFB gasifiers at 700–750°C in three pilot or demonstration gasification units in Denmark. The biomass feedstocks included wheat straw (STR and STR1–4), shea nut shells (NUT), poultry manure (POUL), and two mixtures of dry sludge pellets and wheat straw (SSA and SSB). Two different batches of straw were used to produce STR and STR1–4 but under the same conditions. Selected properties and application rates of the GBs in the incubation experiment are presented in **Table 1**. All materials were in a very fine powder form. The SSA, SSB, and POUL biochars were applied in dry form, while the STR and NUT contained 57 and 54% water, respectively. More detailed descriptions of the GBs applied in this study can be found in Li et al. (2017). The gasification process was described in detail in Ahrenfeldt et al. (2013) and Thomsen et al. (2015).

Soil for the Incubation Experiment

Three Danish soils with contrasting clay contents and pH (**Table 2**) were used in an incubation experiment to study the P availability after application of GBs. The soils were all sampled from the plough layer of arable fields. The Aarup soil contained 17% clay and had a pH (H₂O) of 5.8. The Jyndevad E and Jyndevad P soils were from different treatments of a long-term experiment that explores the effects of liming and P fertilisation at St. Jyndevad Experimental Station in southern Denmark (Rubæk, 2008). The Jyndevad E soil was from plots that had not received P fertiliser since 1942, but lime every 6–9 years to achieve a soil pH of 5.4 (low lime). The Jyndevad P soil was from plots that had received triple superphosphate (TSP) at 15.6 kg P ha⁻¹ year⁻¹ and lime regularly (target pH 6.7, high lime) (Rubæk, 2008). The soil at Jyndevad is a coarse sand soil with 3.2% clay and has been continuously cultivated with spring barley for many decades. The Jyndevad soils were sampled about 10 months after a liming event

TABLE 1 | Selected properties and application rates of gasification biochars (GBs) or mineral fertilisers in the incubation experiment (Li et al., 2017).

Treatment ^a	Concentration							WSP ^b %	Molar ratio			Application rate	
	C	N	P	K	Ca	Mg	Fe		Ca:P	Mg:P	Fe:P	Fertiliser/GB g kg ⁻¹	P mg kg ⁻¹
	g kg ⁻¹ DM												
Con	–	–	–	–	–	–	–	–	–	–	–	0	0
ConK	NA	NA	0	524	NA	NA	NA	0	–	–	–	1.9	0
ConP	NA	NA	216.6	NA	NA	NA	NA	90.2	–	–	–	0.6	138.5
STR	372	3.0	4.0	55	27	5.7	2.0	8.61	5.3	1.8	0.3	17.3	69.3
NUT	594	12.9	8.7	78	15	10.4	3.5	22.6	1.4	1.5	0.2	15.8	138.5
POUL	93	8.3	57.1	91	156	16.2	8.6	0.06	2.1	0.4	0.1	2.4	138.5
SSA	274	7.8	26.0	51	52	8.5	23.5	0.51	1.6	0.4	0.5	5.3	138.5
SSB	222	3.1	26.0	84	53	8.4	30.8	3.92	1.6	0.4	0.7	5.3	138.5

^aCon, control without any amendment; ConK, reference treatment amended with potassium chloride (KCl); ConP, reference treatment amended with triple superphosphate (TSP); STR, straw GB; NUT, shea nut shell GB; POUL, poultry manure GB; SSA, straw-sewage sludge A GB; SSB, straw-sewage sludge B GB. All GBs were produced at ca. 730°C.

^bFraction of water-soluble P to total P (%).

TABLE 2 | Selected properties of the soils used for the incubation and field mini-plot experiments.

Soil	Unit	Incubation			Field mini-plot ^a Foulum
		Aarup	Jyndeved E	Jyndeved P	
Texture		Loam soil	Coarse sand soil	Coarse sand soil	Loamy sand soil
Clay (<2 μm)	%	17.0	3.2	3.2	8.6
Silt (2–20 μm)	%	22.0	2.8	3.3	12.0
Coarse silt (20–63 μm)	%	23.0	2.4	2.6	–
Fine sand (63–200 μm)	%	19.0	14.1	15.7	46.6 ^b
Coarse sand (200–2,000 μm)	%	15.2	75.5	73.4	32.8
Total C	%	1.9	1.2	1.1	1.8
Total N	%	0.2	0.1	0.1	0.2
Olsen P	mg kg ⁻¹	14.4	19.6	25.3	24.0
WSP ^c	mg kg ⁻¹	3.3	5.4	8.7	NA
Resin P	mg kg ⁻¹	9.3	10.8	15.9	NA
pH (H ₂ O)	–	5.8	5.8	7.8	5.4 ^d

^aSoil property data from Li et al. (2015).

^bIncluding coarse silt and fine sand (20–200 μm).

^cWater-soluble P.

^dMeasured before the experiment in this field.

resulting in soil pH values higher than the target values (Table 2). The pH values at the start of the incubation were 5.8 and 7.8 for the two soils with low and high lime, respectively. All soils were air-dried and sieved to ≤4 mm before use.

Laboratory Incubation

The incubation experiment was organised in a completely randomised design with two factors, i.e., biochar type and soil type. For each of the three soil types there were eight treatments: one control without any amendment (Con), one reference with TSP (ConP), one reference with potassium chloride (KCl, ConK), and the five GBs (Table 1). The 24 treatment combinations were replicated 12 times to allow destructive sampling of three replicates each time at week 1, 4, 8, and 16 after the start of the incubation.

The GB or TSP was added at 138.5 mg P kg⁻¹ soil, equivalent to 90 kg P ha⁻¹ assuming a distribution in 5-cm topsoil with a bulk density of 1.3 g cm⁻³. An exception was the STR treatment, where the material was applied at 69.3 mg P kg⁻¹ soil to limit the amount of K added because of its high K-to-P ratio. The KCl was applied in a solution at 1,000 mg K kg⁻¹ soil. The GB or fertiliser was mixed with 200 g soil (dry weight basis) in polyethylene bags. After thorough mixing, the soil was moistened with deionised water to 60% of the water-holding capacity and kept at 10°C for 16 weeks in a dark room. During the whole period, the bags were kept open to allow gas exchange, and deionised water was added regularly to maintain the soil moisture on a weight basis. The sampled soil was analysed for water-, bicarbonate-, and resin-extractable inorganic P and pH in the soil (see below).

To evaluate the P availability after different wetting–drying treatments of the GB during storage, four additional treatments were included using a straw GB that was originally sampled in dry form. Mixing of the straw GB with water was done on a weight:weight basis. The treatments were as follows: STR1, the straw GB was mixed with water at 1:1 and stored in moist form at 20°C in a closed container before initiation of the incubation experiment; STR2, the GB was mixed with water at 1:1, stored at 20°C in a closed container for 4 weeks, then air-dried at 20°C for 4 weeks and rewetted again (1:1) before the incubation; STR3, the GB was mixed with water at 2:1 and treated similarly as STR1; and STR4, the GB was applied directly in dry form. The straw GB for these extra treatments was from a batch different from STR and showed a slightly higher P concentration 4.3 g P kg⁻¹ DM. The straw GB in these treatments was also applied at 69.3 mg P kg⁻¹ soil. The incubation, sampling, and analyses were the same as in the main incubation experiment.

Mini-Plot Field Experiment

The mini-plot field experiment was conducted at Research Centre Foulum, Aarhus University, Denmark. The field had previously been a grass-clover ley without P fertilisation for more than 5 years. The texture of the top 25 cm of this soil was loamy sand with 8.6% clay, bicarbonate extractable Olsen P was 24 mg P kg⁻¹ (Olsen et al., 1954), and pH (H₂O) was 5.4 at the start of the

experiment (Table 2). According to our experience, this soil was a medium-to-low P status soil in Denmark, having a P status in the lowest 25% quantile based on two surveys across Danish agricultural soils (Rubæk et al., 2013). In spring 2014, PVC cylinders (30 cm height and inner diameter) were inserted to 25 cm depth in the field with 45 cm intervals in four blocks.

The mini-plot experiment was organised in a completely randomised block design consisting of 4 blocks and 11 treatments: 1 control without any amendment (Con), 2 reference treatments with monopotassium phosphate (KH_2PO_4 , MP) at 30 and 60 kg P ha^{-1} , respectively, and 4 GBs (POUL was excluded due to insufficient amounts available) at the same P rates as in the MP treatment (Table 3). The top 15 cm soil in each plot was removed and mixed thoroughly with the GB/fertiliser in a bucket. The soil was divided into three portions of approximately the same size. Each portion was then returned to the mini-plot separately and compacted slightly. Twenty-eight seeds of spring barley (*Hordeum vulgare* L.; cv. Quench) were sown in two rows on 29 April 2014. Barley was also sown around the plots at the same rate the same day. After sowing, all plots were supplied with nutrient solutions (without P), containing 150 kg N ha^{-1} as ammonium nitrate (NH_4NO_3), 150 kg K ha^{-1} as KCl and sufficient amounts of S, Mg, Ca, Mn, Zn, B, Cu, and Mo. The barley was hand-cut at maturity on 7 August 2014 and dried (80°C) for determination of total DM. Grain and straw were separated for determination of grain and straw DM yield. After harvest, three soil cores (dia. 2 cm) were taken from each plot at 0–13 cm depth and pooled to one sample for analysis of pH, water-, bicarbonate-, and resin-extractable inorganic P (see below).

Biochar, Soil, and Plant Analysis

Total C and N in the GB or soil were determined by an elemental analyser (LECO Corporation, MI, USA). Total P, K, Ca, Mg, and Fe in the GB were measured by inductively coupled plasma-optical emission spectroscopy (ICP-OES, iCAP 6000 series, Thermo Fisher Scientific, UK) after sample digestion in a mixture of nitric acid (HNO_3), hydrogen peroxide (H_2O_2), and hydrofluoric acid (HF) in a microwave digestion system at 210°C (Li et al., 2016).

TABLE 3 | Treatment description and application rates of the gasification biochars (GBs)/fertiliser in the field experiment.

Treatment	Description	Fertiliser/GB Mg ha^{-1}	P rate kg ha^{-1}
Con	No amendment	0	0
MP30	KH_2PO_4	0.13	30
MP60	KH_2PO_4	0.26	60
STR30	Straw GB	7.50	30
STR60	Straw GB	15.00	60
NUT30	Shea nut shell GB	3.43	30
NUT60	Shea nut shell GB	6.86	60
SSA30	Straw-sewage sludge A GB	1.16	30
SSA60	Straw-sewage sludge A GB	2.31	60
SSB30	Straw-sewage sludge B GB	1.15	30
SSB60	Straw-sewage sludge B GB	2.31	60

Selected GB properties can be found in Table 1.

The total P in the barley straw and grain was measured using the same procedure but without HF in the digestion process.

For water-extractable P in GB or soil, 1 g sample was extracted in 50 mL deionised water for 1 h (20°C) and then separated by centrifugation for 10 min ($1,831 \times g$). 1 mL of the 50 mL water was applied to the GB sample 24 h before the extraction. In soil samples, P was also extracted with 0.5 M NaHCO_3 (Olsen et al., 1954) and with anion exchange resin membranes (resin-extractable P). For resin-extractable P, two resin strips (60 \times 10 mm, in bicarbonate form) were mixed with 30 mL deionised water and soil (equivalent to ca. 2 g dry soil) in a 50 mL centrifuge tube. The tube was shaken end over end at 25°C for 17 h. The resin strips were removed and rinsed with water before the extracted P was eluted with 0.5 M HCl (modified from Kouno et al., 1995). All P concentrations were then measured by spectrophotometry using the molybdate blue method essentially as described for water analysis (ISO 6878:2004, 2004). Soil pH was measured after mixing of soil with water (1:2.5, w:v) for 1 h. Soil pH and resin-extractable P were measured on moist soil samples right after sampling while the other P analyses were carried out with air-dried soil.

The recovery rate (%) of applied P in the soil-extractable P pools was calculated by subtracting the mean extractable P concentration in the control soil from that in the amended soil at the same sampling, which was then divided by the corresponding P application rate. Additionally, by taking the ratio between the P recovery rate from biochar and that from the corresponding TSP treatment, relative extractability (%) of P by resins was calculated to indicate the fertiliser value of added GBs. The P recovery rate (%) in the spring barley total aboveground biomass in the mini-plot experiment was calculated accordingly.

Statistical Analysis

Statistical analyses were performed using the R software (R Core Team, 2015) at significance level $\alpha = 0.05$. Two-way analysis of variance (ANOVA) revealed a significant interaction effect of biochar and soil type on the measured soil pH, water-, bicarbonate-, and resin-extractable P in the incubation experiment. To facilitate interpretation of the biochar effect on soil pH and extractable P, results of one-way ANOVA for the soil pH and P data for each soil type were presented. Because the two-way ANOVA in the mini-plot experiment indicated no significant effect of either the biochar or the application rate on crop measurements, results presented were from one-way ANOVA of all biochar-rate treatment combinations. Treatment effects were then analysed by multiple comparison with the *glht* function from the *multcomp* package (Hothorn et al., 2008). The potential relation between the soil resin-extractable P concentration and pH was explored using linear regression in R.

RESULTS

Varying N, P, K, and C Contents in GBs

As expected, the concentrations of macronutrients (N, P, and K) and C in GBs varied among the types of feedstocks (Table 1). The total N in all GBs was 0.3–1.3%. NUT had the highest total N content. The highest P concentration (57 g kg^{-1} DM) was measured in

POUL, and POUL also had the smallest fraction of water-soluble P (WSP) (< 0.1% of the total P) of the five GBs. The lowest P concentration was observed in STR at 4 g kg⁻¹ DM, of which 8.6% was water-soluble. NUT contained 9 g total P kg⁻¹ DM and had the highest fraction of WSP (23%) of all GBs. No difference in total P was seen for the two sludge GBs (26 g kg⁻¹ DM for both SSA and SSB) despite the different methods used to precipitate P during wastewater treatment, but the fraction of WSP in SSB (4%) was about eight times higher than in SSA (0.5%). The total K concentration was in the range 51–91 g K kg⁻¹ DM. The total C content ranged from 9% in POUL to 59% in NUT. Additionally, POUL showed a much higher Ca concentration than the other GBs, 156 vs. 15–53 g Ca kg⁻¹ DM, but STR had the highest Ca:P molar ratio at 5.3. SSA and SSB showed higher Fe concentrations than the others, 24–31 vs. 2–9 g Fe kg⁻¹ DM, as well as high Fe:P molar ratios of 0.5–0.7.

pH Increase in GB-Amended Soils

The different GBs were able to increase pH in the three soils to varying extents (Table 4). In general, the increase in pH was more pronounced when adding GBs to the acid sandy soil than to the more clayey or alkaline soils. STR and NUT were added in much larger amounts than the other GBs (Table 1) and these two treatments significantly increased soil pH ($p < 0.05$) in the first week by 0.9–1.1, 1.8–2.0 and 0.6–0.9 units in the Aarup, Jyndeved E, and Jyndeved P soil, respectively. A weaker, but significant, pH increase was also seen after addition of POUL, SSA, and SSB to the acid Aarup and Jyndeved E soil ($p < 0.05$), but not in the alkaline Jyndeved P soil. Both sludge biochars displayed the same effect on pH in all soils. In contrast to biochars, added mineral fertilisers tended to reduce soil pH. The differences among treatments in soil pH in the first week persisted throughout the 16-week incubation with little variation.

Available P in the Three Soils

The resin-extractable P (resin-P) was compared with the P extracted by 0.5 M NaHCO₃ (Olsen P) or by water (WSP) in this study. Only the resin-P data are presented in Figure 1,

while the Olsen P and WSP can be found in Figures S1 and S2 in Supplementary Material. After 1 week, 68, 68, and 71% of the P applied as TSP was recovered as resin-P in the two acid soils and the alkaline soil, respectively (Figure 1). The recovery in all TSP fertilised soils then declined and reached a stable level. In the Aarup soil, the recovery declined from 68 to 55% (from week 1 to 4) and then declined further and stabilised at 48% in weeks 8 and 16. In the Jyndeved E and P soils, the decline of resin-P was rapid during the first 4 weeks, and the recovery rates were 43 and 52% after 16 weeks, respectively.

Both the absolute concentration and the recovery rate of resin-P in the amended soils varied among the different biochars on all three soil types (Figure 1). Similar to the TSP treatment, an initial decline in resin-P recovery was seen in the POUL treatment within the first 4 weeks, which subsequently (i.e., weeks 4–16) stabilised at 27–31, 20–24, and 23–28% for the Aarup, and Jyndeved E and P soils, respectively. The resin-P recovery from SSA and SSB was similar and constantly low, i.e., <14% throughout the incubation in all three soils, although they contained the second highest amount of P after POUL of the five GBs (Table 1). The P recovery from shea nut shell GB (NUT) developed differently over time across the three soils. It stayed constant at 22% in the Aarup soil, declined from 29 to about 20% in the Jyndeved E soil, but increased from 28 to 38% in the alkaline Jyndeved P soil during the 16-week incubation. The biochar from gasified straw (STR) showed a slightly increasing resin-P recovery in Aarup and Jyndeved E soil and strongly increasing rates in the alkaline Jyndeved P soil, from 34 to 49% over 16 weeks, which almost matched the recovery rate of P from TSP, at 52%.

Resin-P extractability in soils amended with GBs (relative to TSP) was affected by soil types, in particular for STR and NUT (Figure 2). The relative P extractability in the STR treatment in the alkaline sandy soil (Jyndeved P) was significantly higher than in the two acid soils and increased from 49 to 94% from week 1 to 16. A significantly greater relative P extractability was also observed for NUT 16 weeks after being applied to the alkaline soils with a pH of 7.8 compared to the two acid soils with pH 5.8 (Figure 2B). In the POUL treatment, the relative P

TABLE 4 | Soil pH (H₂O) measured at 1, 4, 8, and 16 weeks after application of gasification biochars/fertiliser to the three soils in the incubation experiment.

Soil	Aarup				Jyndeved E				Jyndeved P			
	1	4	8	16	1	4	8	16	1	4	8	16
Treatment												
Con	5.9 (e)	5.7 (e)	5.8 (d)	5.7 (e)	5.6 (c)	5.7 (cd)	5.4 (cd)	5.9 (cd)	8.1 (c)	7.7 (cd)	8.1 (c)	8.0 (c)
ConK	5.5 (f)	5.4 (g)	5.4 (e)	5.3 (f)	5.5 (c)	5.5 (d)	5.2 (d)	5.6 (d)	7.5 (d)	7.4 (d)	7.8 (d)	7.7 (d)
ConP	5.8 (e)	5.6 (f)	5.8 (d)	5.6 (e)	5.5 (c)	5.5 (d)	5.5 (c)	5.7 (cd)	7.5 (d)	7.3 (d)	7.5 (e)	7.4 (e)
STR	7.0 (a)	6.4 (b)	6.9 (a)	6.4 (b)	7.4 (a)	7.1 (ab)	7.5 (a)	7.3 (a)	8.7 (b)	8.6 (a)	8.6 (b)	8.5 (b)
NUT	6.8 (b)	6.6 (a)	6.8 (a)	6.5 (a)	7.6 (a)	7.7 (a)	7.6 (a)	7.6 (a)	9.0 (a)	9.0 (a)	8.9 (a)	8.9 (a)
POUL	6.4 (c)	6.1 (c)	6.6 (b)	6.2 (c)	6.4 (b)	6.5 (b)	6.7 (b)	6.6 (b)	8.2 (c)	8.1 (b)	8.2 (c)	8.0 (c)
SSA	6.2 (d)	5.9 (d)	6.2 (c)	5.9 (d)	6.1 (b)	6.4 (bc)	6.5 (b)	6.2 (bc)	8.1 (c)	7.9 (bc)	8.3 (c)	7.8 (cd)
SSB	6.2 (d)	5.9 (d)	6.2 (c)	5.9 (d)	6.2 (b)	6.5 (bc)	6.4 (b)	6.6 (b)	8.2 (c)	8.0 (bc)	8.1 (c)	8.0 (c)
Max ^a	1.1	0.9	1.1	0.8	2.0	2.0	2.2	1.7	0.9	1.3	0.8	0.9
Min ^b	0.3	0.2	0.4	0.2	0.5	0.7	1.0	0.3	0.0	0.2	0.0	−0.2

For treatment details see Table 1. Same letters within the same column indicate no significant differences ($p > 0.05$, $n = 3$).

^aMaximum increase in soil pH compared to Con among the five biochar treatments.

^bMinimum increase in soil pH compared to Con among the five biochar treatments.

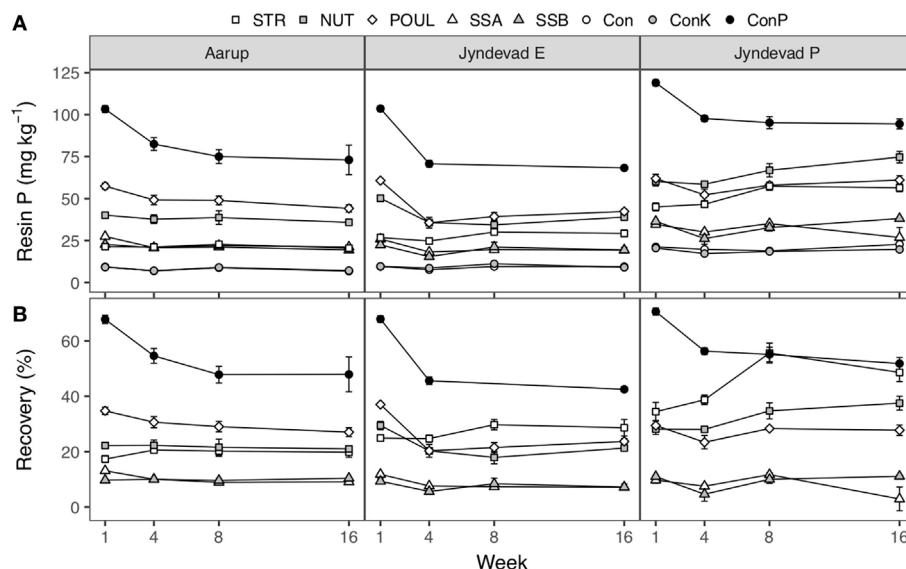


FIGURE 1 | Concentration **(A)** of resin-extractable P and recovery rate **(B)** as resin-P from applied gasification biochars/fertilisers in the three soils in the incubation experiment. Bars are SEs ($n = 3$). For treatment details see **Table 1**.

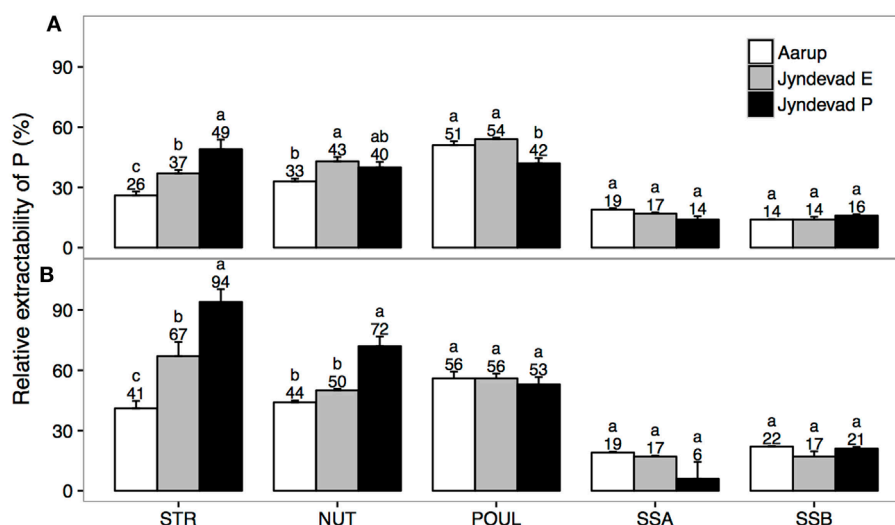


FIGURE 2 | Relative extractability of P by resins in soils amended with gasification biochars (GBs) in week 1 **(A)** and 16 **(B)** in the incubation experiment. It is calculated as the ratio (%) of recovery of applied P between GB and corresponding triple superphosphate treatments. The values and error bars are mean and SEs ($n = 3$). Same letters above each GB indicate no significant differences among the soils ($p > 0.05$). For treatment details see **Table 1**.

extractability was significantly lower in the alkaline soil than in the acid soils in week 1 but had caught up with the latter after 16 weeks. Furthermore, for STR and NUT significantly higher P extractability was seen in the sandy Jynde vad E soil compared with the clay-rich Aarup soil (**Figure 2A**).

No Effect of Moisture in GB on P Availability

The resin-P recovery rates from soils amended with the straw GB subjected to different wetting and drying treatments did not differ significantly from each other at most of the measurement dates

(**Figure 3**). Moreover, the temporal trends of both the absolute values of resin-P and the recovery rates in these treatments were similar to the observations for the STR treatment in the main incubation experiment (**Figure 1**).

Positive Correlation: Soil pH and Resin-P

A significant positive relationship was identified between soil pH and resin-P concentrations in each incubated soil, especially after amendment with GBs with low P contents, i.e., STR, NUT, SSA, and SSB (**Figure 4**). The resin-P concentration tended to increase more rapidly with pH in the alkaline Jynde vad P soil.

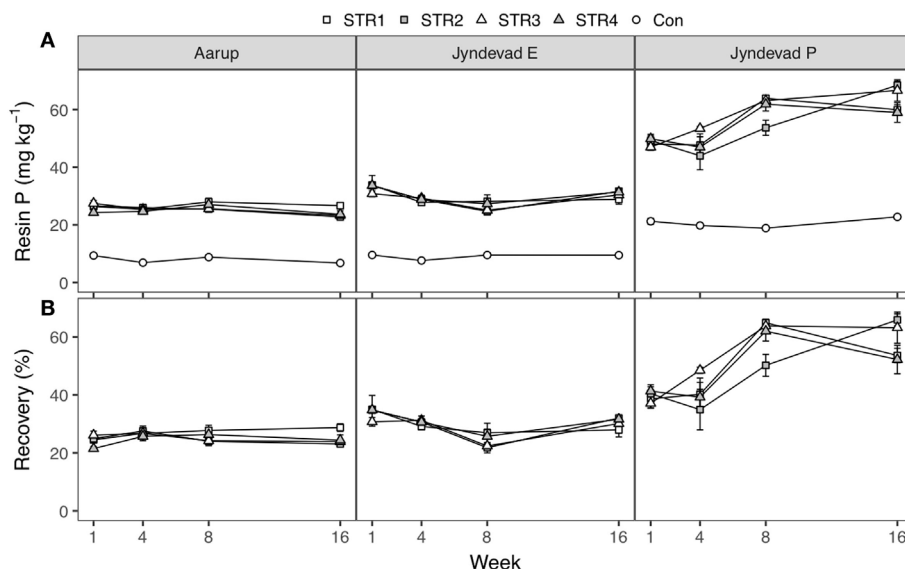


FIGURE 3 | The concentration (A) of resin-extractable P and the recovery rate (B) as resin-P from applied straw gasification biochars (GBs) in the three soils in the extra wetting–drying treatments of the incubation experiment. STR1–4 refer to the straw GBs that were subject to different wetting and drying treatments: STR1, the biochar was mixed with water at 1:1 (w:w) before initiation of the incubation; STR2, the biochar was mixed with water at 1:1, air dried, and watered again at 1:1; STR3, the biochar was mixed with water at 2:1; and STR4, the original dry GB. The biochars were applied at 69.3 mg P kg⁻¹ soil. Con is the control without amendment. Bars are SEs ($n = 3$).

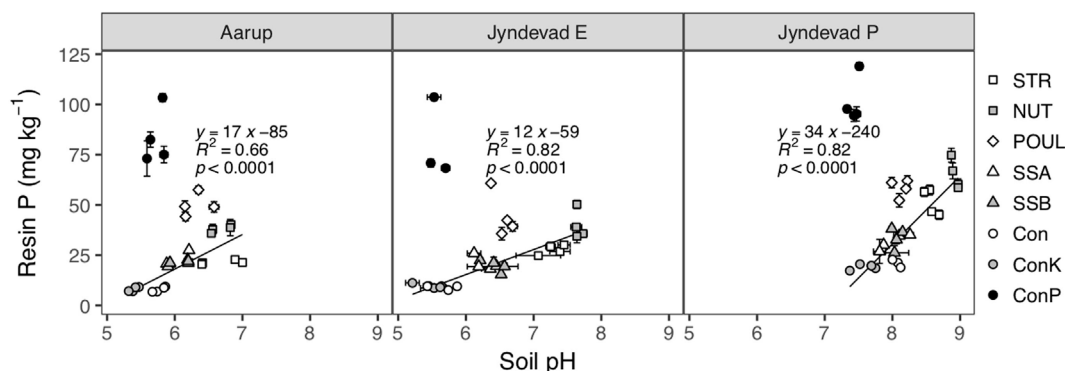


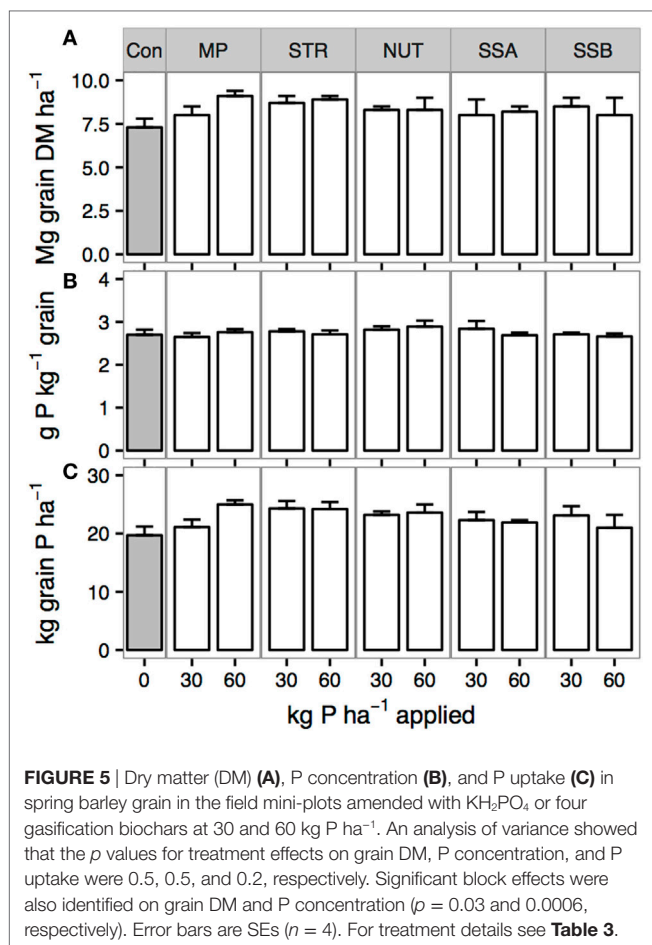
FIGURE 4 | Correlation between the concentration of resin-extractable P and pH in the three soils after gasification biochar application. Two treatments, i.e., mineral P fertiliser (ConP, black circles) and the poultry manure biochar (POUL, diamonds), were excluded from the regression analysis, and data from all four sampling dates are included. Error bars are SEs ($n = 3$). For treatment details see Table 1.

The coefficient of determination for such regression was 0.66–0.82 in the three tested soils (Figure 4) compared to only 0.26–0.39 for the regression between the Olsen P and pH (data not shown).

No Adverse Effect of GB on Barley Growth

In the mini-plot experiment, only a slight increase was observed in crop yield, P uptake, soil pH, and available P after harvest in either GB or mineral fertiliser treatments, and few of these were statistically significant compared with the control (Figures 5–7; $p > 0.05$). Compared to the control, we only observed a significantly higher soil resin-P level after barley in the SSA treatment (applied at 60 kg P ha⁻¹, Figure 7A), higher Olsen-P in the

two mineral P fertiliser treatments (Figure 7B) and higher soil pH in the STR treatment (applied at 30 kg P ha⁻¹, Figure 7C). Additionally, soil pH was raised to a level significantly higher in STR than in MP and SSB treatments when applied at the same P dose of 30 kg P ha⁻¹. Significantly higher levels of soil resin-P were also seen when NUT and SSA were applied at a high dose (60 kg P ha⁻¹) compared to the STR treatment at a lower dose (30 kg P ha⁻¹), which was however not clearly observed in the Olsen P (Figures 7A,B). The grain yield was 7.3 and 8.0–9.1 Mg ha⁻¹ in the control and the amended treatments, respectively, but not significantly different from each other (Figure 5). The variation in grain P concentration was very small, 2.65–2.89 g P kg⁻¹.

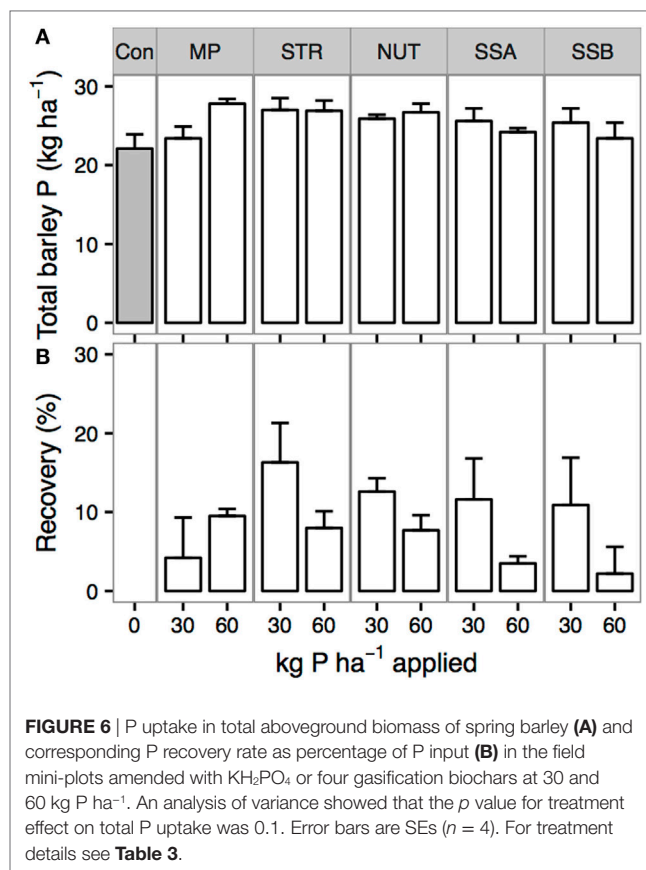


Due to the small variation in P concentration, the trend of total grain P was similar to that of the grain yield, 20 kg P ha^{-1} in the control and 21–25 kg P ha^{-1} in the amended treatments, and the difference was not statistically significant.

DISCUSSION

Composition of GBs

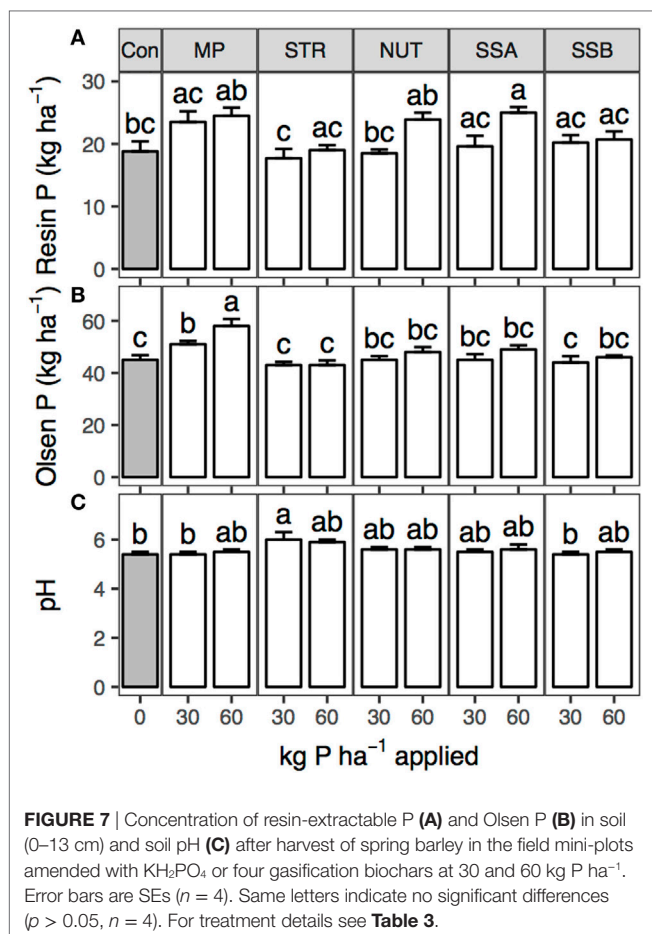
Biochars contain considerable amounts of mineral nutrients and C, as shown in **Table 1**. It has been reported that biochars can be a direct source of macronutrients and micronutrients for crop production, such as P (Wang et al., 2012; Vassilev et al., 2013). However, the total P concentration varied considerably among the biochars of different origin. The review by Spokas et al. (2012) compiled total P concentrations in a number of biochars derived from feedstocks like agricultural residues, woody biomass, and animal manures ranging from 0.1 to 59 g P kg^{-1} . Differences in total P in biochars might not only depend on the nutrient composition of the feedstock but also on the thermal conversion conditions (Spokas et al., 2012). The K concentration in these GBs was generally much higher than the P concentration, particularly in STR (**Table 1**). Therefore, not only P but also K needs to be considered when planning soil application of biochars to ensure sustainable utilisation of both P and K. The tested GBs contained



furthermore 0.3–1.3% total N, which might be mineralised and used by crops, but Wang et al. (2012) showed decreasing N availability in biochars with increasing pyrolysis temperature. Due to the nature of incomplete combustion, there was 9–57% of total C in the tested GBs. Hansen et al. (2016) showed that application of biochars derived from the gasification of straw by the LT-CFB technology has the potential to increase C sequestration in soil.

Effects of Moisture during GB Storage on P Availability

Addition of water to the GB during storage can have large benefits by lowering the risk of self-ignition and minimising dusting during storage and spreading. One 16-week study reported that during different types of storage (dry/wet, open/airtight) of a CaO-rich wood ash, the main chemical reaction was the hydration of CaO with water to form Ca(OH)_2 (Supancic et al., 2014), but it is unclear how different storage types affect P availability in biochar or ash residues. We hypothesised that wetting and drying of the GB could also influence P solubility and the following availability of P in soil through possible recombination of ions in the wet phase followed by precipitation. This was clearly not the case in our study, since we observed almost no differences in P availability after either dry or wet storage including wetting–drying events (**Figure 3**). The results might be different for other GBs, depending on their chemical composition, and this still needs further testing.



Effect of GBs on Soil pH

Soil pH increased to different extents in the three soils amended with the five GBs (Table 4). The increase in pH was most pronounced in the acid sandy soil with STR and NUT. The greater liming effect in these two treatments compared to the others was probably due to the higher application rates of STR and NUT (15.8–16.5 vs. 2.4–5.3 g DM kg^{-1}) as we aimed for comparable P application rates with the biochars. Previous studies have reported that pH increased with the application rates of biochars (Xu et al., 2014). In addition, the relatively higher increase in pH in the acid sandy soil than in soils with higher clay and pH levels might be due to a lower buffering capacity of the acid sandy soil. Equal doses of biochar increased soil pH more in acidic soils than alkaline soils in a meta-analysis (Biederman and Harpole, 2013). In contrast to the significant pH increase in the incubation experiment, the pH in our mini-plots only increased significantly (i.e., by 0.6 units) in one treatment amended with STR applied at 30 kg P ha^{-1} (Figure 7). The insignificant pH increase in most treatments in this loamy sand soil with a low pH may be due to relatively lower application rates of GBs compared to those in the incubation study and because GBs were well mixed with the top 15 cm soil layer. In practice, biochars will not be so well mixed with soil under normal agricultural conditions and locally concentrations will

be higher. Additionally, to ensure that N was not limiting the growth of spring barley, NH_4NO_3 was supplied at 150 kg N ha^{-1} in the mini-plot experiment, and it is well known that NH_4^+ application may cause soil pH to decrease due to nitrification of ammonium (Barak et al., 1997). This may have counterbalanced the liming effect of the biochars.

Effect of GBs on Soil Available P

Total P in GBs varied from 4 (in STR) to 57 g kg^{-1} (in POUL), and the fraction of WSP ranged from <0.1% (in POUL) to 22.6% (in NUT). However, both the total P and the WSP concentration do not necessarily reflect P availability after soil application (Spokas et al., 2012), as shown with POUL, where almost 40% of the applied P was recovered as resin-P in all three soils after 1 week despite its lower fraction of WSP (Figure 1; Table 1). Compared to direct analysis of P availability in the products by simple extractions, mixing biochar/ash residues with soil introduces important chemical and microbiological conditions for dissolution of P in the products. When evaluating the P availability after addition of P-containing materials, the P already present in the soil can be taken into account by including a control treatment and calculating apparent recoveries of the added P.

The method of chemical extraction from soil samples with 0.5 M NaHCO_3 (known as Olsen P) is widely used to indicate soil P availability (Jordan-Meille et al., 2012). Later developed soil P test methods such as anion exchange membrane (i.e., resin) extractable P may be more suitable to indicate plant-available P, because the extraction process by resins resembles the plant–soil interactions by plant P uptake in soil, and the method is less dependent on soil types (Schoenau and Huang, 1991). In our study, the results by the resin-P method were more differentiated among treatments than Olsen P and water-extractable P regardless of soil types, although the general response pattern was the same for all three methods (Figure 1; Figures S1 and S2 in Supplementary Material). This may be related to the fact that the resin extraction was done on moist samples but the other two on dried samples. Therefore, only the resin-P data are discussed further to represent the available P in this study.

There was a clear effect of the gasification feedstock types on the P availability in soil. Generally, high recovery rates were achieved with the GB derived from manure, which accords well with other studies on pyrolysis biochar (Ippolito et al., 2015) and GB (Kuligowski et al., 2010). Wang et al. (2012) found constantly higher P recovery rates from biochars originating from manure compared to those produced from sewage sludge, which was confirmed in our study. This could be due to the presence of different chemical P compounds in the GB. The P is probably dominated by Mg/Ca-P in manure-based biochars (Wang et al., 2012, 2015) and by Fe/Al-P in GB originating from sewage sludge (Parés-Viader et al., 2016). This was also reflected in the relatively high Mg:P molar ratio in POUL and in the high Fe contents and Fe:P molar ratios in SSA and SSB compared to the other GBs (Table 1).

The recovery rates and the relative extractability of P from NUT and STR varied among the different soils after the 16-week incubation, being significantly lower in the Aarup and Jyndeved

E soils and higher in the alkaline Jyndeved P soil (Figure 2). Contrarily, those from POUL, SSA, and SSB seemed not to be strongly affected by the soil type. This implies that the fertiliser value of GBs may be dependent on both the feedstock and soil type.

Biochars can generally increase plant-available P in soil not only by the mere increase in available P introduced by P released from the material into the soil solution but also by altering the physico-chemical conditions of the soil, such as pH, which in turn can influence the P availability in the native soil P pools. An increase in soil pH may not have the same effect on P availability in different soil types because of their different conditions for binding P arising from their mineralogy, organic matter content, nutrient status, and initial pH as shown in many studies (e.g., Haynes, 1982; Weng et al., 2011). In the three soils tested here, we found a significant positive and soil type-specific relationship between the resin-P concentration and pH in soil after addition of biochars with relatively low P contents (Figure 4). One possible explanation for this positive correlation is that the surfaces of the soil particles become more negatively charged with increased pH (deprotonation) and hence phosphate adsorption decreases with increasing pH (Devau et al., 2009; Murphy and Stevens, 2010). As pH increases following biochar application in the acid Aarup and Jyndeved E soils, P may become released from its tight binding with Fe and/or Al oxides (Cui et al., 2011; Macdonald et al., 2014) due to reduced solubility of the hydroxy-Al or -Fe species at around the neutral pH range (Haynes, 1982). In the alkaline soil (Jyndeved P) with a history of intensive lime applications, high contents of exchangeable calcium ions are expected to be present in the soil which might associate with the applied biochar. It has been shown previously that straw GB is able to increase cation exchange capacity of soils (Hansen et al., 2016). Therefore, the adsorption of Ca to biochars may weaken the binding between Ca and phosphate in this soil and thereby increase the P availability in combination with the effect of deprotonation induced by the higher pH (Devau et al., 2009). The rapid increase of resin-P with rising pH in the Jyndeved P soil was consistent with several previous studies, which also reported increasing P concentrations in the soil solution with elevated pH (>7) in several soil types, and demonstrated that pH is an important factor controlling the availability of P in soil (Haynes, 1982; Devau et al., 2009; Weng et al., 2011; Xu et al., 2014).

The different temporal patterns of available P that occurred for different GBs when added to different soils, especially the changes in alkaline Jyndeved P soil (Figure 1), could probably be explained by the various processes governing adsorption/immobilisation and desorption/mobilisation of P needing varying lengths of time to reach equilibrium. For instance, there might be a net adsorption/immobilisation in soil in the 4–8 weeks after applying TSP as it readily dissolves (Figure 1). For STR in the Jyndeved P soil, there might be a net desorption/mobilisation of P during the first 8 weeks because STR may dissolve more slowly considering its higher Ca:P ratio (Table 1), after which equilibrium might have been reached (Figure 1).

Crop and Soil Response to GBs

It has been acknowledged that biochar may have positive, negative, or no effect on crop production as influenced by many complex factors (Spokas et al., 2012; Biederman and Harpole, 2013; Macdonald et al., 2014). We demonstrated that there was no adverse impact on crop growth of the added biochars in doses (e.g., 30 and 60 kg P ha⁻¹) corresponding to relevant P doses typically given as mineral P fertiliser to the studied soil. The P uptake (Figure 6) and grain yield (Figure 5) in the mini-plot experiment tended to be higher in the plots amended with mineral fertiliser or biochars than in the control, but were not significantly different. One reason for the insignificant response might be that the P status of the soil at 24 mg Olsen-P kg⁻¹ was not low enough to limit the barley growth. Compared to this soil, a similar or higher level of Olsen-P is found in the majority (ca. 75%) of Danish agricultural soils (Rubæk et al., 2013), where P fertilisers have been applied for several decades to build up and to maintain a sufficient level of available P in soil.

P fertilisers are applied by farmers to maintain a sufficient level of available P in soil, also in the longer term. A general positive recovery rate of P applied with biochars (Figure 6) indicates that they also contribute positively to available P in soil. After harvest, the resin-P level increased in the soil amended with mineral fertiliser, and the NUT and SSA biochar treatments (high rate) compared to the control, though only significantly in the SSA treatment. We therefore conclude that the tested biochars and the mineral fertiliser had only minor and barely significant impacts on the crop performance in this experiment, but the tested biochars will contribute to maintaining the level of available P in soil.

CONCLUSION

In this study, we evaluated plant P availability in contrasting soils amended with five biochars derived from biomass gasification *via* the LT-CFB technology. In a mini-plot experiment on a loamy sand soil, biochar application showed only insignificant positive effects on P uptake and yield of spring barley, probably due to sufficient soil P availability. In a 16-week incubation, considerable though varying amounts of P (about 10–50%) from applied biochars were recovered as resin-extractable P in three different soils, demonstrating the varying ability of GBs to replace mineral P fertilisers on agricultural land. A strong influence of the GB feedstock on P availability was observed. GBs derived from poultry manure, wheat straw and shea nut residues showed higher P extractability relative to TSP (indicating the fertiliser value) than sewage sludge-based GBs, i.e., 41–94% vs. 6–22% after 16 weeks. A significant pH increase was observed after biochar application, which was dependent on the dose applied, the initial soil pH, and probably the buffering capacity of the soil. There was a significant positive correlation between available P and pH in soils amended with certain GBs with relatively low P contents (i.e., STR, NUT, SSA, and SSB), which proved the importance of soil pH in determining P availability. Wetting and drying of a straw GB during storage had no significant influence on the P availability following soil application, showing that wet storage is suitable in the practical handling of GBs. These results suggest that feedstock types

and soil properties, e.g., soil pH, are important for P availability after application of GBs.

AUTHOR CONTRIBUTIONS

GR, DM-S, and PS designed and performed the experiments. XL analysed the data and wrote the manuscript. GR, DM-S, and PS contributed to discussion and revision of the manuscript. TT and JA provided some biochar materials and information concerning the LT-CFB gasification and contributed to revision of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at <http://www.frontiersin.org/articles/10.3389/fsufs.2017.00002/full#supplementary-material>.

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Transformation of Phosphorus in Speciation and Bioavailability During Converting Poultry Litter to Biochar

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Converting poultry litter (PL) to biochar and applying the biochar to cropland as a soil amendment may be a best approach for recovering nutrients from solid biowastes while minimizing nutrient runoff losses from the treated field. To evaluate the potential of PL-derived biochar as a slow-release phosphorus (P) fertilizer, the speciation, lability, and bioavailability of P in PL and the derived biochars were examined and compared. Raw PL and its derived biochars through 300–600°C slow pyrolysis were analyzed for total P (TP), inorganic P (IP), and organic P (OP) contents. The TP was fractionated into readily, generally, moderately, low, and non-labile pools by sequential extraction with different solutions. The TP was further assessed for bioavailability using batch extraction by water, Olsen, Bray-1, Mehlich-3, and 1 M HCl extractants. The P species in biochars were characterized using solid-state ³¹P nuclear magnetic resonance (NMR) techniques. The results indicate that during pyrolysis OP in PL was transformed to IP and water-soluble P to low labile forms such as hydroxyapatite and oxyapatite especially at higher temperature. Bray-1 and Mehlich-3 were appropriate extractants for evaluating the immediate to medium-term available and the long-term available P in biochar, respectively. Converting PL to biochar through ≤450°C pyrolysis significantly reduced the water-soluble proportion and the lability of P but did not compromise the long-term P bioavailability, resulting in a P-enriched, slow release soil amendment that would minimize the P runoff risks following field application. The promising results need to be further validated in soil-biochar-plant systems.

Keywords: poultry litter biochar, phosphorus speciation, hydroxyapatite, lability, chemical extraction

INTRODUCTION

Poultry production (domestic fowl rearing for meat and eggs) is a crucial economic activity in nearly all countries to furnish affordable diet protein and suppress hunger. Intensive and concentrated bird rearing, however, generates vast volumes of litter waste (mixture of feces, sheddings, and bedding materials) that requires appropriate disposal (Bolan et al., 2010). The U.S. poultry industry, for example, generates annually more than 550 million dry tons of litter waste (Coker, 2017). Poultry litter (PL) contains high contents of organic carbon (OC, ~380 g kg⁻¹) and the plant nutrients nitrogen (N, ~40 g kg⁻¹), phosphorus (P, ~15 g kg⁻¹), and potassium

(K, $\sim 38 \text{ g kg}^{-1}$) (Guo et al., 2009a) and is predominantly disposed of through land application as an organic fertilizer. The P:N ratio (e.g., 1:2–1:3) of PL is much higher than the typical crop nutrient requirement (e.g., 1:6–1:8) (Sadras, 2005) and therefore, application of PL at N-based agronomic rates would result in over-fertilization of P, which is subject to runoff losses to natural water bodies. In regions with concentrated poultry production, repeated and excess application of PL to cropland has introduced substantial loads of P and N nutrients to local water systems, causing eutrophication, and general water quality degradation issues (NASA, 2016). Phosphorus exists in PL principally in labile forms (e.g., water-soluble, 0.5 M NaHCO_3 -extractable, and organic P) (Dou et al., 2000; Li et al., 2014). Up to 50% of the P in PL is water extractable (Dou et al., 2000) and can be rapidly released into the soil water via rainfall following land application (Guo et al., 2009b). To reduce the risks of P runoff from land-applied PL, it is critical to decrease the P water extractability and release rate of the organic fertilizer.

Biochar is a promising soil amendment capable of persistently enhancing soil quality through ameliorating soil physical, chemical, and biological properties (Guo et al., 2016). Converting agricultural byproducts and other organic residues to biochar and utilizing the biochar as a soil amendment may be a best management practice for recovering nutrients and sustaining soil quality. Research demonstrates the feasibility of manufacturing nutrients-enriched biochar by pyrolysis of PL for promoting crop growth (Chan et al., 2008; Revell et al., 2012; Mierzwa-Hersztek et al., 2016). Depending on the pyrolysis conditions especially the temperature and time duration, the yield of biochar ranged from 45 to 60 mass% of the feedstock PL (Song and Guo, 2012). Nearly 100% of the PL-P was recovered in biochar (Song and Guo, 2012). Pyrolysis significantly transformed the P in PL and reduced the water extractable portion of P from 19.5% of total P in raw PL to $<7.0\%$ in biochar. As the pyrolysis temperature was elevated from 300 to 600°C , the portion of 0.01 M HCl-extractable P in the PL-derived biochar products decreased from $>55\%$ of total P to $<16\%$ (Song and Guo, 2012). Pyrolysis at 400°C converted the labile P in raw PL to Mg/Ca phosphate minerals in biochar and reduced the water-soluble P from 2.95 g kg^{-1} in raw PL to 0.17 g kg^{-1} in biochar (Wang et al., 2015). In PL biochar, orthophosphate was predominant while organic phosphate (a major form of P in raw PL) was barely present. Furthermore, release of P from PL biochar in water and neutral soils was significantly slower and steadier than from raw PL (Wang et al., 2015). Relative to raw PL, the derived biochar is a slow P release fertilizer (Dai et al., 2016). To date the transformation processes of P in PL during pyrolysis have not been fully understood. The existing forms of P in PL-derived biochar are not clear, and the bioavailability of the biochar P lacks assessment. The objective of this study was to examine the transformation of P in speciation and bioavailability during converting PL to biochar under varying pyrolysis conditions, aiming to optimize the pyrolysis operation for producing a slow release yet efficiently available P soil amendment from animal wastes.

MATERIALS AND METHODS

Poultry Litter

Poultry litter was procured from an industrial facility in Seaford, DE that processes raw PL collected from local broiler farms into a marketable fertilizer product via a series of physical and mechanical operations. The PL predominantly in $<4 \text{ mm}$ granules contained 92.3 wt% of dry matter and 7.7 wt% of moisture. The dry matter was comprised of 71.5 wt% organic constituents and 28.5 wt% ash minerals. On the dry mass basis the PL contained total N 30.7, total P 13.7, and total K 41.8 g kg^{-1} . More nutrient composition information of the PL can be found in Table 1 of Song and Guo (2012). The PL was used as received.

Converting PL to Biochar

The PL was converted to biochar through slow pyrolysis at varied peak temperatures, with duplicate trials performed at each selected temperature. Briefly, 650 g of the granular PL were weighed into a metal canister (11 cm i.d. \times 13 cm height) to its volume at loose packing. The canister was covered by its metal lid that had a 5-mm hole in the center. The canister was placed in an Isotemp muffle furnace (Thermo Fisher Scientific, Inc., Suwanee, GA) and heated at a pre-determined temperature ($300\text{--}600^\circ\text{C}$) until the pyrolysis was complete, as indicated by no further visible smokes escaping from the furnace. The furnace was able to raise its internal temperature at $20^\circ\text{C}/\text{min}$ and maintain a pre-set constant temperature with continuous power supply. Pyrolysis of PL inside the canister started when the furnace temperature reached above 250°C , signified by visible smokes (pyrolysis vapors) emitted out of the 4-cm gas vent in the top panel of the furnace. It took 130–370 min for the pyrolysis of PL in the canister to be complete, the time shortened as the peak pyrolysis temperature increased in the range of $300\text{--}600^\circ\text{C}$.

Once the complete pyrolysis was achieved, the furnace was switched off, and the canister was taken out and cooled to the room temperature with immediate sealing of the lid hole with a piece of metal tape. The biochar in the canister was then transferred into a Ziploc plastic bag and stored in a dark cabinet at 22°C prior to further characterization. More details of the biochar preparation methods can be found in Song and Guo (2012). The peak temperatures 300, 350, 400, 450, 500, 550, and 600°C were used to produce biochars from PL through pyrolysis operations varying in temperature, the most important parameter that controls the yield and quality of biochar derived from a specific feedstock (Guo et al., 2012). The PL-derived biochar samples are hereafter referred to as C300, C350, C400, C450, C500, C550, and C600, respectively.

Chemical Characterization of P in PL and Biochar Samples

The PL and the derived biochars were ground to $<0.15 \text{ mm}$ and stored in brown glass vials. Total P (TP) and its constituents inorganic P (IP) and organic P (OP) of the samples were analyzed. The contents of TP were determined by acid digestion and colorimetric P measurement (Song and Guo, 2012). For each sample, duplicate measurements were conducted. All the laboratory wares were of glass or Teflon and were acid-washed.

TABLE 1 | Contents of total phosphorus (TP) and its inorganic phosphorus (IP) and organic phosphorus (OP) fractions in poultry litter (PL) and the derived biochars.

	TP	IP		OP	
	g kg ⁻¹	g kg ⁻¹	% of TP	g kg ⁻¹	% of TP
PL	13.70 ± 0.90	9.26 ± 0.026	67.62 ± 1.77 ^a	4.44 ± 0.93	32.38 ± 1.77 ^a
C300	22.73 ± 0.15	19.78 ± 0.12	87.03 ± 0.18 ^b	2.95 ± 0.27	12.97 ± 0.18 ^b
C350	24.02 ± 0.086	23.01 ± 0.12	95.79 ± 0.13 ^d	1.01 ± 0.21	4.21 ± 0.13 ^d
C400	26.29 ± 0.42	25.58 ± 0.048	97.31 ± 0.31 ^e	0.71 ± 0.47	2.69 ± 0.31 ^e
C450	26.59 ± 0.77	26.52 ± 0.048	99.75 ± 0.56 ^f	0.07 ± 0.82	0.25 ± 0.56 ^f
C500	27.87 ± 0.69	26.91 ± 0.024	96.56 ± 0.47 ^{d,e}	0.96 ± 0.71	3.44 ± 0.47 ^{d,e}
C550	29.84 ± 0.52	27.04 ± 0.024	90.63 ± 0.32 ^c	2.80 ± 0.54	9.37 ± 0.32 ^c
C600	30.54 ± 0.86	27.25 ± 0.024	89.23 ± 0.51 ^c	3.29 ± 0.88	10.77 ± 0.51 ^c

Data are means ± standard deviations of duplicate measurements. Superscript letters denote significance of difference between treatment levels.

In operation, 0.20 g of a sample were transferred into a 45-mL Teflon digestion cylinder (Parr Instrument Company, Moline, IL), followed by addition of 5.0 mL Milli-Q water and 5.0 mL concentrated HNO₃. Procedure blanks without sample addition were included. After gentle swirling for mixing, the cylinder was capped and placed into a digestion case and microwaved at 0.6 KW for 2.5 min. The digestion bomb was left in the microwave oven to cool to nearly room temperature, then opened, and the digestate in the cylinder was fully transferred into a 100-mL volumetric flask with several times of Milli-Q water rinsing. The digestate in the flask was brought to 100 mL by adding Milli-Q water, well-mixed, and passed through a 0.45-μm glass fiber filter to remove any particulates. The clear digestate was then analyzed for TP concentrations using the standard phosphomolybdate blue method (Murphy and Riley, 1962). The IP contents were determined by extracting the samples with 1 M HCl at 1:50 solid/solution ratio under room temperature rotary shaking for 24 h, centrifuging the extract at 4,080 × g for 20 min., passing the supernatant through a 0.2-μm syringe filter, and measuring the P content of the filtered extract using the phosphomolybdate blue method (de Jonge et al., 1993). The OP contents were computed as the difference between TP and IP of a sample. The method detection limit was 3.0 mg kg⁻¹.

To examine the fractionation of TP in different pools showing distinct leachability and bioavailability, the PL and biochar samples in duplicates were sequentially extracted by Milli-Q water, 0.5 M NaHCO₃, 0.1 M NaOH, and 1 M HCl, each at 1:50 solid/solution ratio (Qian and Jiang, 2014). At each sequential extraction step, the solid/solution slurry was continuously agitated by 30 rpm rotary shaking at room temperature for 24 h and centrifuged at 4080 × g for 20 min to separate the mixture into supernatant and pellet. The supernatant was passed through a 0.2-μm syringe filter and collected in a 50-mL volumetric flask; the pellet was washed twice each by 10 mL Milli-Q water and the rinsate was combined with the supernatant. The next sequential extraction step was then started by adding a new extractant solution and re-suspending the pellet. Any P remaining in the pellet after the sequential 1 M HCl extraction was treated as residual P (non-labile, non-bioavailable) of the original sample. The extract in the volumetric flask was brought to volume with Milli-Q water and

analyzed for concentrations of total dissolved P (P_T), dissolved organic P (P_O), dissolved inorganic P (P_i), dissolved inorganic orthophosphate P (P_r), and dissolved inorganic polyphosphate P (P_x). Prior to bringing to the volume, the NaHCO₃- and NaOH-extracts were adjusted to pH just below 8 by drop-adding concentrated HCl with phenolphthalein as the indicator. The P_T, P_i, and P_r concentrations of the sequential extracts were measured following the phosphomolybdate blue method after acidic K₂S₂O₈ autoclave digestion, H₂SO₄ autoclave digestion, and no pretreatment of the extracts, respectively (Wang et al., 2015). Once again, procedure blanks without sample addition were included. All standard P solutions for establishing the calibration curves were subject to the same digestion processes as the extracts. The method detection limit was 0.91 mg kg⁻¹. The P_O was computed as the difference between P_T and P_i (P_O = P_T - P_i) and P_x as the difference between P_i and P_r (P_x = P_i - P_r). For the HCl-extracts, only P_r was measured; no organic P or inorganic polyphosphate P was expected in this extract and therefore, P_r = P_i = P_T was assumed (Qian and Jiang, 2014).

To further estimate the bioavailability of P in the PL and biochar samples, the fractions of Mehlich-3 P, Bray-1 P, and Olsen P were measured by batch extraction methods (Pierzynski, 2000). Briefly, 0.5-g aliquots of the samples were extracted separately by 25 mL of Mehlich-3 extractant (0.2 M CH₃COOH + 0.015 M NH₄F + 0.013 M HNO₃ + 0.001 M EDTA + 0.25 M NH₄NO₃), Bray-1 extractant (0.025 M HCl in 0.03 M NH₄F), and Olsen extractant (0.5 M NaHCO₃, pH adjusted to 8.5) at room temperature for 24 h under continuous rotary shaking and the extracts were analyzed for P_T concentrations following the phosphomolybdate blue method after acidic K₂S₂O₈ autoclave digestion. For each sample, duplicate measurements were conducted. Procedure blanks without sample addition were included. The method detection limit was 0.61 mg kg⁻¹.

Spectroscopic Characterization of P in PL-Derived Biochar Samples

To identify the existing species of P in PL-derived biochar, solid-state ³¹P single-pulse (SP) and ³¹P{¹H} cross-polarization (CP) magic-angle spinning (MAS) nuclear magnetic resonance (NMR) spectra of the biochar samples were collected on a 400 MHz Varian Unity Inova spectrometer (Varian, Inc., Palo Alto, CA) at

operating frequencies of 161.8 and 399.8 MHz for ^{31}P and ^1H , respectively. Spectra were collected using a Varian/Chemagnetics T3-type probe, with samples contained in 7.5 mm (o.d.) normal wall ZrO_2 rotors. The $^{31}\text{P}\{^1\text{H}\}$ CP/MAS spectra were obtained at the spinning rate of 5 kHz with CP contact time of 1 ms using the same probe. The CP kinetic curves were measured at a spinning rate of 5 kHz with contact times varying from 0.3 to 5 ms and irradiation under the $n = -1$ sideband match condition. For all CP/MAS spectra, the transverse ^1H field ($\gamma\text{B}_{1,\text{H}}$) was ramped over approximately 5 kHz, centered near the first sideband match at a 42 kHz ^1H field. Proton decoupling (CW) was employed during acquisition of all $^{31}\text{P}\{^1\text{H}\}$ CP/MAS spectra. The ^{31}P chemical shifts ($\delta_{\text{iso,P}}$) were reported relative to external 85% H_3PO_4 solution, using hydroxyapatite as a secondary reference set to $\delta_{\text{iso,P}} = 2.65$ ppm.

Statistical Data Analysis

All chemical characterization data are expressed as means of duplicate measurements, with standard deviations showing the analytical precision. The results of analytical procedure blanks were incorporated in sample data processing. The relative abundance of various P forms is articulated as the percentage relative to the TP concentrations of the PL and biochar samples. Variations in speciation and bioavailability of P in PL and the derived biochars from different pyrolysis temperatures were statistically evaluated at the level of significance $\alpha = 0.05$ following the analysis of variance (ANOVA) and the Fisher least significant difference (LSD) methods.

RESULTS

Transformation of Organic P During Pyrolysis of PL to Biochar

The PL contained 13.7 g kg^{-1} TP, of which 67.6% was IP and 32.4% belonged to OP. In the biochar products, the TP content was elevated to 22.7 g kg^{-1} for C300 and 30.5 g kg^{-1} for C600, increasing steadily as the pyrolysis temperature was raised (Table 1). The proportion of IP in the TP was also elevated to 87.0% for C300 and further to 99.7% for C450. When the pyrolysis temperature was increased to 500°C and above, the “nomial” proportion of IP (extractable by 1 M HCl) decreased slightly from the peak value of nearly 100 to 96.6% in C500 and to 89.2% in C600 (Table 1). Correspondingly, the OP proportion decreased from 32.4% in raw PL to 13.0% in C300 and further to nearly null (0.25%) in C450. As the pyrolysis temperature was elevated to 500°C and higher, the “nomial” OP proportion showed a slightly increasing trend in the resulting biochar products, reaching 10.8% in C600 (Table 1). The changes in IP and OP proportions between the biochars generated at 300–600°C with a 50°C interval were statistically significant, especially when the pyrolysis temperature was $\leq 450^\circ\text{C}$ (Table 1).

Redistribution of Labile P Fractions During Pyrolysis of PL to Biochar

In raw PL, water-soluble P accounted for nearly half (49.5%) of the TP, while the remaining half was shared by NaHCO_3 -extractable, NaOH-extractable, HCl-extractable, and residual P at

9.0, 5.5, 17.1, and 18.9%, respectively (Figure 1). In PL-derived biochars, the portion of water-soluble P decreased radically to 11.7% of TP in C300, 8.7% in C350, 5.5% in C400, and further to $\sim 2.4\%$ in biochars produced at $\geq 450^\circ\text{C}$ pyrolysis temperatures. The proportion of NaHCO_3 -extractable P, on the contrary, increased significantly to 20.4% in C300, fluctuated between 17.7 and 19.2% in C350, C400, C450, and C500. The proportion then descended to 10.8% in C550 and 9.7% in C600. Similarly, the NaOH-extractable P increased from 5.5% of TP in raw PL to the peak proportion of 13.2% (of TP) in C300 and then declined gradually to 9.2% in C350, 7.5% in C400, 4.2% in C450, 1.6% in C500, and to $<0.2\%$ in biochars generated at $\geq 550^\circ\text{C}$ pyrolysis temperatures (Figure 1). The sequentially HCl-extractable P increased its proportion from 17.1% of TP in raw PL to 33.9% in C300, 52.2% in C350, and further to 60–72% in biochars produced from $\geq 400^\circ\text{C}$ pyrolysis. The residual P in C300 accounted for 20.8% of the TP, a percentage close to that of raw PL (18.9%). The proportion decreased to 10.8% in C350 and reached the bottom level of 5.4% in C450. Further elevation of the pyrolysis temperature caused back increases of the residual P proportion in the resulting biochar products, to 16.1% in C600 (Figure 1). The residual P proportion profile is consistent with that of “nominally” OP (Table 1). In general, converting to biochar through pyrolysis significantly reduced the highly labile, highly leachable, and highly bioavailable (primarily water-soluble) P in PL.

Inorganic P (P_i , including orthophosphate-P (P_r) and polyphosphate-P (P_x)) dominated the water-soluble P in PL and the derived biochars, accounting for $>89\%$ of the total dissolved P (P_t) extractable by water (Table 2). In PL, for example, the water-soluble P consisted of 84.5% P_r , 4.5% P_x , and 11.0% P_o (dissolved organic P). The proportion of P_o in water-soluble P from C300 decreased to 2.9%, whereas the proportion of P_x increased to 10.4% and P_r to 86.7% (P_i 97.1%). The P_o proportion

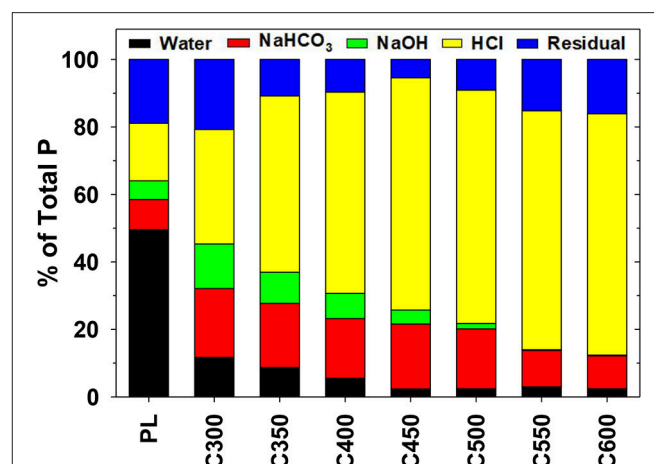


FIGURE 1 | Fractionation of phosphorus (P) in poultry litter (PL) and the derived biochars into water-extractable, NaHCO_3 -extractable, NaOH-extractable, HCl-extractable, and residual forms. Data are means of duplicate measurements. The coefficients of variation of the duplicate measurements are within 3%.

TABLE 2 | Composition of water-soluble, NaHCO₃-extractable, and NaOH-extractable phosphorus (P) fractions in poultry litter (PL) and the derived biochars.

	Water-soluble P			NaHCO ₃ -extractable P			NaOH-extractable P		
	P _r	P _x	P _o	P _r	P _x	P _o	P _r	P _x	P _o
	% of water-soluble P _t			% of NaHCO ₃ -extractable P _t			% of NaOH-extractable P _t		
PL	84.5 ± 0.66 ^a	4.45 ± 0.57 ^{c,d}	11.0 ± 1.23 ^a	75.2 ± 0.32 ^a	6.82 ± 1.10 ^b	17.9 ± 0.77 ^a	14.6 ± 0.81 ^a	19.8 ± 0.38 ^b	65.6 ± 1.19 ^a
C300	86.7 ± 0.11 ^b	10.4 ± 0.50 ^a	2.90 ± 0.61 ^b	86.7 ± 0.41 ^b	13.3 ± 0.41 ^a	0.00 ± 0.00 ^b	71.7 ± 0.63 ^b	24.4 ± 0.38 ^a	3.94 ± 0.25 ^b
C350	92.5 ± 0.19 ^c	5.46 ± 0.02 ^b	2.06 ± 0.22 ^b	96.8 ± 0.36 ^c	3.21 ± 0.36 ^c	0.00 ± 0.00 ^b	91.4 ± 0.40 ^c	7.72 ± 0.57 ^c	0.85 ± 0.17 ^c
C400	93.9 ± 0.17 ^d	4.75 ± 0.13 ^c	1.40 ± 0.04 ^b	99.0 ± 0.17 ^d	1.00 ± 0.17 ^d	0.00 ± 0.00 ^b	98.8 ± 0.21 ^d	1.15 ± 0.21 ^d	0.00 ± 0.00 ^d
C450	96.7 ± 0.26 ^e	2.83 ± 0.25 ^{d,e}	0.51 ± 0.01 ^c	100 ± 0.00 ^e	0.00 ± 0.00 ^e	0.00 ± 0.00 ^b	100 ± 0.00 ^e	0.00 ± 0.00 ^e	0.00 ± 0.00 ^d
C500	98.3 ± 0.31 ^f	1.69 ± 0.31 ^e	0.00 ± 0.00 ^d	100 ± 0.00 ^e	0.00 ± 0.00 ^e	0.00 ± 0.00 ^b	100 ± 0.00 ^e	0.00 ± 0.00 ^e	0.00 ± 0.00 ^d
C550	100 ± 0.00 ^g	0.00 ± 0.00 ^f	0.00 ± 0.00 ^d	100 ± 0.00 ^e	0.00 ± 0.00 ^e	0.00 ± 0.00 ^b	100 ± 0.00 ^e	0.00 ± 0.00 ^e	0.00 ± 0.00 ^d
C600	100 ± 0.00 ^g	0.00 ± 0.00 ^f	0.00 ± 0.00 ^d	100 ± 0.00 ^e	0.00 ± 0.00 ^e	0.00 ± 0.00 ^b	100 ± 0.00 ^e	0.00 ± 0.00 ^e	0.00 ± 0.00 ^d

Data are means ± standard deviations of duplicate measurements. Superscript letters denote significance of difference between treatment levels. P_t, total dissolved phosphorus (P); P_r, dissolved inorganic orthophosphate-P; P_x, dissolved inorganic polyphosphate-P; P_o, dissolved organic phosphorus.

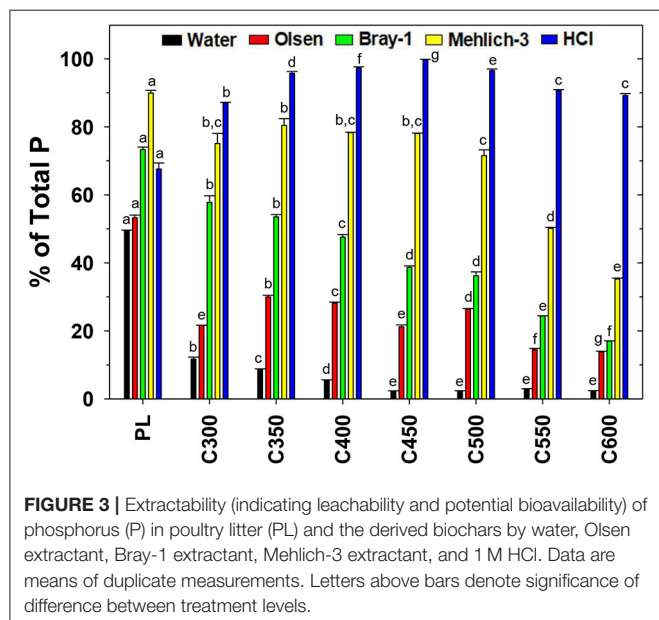
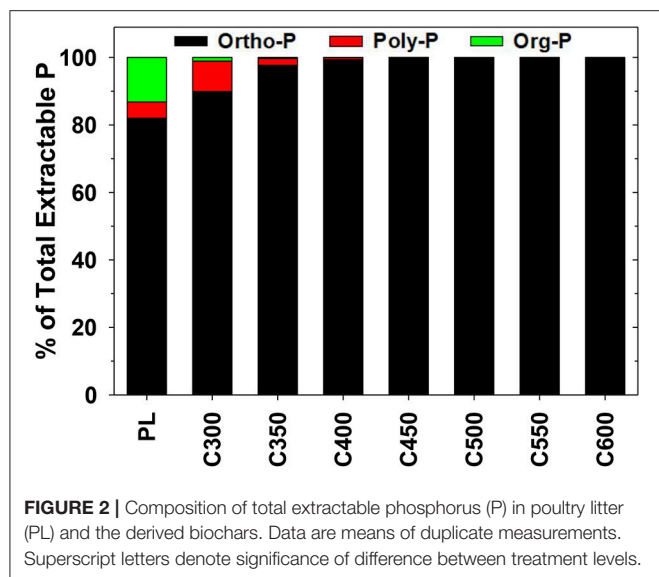
of water-soluble P in biochar products decreased gradually as the production temperature was elevated in the range of 300–600°C; the P_x proportion also decreased steadily; yet the P_r proportion and the overall P_i proportion increased accordingly. Organic P disappeared from the C500 water extract; only P_r was present in water-soluble P of biochars produced at ≥550°C pyrolysis temperatures (Table 2). Sequential extraction of PL and the biochars by NaHCO₃ after water showed the composition of 75.2% P_r, 6.9% P_x, and 17.9% P_o in the extractable P from PL and 86.7% P_r, 13.3% P_x, and 0.0% P_o in the extractable P from C300. Organic P completely disappeared from the sequential NaHCO₃ extracts of biochars, while P_x was only present in extracts from low-temperature biochars and decreased its proportion in products from higher temperature pyrolysis. In the sequential NaHCO₃ extracts of C450, P_x nulled and P_r accounted for 100% of P_i and P_t (Table 2). The composition of P in the sequential NaOH extracts demonstrated similar variation trends. The strongly alkaline NaOH solution is rather efficient for extracting organics from solid matrix. It was assumed that all remaining P_o in the PL and biochars after the sequential NaHCO₃ extraction would be recovered in the NaOH extracts. Consequently, P_o was the principal form of P in the NaOH extracts from PL, accounting for 65.6% of the P_t (Table 2). In the NaOH extracts from C300, however, P_o was merely 3.9% of the P_t, and proportion decreased to 0.0% in the extracts from C400. Meanwhile, P_x decreased its proportion of 24.4% in the C300 extracts to 1.1% in the C400 extracts. In the NaOH extracts from C450, P_r became the single form of dissolved P (Table 2).

Of the total extractable P from the four-stage sequential extraction (by water, NaHCO₃, NaOH, and HCl), P_r, P_x, and P_o accounted for 82.0, 4.8, and 13.2%, respectively in PL. The composition changed to 89.9% P_r, 9.0% P_x, and 1.1% P_o in C300 (Figure 2). In biochars produced at higher pyrolysis temperatures, both the extractable P_o and P_x demonstrated a steadily declining trend in proportion. The extractable P_o and P_x disappeared and P_r was the only extractable P form in biochars prepared through ≥500°C pyrolysis (Figure 2). In consistent with Table 1, the results suggest transformation of OP to polyphosphate-P and further to orthophosphate-P during

converting PL to biochar. The transformation was facilitated as the pyrolysis temperature was elevated.

Potential Bioavailability of P in PL-Derived Biochars as Indicated by Extractability

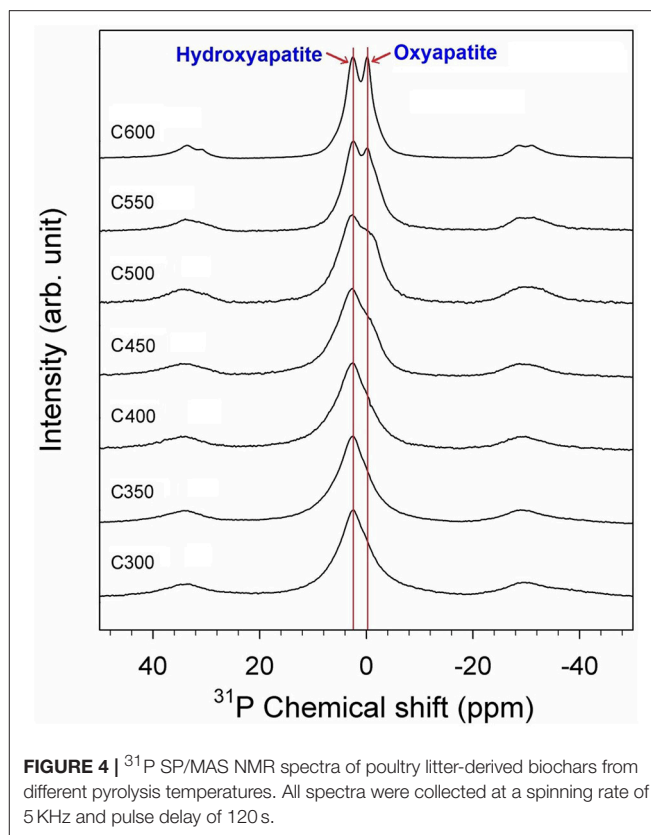
Water was able to extract 2.4–11.7% of TP in PL-derived biochars, the proportion decreasing readily to the minimum as the pyrolysis temperature was elevated in the range of 300°C to 450°C and above (Figure 3). In comparison, water extracted 49.5% of TP from raw PL under the same conditions. Clearly, conversion to biochar dramatically reduced the water solubility of P in PL. The Olsen solution (0.5 M NaHCO₃, pH 8.5) performed slightly better than water in recovering P from PL and the derived biochars. The Olsen P accounted for 53.3% of TP in raw PL and 13.8–30.0% in biochars. Nevertheless, the pyrolysis temperature effect on the proportion of Olsen P in PL-derived biochar was not straightforward. For C300, the proportion was 21.6%. The proportion increased to 30.0% for C350 yet then declined for biochars produced at higher pyrolysis temperatures, to 28.2% for C400 and 13.8% for C600 (Figure 3). The Bray-1 extractant (0.025 M HCl in 0.03 M NH₄F) acted more efficiently than water and the Olsen extractant in recovering P from PL and the biochars. The solution was able to recover 73.3% of TP from raw PL and 17.0–57.8% of TP from the biochars. Furthermore, the proportion of Bray-1 extractable P in biochars decreased gradually as the pyrolysis temperature was elevated in the range of 300–600°C (Figure 3). Compared with the Bray-1 extractant, the more corrosive Mehlich-3 solution (0.2 M CH₃COOH + 0.013 M HNO₃ + 0.015 M NH₄F + 0.001 M EDTA + 0.25 M NH₄NO₃) extracted 89.9% of TP from PL and 35.0–80.4% of TP from the biochars, among which C350 demonstrated the highest proportion (80.4%) of Mehlich-3 P while C550 and C600 possessed the drastically lower (35–50%) fractions (Figure 3). In contrast, the strongly acidic extractant 1 M HCl recovered 67.6% of TP from PL yet much higher proportions (87.0–99.8%) from the biochars. All IP but little OP in soils and soil amendments was assumed extractable by 1 M HCl. The high recovery of P by HCl extraction (Figure 3) implicated that inorganic phosphate



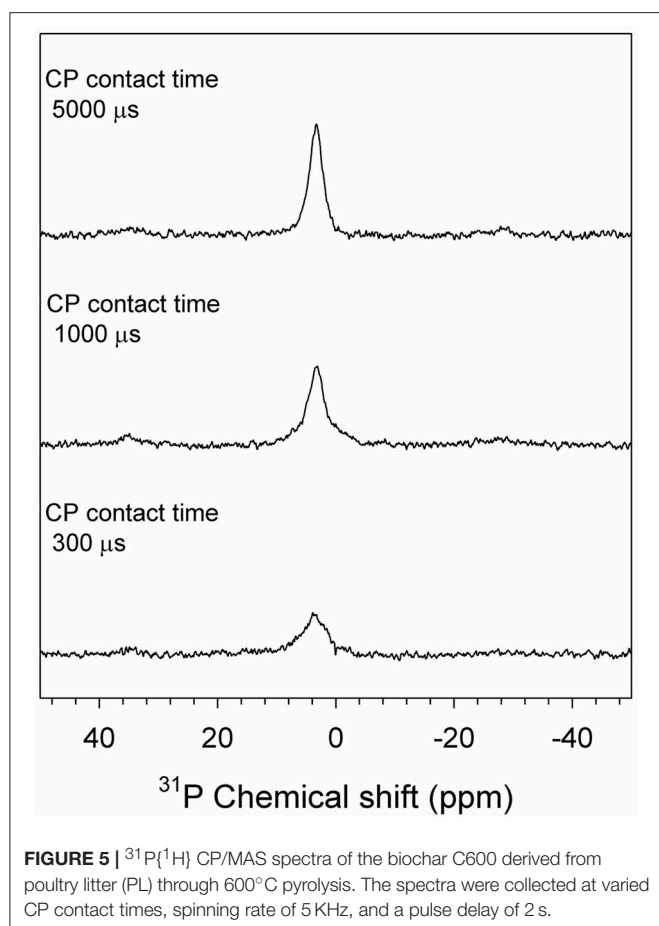
was the predominant P form in biochars. Overall, water, Bray-1, and 1 M HCl extracted significantly different proportions of P from PL and the biochars especially those generated at $\leq 450^\circ\text{C}$, but the alkaline Olsen solution and the corrosive Mehlich-3 agent failed in this function (Figure 3).

Spectroscopic Evidence of P Species in PL-Derived Biochar

The ^{31}P SP/MAS NMR spectra of the PL-derived biochars are illustrated in Figure 4. A broad peak at the chemical shift $\delta_{\text{P}-31} = 2.7$ ppm with full width at half maximum (FWHM) of 6.8 ppm was observed for C300. The chemical shift is fully consistent with that of hydroxyapatite ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$), but the FWHM is much broader than well-crystalline hydroxyapatite. This peak



was therefore assigned to poorly crystalline hydroxyapatite (Jaeger et al., 2006; He et al., 2007; Vyalikh et al., 2017). The same peak was also observed for C350 and C400. As the pyrolysis temperature was elevated to 450°C an additional shoulder at $\delta_{\text{P}-31} = -0.3$ ppm was observed for the biochar product, suggesting some poorly crystalline hydroxyapatite was transformed to a new species. This shoulder became more and more pronounced in biochars produced from higher temperature pyrolysis. Meanwhile, the major peak at $\delta_{\text{P}-31} = 2.7$ turned to be narrower with increasing the pyrolysis temperature, indicating facilitated crystallization of hydroxyapatite under higher temperature heating. At 600°C , two well-separated NMR peaks at $\delta_{\text{P}-31} = 2.7$ and -0.3 ppm were identified (Figure 4). Assignment of the peak at $\delta_{\text{P}-31} = 2.7$ ppm to hydroxyapatite is further supported by the CP kinetics of C600 shown in the $^{31}\text{P}\{^1\text{H}\}$ CP/MAS NMR spectra (Figure 5), since only hydroxyapatite possesses such phosphate/proton environments that would yield stronger signal at a longer CP contact time (i.e., 5,000 μs) than at a shorter CP contact time (e.g., 300 and 1,000 μs). The assignment of the shoulder peak at $\delta_{\text{P}-31} = -0.3$ ppm was not straightforward. The compound from which the peak stemmed was most likely a dehydrated product of hydroxyapatite after heating. In the $^{31}\text{P}\{^1\text{H}\}$ CP/MAS spectra of C600, the peak at $\delta_{\text{P}-31} = -0.3$ ppm shows no CP signals (Figure 5), implicating there are no protons associated with the P species. Though the peak occurred at a chemical shift rather close to that of β -tricalcium phosphate ($\text{Ca}_3(\text{PO}_4)_2$) (Sakka



et al., 2013), formation of tetracalcium phosphate ($\text{Ca}_4(\text{PO}_4)_2\text{O}$) and β -tricalcium phosphate ($\text{Ca}_3(\text{PO}_4)_2$) from dehydrating hydroxyapatite required a much higher temperature (e.g., $>1000^\circ\text{C}$) environment (Greenwood, 2014). At 600°C , however, hydroxyapatite is ready to be dehydrated to form oxyapatite: $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2 \rightarrow \text{Ca}_{10}(\text{PO}_4)_6\text{O} + \text{H}_2\text{O}$ (Greenwood, 2014). Therefore, the $\delta_{\text{P}-31} = -0.3$ ppm peak was assigned to oxyapatite (Figure 4).

DISCUSSION

Pyrolysis of PL enriched the inherent non-volatile elements including P in biochar products. The enrichment became more prominent at higher pyrolysis temperatures in response to the decreasing biochar yield (Song and Guo, 2012). The enrichment factor of P (the ratio of TP content between biochar and PL) for C300 was computed at 1.66. For C450 and C600, the factor increased to 1.94 and 2.23, respectively (Table 1). In addition to enrichment, speciation transformation of P was also evident. In PL, both inorganic P species (e.g., dicalcium phosphate (CaHPO_4), amorphous tricalcium phosphate ($\text{Ca}_3(\text{PO}_4)_2$), and octacalcium phosphate ($\text{Ca}_8\text{H}_2(\text{PO}_4)_6 \cdot 5\text{H}_2\text{O}$)) and organic P species (e.g., phytates, phospholipids, and nucleic acids) are significantly present (Hunger et al., 2004; Turner and Leytem,

2004; Toor et al., 2005; He et al., 2008; Li et al., 2014). Most of the OP in PL was converted to inorganic forms during pyrolysis, as indicated by the drastic decreases in the proportion of OP yet abrupt increases in the proportion of IP in the derived biochars relative to raw PL (Table 1). Transformation of OP to metal/mineral-associated inorganic species was widely observed when converting sewage sludge, manures, and other solid biowastes to biochar through pyrolysis (Huang et al., 2017). Decomposition of OP was promoted by increasing the pyrolysis temperature. At 450°C , nearly all OP in PL was converted to inorganic P species, with the product C450 showing an OP proportion close to 0% (Table 1). The null presence of OP in biochars generated from $\geq 450^\circ\text{C}$ pyrolysis was validated by the sequential extraction analyses, in which OP was barely recovered from these biochars by the extractants water, NaHCO_3 , and NaOH (Table 2; Figure 2). In Table 1, the unexpected increasing OP proportion for biochars produced at higher pyrolysis temperatures (C500, C550, and C600) was calculated based on the difference between TP and IP. As the pyrolysis temperature was elevated to 450°C and above, however, IP in the biochar products became more recalcitrant and less extractable by 1 M HCl. This is supported by the sequential extraction results, showing increased proportions of residual P in biochars generated from $\geq 450^\circ\text{C}$ pyrolysis (Figure 1). The “nominal” OP in C500, C550, and C600 (Table 1) was actually residual P (unextractable by 1 M HCl).

Indeed, transformation of OP to IP occurred during pyrolysis of PL to biochar. The transformation was initially to inorganic polyphosphates (i.e., condensed P forms including pyrophosphates, polyphosphates, and metaphosphates) and further to inorganic orthophosphates at higher pyrolysis temperature. Using solution ^{31}P NMR spectroscopic techniques, polyphosphates were detected in raw PL (Turner and Leytem, 2004; Dou et al., 2009), sewage sludge (Qian and Jiang, 2014; Huang and Tang, 2015), and other solid biowastes (Huang et al., 2017). Relative to raw PL, biochars generated at low pyrolysis temperature (e.g., C300) showed notably reduced proportion of extractable OP and correspondingly increased proportion of polyphosphate-P (Figure 2), suggesting transformation of OP to initially inorganic polyphosphates during pyrolysis. Elevating the pyrolysis temperature resulted in biochar products possessing decreased proportions of extractable polyphosphate-P yet increased proportions of orthophosphate-P (Figure 2), implicating further conversion of polyphosphates to orthophosphates. Raw PL is abundant in ash minerals, with the molar Ca/P ratio greater than 2.0 (Song and Guo, 2012). Seemingly, high temperature and sufficient metal ion supply facilitates transformation of polyphosphates to orthophosphates. Different forms of P vary in water solubility, mobility, and phytoavailability. Converting PL to biochar would substantially alter the environmental fate and transport of P following land application.

Conversion of PL to biochar greatly reduced the water-soluble P fraction (Figure 1), which is the most mobile and plant available P portion in PL. Runoff P losses are largely controlled by and directly correlated with the water-soluble P

content of land-applied solid wastes (Shreve et al., 1995; Hart et al., 2004; White et al., 2010). Evidently, soil amendment with biochar instead of raw PL would minimize the highly-concerned P runoff risks. The sequentially-used extractants water, NaHCO_3 , NaOH , and HCl were designed to recover readily labile P, generally labile P (adsorbed on crystalline mineral surfaces), moderately labile P (associated with carbonates and Fe/Al oxides or in organic particulates), and low labile P (bound in Ca-minerals), respectively, in soils and solid residues (Hedley et al., 1982; Dou et al., 2000; He et al., 2006). Likely, the readily labile, water-soluble P in PL was transformed mainly to low labile and residual P (e.g., calcium phosphate minerals such as hydroxyapatite and oxyapatite) and marginally to generally/moderately labile P (e.g., amorphous $\text{Ca}_3(\text{PO}_4)_2$ surface precipitate on calcium carbonate and phosphate surface complexes on Fe/Al oxides) (**Figure 1**). Phosphate surface precipitates and Fe/Al-oxide complexes were identified in PL using solid state MAS and CP-MAS ^{31}P NMR techniques (Hunger et al., 2004). Transformation of these two phases of phosphate to Ca-bound phosphates (e.g., hydroxyapatite) and further to residual P (e.g., oxyapatite) occurred possibly at higher temperature in the presence of available calcium (**Figure 1**). Overall, the labile portion of P (sequentially extractable by water, NaHCO_3 , and NaOH ; readily to moderately labile; and immediately to medium-term plant available) was significantly reduced during converting PL to biochar; and the reduction became greater as the pyrolysis temperature was elevated in the range of 300–600°C. Similar P transformation trends were observed for pyrolysis of sewage sludge to biochar at different temperatures ranging from 400°C to 800°C (Qian and Jiang, 2014).

The labile portion of P in PL and the biochars was dominated by orthophosphates, with polyphosphates and organic P as minor forms (**Table 2**; **Figure 2**). For PL, all the three forms of P (P_r , P_x , and P_o) were present in the water-soluble, NaHCO_3 -extractable, and NaOH -extractable fractions. Organic P was the principal form of P in the NaOH -extractable fraction, suggesting significant existence of P-containing particulate organic matter in PL (**Table 2**). Due to the thermal decomposition of OP to IP, P_o was detected only in the water-soluble and NaOH -extractable fractions from the biochars generated at lower pyrolysis temperature ($\leq 450^\circ\text{C}$). Polyphosphates existed predominantly in the water-soluble P pool of biochars and fully disappeared in products from $\geq 550^\circ\text{C}$ pyrolysis. Using coupled sequential extraction and solution ^{31}P NMR techniques, Qian and Jiang (2014) detected polyphosphates in sewage sludge and low pyrolysis temperature (400–600°C) biochar products but not in high temperature (800°C) biochars.

The bioavailability of P in soil is commonly estimated by batch extraction using a specific chemical extractant (Pierzynski, 2000). For example, the Olsen solution (0.5 M NaHCO_3 , pH 8.5) was introduced to extract phytoavailable P from neutral, alkaline, and calcareous soils by enhancing the dissolution of Ca-phosphates. The Bray-1 extractant was designed to recover water-soluble and adsorbed forms of P in pH < 7.5 soils. The Mehlich-3 extractant were developed to remove adsorbed and Fe/Al oxides-complexed phosphates and other elements from

acidic and neutral soils (Elrashidi, 2001). Lucero et al. (1998) found that both Bray-1 and Mehlich-3 extracts were effective to evaluate excess P in PL-fertilized soils. In the present study, the raw PL had a pH value of 7.1 and the biochars of 9.5–11.5, increasing with the pyrolysis temperature (Song and Guo, 2012). It is notable that all the chemical extractants recovered more P from PL and the biochars than water, and their effectiveness for extracting P from biochars followed the order: Olsen < Bray-1 < Mehlich-3 < HCl (**Figure 3**). The Mehlich-3 solution was more efficient than HCl in extracting P from raw PL, probably due to the abundance of OP (**Table 1**) that would be precipitated in 1 M HCl. The Bray-1 extractable P demonstrated a variation trend highly consistent with that of the labile P fraction (extractable by water, NaHCO_3 , and NaOH) among the PL and the derived-biochars (**Figure 1**), suggesting the solution is an appropriate extractant for evaluating the labile P content of PL-derived biochars. A large portion of HCl-extractable, Ca-bound P is low labile yet long-term available to plants (Qian and Jiang, 2014) and therefore, Mehlich-3 was considered a proper solution for recovering overall bioavailable P from biochar. The Mehlich-3 extraction was able to reflect the changes in residual P fraction and general P recalcitrance of biochars produced at different pyrolysis temperatures (**Figure 1**). It is noteworthy that chemical extraction cannot reflect the interactions among soil, biochar, and plants that influence the P bioavailability of field applied biochar and therefore, the lability and bioavailability of P in PL-derived biochars as evaluated by the present batch and sequential extraction methods need to be validated in soil-biochar-plant systems.

To date most phosphate compounds and organic P species in soil, solid biowastes, and biochars remain unknown due to the matrix complexity, P chemical diversity, and technology restriction. In addition to solid-state ^{31}P NMR techniques, other methods such as liquid ^{31}P NMR, X-ray absorption near edge structure (XANES) spectroscopy, Fourier transform infrared spectroscopy (FTIR), and X-ray diffraction (XRD) spectroscopy have been used to characterize P compounds in environmental samples (Toor et al., 2005; He et al., 2007; Dou et al., 2009; Li et al., 2014; Qian and Jiang, 2014; Huang and Tang, 2015; Huang et al., 2017). Identified P chemicals are limited to phytate, dicalcium phosphate, tricalcium phosphate, struvite ($\text{NH}_4\text{MgPO}_4 \cdot 6\text{H}_2\text{O}$), hydroxyapatite, and broadly pyrophosphates (Hunger et al., 2008; Qian and Jiang, 2014; Huang and Tang, 2015; Huang et al., 2017). Hydroxyapatite does not exist in raw PL (Hunger et al., 2004). Instead, octacalcium phosphate, a precursor of hydroxyapatite, is present in PL (Hunger et al., 2008). The present ^{31}P NMR analysis indicates that hydroxyapatite was formed during pyrolysis of PL to biochar. Improved crystallization of hydroxyapatite occurred as the pyrolysis temperature was elevated. At 450°C and above, a portion of hydroxyapatite was dehydrated and transformed to possibly oxyapatite (**Figure 4**). Both hydroxyapatite and oxyapatite are water insoluble and belongs to the low labile P pool in sequential extraction. The speciation transformation supports the chemical extraction results that the solubility, lability, and bioavailability of P generally decreased in biochars produced from higher temperature pyrolysis.

CONCLUSIONS

Nearly all P in the feedstock was recovered during pyrolytic conversion of PL to biochar. Relative to raw PL, the derived biochar was substantially P-enriched and might demonstrate a total P content doubling that of the original feedstock. During pyrolysis, OP in PL was decomposed to IP, initially to polyphosphates and subsequently to orthophosphates at higher temperature. Orthophosphate-P was the predominant form of P in PL-derived biochars. In the products manufactured at $\geq 450^{\circ}\text{C}$ pyrolysis temperature, OP fully disappeared and polyphosphate-P became barely detectable. Overall, pyrolysis transformed the P in raw PL to much less labile forms (e.g., hydroxyapatite and oxyapatite) in biochar. The lability of P in biochars decreased as the pyrolysis temperature increased in the range of $300\text{--}600^{\circ}\text{C}$. Among the biochars generated at different temperatures, C450 possessed the smallest pool of residual P, suggesting 450°C is the optimal temperature for converting PL to biochar with maximum P bioavailability. The Bray-1 extractant was an appropriate solution for recovering the immediately to medium-term available P in biochar, while the Mehlich-3 solution was suitable for evaluating the overall P bioavailability of soil-applied biochar. The bioavailability of P in PL-derived biochars

needs to be further assessed in soil-biochar-plant systems. In addition to P lability and bioavailability, the remarkable N losses and potential stable C recovery should also be considered in selecting an optimal pyrolysis temperature for converting PL to biochar.

AUTHOR CONTRIBUTIONS

MG designed the experiments, synthesized the data, and drafted the paper. WL developed the research topic, participated in sample analysis, interpreted the spectroscopic results, and helped prepare the manuscript. XF carried out the spectroscopic analysis of the biochar samples, processed the spectral information, and reviewed the manuscript. WS conducted chemical characterization of the samples and reviewed the draft.

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Recovery of Ammonia in Raw and Co-digested Swine Manure Using Gas-Permeable Membrane Technology

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Anaerobic digestion of agro-industrial and livestock waste generates considerable digestate volumes that are important sources of nitrogen (N). However, on some occasions, the high concentrations of N present in the digestates may represent an obstacle to its use locally as fertilizer, since it can cause an environmental impact (European Community, 2000). This study analyzes the efficiency of gas-permeable membranes (GPM) in the recovery of the ammoniacal nitrogen (NH_4^+) present in the swine manure (SM, Control) and three digestates generated from the anaerobic co-digestion of mixtures of SM, fruit and vegetable sludge (FVS) from the vegetable industry (peppers and artichokes), and by-products of the tomato processing industry (TW) (skins and seeds), which were mixed at three different proportions [TW + FVS]: [SM]. Their NH_4^+ content was 2,240 mg L⁻¹ for the SM and 4,670–5,370 for the digestate mixtures. Throughout the duration of the Recovery experiment (96 h), the percentages of NH_4^+ removal and recovery achieved were consistent among treatments, approximately 78 and 96%, respectively. The recovery of NH_4^+ for the digestate mixtures seems to be dependent on the treatment time, since the potential of N recovery was higher when the treatment time increased. However, for the control experiment (SM), with lower N concentration, the maximum N recovery was obtained at the experimental time established, without increasing the treatment time.

Keywords: ammonium, anaerobic digestion, nitrogen recovery, semipermeable membrane, sludge, vegetable sludge

INTRODUCTION

Livestock production is a sector that contributes significantly to global greenhouse gas (GHG) emissions, with annual values estimated at 7.1 Gton of the CO₂ equivalent, accounting for 14.5% of all anthropogenic emissions (Food and Agriculture Organization of the United Nations (FAO), 2013). In the specific case of pig production, this activity has the potential to emit about 4.87 kg of CO₂ equivalent per kg of carcass produced (Philippe and Nicks, 2015). The accumulation of swine manures (SM) is responsible for the emission of various gases into the atmosphere. Among them,

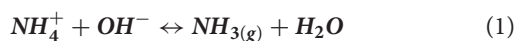
ammonia (NH_3) stands out for its high release ($86.8 \mu\text{g NH}_3 \text{ s}^{-1} \text{ m}^{-2}$) and high environmental impact potential (Dai et al., 2015).

Anaerobic co-digestion (ACoD) has been used as a technology to minimize the negative effects of the accumulation of SM and other organic residues on rural areas, generating biogas (renewable energy) and digestate, which can be used as agricultural fertilizer (Mata-Alvarez et al., 2014; Akhiar et al., 2017). The ACoD has been used as a way to improve the efficiency of biogas production systems that use a single manure substrate. Molinuevo-Salces et al. (2013) indicated that ACoD of SM with vegetable processing residues increases methane production by 219%, while De Vries et al. (2012) the ACoD of SM with fruits and vegetable wastes (FVW) (skin, seeds and slurry from fruit washing) can increase bioenergy production by 568%. Furthermore, Muscolo et al. (2017) confirmed that the agronomic properties improved (organic matter, nutrient balance) in the soils where digestates were applied coming from the ACoD of the mixture of SM, olive waste and citrus pulp compared with chemical fertilizers.

However, high concentrations of nitrogen (N) in the ACoD effluents (digestate) can complicate its management and cause negative impacts to the environment due to its application in local agricultural areas (European Community, 2000). Some technologies have been used to reduce the excess N content in livestock wastewater, promoting its recovery in the mineral form (NH_4^+) and allowing its reuse as agricultural fertilizer in areas far from the livestock concentration with greater needs for the N nutrient. Dube et al. (2016) and Garcia-Gonzalez et al. (2016) used a new process utilizing gas-permeable membranes (GPM) in low pressure systems to capture the N present in the digestate from the anaerobic mono-digestion of SM and store it in a concentrated form in an acid solution of 0.5 M H_2SO_4 .

The new process using GPM consists of submerging the gas membranes in the organic liquid residue causing the $\text{NH}_{3(g)}$ to penetrate into its pores and is captured by an acidic solution circulating in the membrane interior. The combination of the $\text{NH}_{3(g)}$ with H^+ ions from H_2SO_4 inside the membrane generates a NH_4^+ concentrated solution with potential to be used as a nitrogen fertilizer (**Figure 1**; Garcia-Gonzalez and Vanotti, 2015; García-González et al., 2015; Vanotti and Szogi, 2015).

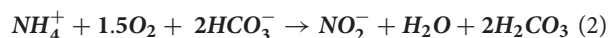
To carry out this process, it is necessary to increase the pH of the wastewater to be treated to favor the transformation of NH_4^+ into $\text{NH}_{3(g)}$ (Equation 1) and enable its passage through the gas-permeable hydrophobic membrane where an acidic solution circulates (**Figure 1**). This solution dissolves the ammonia in the form of ammonium, which is the predominant species at pH values below 7.



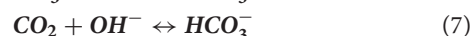
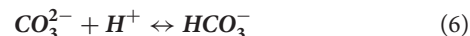
Previous studies have shown that the efficiency of the removal and recovery of NH_4^+ depends on the design and type of system used. Thus, different authors have reported better results using hydrophobic GPM submerged in the effluent, since this system can conduct the gaseous species toward the acidic stripping

solution placed on the other side of the membrane (Lauterböck et al., 2013; Boehler et al., 2015; Vanotti and Szogi, 2015).

In addition, the dynamics of the chemical and biochemical reactions that may occur in the wastewater environment have an impact on the efficiency of the process. The recovery of NH_4^+ is reduced when there is microbial activity capable of (1) oxidizing the ammonium to nitrites (Equation 2) and nitrates (Equation 3), and (2) reducing the carbonate alkalinity (Equation 2). Moreover, the ammonium recovery through the gas-permeable membrane (GPM) is affected by the pH of the environment, and the environments could be affected by the presence and concentration of the different forms of inorganic carbon ($\text{CO}_2/\text{HCO}_3^-/\text{CO}_3^{2-}$); among which, the bicarbonate ion is the dominant species in livestock wastewater and the transformations of both the CO_2 (Equation 4) and the CO_3^{2-} (Equation 5) are shifted to the right.

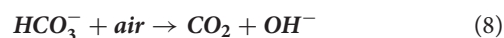


Additionally, a reaction (Equation 6) between the CO_3^{2-} present and the H^+ of the balance of Equation (4) may occur, and also a reaction (Equation 7) between the CO_2 present and the OH^- of the balance (Equation 5).



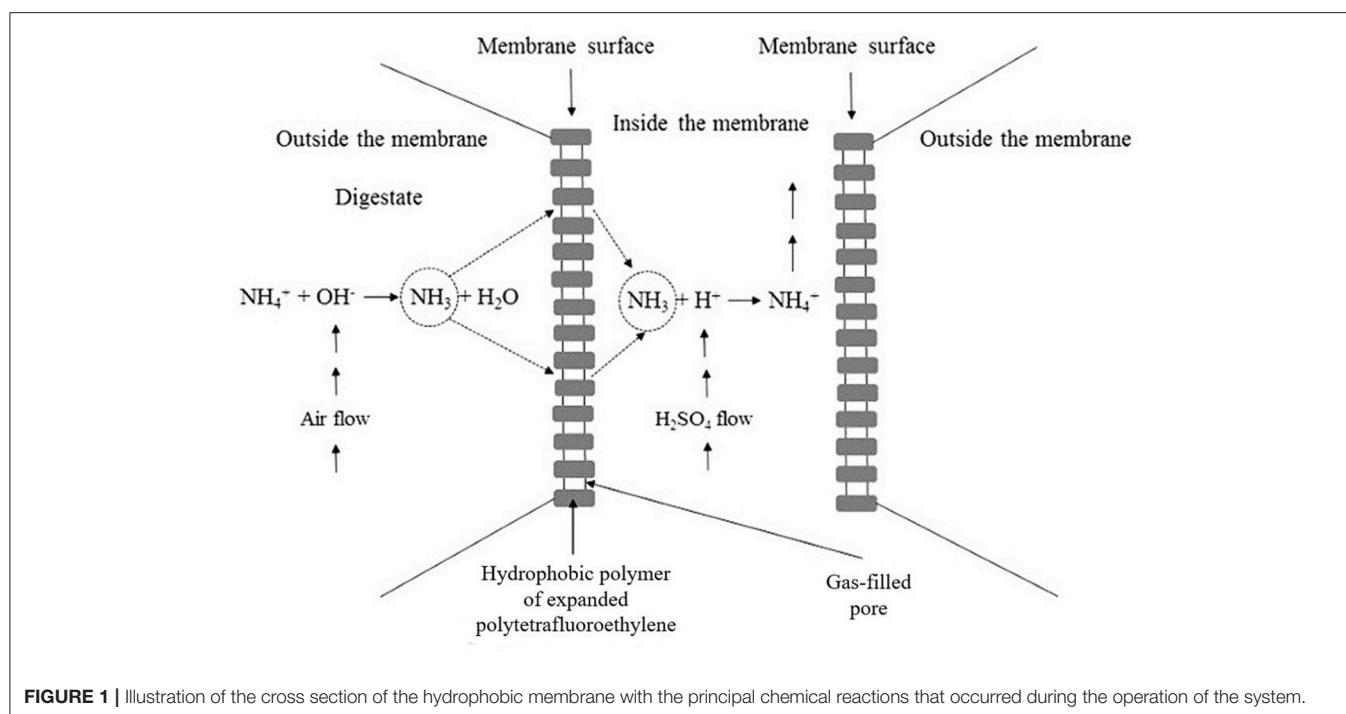
Taking into account all of the above, the presence of carbonates favors the basic environment (according to Equation 5); whilst nitrification processes reduce the concentration of ammonium and the pH (according to Equations 2–4, Magrí et al., 2012).

García-González et al. (2015) and Vanotti and Szogi (2015) have proposed the use of nitrification inhibitors and the application of low-rate aeration to increase the pH without the need of adding alkaline chemical agents, in this way reducing the economic and environmental costs involved in the treatments of effluents rich in ammonium. During low-rate aeration, the CO_2 that is in equilibrium in the solution is stripped (Equation 7) and the reaction (Equation 8) is shifted to the formation of OH^- , increasing the pH and reducing the bicarbonate in the environment. All these factors cause an increase in the formation of gaseous $\text{NH}_{3(g)}$ (Equation 1), which accelerates its uptake by the membrane (**Figure 1**).



According to Equation (9), the recovery of $\text{NH}_{3(g)}$ through the membrane causes an increase of acidity in the environment being treated and a decrease of the bicarbonate (Equations 1, 8) and, therefore, it is necessary that the operating conditions allow to continually raise the pH levels in order to maintain recovery efficiency (García-Gonzalez and Vanotti, 2015).





The aeration effect is double, on the one hand, it promotes the increase in pH values and, on the other hand, promotes the increase in the concentration of the free ammonium which favors the passage of $\text{NH}_3(\text{g})$ through the membrane (Vanotti et al., 2017). In the studies conducted by Dube et al. (2016), the GPM system with low-rate aeration promoted removals of 97–99% of the initial NH_4^+ contents in the digestate in 5 days of operation, obtaining an accumulation of 11,900 mg of $\text{NH}_4^+\text{-N L}^{-1}$ in the acid solution. In the studies conducted by Daguerre-Martini et al. (2018), the GPM system with low-rate aeration removed 65–85% of the initial NH_4^+ contents in swine manure in 4 days of operation, obtaining concentration of $\text{NH}_4^+\text{-N}$ in the acid tank of 37,400 mg L^{-1} .

Successful N recovery using GPM has already been performed on different organic wastes such as poultry litter (Rothrock et al., 2010, 2013), swine manure (Garcia-Gonzalez and Vanotti, 2015; García-González et al., 2015) and digestate from mono-digestion of swine manure (Dube et al., 2016; Garcia-Gonzalez et al., 2016; Vanotti et al., 2017) with NH_4^+ recovery above 90% of the total content of this form of the nutrient in the residues. However, recovery of N in the digestate from systems operating in ACoD has not yet been performed.

In this study, a low pressure GPM system operated under low-rate aeration was evaluated to recover the ammonia in the digestates generated by an AcoD pilot system, aiming for the production of N fertilizer concentrates. SM, FVS, and tomato waste (TW) mixtures were used as co-substrates in three different mixing ratios for AcoD, which gave rise to three different digestates to evaluate N recovery. The recovery of N in raw SM was also performed and served as a control treatment.

MATERIALS AND METHODS

Substrates for Anaerobic Co-digestion (AcoD)

TW mainly constituted by skins and seeds, was collected from a tomato processing industry to obtain tomato cans. FVS was obtained from three fruit and vegetable processing plants in Murcia (Spain): a canning company and two companies producing frozen artichoke and pepper. SM was collected from a receiving pit in a pig farm located in Alicante (Spain). Immediately after sampling, SM and FVS were preserved at 4°C, while TW was preserved at −20°C. The main characteristics of the feedstocks are presented in Table 1.

AcoD Process

A cylindrical anaerobic reactor with a working volume of 300 L was used. The reactor was kept under agitation (8–10 rpm) and constant temperature ($35 \pm 1^\circ\text{C}$) and was operated in a semi-continuous mode three times a week with the manual addition of 10 kg of substrate mixture (fresh weight), with 6% TS, through the reactor feed intake (30 kg wk^{-1} or 4.3 kg d^{-1}), followed by the removal of 10 kg of digestate, through the sample opening of the reactor, to maintain a constant working weight of 245 kg (fresh weight). Our contribution is enclosed in a complex experiment (Journal of Cleaner Production, 156: 757–765, 2017) that included in a 4 month period of optimization of biogas production using these wastes. We sampled the digestate at the end of stage III to obtain a pseudo-steady-state inside the reactor (Figure 2).

TW and FVS were initially homogenized and then mixed in different proportions to SM. Three mixing percentage ratios [TW + FVS]:[SM] were established: 7/3; 5/5; and 4/6. Each mixture

TABLE 1 | Chemical characterization of feedstocks (TW, SM, and FVS)^a.

	TW	SM	FVS
pH	7.6 (0.1) ^b	7.8 (0.4)	6.4 (0.4)
EC (dS m ⁻¹)	14.9 (0.1)	21.2 (0.4)	3.4 (0.2)
TS (g L ⁻¹)	23.3 (1.2)	10.5 (1.0)	0.8 (0.0)
VS (g L ⁻¹)	10.9 (0.9)	6.7 (0.6)	0.05 (0.0)

^a TW, tomato waste; SM, swine manure; FVS, fruit and vegetable sludge; EC, electrical conductivity (EC); TS, total solids; VS, volatile solids.

^b Each value represents the mean of 2 replicates (\pm standard deviation).

gave rise to a specific digestate as described below: Digestate 1: 7/3; Digestate 2: 5/5; and Digestate 3: 4/6. The reactor was fed with each mixture for 15 days. After this period, the digestate for each mixture was collected, chemically analyzed and immediately taken to the NH₃ recovery system.

Ammonia Recovery System

The elements that constituted the NH₃ recovery system follow the diagram of Dube et al. (2016) and are identified in **Figure 2** (showing four identical units). Specifically, it consisted of the following parts: (A) 2 L wastewater reaction vessels with an effective volume of 1.5 L SM or digestate; (B) glass vessel (Erlenmeyer) containing 200 mL of 1N H₂SO₄ solution; (C) peristaltic pump (Watson-Marlow 120S) used to continuously recirculate the H₂SO₄ through the membrane lumen; (D) aeration pump (Sera Precision Air 110 Plus: 1.8 L min⁻¹); (E) airflow meter; (F) tubular GPM made of expanded polytetrafluoroethylene (ePTFE) that were microporous and hydrophobic (Phillips Scientific Inc., Rock Hill, SC), and (G) pH meter for pH monitoring during the experiment. Characteristics of the membrane are provided by Dube et al. (2016).

The experiment was conducted under laboratory conditions and under controlled temperature (25 \pm 1°C) with a total duration of 4 days (93 h). During this period, SM and digestate samples were taken daily for NH₄⁺ and pH determinations both at the beginning (9 a.m.) and end (8 p.m.) of the day.

The process of ammonia recovery consisted of submerging the tubular membrane in the wastewater and circulating the acidic solution through the inside using the peristaltic pumps. The hydrophobic material of the membrane prevents both the acid solution from flowing out of it and the wastewater to penetrate into the membrane lumen. The manure was aerated continuously in order to raise its pH and promote NH₄⁺ transformation into gaseous NH₃. The NH_{3(g)} formed penetrates in the membrane gas pores and is immediately captured by the H₂SO₄ solution circulating inside. Finally, a concentrated NH₄⁺ acid solution was formed which returned to the acid reservoirs (**Figure 1**).

The peristaltic pumps circulated the acid solution constantly at a rate of 5.8 L day⁻¹ throughout the experimental period. The air flow was constantly applied at a rate of 0.24 L⁻¹ air L⁻¹ residue min⁻¹. The membranes had a length of 60 cm with an outer diameter of 10.25 mm and a wall thickness of 0.75 mm. The pores open to gas passage had a mean diameter of 2.5 μ m and had a bubble point of 210 kPa. At the beginning of the experiment, 10 mg L⁻¹ of the n-allylthiourea 98% (nitrification inhibitor) was

added to each vessel to avoid transformation of NH₄⁺ to NO₃⁻ according to Equations (2) and (3) (Vanotti and Szogi, 2015).

The buffer capacity of the liquid was measured according to the TAC method of McGhee (1968) by titration with 0.1N H₂SO₄ to pH 5.0 and expressed in terms of mg CaCO₃ L⁻¹. The pH of digestate was measured directly with a Consort C860 multiparameter analyzer. Total Solids (TS), Volatile Solids (VS) and ammonium were determined at the beginning and end of the experiment according to American Public Health Association/American (1998) 2540B-2540E-4500B and C methods, respectively.

RESULTS AND DISCUSSION

The chemical characterizations of SM and digestates before and after treatment are presented in **Table 2**. For both the SM and the digestates, an increase in pH and a reduction of values of TS, VS, NH₄⁺ and buffer capacity were observed. The increase of pH values and the reduction of carbonate buffer capacity and NH₄⁺ concentration were expected and indicated a correct functioning of the system.

In the specific case of the reduction of the buffer capacity TAC in the residues, this characteristic is the result of the application of the aeration that consumes HCO₃⁻ in order to achieve the generation of OH⁻ and subsequent increase of the pH of the residues according to Equations (7) and (8). As a consequence of the increase in pH values, an active NH₄⁺ transformation to NH_{3(g)} occurs in the residue according to Equation (9). The NH_{3(g)} formed can then be recovered by the membrane submerged in the residue. A lower loss of the NH_{3(g)} to the environment contributes to a higher NH_{3(g)} recovery efficiency of the system.

In our case, the recovery efficiencies were high (about 96%) in the four treatments and the unaccounted N fraction was generally low (around 4%). This means that almost all the NH₄⁺ that was removed from the manure and digestates with the GPM has been recovered in the acidic solution (**Table 3**). The efficiency of this system is affected by the concentration of NH₄⁺ and the carbonate alkalinity in the wastewater. Thus, raising such parameters means a higher removal and efficiency in the recovery of NH₄⁺ (Garcia-Gonzalez et al., 2009; Garcia-Gonzalez and Vanotti, 2015; Daguerre-Martini et al., 2018), which is consistent with the results obtained in this study.

Effect of Aeration on pH Value

The dynamics of the pH values of SM and of the different digestates during the experimental period are presented in **Figure 3**. All the residues presented an increase of the pH with time. The initial pH of the SM was 7.83 and after 51 h of aeration reached the maximum pH value (8.93), increasing by 1.10 pH units. After this period and with continued aeration up to the end of the experiment at 93 h, the SM pH at the end of the experiment was 8.8. García-González et al. (2015) and Dube et al. (2016) reported similar results in experiments of recovery of ammonia from swine manure and swine manure derived anaerobic digestate, respectively.

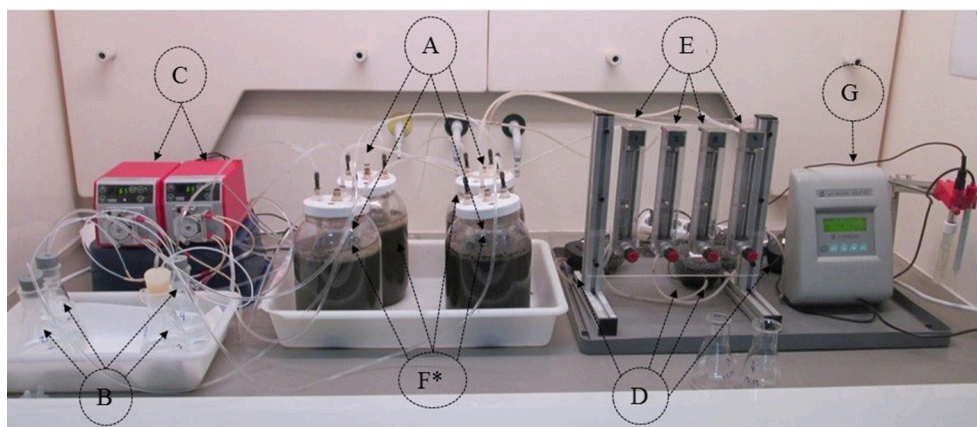


FIGURE 2 | Components of the ammonia recovery system (4 batch units). **(A)** N removal vessels with the wastewater. **(B)** Acid solution tanks. **(C)** Pumps for acid recirculation. **(D)** Air pumps. **(E)** Airflow meters and valves. **(F)** Tubular membranes submerged in the wastewater. **(G)** pH meter. *Duplicate reactors.

TABLE 2 | Chemical characterization of the swine manure (SM) and the digestates at the start and end of the N recovery experiment.

	SM	Digestate 1	Digestate 2	Digestate 3
INFLUENT				
pH	7.8 (0.0) ^a	7.9 (0.0)	7.9 (0.1)	7.8 (0.0)
TS (g L ⁻¹)	10.5 (0.4)	3.9 (0.2)	4.2 (0.3)	4.9 (0.3)
VS (g L ⁻¹)	6.7 (0.1)	2.7 (0.1)	2.9 (0.3)	3.1 (0.1)
NH ₄ ⁺ (mg L ⁻¹)	2,242 (12.7)	4,674 (12.7)	5,034 (12.7)	5,366 (25.5)
Buffer capacity (mg CaCO ₃ L ⁻¹)	10,600 (35.4)	11,513 (8.8)	10,575 (35.4)	11,875 (106.1)
EFFLUENT				
pH	8.8 (0.1)	9.1 (0.1)	9.0 (0.0)	9.2 (0.1)
TS (g L ⁻¹)	9.3 (0.5)	3.8 (0.1)	3.9 (0.4)	3.8 (0.3)
VS (g L ⁻¹)	4.2 (0.6)	2.5 (0.1)	2.6 (0.3)	2.8 (0.1)
NH ₄ ⁺ (mg L ⁻¹)	396 (25.5)	1,027 (127)	1,324 (12.7)	1,233 (38.2)
Buffer capacity (mg CaCO ₃ L ⁻¹)	3,538 (88.4)	2,475 (17.7)	1,388 (17.7)	2,788 (17.7)

^aValues are means and standard deviations of duplicate reactors.

TABLE 3 | Mass balance for the NH₄⁺ recovery from swine manure (SM) and co-digested effluents using gas-permeable membranes^a.

Waste	Initial NH ₄ ⁺	Removed NH ₄ ⁺	Remaining NH ₄ ⁺	NH ₄ ⁺ recovered ^b	Unaccounted NH ₄ ⁺ ^c	NH ₄ ⁺ Removal efficiency ^d	NH ₄ ⁺ Recovery efficiency ^e
	mg NH ₄ ⁺					(%)	
SM	3,363	2,769	594	2,639	130	82.3	95.3
Digestate 1	7,011	5,470	1,541	5,255	215	78.0	96.1
Digestate 2	7,551	5,565	1,986	5,285	280	73.7	95.0
Digestate 3	8,049	6,199	1,850	5,955	244	77.0	96.1

^aData are average of duplicate reactors. The N recovery system contained 0.6 m of tubular membrane with a surface area of 193 cm². NH₄⁺ recovered during a 4-d period from 1.5 L wastewater influent into 0.2 L of N trapping solution (1 N H₂SO₄).

^bMass of NH₄⁺ recovered in the acidic solution.

^cUnaccounted NH₄⁺ mass = Initial NH₄⁺ – remaining NH₄⁺ – recovered NH₄⁺.

^dRemoval efficiency = (removed NH₄⁺/initial NH₄⁺) × 100.

^eRecovery efficiency = (NH₄⁺ recovered/NH₄⁺ removed) × 100.

In the three digestates, the pH increase during the treatment was similar. The digestates presented initial pH values of 7.8–7.9 (Table 2). In digestate 1 and 2, the maximum pH value (9.19 and

9.17) was observed after 51 h of treatment and in digestate 3, the maximum pH value (9.25) was observed at 30 h of treatment (Figure 3). Aeration provided an increase of 1.23, 1.26, and 1.45

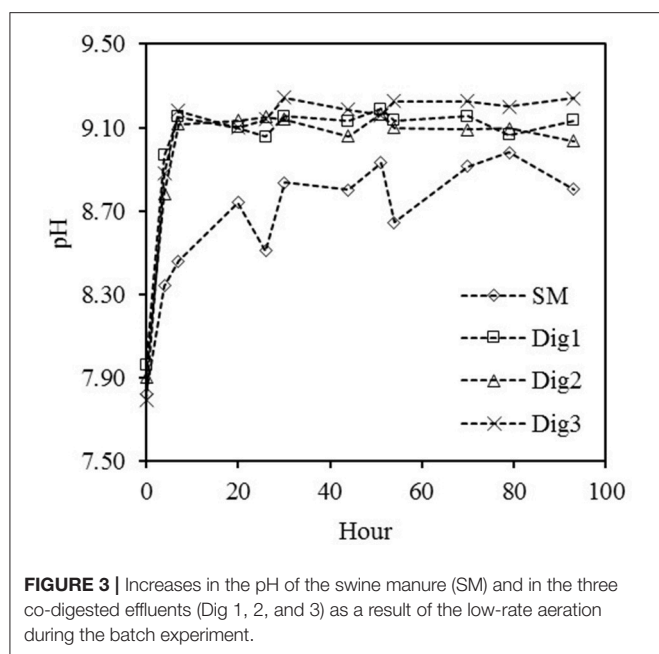


FIGURE 3 | Increases in the pH of the swine manure (SM) and in the three co-digested effluents (Dig 1, 2, and 3) as a result of the low-rate aeration during the batch experiment.

pH units for digestates 1, 2, and 3 respectively. After 93 h of treatment with active N removal, the aeration of the manure still maintained a high pH (9.14, 9.04, and 9.24, respectively). Therefore, the modified wastewater environment promoted NH_3 formation.

In our case, as the experiment evolves, the increase in pH is smoothed in all four tests, as a result of acidification by the NH_3 crossing the membrane and being removed from the wastewater environment; the balance moves toward the formation of H^+ (Dube et al., 2016). The consumption of TAC varied between the SM and the digestate treatments due to the differences in amounts of N removed from the wastewater by the GPM system. In the case of SM, 7062 mg $\text{CaCO}_3 \text{ L}^{-1}$ was consumed, while 9038, 9187, and 9087 mg $\text{CaCO}_3 \text{ L}^{-1}$ were consumed in digestates 1, 2, and 3, respectively.

The use of aeration in the waste with the objective of raising the pH about 1 unit was proposed by Vanotti and Szogi (2015) to accelerate membrane uptake of the N without chemicals. In experiment with swine manure wastewater, García-González et al. (2015) determined that the increase of pH with the aeration approach is equivalent to the addition of 2.14 g NaOH L^{-1} to the wastewater without aeration.

Removal of N From Digestates and NH_4^+ Recovery in the Acidic Solution

Data in Figure 4 shows the decrease of NH_4^+ in SM and the digestates during the experiment and the corresponding concentrations of NH_4^+ being accumulated in the acid solution over time. The initial concentration of NH_4^+ in SM was 2242 mg $\text{NH}_4^+ \text{ L}^{-1}$ and after 4 days of treatment, it decreased to 396 mg $\text{NH}_4^+ \text{ L}^{-1}$. This represents a removal efficiency of NH_4^+ of 82.3% (Table 3). The digestates 1, 2, and 3 initially contained 4674, 5034, and 5366 mg $\text{NH}_4^+ \text{ L}^{-1}$ and after 4 days of treatment, it decreased to 1027, 1324, and 1233 mg $\text{NH}_4^+ \text{ L}^{-1}$ respectively,

with corresponding removal efficiencies of N of 78, 73.7, and 77%.

It is probable that greater N removals would have been possible with a longer treatment time, since substantial amounts of CaCO_3 (buffer capacity) still remained in the four effluents after 4 days of treatment (3,538, 2,475, 1,388, and 2,788 mg $\text{CaCO}_3 \text{ L}^{-1}$ in SM, and digestates 1–3, Table 2), with the potential to be transformed into OH^- and maintain high pH values, according to equation 8.

In the case of NH_4^+ recovery in the SM acid tank, a maximum accumulation of 13,195 mg $\text{NH}_4^+ \text{ L}^{-1}$ was observed (Figure 5). This accumulation represented a recovery efficiency of 95.3% (Table 3). This means that nearly all of the NH_4^+ that was removed from the liquid (2,769 mg) passed through the membrane and was captured by the acidic solution, forming the mineral nitrogen fertilizer. Only a small amount of the NH_4^+ (130 mg) was unaccounted and probably lost in the air. In the case of NH_4^+ recovery in the digestates acid tanks, higher concentration recoveries were obtained (26,275, 26,425, and 29,775 mg $\text{NH}_4^+ \text{ L}^{-1}$, Figure 5), representing recovery efficiencies >95% (Table 3). In quantitative terms, with 4 days of treatment using GPM technology and low-rate aeration, it was possible to form 5,255, 5,285, and 5,955 mg of NH_4^+ as fertilizer salt from co-digested effluents 1, 2, and 3 with NH_4^+ concentrations of about 2.6–3% NH_4^+ . The lower NH_4^+ recovery observed in SM was due to the lower NH_4^+ initial content. Compared with the SM, the digestates were almost double the initial NH_4^+ content and doubled the recovery of NH_4^+ in the acidic solution. The ammonia fluxes (N recovery per unit of membrane area) were: 2.66 mg N per cm^2 of membrane per day for SM (initial $\text{NH}_4^+ = 2,242 \text{ mg L}^{-1}$), and 5.29, 5.32, and 6.00 mg $\text{N cm}^{-2} \text{ d}^{-1}$ for digestates 1, 2, and 3 (initial $\text{NH}_4^+ = 4,674, 5,034, \text{ and } 5,366 \text{ mg L}^{-1}$). With even higher effluent NH_4^+ concentrations (initial $\text{NH}_4^+ = 6,350 \text{ mg L}^{-1}$), Daguerre-Martini et al. (2018) obtained higher ammonia fluxes (8.9 mg $\text{N cm}^{-2} \text{ d}^{-1}$). Therefore, the transport efficiency of the GPM is positively affected by the initial concentration of NH_4^+ in the effluent.

The control of the pH of the acid solution used in $\text{NH}_3(\text{g})$ recovery must be rigorous, maintained at between 1 and 2 units, so as to guarantee the total recovery of $\text{NH}_3(\text{g})$ with an acid trapping system (Lahav et al., 2008). As the acidic solution recovers $\text{NH}_3(\text{g})$, its pH increases substantially, reducing its ability to form NH_4^+ . The recommendation is to add concentrated acid to the acid tank to an end-point pH of 1 with a pH controller when pH in the acidic solution reaches 2.

The recovery of NH_4^+ by the acid solution of the four treatments followed a second order curve (Figure 5). In the case of SM, NH_4^+ recovery showed a higher recovery of N in the first hours of the experiment and decreasing rates afterwards. In the case of the digestate mixtures, the NH_4^+ recovery kinetics indicated that the potential of the N recovery process was higher with an increase in treatment time, whereas in the case of the control experiment (SM) with lower N concentration, the maximum N recovery was observed within the experimental time imposed.

In the NH_4^+ recovery system used in this study, all effluents presented consistent values of removal (average 78%) and

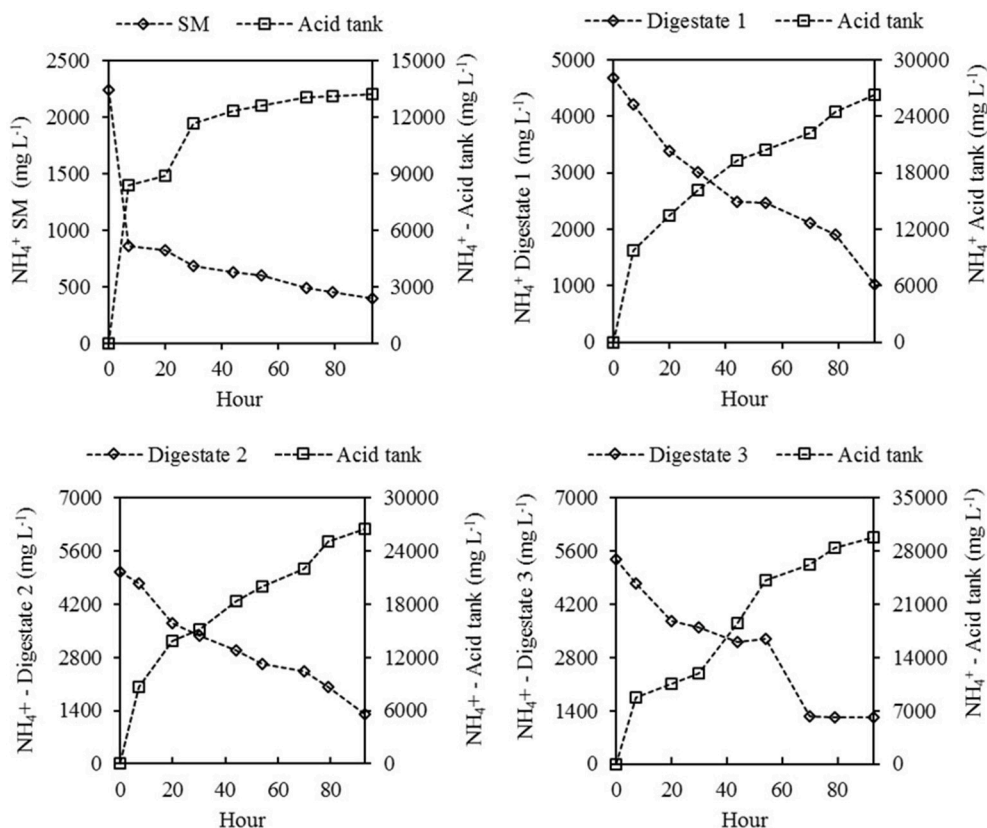


FIGURE 4 | Decrease of NH_4^+ concentration in the swine manure (SM) and the three digestates (Left axis) and corresponding increase of NH_4^+ concentration in the acidic solutions (Right axis) during the N recovery experiment.

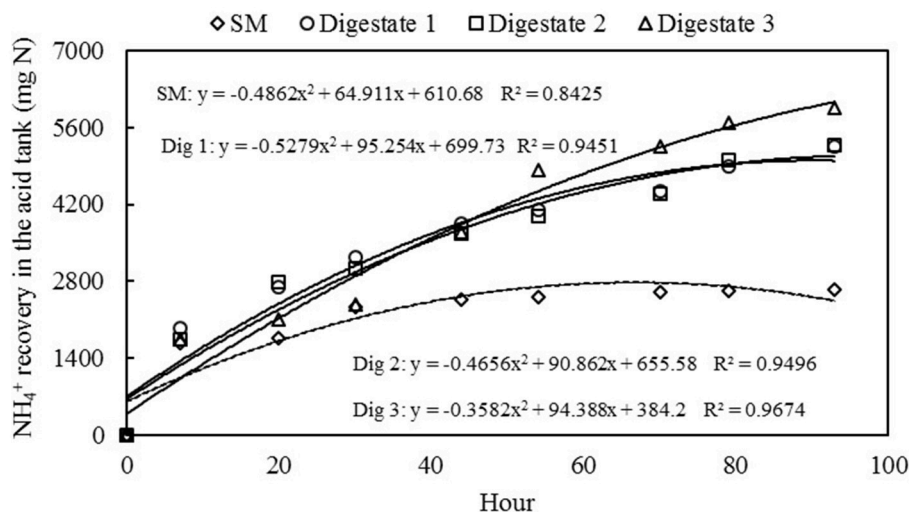


FIGURE 5 | Evolution of NH_4^+ mass recovered in the acidic solution obtained from the swine manure (SM) and three digestates during the experimental period.

recovery (average 96%). These results indicate that it is possible to use GPM with digestates from biogas plants operating in the ACoD system for production of nitrogen fertilizer solutions. This

combination of AcoD and GPM will generate renewable energy and nitrogen fertilizer (NH_4^+) from the same waste feedstock. Furthermore, the reduction of the N content in the digestate

may facilitate its use for other subsequent treatments such as the chemical precipitation of phosphate minerals in the form of newberyite (MgHPO_4), which can also be used as agricultural fertilizer (Vanotti et al., 2017).

CONCLUSIONS

The GPM system with low-rate aeration was efficient to recover the NH_4^+ present in the digestates from biogas plants operating in anaerobic co-digestion systems. The physico-chemical characteristics of the digestates (high concentrations of NH_4^+ and buffer capacity and low percentage of total solids) created a favorable environment for the recovery of NH_4^+ using the technology. The percentage of NH_4^+ removal was consistent among co-digested effluents, about 78%. Also the percentage of NH_4^+ recovery, about 96%. The ammonia fluxes (N recovery per unit of membrane area) were $5.3\text{--}6.0\text{ mg N cm}^{-2}\text{ d}^{-1}$ for digestate effluents containing $4,700\text{--}5,400\text{ mg NH}_4^+\text{ L}^{-1}$. In quantitative terms, with 4 days of treatment using GPM technology and low-rate aeration, it was possible to form liquid fertilizer salt solution from the co-digested effluents with NH_4^+ concentrations of about $26,000\text{--}30,000\text{ mg NH}_4^+\text{ L}^{-1}$. This means that, with the operating conditions imposed, the joint treatment of the waste using AcoD and N recovery doesn't affect, adversely, the efficacy of this type of technology in terms of depuration and ammoniacal nitrogen recovery in the environment.

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AUTHOR CONTRIBUTIONS

JO developed the experiments of ammonia capture and monitored all the batches and replications. Also contributed to data collection and data results summary. SD-M produced the experimental set-up, including all mechanical and engineering tests for fluxes, pumps, membranes. Also maintained the set-up and analyzed gaseous measurements. MV supervised the set-up and the general design of batch experiments, collaborated in discussion and conclusions. JS-T developed the chemical analyses and the monitoring parameters during the experiments. AR collaborated at engineering scale, mass fluxes calculations and analysis of raw data. MP-M analyzed the results at statistical and reproducibility scales. MB collaborated in discussion of the results and documentation. RM coordinated all the research, collaborated in discussion and conclusions.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Gas-Permeable Membrane Technology Coupled With Anaerobic Digestion for Swine Manure Treatment

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This study was aimed at evaluating gas-permeable membrane technology (N-recovery) coupled with anaerobic digestion (AD) for the treatment of swine manure (SM). For this purpose, 66.7% of the initial total ammoniacal nitrogen (TAN) contained in centrifuged SM was first recovered by an e-PTFE gas-membrane as an ammonium sulfate solution. The resultant manure effluent with reduced ammonia (ammonia-trapped manure, ATM) was evaluated as AD substrate. It was compared with AD using the initial swine effluent (SM) without the N-recovery step (control). An organic loading rate (OLR) of 2.8 ± 0.5 g total chemical oxygen demand (TCOD) $L^{-1} day^{-1}$ was established to ensure a stable process when working at semi-continuous mode. Regardless of the operation mode, methane yields of 105 ± 2 mL CH_4 g TCOD⁻¹ were obtained for ATM. The combined treatment resulted in an organic matter removal efficiency of 68.6%. Initial TCOD accounted for 54.69 g L^{-1} . The results prove that it is feasible to combine gas-permeable membrane technology and AD for the treatment of SM, contributing to ammonia emissions mitigation, and sustainable livestock waste treatment. Moreover, by means of this technology combination, a variety of valuable products is obtained, namely sustainable energy in the form of methane and fertilizers.

Keywords: swine manure, ammonia recovery, gas-permeable membranes, biogas, CSTR, fertilizer

INTRODUCTION

Spain is the second European country with the highest pig population, accounting for 29,970,000 swine heads in 2017. Estimating a production of 2.5 m³ manure per head per year, the calculated swine manure (SM) production would be around 75 million m³ of manure every year (Spanish Royal Decree 324, 2000; EUROSTAT, 2018). SM has been traditionally applied for agricultural purposes close to the farm. However, this is not always possible in recent times, due to the size increase in livestock farms together with their location in concentrated areas. Moreover, high transportation costs due to the high water content of SM makes its transportation economically unfeasible (Flotats et al., 2009). Since agriculture is nowadays the largest source of ammonia (NH₃) emissions (EEA, 2015), which are closely related to a variety of environmental problems and to human health risks, an improved management of SM with reduced NH₃ emissions is urgently required. In this vein, a new European Directive was proposed in 2016 to improve the quality of the air (EC-European Commission, 2016). In the case of Spain, the reduction commitment for NH₃ annual emissions accounts for

3% for any year from 2020 to 2029 and for 16% for years after 2030, compared to 2005, which was selected as the base year. Reducing nitrogen content in SM could help to balance nutrient composition and fertilizing value while minimizing NH_3 emissions. Different technologies are being successfully applied to reduce nitrogen from livestock wastes. These technologies are mainly based on biological methods, as nitrification–denitrification (Riaño and García-González, 2014), microbial fuel cells (Kuntke et al., 2012) or anammox process (Molinuevo et al., 2009) and physical-chemical approaches, as ammonia stripping (Bonmatí and Flotats, 2003), ion exchange (Milan et al., 1997), struvite precipitation (Laridi et al., 2005), reverse osmosis (Masse et al., 2010), or, more recently, gas-permeable membrane technology (García-González et al., 2015; Vanotti and Szogi, 2015). In most of the cases, nitrogen is eliminated from the waste and either released to the atmosphere (as N_2) or trapped in an inert material and, therefore, not recovered. However, nitrogen should be considered as a resource and technologies should be aimed at recovering this compound.

Besides the capacity of recovering NH_3 , gas-permeable membrane technology presents diverse advantages over traditional technologies such as its large contact area between the liquid waste and NH_3 -trapping solution, the low-pressure performance or no need of additives in the liquid waste. More specifically, just a diluted acid is used to recover the nitrogen, which is further valorized as fertilizer. pH control is carried out using low-rate aeration, reducing operational costs by 57% when compared to alkali chemical addition (García-González et al., 2015). Moreover, the energy consumption is lower than in other nitrogen recovery technologies. For instance, energy demand is 18 times lower than for air stripping (Zarebska et al., 2015). Ammonia passes through the microporous hydrophobic membrane by diffusion. Mass transfer depends on the differences in NH_3 concentration between the wastewater and a concentrated acidic solution, which is circulating inside the membrane lumen and it is used to recover nitrogen as an ammonium sulfate solution. The efficiency of the process is dependent on the pH and temperature in the manure, which determines free NH_3 concentration. Total ammoniacal nitrogen (TAN) recovers up to 82% of the initial TAN have been reported with pH adjustment. This value decreased to 55% without pH control (García-González and Vanotti, 2015). Moreover, organic matter is partially removed during SM treatment by gas-permeable membrane technology at low-aeration rates. Percentages between 26 and 65% of the initial total chemical oxygen demand (TCOD) in SM (initial TCOD between 16.7 and 67.1 g TCOD $\text{L}^{-1}_{\text{manure}}$) have been reported (García-González et al., 2015; Riaño et al., submitted). Unfortunately, none of these studies has presented an explanation for this fact.

The resultant effluent after treating manure by gas-membrane technology, from now on called ammonia-trapped manure (ATM), which is characterized by a low TAN content, still needs stabilization. Similar to fresh manure, the high buffer capacity together with the high content of organic matter makes ATM a potential substrate for anaerobic digestion (AD). Anaerobic digestion is a well-established stabilization technology by which organic matter is converted into renewable energy.

Additionally, AD contributes to greenhouse gas mitigation, odor and pathogen reduction, and organic nitrogen mineralization into available nitrogen for plant growth (Cantrell et al., 2008). Moreover, coupling gas-permeable membranes with AD could contribute to reduce potential NH_3 -mediated inhibitions of the acetoclastic methanogenic biomass, which would result in an enhanced methane production. The use of gas-permeable membrane technology in order to counteract the NH_3 inhibition in AD has been minimally studied (Lauterböck et al., 2012, 2014) and further research is needed. Lauterböck et al. (2012) proposed the introduction of a membrane module inside AD reactors. They reported acid leakage and low pHs (only 10% of the total TAN was dissociated to free NH_3), resulting in low nitrogen recovery rates. The present study is aimed at evaluating gas-permeable membrane technology coupled to AD for the treatment of SM. In order to avoid possible operational issues and with the objective of optimizing nitrogen recovery (N-recovery), both processes were separated. For this purpose, nitrogen from fresh SM was first recovered by an e-PTFE gas-membrane. ATM was evaluated as substrate for AD and its stabilization was studied. Hence, batch and semi-continuous AD at increasing organic loading rates (OLRs) were investigated for ATM compared to a control treatment (i.e., AD of SM). Finally, the nutrient removal efficiencies and the obtained products (i.e., fertilizers and methane) were assessed for the combined treatment compared to the control.

MATERIALS AND METHODS

Origin of the Two Substrates, Swine Manure (SM) and Ammonia-Trapped Manure (ATM), and the Inoculum

Centrifuged SM was collected from a farm located in Narros de Cuellar (Segovia, Spain). The manure was transported in coolers to the laboratory and subsequently stored at 4°C for further use. ATM was obtained from the N-recovery experiments described in section N-Recovery Step. The inoculum used for the AD experiments (AD inoculum) was a mesophilic anaerobic sludge that was obtained from the municipal wastewater treatment plant (WWTP) in Valladolid, Spain.

N-Recovery Step

Nitrogen recovery was conducted in semi-continuous experiments as described by Riaño et al. (submitted). Ammonia separation tanks contained a total working volume of SM of 2 L. A tubular gas-permeable membrane, made of expanded polytetrafluoroethylene (e-PTFE) (Zeus Industrial Products Inc., Orangeburg, SC, USA), was submerged and fixed in horizontal position in the bottom of the NH_3 separation tank. Continuous stirring was provided and a nitrification inhibitor was added in order to avoid nitrification processes in the SM. Continuous aeration was supplied with an airflow rate of 0.24 $\text{L}_{\text{air}} \text{L}^{-1}_{\text{manure}} \text{min}^{-1}$. Sulfuric acid 1N was continuously recirculated through the tubular membrane. Initial pH in the manure accounted for 7.6. It rapidly increased during the five first days of operation, being in the range of 8.4–8.6. pH in the acidic solution was

maintained below 2. A protocol was established: concentrated H_2SO_4 (96–98%) was added to the acidic solution to an endpoint of $\text{pH} < 1$ whenever the pH of the acidic solution increased to 2. The NH_3 separation tank was fed once a day each weekday at a hydraulic retention time (HRT) of 7 d during 30 d (period I) and a HRT of 5 d for 20 d (period II). These HRTs corresponded to ammonium loading rates (ALR) of 491 and 696 $\text{mg TAN L}^{-1} \text{d}^{-1}$, respectively. TAN removal reached 79% for period I and 56% for period II, with 90% of recovery by the membrane in both periods. TCOD removal reached 37% for period I and 27% for period II, respectively (initial TCOD in SM was $67.1 \pm 10.1 \text{ g TCOD L}^{-1}$) (Riaño et al., submitted). Moreover, a solution of up to 19 g TAN L^{-1} was obtained, since NH_3 was converted to ammonium sulfate. N-recovery experiments were carried out in semi-continuous mode and different operational conditions were tested. In this manner, different ATM batches were obtained during the experimental time. ATM effluents from the whole experiment were utilized for the AD experiments.

Biochemical Methane Potential (BMP) Experiments

The biochemical methane potential (BMP) of the different substrates was carried out in bottles with a total volume of 0.57 L. Two different experiments (batch 1 and batch 2) with different substrate (S_0) to inoculum (X_0) ratios were run. Thus, batch 1 and batch 2 were performed with S_0/X_0 ratios of 1 and 3, respectively. The units for S_0 were g TCOD L^{-1} and the units for X_0 were g VS L^{-1} . These ratios were chosen based on substrate characteristics (González-Fernández and García-Encina, 2009; Raposo et al., 2011). The S_0/X_0 ratio of 1 was chosen to study the maximum BMP that can be obtained from the substrates, while ensuring a stable process. The S_0/X_0 ratio of 3 was used to investigate the potential inhibitors during AD of the substrates. More specifically, a S_0/X_0 ratio of 3 in SM AD would result in reactor imbalances due to acetate and propionate accumulation (González-Fernández and García-Encina, 2009). The composition of ATM was similar

to SM but with more recalcitrant organic matter, so that a S_0/X_0 ratio of 3 was chosen in this case to study the possible imbalances caused by VFA accumulation. Two different substrates were studied in each batch experiment, namely ATM and SM. Anaerobic sludge was used as inoculum (AD inoculum). Chemical characteristics of AD inoculum, SM, ATM1 (ATM effluent used for batch 1) and ATM2 (ATM effluent used for batch 2) are shown in **Table 1**. Quantities of substrates and inoculum were calculated to get the desired S_0/X_0 ratio in each case. More specifically, in batch 1, 87 and 51 g of fresh ATM1 and fresh SM were added to the bottles corresponding to BMPs of ATM1 and SM, respectively. Concerning batch 2, 130 and 76 g of fresh ATM2 and fresh SM were added to the bottles corresponding to BMPs of ATM2 and SM, respectively. The amount of AD inoculum in each bottle was 200 and 100 g for bottles in batch 1 and bottles in batch 2, respectively. In every bottle, water up to a final amount of 300 g of liquid mixture was added, thus allowing headspace for the gas of approximately 0.27 L. For the determination of endogenous methane production, blanks containing only AD inoculum were run. The BMP assays were run in triplicates using the method of Molinuevo-Salces et al. (2013). After the set-up of each bottle, the headspace was flushed with nitrogen in order to ensure anaerobic conditions. Then, the bottles were placed in an incubator at $36 \pm 1^\circ\text{C}$ and continuous agitation was provided by a shaker. The incubation time was 35 days. The volume of biogas produced by the different substrates was calculated by measuring the pressure of the bottle's headspace. Biogas composition was analyzed twice per week. Methane yield, expressed as CH_4 per gram of TCOD added, was calculated.

Anaerobic Digestion Step: CSTRs Set-Up

In order to study the feasibility of treating ATM effluents in AD plants, the results obtained in the batch experiments were scaled up in a semi-continuous system. Two semi-continuous stirred tank reactors (CSTR) with a working volume of 2 L were utilized: one reactor (R1) was used for AD of diluted swine manure (DSM) and the other for AD of ATM (R2). Fresh SM was

TABLE 1 | Chemical characteristics of the different substrates and inoculum used in batch 1 and batch 2.

Parameters	Unit	AD inoculum		SM		ATM1		ATM2	
		Average	STDEV	Average	STDEV	Average	STDEV	Average	STDEV
pH		7.61	0.02	7.70	0.10	8.09	0.00	7.93	0.23
TS	g L^{-1}	22.24	0.54	29.51	0.51	27.47	0.14	27.61	0.23
Humidity	%	97.78	0.00	97.05	0.05	97.25	0.01	97.24	0.02
VS	g L^{-1}	13.84	0.52	20.29	0.57	19.18	0.08	19.19	0.18
TCOD	g L^{-1}	12.18	0.67	54.69	2.23	31.94	1.34	33.73	0.83
SCOD	g L^{-1}	n.d.		34.11	0.02	12.26	0.02	16.80	0.76
TVFA	g COD L^{-1}	n.d.		15.80	2.27	1.50	0.19	2.27	0.01
TAN	g L^{-1}	0.86	0.01	3.36	0.01	0.56	0.00	0.70	0.01
TKN	g L^{-1}	1.78	0.00	4.27	0.01	1.67	0.00	1.69	0.03
Ratio TCOD/TKN				12.82		19.17		20.02	

ATM1 and ATM2 correspond to the ATM effluents used for batch 1 and batch 2, respectively. TS, VS, TCOD, SCOD, TVFA, TAN, and TKN stand for total solids, volatile solids, total chemical oxygen demand, soluble chemical oxygen demand, total volatile fatty acids, total ammoniacal nitrogen and total Kjeldahl nitrogen, respectively. STDEV stands for standard deviation. n.d., not determined.

diluted with water, resulting in DSM. The high organic matter removals during N-recovery made it necessary to dilute the fresh SM. This was done in order to keep a constant and equal influent concentration of TCOD in both reactors and compare the AD performance of both substrates (DSM and ATM) at exactly the same OLR. **Figure 1** presents a scheme of the experimental set-up. Agitation was magnetically provided in both reactors (400 rpm). The temperature was maintained at $37 \pm 1^\circ\text{C}$ using a water jacket connected to a temperature-controlled water bath. The reactors were initially filled with 2 L of AD inoculum (**Table 1**). After one day, manual feeding of the reactors was started once per day, every weekday. Substrates for R1 and R2 were daily prepared by diluting the substrate in water when necessary. Three different stages were established for the semi-continuous operation of the reactors. The first stage corresponded to an acclimation period which lasted 20 days. The second stage (period I) was started by fixing OLR and HRT for both reactors at $2.7 \pm 0.4 \text{ g TCOD L}^{-1} \text{ d}^{-1}$ and 11 days, respectively. Finally, for period II, OLR and HRT were set for both reactors to $7.0 \pm 1.3 \text{ g TCOD L}^{-1} \text{ d}^{-1}$ and 5 days, respectively. **Table 2** presents the operational parameters, influents chemical composition, effluents chemical composition, and biogas characteristics for R1 and R2 during both periods. Biogas production (quantified by water displacement) and pH were measured every weekday. Influent and effluent samples were taken and analyzed for total alkalinity (TA), partial alkalinity (PA), total solids (TS), VS, TCOD, soluble chemical oxygen demand (SCOD), total volatile fatty acids (TVFA), TAN, and total Kjeldahl nitrogen (TKN) twice a week. Biogas composition was measured once per week.

Mass Balances

Mass balances in terms of TAN, TKN, SCOD, and TCOD were carried out both for the combined SM treatment (N-recovery from SM by gas-permeable membrane technology and AD of ATM) and for the control treatment (AD of SM). These mass balances were performed with data corresponding to the best scenarios in semi-continuous mode for N-recovery

from SM (section N-Recovery Step) and AD (section Anaerobic Digestion Step: CSTRs Set-Up). More specifically, the mass balances used data corresponding to period I in the N-recovery by gas-permeable membranes (Riaño et al., submitted) and data corresponding to period I for the AD-CSTRs experiments.

Analytical Methods

Analyses of TS, VS, TCOD, SCOD, TAN, and TKN were performed in duplicate in accordance with APHA (2005). TS content was determined by drying the sample to a constant weight at $103\text{--}105^\circ\text{C}$. The TS residue was ignited at 550°C to constant weight and the weight lost on ignition was the VS content. TCOD and SCOD were determined following closed reflux colorimetric method. TKN was measured according to the Kjeldahl digestion, distillation, and titration method. TAN was measured according to the distillation and titration method. Total alkalinity, PA, and pH were monitored using a pH meter Crison Basic 20 (Crison Instruments S.A., Barcelona, Spain); TA and PA were obtained by measuring the amount of $0.1 \text{ N-H}_2\text{SO}_4$ needed to bring the sample to a pH of 4.3 and 5.75, respectively. IA and IA/PA ratio were determined as proposed by Ripley et al. (1986). Intermediate alkalinity (IA) is the result of subtracting PA from TA.

Biogas composition was analyzed using a gas chromatograph (Agilent 7890A, USA) with a thermal conductivity detector, provided by a HP-Plot column (30 m $0.53 \text{ mm } 40 \mu\text{m}$) followed by a HP-Molesieve column (30 m $0.53 \text{ mm } 50 \mu\text{m}$). Helium (7 mL min^{-1}) was used as the carrier gas. The injection port temperature was set at 250°C and the detector temperature was 200°C . The temperature of the oven was set at 40°C for 4 min and thereafter increased to 115°C . Methane values were expressed at normal conditions (i.e., 0°C and 1 atm). The concentrations of acetate, propionate, butyrate, iso-butyrate, valerate, iso-valerate, and caproate were determined using a gas chromatograph (Agilent 7890A, USA) equipped with a Teknokroma TRB-FFAP column of 30 m length and 0.25 mm i.d. followed by a flame ionization detector (FID). The carrier gas

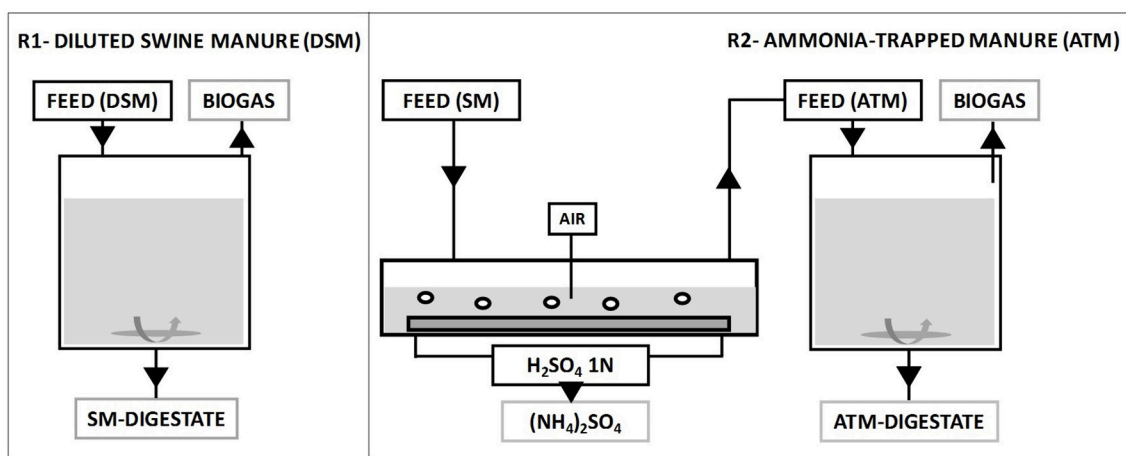


FIGURE 1 | Experimental set-up for CSTR experiments.

TABLE 2 | Operational parameters, influents chemical composition, effluents chemical composition, and biogas characteristics for reactors R1 and R2.

Parameter	Unit	R1-DSM				R2-ATM			
		Period I		Period II		Period I		Period II	
		Average	STDEV	Average	STDEV	Average	STDEV	Average	STDEV
OPERATIONAL PARAMETERS									
T°	°C	37.7	0.7	37.3	0.6	37.5	0.7	37.3	0.5
HRT	d	11.2	2.3	4.5	0.0	11.1	2.9	4.7	0.2
OLR	g TCOD L ⁻¹ _{reactor} d ⁻¹	2.6	0.3	7.9	0.9	2.8	0.5	6.1	0.4
INFLUENT COMPOSITION									
pH		7.37	0.18	7.41	0.12	7.80	0.16	7.76	0.10
TAN	g L ⁻¹	1.59	0.22	1.83	0.15	0.87	0.09	0.95	0.06
TKN	g L ⁻¹	2.09	0.32	2.42	0.29	1.65	0.11	1.56	0.12
TS	g L ⁻¹	15.89	3.41	18.69	1.82	21.62	187	19.35	2.41
VS	g L ⁻¹	11.39	2.55	13.21	1.75	15.06	1.00	13.62	1.70
TCOD	g L ⁻¹	30.28	6.73	34.16	4.75	31.35	4.34	27.52	3.48
SCOD	g L ⁻¹	17.75	3.19	15.16	4.26	11.83	2.55	12.84	1.93
TVFA	g COD L ⁻¹	9.82	1.84	13.19	3.78	1.31	0.57	1.35	0.61
Ratio SCOD/TCOD		0.59		0.44		0.38		0.47	
Ratio TCOD/TKN		14.47		14.11		19.00		17.69	
EFFLUENT COMPOSITION									
pH		7.92	0.11	7.92	0.11	7.78	0.11	7.81	0.16
TAN	g L ⁻¹	1.63	0.16	1.84	0.23	0.99	0.04	1.06	0.13
TKN	g L ⁻¹	2.04	0.22	2.27	0.33	1.59	0.08	1.64	0.14
TS	g L ⁻¹	12.49	2.19	14.41	1.55	16.30	0.76	17.29	0.91
VS	g L ⁻¹	8.01	1.62	9.59	1.00	10.17	0.50	11.18	0.64
TCOD	g L ⁻¹	14.06	4.96	19.90	5.37	17.17	1.59	21.51	1.40
SCOD	g L ⁻¹	4.95	0.96	8.03	1.30	4.05	0.61	7.03	1.50
TVFA	g COD L ⁻¹	1.20	0.56	5.62	1.34	0.59	0.25	2.84	2.00
Acetate	g COD L ⁻¹	0.50	0.27	1.59	0.71	0.43	0.21	1.91	1.24
Propionate	g COD L ⁻¹	0.69	0.44	2.73	0.60	0.16	0.09	0.18	0.04
Butyrate + Isobutyrate	g COD L ⁻¹	0.01	0.00	0.29	0.14	BDL		0.29	0.15
Valerate + Isovalerate	g COD L ⁻¹	0.05	0.02	0.82	0.39	BDL		0.40	0.37
Caproate	g COD L ⁻¹	BDL		0.09	0.02	BDL		0.05	0.05
TA	g CaCO ₃ L ⁻¹	8.06	0.59	9.22	0.85	7.57	1.22	7.09	0.51
PA	g CaCO ₃ L ⁻¹	6.26	0.48	6.48	0.41	5.82	0.99	5.17	0.70
IA	g CaCO ₃ L ⁻¹	1.80	0.28	5.17	0.70	1.71	0.22	1.93	0.31
IA/TA ratio		0.29	0.05	0.44	0.06	0.31	0.05	0.41	0.12
BIOGAS CHARACTERISTICS									
Biogas yield	mL g TCOD ⁻¹ _{added}	200	28.90	70	45.00	155	30.00	107	41.32
Methane content	%	71.68	2.63	75.84	0.77	66.66	3.38	65.58	3.21
Methane yield	mL g TCOD ⁻¹ _{added}	145	17.26	56	32.57	105	17.11	71	18.83

BDL, Below Detection Limit.

was helium (1 mL min⁻¹). The temperature of the detector and the injector was 280°C. The temperature of the oven was set at 100°C for 4 min, then increased to 155°C for 2 min and thereafter increased to 210°C. TVFA were calculated as the sum of those acids.

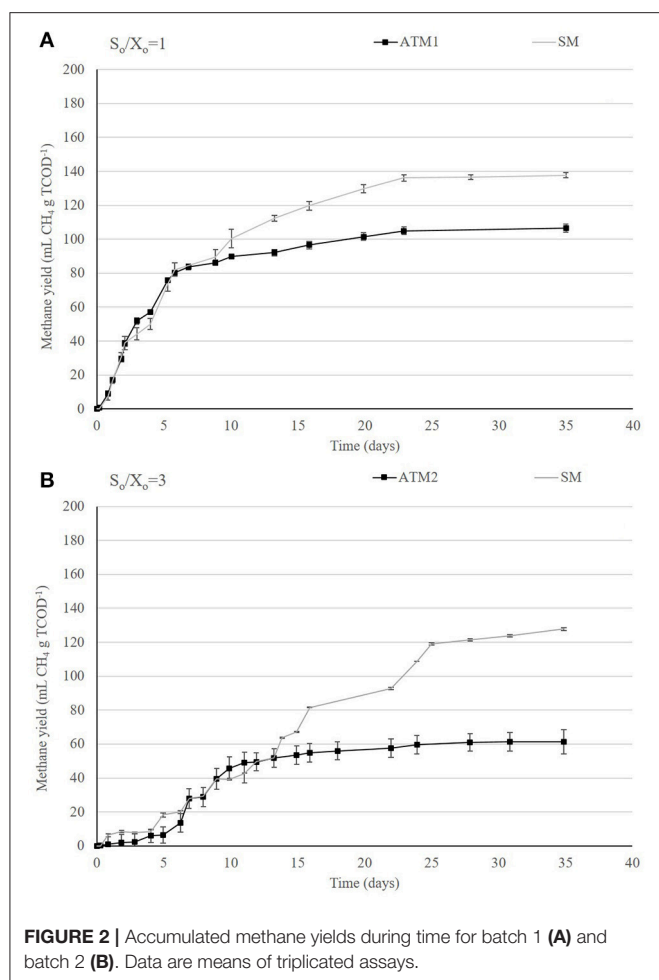
RESULTS

Anaerobic Digestion of ATM Effluents

BMP Tests

The accumulated methane yields, as mL of CH₄ per gram of TCOD added, are presented in **Figure 2**. **Figure 2A** corresponds

to a S₀/X₀ ratio of 1 (batch 1). **Figure 2B** corresponds to a S₀/X₀ ratio of 3 (batch 2). In both S₀/X₀ ratios, ATM achieved lower methane yields than SM and the differences were smaller when S₀/X₀ ratio was 1. More specifically, in the case of a S₀/X₀ ratio of 1, the difference in final CH₄ yields between ATM1 and SM was approximately 30% (106 ± 2 and 138 ± 2 mL CH₄ g TCOD⁻¹, respectively). However, with a S₀/X₀ ratio of 3, the performance of ATM was affected, with a difference in final methane yields higher than 100% (61 ± 7 and 128 ± 1 mL CH₄ g TCOD⁻¹ for ATM2 and SM, respectively). As expected, a lag phase during the first week in batch 2, corresponding to a S₀/X₀ ratio of 3, was evidenced affecting AD for both ATM2 and SM (**Figure 2B**).



Anaerobic Digestion in Semi-continuous Mode: CSTRs

Two AD CSTRs, namely R1 and R2, were run with DSM and ATM as substrate, respectively. Substrates were prepared daily prior to feeding the CSTRs to keep a constant HRT and equal influent concentration of TCOD in both reactors. This was achieved by diluting the substrate in water when necessary. The influent compositions are provided in Table 2. Figure 3 presents CH₄ yields and OLR of both reactors during this study. After an adaptation phase of approximately 20 days, both reactors reached steady state conditions. Regarding period I, averaged CH₄ yields for DSM (R1) were 1.4-fold higher than for ATM (R2), accounting for 145 ± 17 and 105 ± 17 mL CH₄ g TCOD⁻¹, respectively. An increase in the OLR from 2.7 to 7 g TCOD L⁻¹ reactor d⁻¹ resulted in a decrease in methane yield during period II. More specifically, yields of 56 ± 33 and 71 ± 20 mL CH₄ g TCOD⁻¹ were obtained for DSM (R1) and ATM (R2) during period II, respectively. Besides methane yield, the stability of the AD process was evaluated in terms of TVFA concentration, pH, and ratio IA/PA. As seen in Figure 4 and Table 2, after the adaptation of the microbial community to the new substrates, the concentration of TVFA remained stable and at low levels

during period I. The change in OLR resulted in a variation of AD performance. Thus, TVFA concentration increased and its composition varied (Table 2). The ratio IA/PA is used as a stability parameter in AD, where ratios below 0.3 indicate a good state of the anaerobic process (Ripley et al., 1986). The fate of IA/PA ratio and pH over time is presented in Figure 4. Although pH remained stable during the complete experimental time, an increase in IA/PA ratio was observed during both the adaptation period and period II.

Membrane Technology Coupled With AD: Mass Removal Efficiencies and Evaluation of the Obtained Products

Nitrogen recovery and organic matter conversion were studied both for the combined treatment of SM, namely N-recovery by gas-permeable membrane technology followed by AD, and for the control treatment (AD of SM). Figure 5 presents mass balances for nitrogen in terms of TAN and TKN for the combined treatment (Figure 5A) and the control treatment (Figure 5B). Figure 6 presents mass balances for carbon in terms of TCOD and SCOD for the combined treatment (Figure 6A) and the control treatment (Figure 6B). The combined treatment of membranes and AD resulted in TAN recoveries as (NH₄)₂SO₄ of 66.7%. TAN concentration in the ATM-Digestate accounted for 29.5% of the initial TAN. In the case of the control treatment of DSM, all the initial TAN was present in the DSM-Digestate. Concerning TKN, recovery efficiencies accounted for 92.4 and 97.5% for the combined and the control treatment, respectively. In the case of the combined SM treatment, 55.2% of the initial TKN was recovered as (NH₄)₂SO₄. 31.4 and 11.9% of the initial TCOD and SCOD, respectively, were found in the ATM-Digestate. These values were increased in the control treatment to 46.4 and 27.9% for TCOD and SCOD, respectively (Figure 6). Moreover, organic matter was converted in methane during AD. From the initial TCOD, 23.3 and 48.2% were recovered as methane for the combined treatment and the control treatment, respectively.

Three products were obtained by the combined SM treatment, namely renewable energy in the form of methane, a (NH₄)₂SO₄ solution and ATM-Digestate. Methane content in the biogas produced with ATM as substrate accounted for 66–67% (R2; Table 2). Table 3 presents the chemical characteristics of the obtained (NH₄)₂SO₄ and ATM-Digestate.

DISCUSSION

Anaerobic Digestion of ATM Effluents BMP Tests

It is feasible to use ATM as a substrate for AD. Anaerobic digestion of ATM resulted in CH₄ yields in the range of 61–106 mL CH₄ g TCOD⁻¹. Regardless of the S_0/X_0 ratio applied, CH₄ yields corresponding to the control (SM) were higher than CH₄ yields for ATM (Figure 2). This difference could be explained by the higher concentration of easily degradable organic matter in SM, when it is compared to ATM (Table 1). Consequently, the higher concentration of available easily

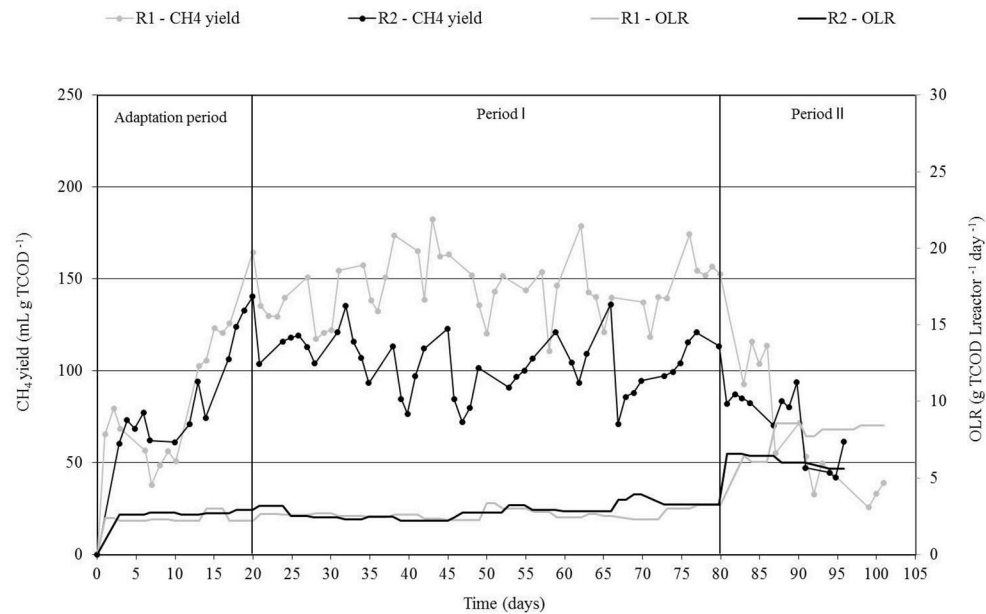


FIGURE 3 | Methane yields and OLR during semi-continuous operation for R1 (DSM) and R2 (ATM).

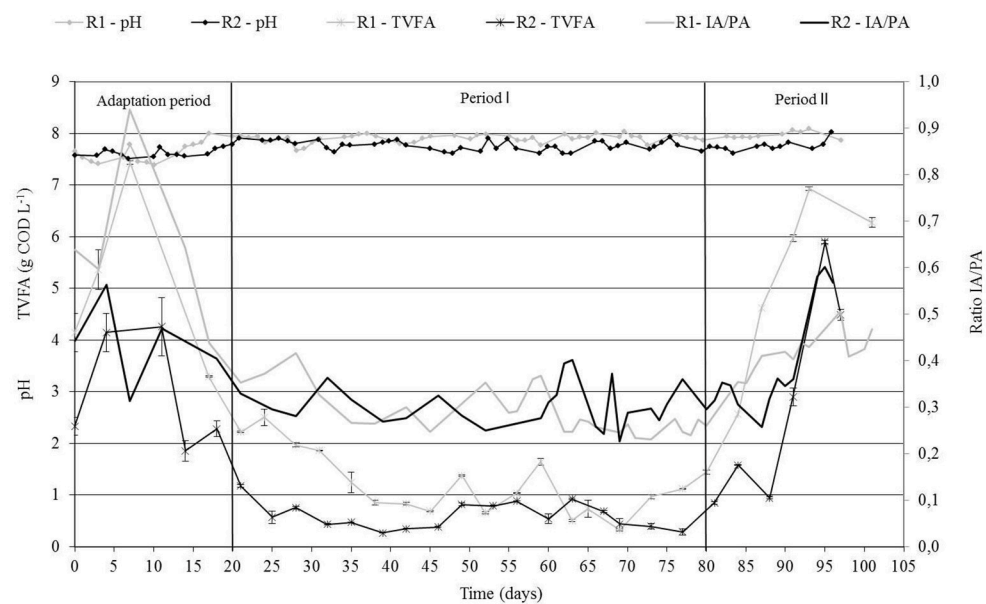


FIGURE 4 | Stability parameters during semi-continuous operation of R1 (DSM) and R2 (ATM).

degradable organic matter in SM resulted in higher CH₄ yields than those obtained for ATM. The difference in concentration is the result of the partial degradation of organic matter that took place during the N-recovery process, resulting in TCOD removal values of 27–37% of the initial TCOD in SM. This degradation was probably due to the biological activity that takes place in SM at low HRT at room temperature. Moreover, this degradation could have been enhanced by the aerobic

conditions caused by aeration. A previous study dealing with N-recovery by gas-permeable membranes using low-aeration rates reported TCOD removals up to 65%. On the contrary; removed TCOD was very little when chemically adjusting wastewater to high pHs (García-González et al., 2015). Therefore, COD removal was most likely due to a mixture of both aerobic bacteria oxidation and COD volatilization during air bubbling.

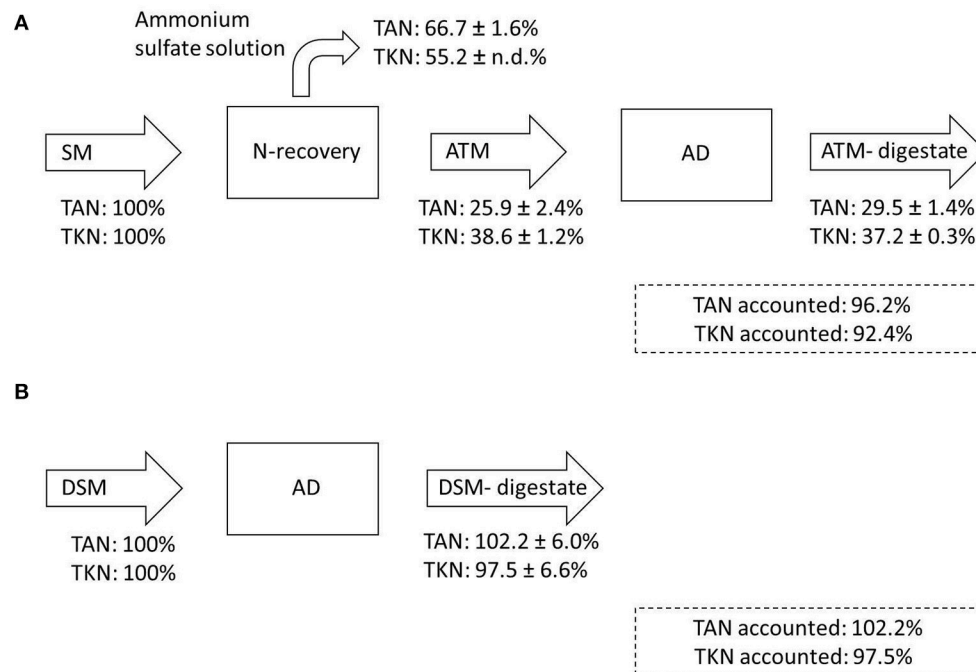


FIGURE 5 | Mass balance for nitrogen for the combined treatment **(A)** and the control treatment **(B)**. TAN accounted is the addition of TAN in the ammonia concentrator tank and TAN in the ATM-Digestate. TKN accounted is the addition of TKN in the ammonia concentrator tank and TKN in the ATM-Digestate.

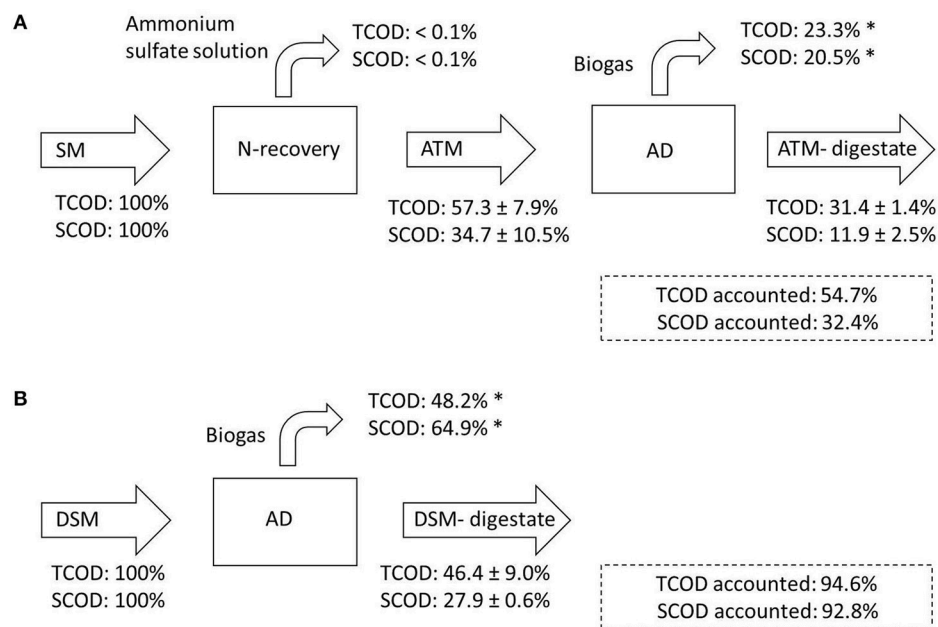


FIGURE 6 | Mass balance for carbon for the combined treatment **(A)** and the control treatment **(B)**. *It is assumed that the decrease of COD from ATM to ATM-Digestate was converted into biogas, except a 10% of that COD that was utilized for microbial growth during AD and not converted into biogas (Metcalf and Eddy, 2014).

Two parameters affecting AD stability, namely TAN concentration and organic load, were studied. First, the potential toxic effect of TAN during AD of ATM was not observed during

these experiments, probably due to the high amount of inoculum utilized that diluted the TAN content during BMP incubation. Ammonium inhibitory levels for AD are established in the range

TABLE 3 | Chemical characterization of fertilizers.

Parameters	Unit	Combined treatment				Control treatment	
		(NH ₄) ₂ SO ₄		ATM-Digestate		DSM-Digestate	
		Average	STDEV	Average	STDEV	Average	STDEV
pH		<2		7.75	0.11	7.92	0.11
Conductivity	mS cm ⁻¹	90.00		11.22	0.11	10.02	0.00
TS	g L ⁻¹	n.d.		16.30	0.76	12.49	2.19
VS	g L ⁻¹	n.d.		10.17	0.50	8.01	1.62
TCOD	g L ⁻¹	0.139	0.01	17.17	1.59	14.06	4.96
SCOD	g L ⁻¹	n.d.		4.05	0.61	4.95	0.96
TVFA	g COD L ⁻¹	0.064	0.03	0.59	0.25	1.20	0.56
TAN	g L ⁻¹	19		0.99	0.04	1.63	0.16
TKN	g L ⁻¹	19		1.60	0.08	2.04	0.22
Ratio C/N		0.01		10.73		10.41	
S	% dw	24		0.08		0.01	
K ⁺	mg L ⁻¹	28		1,898	31.31	1,356	136
Mg	mg L ⁻¹	BDL		67.04	1.92	77.25	9.74
Ca	mg L ⁻¹	BDL		500	15	305.75	41.36
Zn	mg L ⁻¹	BDL		32.02	0.25	26.00	3.56
Cu	mg L ⁻¹	BDL		10.74	0.22	7.75	0.96
Fe	mg L ⁻¹	BDL		34.44	0.16	28.50	6.35
<i>E. coli</i>	CFU mL ⁻¹	<50		<1		<1	
<i>Salmonella</i>	CFU mL ⁻¹	BDL		BDL		BDL	

DSM-Digestate and ATM-Digestate stand for the obtained liquid fractions after AD of DSM and ATM, respectively. BDL, Below Detection Limit; n.d., not determined.

of 4–10 g TAN L⁻¹ (Angelidaki and Ahring, 1993; Rajagopal et al., 2013). In this case, TAN concentrations were far below those inhibitory levels for both ATM and SM ADs (up to 1.4 g TAN L⁻¹). Second, and in order to investigate the effect of organic load over CH₄ yield, two different S₀/X₀ ratios were studied. A lag phase was observed during the first week at a S₀/X₀ ratio of 3 (**Figure 2B**). This lag phase was probably due to the accumulation of TVFA after hydrolysis, which resulted in a delay in CH₄ production. However, a relatively high CH₄ yield together with an absence of TVFA and a stable pH (i.e., 7.49 in all the cases) after 35 days of AD indicates that the inhibition was partially overcome during the experimental time. On the contrary, a S₀/X₀ ratio of 1 avoided reactor imbalances due to TVFA accumulation. For this reason, the S₀/X₀ ratio of 1 was selected as the optimum and any limiting effect in the hydrolysis due to a lack of organic matter for anaerobic bacteria was discarded. The obtained results in the batch experiments were used to select a proper HRT for the semi-continuous operation. As seen in **Figure 2**, CH₄ yields were stable after ~11 days of AD, so that the starting HRT in semi-continuous mode (section Anaerobic CSTRs) was fixed at 11 days to maximize CH₄ production. In order to ensure a stable AD process, the OLR was established at ~3 g TCOD L⁻¹ d⁻¹ (Banks and Heaven, 2013). For Period II, an HRT of 5 days was chosen to investigate if ATM could be used as AD substrate at lower HRTs than SM. Organic matter availability for AD microorganisms was different in SM and ATM, because the more degradable organic matter was eliminated during the N-recovery step. Even though the

minimum generation time of acetoclastic methanogens is in the range of 2.7 days, and 5 is very close to this time, it has been demonstrated that AD of SM is feasible working at HRTs of 3–5 days and OLR between 3 and 5 g VS L_{reactor}⁻¹ d⁻¹ (Hill and Bolte, 2000). Although OLRs was slightly higher in our case, a HRT of 5 days was chosen based in the results obtained in batch mode, where 80% of the final methane yield was obtained in the first 5 days (**Figure 2A**).

Anaerobic CSTRs

In order to obtain the same TCOD (i.e., the same OLR) in both R1 and R2, SM was diluted 1.6–1.8 times to reach the same TCOD concentration than ATM. This was done because an average of 32% of the initial TCOD in SM was lost during the N-recovery step that produced the ATM (Riaño et al., submitted). Process performance during experimental AD of DSM (R1) and ATM (R2) is presented in **Figure 3**. **Table 2** illustrates operational parameters, influent and effluent composition and biogas characteristics for each experimental period. The main difference between both substrates was that ATM substrate presented 10-times lower concentration of organic matter in the form of TVFA than DSM. Moreover, the initial TAN concentration accounted for 1.59 ± 0.2 and 0.87 ± 0.1 g L⁻¹ for DSM and ATM during period I, respectively. Concerning period II, the initial TAN concentration accounted for 1.83 ± 0.2 and 0.95 ± 0.1 g L⁻¹ for DSM and ATM, respectively. Similar to the results achieved in batch mode, maximum CH₄ yield for ATM (R2) at an OLR of 2.8 ± 0.5 g TCOD L_{reactor}⁻¹ d⁻¹ was 105 ±

17 mL CH₄ g TCOD⁻¹. This value increased to 145 ± 17 mL CH₄ g TCOD⁻¹ in the case of DSM (**Figure 3**). Thus, the higher concentration of easily biodegradable organic matter in DSM when it was compared to ATM, resulted in higher CH₄ yields for DSM (**Table 2**).

An increase in OLR during period II from 2.7 to 7.0 g TCOD L⁻¹ reactor d⁻¹ resulted in an organic overload as shown by the large reduction in CH₄ yield (**Figure 3**). **Figure 4** presents TVFA concentration, pH and IA/PA ratio for both reactors during the experimental time. Volatile fatty acids (TVFA) are produced during the acidogenic phase of AD. If hydrolysis and methanogenesis are balanced, TVFA concentration in the reactor should be stable and generally low. TVFA remained stable and at low levels (0.59 g TCOD L⁻¹ for R2) during period I (**Figure 4**). When there is any disturbance in AD performance (i.e., an OLR increase in this case), TVFA concentration increases and TVFA composition varies. More specifically, the perturbation resulted in an increase in long TVFA, as propionate, butyrate, valerate, and caproate (**Table 2**). Similar to TVFA concentration, the ratio IA/PA increased with OLR. Total alkalinity measures the buffering capacity of a solution and it is expressed as milligrams of calcium carbonate per liter of solution. Regarding AD, PA is usually associated to OH⁻, NH₃, HCO₃⁻, and CO₃ while intermediate alkalinity (IA = TA - PA) is related to TVFA presence in the reactor. The ratio IA/PA is used as a stability parameter in AD, where ratios below 0.3 indicate a good state of the anaerobic process (Ripley et al., 1986). An increase in IA/PA ratio was observed during both the adaptation period and period II, which is in accordance to TVFA fate in the reactors. Opposite to that and regardless of the applied disturbances in the reactors, pH remained stable in the range of 7.8–8.0 during the complete experimental time. The high alkalinity registered in both reactors provided enough buffer capacity for preventing pH drop. Finally, TAN levels in the range of 4–10 g TAN L⁻¹ have been reported to diminish anaerobic bacteria activity during AD leading to process failures (Angelidaki and Ahring, 1993; Rajagopal et al., 2013). In this case, maximum TAN concentrations of 1.84 and 1.06 g TAN L⁻¹ were achieved after AD of DSM and ATM, respectively (**Table 2**). Therefore, TAN levels in the reactors were below the inhibitory levels, so that no NH₃-mediated inhibition was detected.

To sum up, an OLR of around 3 g TCOD L⁻¹ reactor d⁻¹ is recommended to achieve a stable AD process when anaerobically digesting ATM. Besides the demonstrated feasibility of using ATM as a substrate for AD, a stabilized digestate, similar to that obtained by AD of SM, would be achieved.

Membrane Technology Coupled With AD: Mass Removal Efficiencies and Evaluation of the Obtained Products

The treatment of SM by a combined treatment of N-recovery by gas-permeable membranes coupled with AD resulted in TAN and TKN recoveries of up to 96.2 and 92.4%, respectively (**Figure 5**). Initial concentrations of TAN and TKN were 3.36 and 4.27 g L⁻¹, respectively (**Table 1**). During the first step of the combined treatment (N-recovery), TAN removals of up to 78%

were obtained at a loading rate of 491 mg TAN L⁻¹ d⁻¹ by gas-permeable membranes. Up to 90% of this TAN was recovered in the acidic solution as (NH₄)₂SO₄, resulting in a solution of up to 19 g TAN L⁻¹. On the other hand, TAN and TKN were not removed during AD of DSM (**Figure 5B**). More specifically, TAN concentration slightly increased due to N mineralization during protein break down in AD.

Chemical composition of the (NH₄)₂SO₄ solution is shown in **Table 3**. This (NH₄)₂SO₄ solution contained a nitrogen concentration of 19 g TAN L⁻¹, presenting several advantages as fertilizer: (1) nitrogen concentration in an easily spreadable solution, (2) reduction of transportation costs, and (3) reduction of nitrogen contamination and NH₃ emissions. Moreover, the solution presented low concentrations of organic matter (i.e., 0.139 ± 0.01 g TCOD L⁻¹ and 0.064 ± 0.03 g VFA-COD L⁻¹, respectively). The presence of low concentrations of organic matter in the (NH₄)₂SO₄ solution could be attributed to the osmotic distillation taking place during TAN diffusion (Riaño et al., submitted). In this manner, volatile organic compounds are transported across the hydrophobic membranes together with water vapor since they present partial vapor pressures comparable to or higher than water vapor (Xie et al., 2016). Although this (NH₄)₂SO₄ solution presents promising fertilizing properties, very few field studies with this solution have been carried out (Majd et al., 2012). Therefore, this product should be further evaluated as soil amendment in field trials.

With regard to organic matter, 88.1% of the initial SCOD and 68.6% of the initial TCOD were removed during the combined treatment (**Figure 6**). A 20.5 and a 23.3% of the initial SCOD and TCOD recovered as CH₄ during AD, respectively. Methane content in biogas produced with ATM as substrate accounted for 66–67% (R2; **Table 2**). Besides CH₄, a stabilized liquid by-product with possibilities as soil amendment (i.e., anaerobic digestate) was obtained. If compared to raw SM, anaerobic digestate presents outstanding fertilizing properties. More specifically, nitrogen mineralization may result in a higher short-term nitrogen fertilizer value (Cavalli et al., 2016; Baral et al., 2017) while the conversion of easily organic matter in CH₄ results in a higher stability of the anaerobic digestate. In this case, chemical composition of both digestates revealed that ATM-Digestate presented 1.65-fold lower TAN and 1.40-fold higher K⁺ concentrations DSM-Digestate. No differences between the DSM-Digestate (control treatment) and ATM-Digestate COD concentration were found (**Table 3**), concluding that AD of ATM results in a stable ATM-Digestate.

This novel combined treatment resulted in the conversion of a problematic stream, as it is swine manure (SM), into three valuable products namely methane, anaerobic digestate-fertilizer and ammonium sulfate solution.

FUTURE DIRECTIONS

The use of gas-permeable membrane technology in combination with AD has been narrowly studied. Since further research is needed in this area, some points were identified and are listed below:

- Livestock wastes present high TAN concentrations, which may result in a problem for AD processes, due to the inhibitory effect on microorganisms, causing TVFA accumulation and reactor failure. Gas-permeable membrane technology could help to mitigate TAN inhibition during AD, contributing to the economical sustainability of the process. The inhibitory effect of NH_3 on AD microorganisms was not detected in the present study because the SM was diluted to obtain the same TCOD concentration as in the ATM effluents. This resulted in a dilution of the TAN content in SM, avoiding NH_3 -mediated inhibition. A comparison between AD of undiluted SM and ATM effluents (obtained after N-recovery by gas-permeable membrane technology) would be useful to properly assess if the inhibition of AD at high NH_3 concentrations could be diminished.
- On the other hand, TCOD is partially removed during N-recovery in SM by gas-permeable membranes. In this manner, that TCOD is not further converted in biogas during AD. In order to optimize the economy of the combined technology, further investigation is needed to study if the use of gas-permeable technology for N-recovery should be placed before or after AD.
- pH values above 8.5 are necessary to obtain an efficient capture of NH_3 by gas-permeable membranes. Aeration has been proposed as an effective method to increase pH, resulting in TCOD removal. This is not desired if ATM is intended to be used as substrate for CH_4 production by AD. Alternative ways of pH control should be considered in order to maximize CH_4 production from ATM effluents.

CONCLUSIONS

It is feasible to combine N-recovery by gas-permeable membrane technology and AD for the treatment of SM, contributing to ammonia emissions mitigation and sustainable livestock waste treatment. Moreover, by means of this novel technology, a variety of valuable products is obtained, namely sustainable energy in

the form of methane and fertilizers (i.e., ammonium sulfate solution and anaerobic digestate). First, 66.7 % of the initial TAN contained in SM was semi-continuously recovered as an ammonium sulfate solution. The resultant effluent was stabilized by AD. A methane yield of $105 \text{ mL CH}_4 \text{ g TCOD}^{-1}$ with an OLR of $2.8 \pm 0.5 \text{ g TCOD L}^{-1} \text{ day}^{-1}$ was achieved during its semi-continuous AD. Moreover, an stabilized anaerobic digestate with fertilizing properties was obtained, containing 29.5% of the initial TAN. Overall, this combined treatment for SM resulted in a 96.2% TAN recovery and a 68.6% TCOD removal.

AUTHOR CONTRIBUTIONS

All the authors contributed to this work. MG-G and BR obtained research funding. BR and BM-S designed and performed the experiments and worked on data acquisition and interpretation. BM-S analyzed the data and wrote the manuscript. BR, MG-G, and MV revised and provided feedback on drafts of the manuscript. All authors approved the final version of the manuscript.

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Development and Testing of the ARS Air Scrubber: A Device for Reducing Ammonia Emissions from Animal Rearing Facilities

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Ammonia (NH₃), dust and odor emissions from animal feeding operations (AFOs) can cause atmospheric pollution and disputes with neighbors. The objectives of this study were to: (1) re-design the ARS Air Scrubber to improve NH₃ removal efficiency, (2) conduct full-scale testing of the scrubber under controlled conditions, (3) evaluate the efficacy of various acids for scrubbing NH₃, and (4) determine the effects of air flow rate and NH₃ concentration on scrubber performance. A full-scale prototype was constructed and a series of experiments were conducted under various conditions. Acid salts, such as aluminum sulfate (alum), sodium bisulfate, potassium bisulfate, ferric chloride and ferric sulfate were found to work as well as strong acids (hydrochloric, phosphoric and sulfuric) for capturing NH₃. The efficiency of the scrubber for capturing NH₃ decreased as the ventilation rate increased from over 90% at flow rates of 5,097 m³ h⁻¹ to around 55% at 16,141 m³ h⁻¹. However, the mass of N captured by the scrubber tripled as flow rates increased from 5,097 to 16,141 m³ h⁻¹. Similarly, there was a slight reduction in NH₃ removal efficiency as inflow NH₃ levels were increased from 10 to 75 μL L⁻¹, whereas the mass of N captured increased from 25 g N h⁻¹ to around 200 g N h⁻¹. This technology could result in the capture of a significant amount of the N lost from AFOs, while simultaneously reducing emissions of dust and odors, which would improve the social and environmental sustainability of poultry and swine production.

Keywords: ammonia, scrubber, poultry, emissions, air pollution, nitrogen, recycling

INTRODUCTION

During the past 20 years there has been increased scrutiny of atmospheric emissions from AFOs in the U.S. since the National Academy of Science reported that there is a urgent need to collect emission data from these facilities (National Academy of Science, 2002). One of the pollutants of greatest concern is NH_3 , with poultry manure reportedly contributing 27% of the total NH_3 emission in the U.S. (U.S. EPA, 2004).

Moore et al. (2011) evaluated NH_3 emissions from a typical broiler farm with four tunnel ventilated houses in NW Arkansas. They found that NH_3 emissions during the flock, between flocks, during storage and following land application were 28.3, 9.09, 0.18, and 7.91 g NH_3 bird⁻¹, respectively. The total NH_3 emission was 45.5 g bird⁻¹, with the majority (82%) of the emissions occurring while the litter was still in the barn. Hence, technologies that reduce in-house NH_3 should be utilized, when possible. Moore et al. (2011) found the total NH_3 emissions for a typical broiler farm with four houses reported was 15,571 kg N yr⁻¹, which was higher than the amount of N that remained in the house as litter or cake (14,464 kg N yr⁻¹). This not only represents a huge waste of an important natural resource since nitrogen (N) is generally the number one element limiting crop production, it also results in substantial air and water pollution.

Environmental problems linked to NH_3 emissions include soil acidification, formation of fine particulate matter, and N deposition into aquatic and terrestrial ecosystems (Hutchinson and Viets, 1969; van Breemen et al., 1982; Schroder, 1985; Sutton and Fowler, 2002; Behera and Sharma, 2010; Updyke et al., 2012). When NH_3 is deposited via wet or dry fallout, it is converted in the soil to nitrate (NO_3^-) via nitrification reactions, generating acidity in the process (van Breemen et al., 1982). In Holland it was estimated that 45% of the total acid deposition was caused by NH_3 in 1989 and 85% of the total ammonia emission originated from livestock farming (Groot Koerkamp et al., 1998). Ammonia has also been shown to react with NO_x and SO_2 to form the dominant fraction of particulate matter less than 2.5 μm ($\text{PM}_{2.5}$) in the atmosphere (Behera and Sharma, 2010; Updyke et al., 2012). Inhalation of such fine particulate matter can be very detrimental to human health (WHO, 2006). Ammonia deposition can also cause species changes in terrestrial environments (Sheppard et al., 2011).

One technology that has been used to control NH_3 emissions from animal rearing facilities in Europe is the use of acid scrubbers (Melse and Ogink, 2005; Melse et al., 2006, 2009a, 2012; Van der Heyden et al., 2015). With acid scrubbers exhaust air from animal barns is passed through a reactor that has been filled with an inert packing material that has a large porosity or void volume (Melse and Ogink, 2005). Acidified water is then sprayed on top of the reactor. Exhaust air from the animal barn enters the scrubber either horizontally (cross-current scrubber) or upwards (counter-current scrubbers). Mass transfer of ammonia and other compounds occurs from the gas to liquid phase, which is dependent on the concentration gradient, contact time of gas and liquid phase, and the size of the contact area between the gas and water phase (Melse and Ogink, 2005).

Normally the pH of the water in acid scrubbers is kept between 2 and 4 with the addition of acid. In Holland, acid scrubbers typically use sulfuric acid (Melse and Ogink, 2005), which results in the production of a solution that contains $(\text{NH}_4)_2\text{SO}_4$ (ammonium sulfate). Ammonia removal efficiencies reported in several studies of full-scale acid scrubbers in Holland varied from 90 to 99% (Vrielink et al., 1997; Hol and Satter, 1998; Hol et al., 1999; Verdoes and Zonderland, 1999; Wever and Groot Koerkamp, 1999, as cited by Melse and Ogink, 2005; Melse et al., 2012; Van der Heyden et al., 2015). Acid scrubbers also remove odors and dust from exhaust air, with Dutch scrubbers showing an odor removal efficiency of 29–34% (Melse and Ogink, 2005) and a PM-10 (particulate matter less than 10 μm , also called thoracic particles) removal efficiency ranging from 62 to 93% (Melse et al., 2009a).

Currently NH_3 scrubbers are not used on poultry or swine barns in the USA. Although the European scrubbers have exceptionally high NH_3 , dust and odor removal, they are very expensive; often as much as \$250,000 per house for the initial investment. Melse et al. (2009b) reported that acid scrubbers were not considered Best Available Technique (BAT) by the European Union, mainly because of the extremely high cost to build and run them, along with the amount of energy and acid needed, which were not considered sustainable. However, the recent BAT reference document for the intensive rearing of poultry or pigs does list acid scrubbers (Joint Research Centre, 2015). Ogink et al. (2008) indicated that acid scrubbers are mainly used in pig housings in Europe and stated that only a few examples of scrubbers on poultry houses were known. The high dust content of air in poultry houses leads to clogging in the packing of the scrubber, causing high pressure drops and an increase in energy use (Ogink et al., 2008).

During the past decade scientists with USDA/ARS in Fayetteville, AR have conducted scrubber research with the goal of developing an inexpensive (<\$10,000) acid scrubber that can handle heavy dust loadings, which are typical in air exhausted from broiler houses, without clogging (Angus et al., 2006; Moore et al., 2013). Another goal of this work was to develop a scrubber that is both simple and safe, so that any poultry or swine producer would be able to use the technology without outside technical assistance or hazardous material training. To achieve this goal, Moore (2007, 2014) developed and patented the use of acid salts, such as alum ($\text{Al}_2(\text{SO}_4)_3 \cdot 14\text{H}_2\text{O}$), potassium bisulfate (KHSO_4) and sodium bisulfate (NaHSO_4) which can be used as a replacement for strong acids, such as sulfuric acid, to acidify the water. Although one advantage of using acid salts for acid scrubbers is safety, another big advantage is their availability on poultry farms. At present, billions of broiler chickens are grown each year in the USA on litter that has been treated with acid salts, like alum (sold under the tradename Al^+ Clear) or sodium bisulfate (sold under the tradename PLT) in order to control NH_3 levels inside the houses (Moore et al., 1999, 2000; Blake and Hess, 2001; Moore, 2011).

Rather than having an expensive, computer-controlled acid delivery system, the grower would simply use a pH meter or litmus paper every day or two to determine whether the scrubber needs acid and manually add dry acid. Between flocks of birds

the scrubbers would be pumped out and the contents would be directly applied to crops on the farm. Martin et al. (2018) found that yields of forages fertilized with scrubber solutions obtained from acid salts like KHSO_4 or NaHSO_4 were higher than that observed when strong acids were used in the scrubber or when ammonium nitrate was applied at an equivalent N rate (112 kg N ha^{-1}).

The first version of this scrubber was a simple single stage unit that was constructed from wood (Angus et al., 2006). The only moving part in that version was a pump used to circulate the acid. There were multiple problems with this version; (1) high quantities of dust, which have high concentrations of calcium carbonate, would neutralize the acid needed to capture NH_3 , (2) during cold weather, the contents of the reservoir would freeze, (3) feathers in the exhaust air would clog the pump, and (4) within a year the scrubber begin to leak.

The objectives of this study were to: (1) re-design the ARS Air Scrubber to improve NH_3 removal efficiency, (2) conduct full-scale testing of the scrubber under controlled conditions at various ventilation rates, (3) evaluate the practicality and efficacy of various acids for scrubbing NH_3 and (4) determine the effects of air flow rate and NH_3 concentration entering the scrubber on scrubber performance.

MATERIALS AND METHODS

Scrubber Construction

A full-scale NH_3 scrubber was used in this research. The re-designed scrubber was comprised of two separate scrubbers; the first scrubber uses water to remove particulate matter (dust, bacteria, etc.) from the air, and the second scrubber uses a dilute acid solution to capture NH_3 and VOCs (Figures 1, 2). The dimensions of each box are approximately $1.5 \times 1.5 \times 1.5 \text{ m}$. The re-designed scrubber had shells made out of fiberglass. Each scrubber is equipped with a 360 L reservoir. A simple float switch is used to control the water level in both scrubbers. Both scrubbers are also equipped with acid tolerant heaters in the re-designed scrubber, which are made by Vulcan Industries, which have a 75 cm long heating element, which are totally immersed in the reservoirs. These heaters are programmed to turn on at 3.3°C to keep the contents in the reservoirs from freezing.

The dust scrubber can hold up to 8 rows of wooden slats at 45° angles (Figure 2). Wooden slats are used because wood is much cheaper than plastic or fiberglass and experience has shown that wooden slats have a long lifetime. An $\frac{1}{2}$ HP submersible pump is used to recirculate water in the dust scrubber (Little Giant, 6EN-CIA-SFS). Water from the pump is delivered into three 3.5 cm (OD) PVC pipes which form water curtains at the top of the dust scrubber (Figure 2). The pipes have a series of holes (0.1 cm diameter) from which the water is delivered. The design of this scrubber allows it to be utilized for several flocks of broilers without clogging and if clogs occur, then a long handled brush can be passed through the PVC pipe to remove clogs. The dust scrubber is also equipped with a removable screen above the reservoir to catch feathers and other large particulate matter, which should be cleaned after each flock.

The acid scrubber uses a $\frac{1}{3}$ HP magnetically driven pump, which can withstand extremely acidic conditions (IWAKI MK-100RCT), which can deliver acid at a rate of up to 53 L/min. The new design initially included plastic cool cell material (Polypropylene Humipacking made by GEA), which was enclosed in a stainless steel frame on the exhaust end of the scrubber. Although this Humipacking is an effective medium for acid scrubbing (it is used in many European scrubbers), it was found to clog with dust within 3–4 weeks when the scrubber was attached to fans on commercial broiler houses and is no longer being used. This occurred even though the dust scrubber was functioning, indicating small amounts of dust can clog that material.

The experiments reported in this paper were conducted in a USDA/ARS machine shop located at the poultry farm on the main University of Arkansas Agricultural Experiment Station in Fayetteville, AR. The dust and acid scrubbers were connected to a ChoretimeTM 122 cm variable speed poultry house fan via a plenum. This fan has a frequency driver that allows for variable flow rates and is capable of producing air flows up to $33,980 \text{ m}^3 \text{ h}^{-1}$ (20,000 cfm) at 0 Pa.

Airflow measurements in this study were made with a Fan Assessment Numeration System (FANS unit), which was attached to the variable speed fan. The FANS unit measures cumulative outflow of air from the fan throughout the cross-sectional area by measuring air velocities on a transect throughout the fan (Gates et al., 2001, 2004). The FANS unit used for this research was constructed by the University of Kentucky and calibrated by the BESS Laboratory (Dept. of Agri. and Biol. Eng., Univ. of Illinois, Champaign, IL). Static pressure was measured with a Setra 2601MS1 differential pressure sensor.

Effect of Slat Angle on Static Pressure and Air Flow

As part of the scrubber redesign, an experiment was conducted to determine the effect of the angle of the slats in the scrubber on static pressure and air flow. One row of 11 wooden slats (10.2 cm wide and 152.4 cm long) was placed in the dust scrubber only. The slats were all attached to a hinge in which the angle of the slats could simultaneously be varied from 0 to 90° . Air flow and static pressure was determined as described above at angles of 0° , 10° , 20° , 30° , 40° , 50° , 60° , 70° , 80° , and 90° with the variable speed fan set at 40 and 60 Hz.

Effect of Acid Type on Ammonia Scrubbing Efficiency

Ammonia removal efficiencies were measured with various acids at 60 and 40 Hz, which corresponded to air flows of roughly $13,592$ and $8,495 \text{ m}^3 \text{ h}^{-1}$ (8,000 and 5,000 cfm). During this experiment there were a total of five slats in the dust scrubber, while the acid scrubber was equipped with three slats and two cool cells. Anhydrous NH_3 was metered out into the air in front of the fan using a distribution manifold with five openings at a sufficient rate to result in $25 \mu\text{L L}^{-1}$ NH_3 entering the scrubber. Air flow rates during each run were measured with the FANS unit as before and pressure drop was measured using the



FIGURE 1 | Photograph of ARS Air Scrubber.

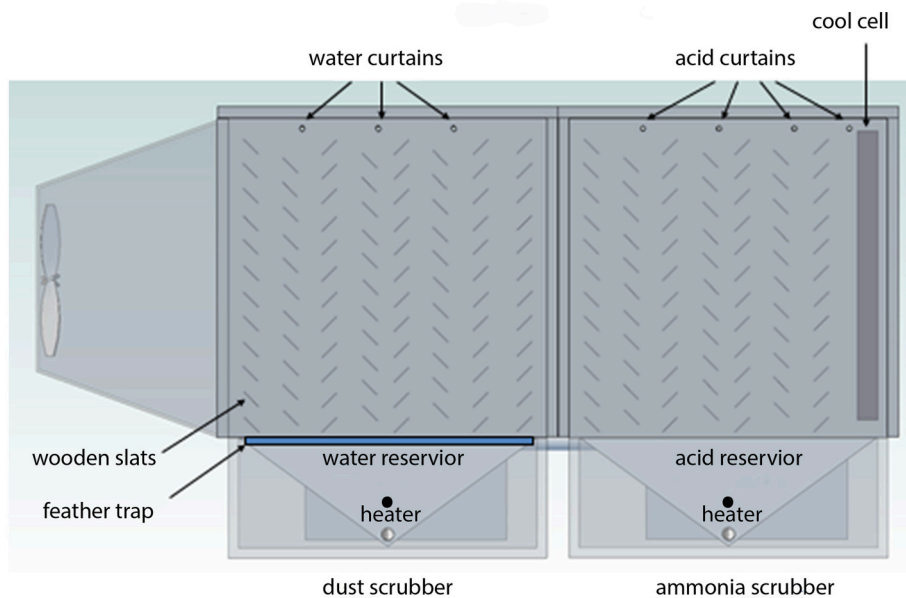


FIGURE 2 | Schematic diagram of the ARS Air Scrubber.

Setra 2601. Ammonia gas concentrations entering and exiting the scrubber were measured every 5 min during the 2 h trials using a photoacoustic multigas analyzer (Innova 1412; Ballerup, Denmark). A tank of $25 \mu\text{L NH}_3 \text{ L}^{-1}$ calibration gas (balance air) was used to test the Innova 1412 before and after each trial.

Three 2-h trials were conducted with solutions of the following chemicals at both 40 and 60 Hz: alum, aluminum chloride (AlCl_3), calcium chloride (CaCl_2), ferric sulfate ($\text{Fe}_2(\text{SO}_4)_3$), ferric chloride (FeCl_3), sodium bisulfate, potassium bisulfate (KHSO_4), sulfuric acid (H_2SO_4), hydrochloric acid

(HCl), phosphoric acid (H_3PO_4), nitric acid (HNO_3) and water. The amount of acid added using strong acids or dry acids was equivalent to two liters of concentrated sulfuric acid (acid equivalent determined by titration with sodium hydroxide). The amount of strong acids added (in ml) to the acid scrubber for H_2SO_4 , HCl, HNO_3 , and H_3PO_4 was 2,000, 5,946, 4,613 and 1,622 ml. This amount of acid was generally sufficient to run 4 of the 6 trials needed for each chemical; after the fourth trial the acid scrubber was cleaned out and fresh acid was added. The amount of dry acid (in grams) added for the same four trials with $\text{Al}_2(\text{SO}_4)_3 \cdot 14\text{H}_2\text{O}$, $\text{Fe}_2(\text{SO}_4)_3$, NaHSO_4 and KHSO_4 was 7,137, 6,744, 8,652, and 9,812. Although calcium chloride is a neutral salt, we wanted to investigate its effect; 3,999 g was used. The amount of acid added for liquid AlCl_3 and liquid FeCl_3 was 1,164 and 1,200 ml, respectively.

Scrubbing efficiency was calculated by measuring the concentration of NH_3 in the scrubber's inflow and exhaust air using the Innova 1412. In order to measure the mass accumulation of $\text{NH}_3 + \text{NH}_4\text{-N}$ in the scrubber solution, 20 ml aliquots of the scrubber solution were taken at time 0, 1, and 2 h from the acid scrubber and analyzed for ammonium colorimetrically with an auto-analyzer using the salicylate-nitroprusside technique (Method 351.2; U.S. EPA, 1979). The pH of these samples was also determined.

Effect of Air Flow Rate and Inlet Ammonia Concentration on Scrubber Performance

In this experiment the effects of varying air flow rate and NH_3 concentration entering the scrubber was evaluated. The number of slats in the dust scrubber was kept at 5 and the number of slats in the acid scrubber was kept at 3, but only one cool cell was used. Sodium bisulfate was used as the acid in the acid scrubber at the same levels as above. Ammonia reduction was evaluated at four fan speeds (10, 20, 40, and 60 Hz) which corresponded to air flow rates of 2,131, 4,557, 10,367, and 15,981 m^3/h . Three 2-h trials were conducted at each fan speed. Anhydrous NH_3 was metered out in front of the fan at sufficient quantities to result in inflow NH_3 levels of 25 $\mu\text{L L}^{-1}$. Ammonia concentrations at the inflow and outflow of the scrubber were measured every 5 min as before. Likewise, the concentration of $\text{NH}_3 + \text{NH}_4$ in the scrubber reservoirs, air flow and pressure drop were measured as described above.

The effect of inflow NH_3 concentration on scrubber performance was evaluated using the identical scrubber configuration as the air flow experiment. The NH_3 levels evaluated in this study were 10, 25, 50, and 75 $\mu\text{L L}^{-1}$. The desired inflow NH_3 levels were maintained by varying the amount of anhydrous NH_3 , as before. Three 2-h trials were conducted at each of the four NH_3 levels at two fan speeds (10,367, and 15,981 m^3/h). Inflow and outflow NH_3 levels were measured with the Innova 1,412 every 5 min. Concentrations of $\text{NH}_3 + \text{NH}_4$ in the reservoirs, air flow, and pressure drop were measured as described earlier.

Statistical analyses of the data were performed using the PROC GLM of SAS Institute (1990). Significant differences

among treatment means were determined using Duncan's new multiple-range test at the $P < 0.05$ level.

RESULTS AND DISCUSSION

Effect of Slat Angle on Static Pressure and Air Flow

Preliminary research on the effect of slat angle on static pressure (SP) showed the SP increased exponentially as the slat angle increased (Figure 3). The greatest increase in SP occurred when the angle was greater than 45° . Air flow was reduced as slat angle increased, with the greatest reduction observed at angles greater than 45° . At 60 Hz air flow varied from 34,362 to 17,367 $\text{m}^3 \text{h}^{-1}$ when slat angles varied from 0 to 90° , whereas at 40 Hz air flow varied from 2,250 to 11,004 $\text{m}^3 \text{h}^{-1}$ when angles from 0 to 90° . Therefore it was decided to minimize pressure drop while maximizing the possibility of particles colliding with a wet surface that 45° the best angle would be the best angle to use.

Effect of Various Acids and Water on Ammonia Removal

Ammonia concentrations in the intake and exhaust of the scrubber when NaHSO_4 was used are shown in Figure 4. Typically NH_3 removal efficiency ($(\text{NH}_3 \text{ inflow} - \text{NH}_3 \text{ outflow})/\text{NH}_3 \text{ inflow}$) was slightly better when the variable speed fan was set on 40 Hz (72%), compared to 60 Hz (70%), due to lower air flow rates at 40 Hz (10,367 $\text{m}^3 \text{h}^{-1}$) than at 60 Hz (15,981 $\text{m}^3 \text{h}^{-1}$).

Ammonia removal efficiency for tap water was much lower than NaHSO_4 or the other acids, as would be expected, although during the first 2 h there was surprisingly good NH_3 removal (Figure 5). These data indicate that the scrubber could be operated without acid, if the residence time of the water in the chemical scrubber was kept to around an hour or less. In other words, a system could be devised in which freshwater was constantly trickled into the scrubber before being discharged into an irrigation reservoir. If the NH_3 concentration of the air exhausted from the poultry barn was around 25 ppm (average annual concentration found by Moore et al., 2011), then the N concentration of that water would be increased to 50–100 mg N/L, which could help meet the N requirements of any crop being irrigated. A system using water like this may be the most sustainable scrubbing system since water is much cheaper than acid. However, to be cost-effective it would only be feasible on farms which are already growing crops that are irrigated. Other issues may also arise. In Arkansas most liquid waste management systems associated with AFO's fall under Regulation 5, which requires a permit for the operation (VanDevender, 2008). If this is the case, it is unlikely poultry producers would be willing to utilize the system, due to an increased burden of reporting requirements. However, it is unclear whether such a system would cause a broiler farm to all under this regulation.

The concentration of NH_3 in the scrubber after an 8 h period with NaHSO_4 in the acid scrubber was over 2,000 mg N/L, whereas when water was used in the acid scrubber the concentration was lower by an order of magnitude (Figure 6).

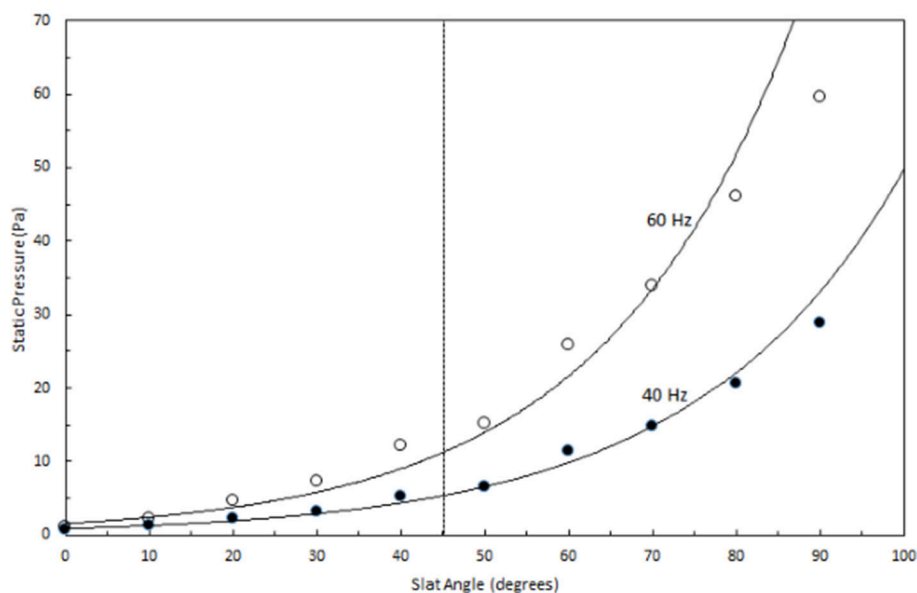


FIGURE 3 | Effect of slat angle on static pressure when one row of slats was placed in the dust scrubber only.

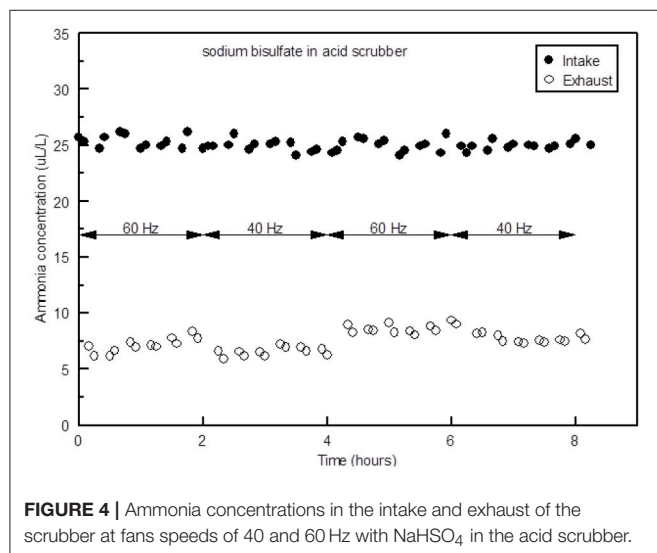


FIGURE 4 | Ammonia concentrations in the intake and exhaust of the scrubber at fans speeds of 40 and 60 Hz with NaHSO_4 in the acid scrubber.

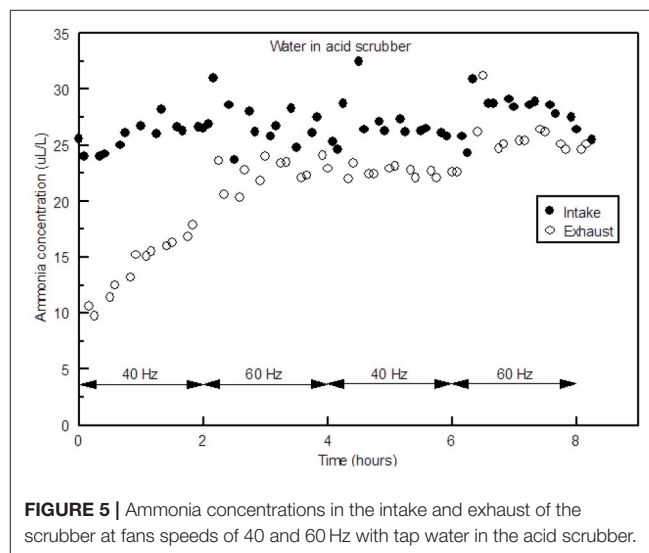


FIGURE 5 | Ammonia concentrations in the intake and exhaust of the scrubber at fans speeds of 40 and 60 Hz with tap water in the acid scrubber.

This difference in NH_3 removal is due to the difference in pH. The pH of tap water started out around 8.4 and increased to over 9.5 after 1 h, whereas when NaHSO_4 was added the pH started out at around 1.1 and increased to around 2.5 after hours (Figure 7). When the pH of a scrubbing solution is acidic, NH_3 in solution is converted to NH_4^+ , which can build up in solution, whereas under very alkaline pH conditions the ammoniacal N stays in the NH_3 form and the amount in solution is limited by the solubility of NH_3 in water.

The effectiveness of the various compounds tested is shown in Table 1. The two acid salts of Fe ($\text{Fe}_2(\text{SO}_4)_3$ and FeCl_3) resulted in slightly better reductions in NH_3 levels in the exhaust than the

other acids. Iron compounds resulted in the formation of iron oxides or hydroxides on the scrubber, which may have increased the surface area. Likewise, the Al compounds formed aluminum oxides or hydroxides on scrubber surfaces. Potassium bisulfate worked as well as the Fe and Al compounds at high air flow rates maintained at 60 Hz ($15,981 \text{ m}^3 \text{ h}^{-1}$) and as well as the Al compounds and FeCl_3 at $10,367 \text{ m}^3 \text{ h}^{-1}$ (40 Hz). Potassium bisulfate performed slightly better than NaHSO_4 at $15,981 \text{ m}^3 \text{ h}^{-1}$ (60 Hz). Potassium bisulfate also performed better than HCl , HNO_3 , and H_3PO_4 .

The amount of N captured in the scrubber with various compounds is shown Table 1. At $15,981 \text{ m}^3 \text{ h}^{-1}$ (60 Hz) KHSO_4

performed as well as all of the strong acids and most of the acid salts, with the exception of AlCl_3 , whereas at $10,367 \text{ m}^3 \text{ h}^{-1}$ (40 Hz) the Al and Fe compounds outperformed KHSO_4 . Although AlCl_3 performed well with respect to capturing NH_3 it formed a gelatinous white mass in the scrubber reservoirs. This did not occur with alum.

From an agronomic point of view, KHSO_4 would probably be the most sustainable acid salt for this purpose, since its use would result in the formation of an ammonium potassium sulfate fertilizer in the scrubber. In a study evaluating the fertilizer effectiveness of various scrubber solutions obtained from ARS Air Scrubbers attached to fans on a commercial broiler farm, Martin et al. (2018) found forage yields were higher when fertilized with scrubber solutions from KHSO_4 and NaHSO_4 than other scrubber solutions or ammonium nitrate applied at an equivalent N rate (112 kg N ha^{-1}). However, at present KHSO_4 is

not produced in North America on an industrial scale, therefore the price is much higher than the other acids.

Effects of Air Flow and Ammonia Concentration on Scrubber Efficiency

As ventilation increased from $2,000$ to $10,000 \text{ m}^3 \text{ h}^{-1}$, static pressure increased from 0 to 30 Pa (Figure 8). This is similar to the 27.5 Pa backpressure reported by Manuzon et al. (2007) for a three stage wet scrubber. As mentioned earlier, the scrubber described in the current study was designed for use on minimum vents, which typically operate at $10,194 \text{ m}^3 \text{ h}^{-1}$ or less. Under such ventilation rates, the static pressure should always be less than 37.4 Pa ($0.15''$ of water), which is far better than pressure drop (74.7 Pa) that is allowed by the USDA/NRCS Conservation Practice Standard 371 for air scrubbers (USDA/NRCS, 2010).

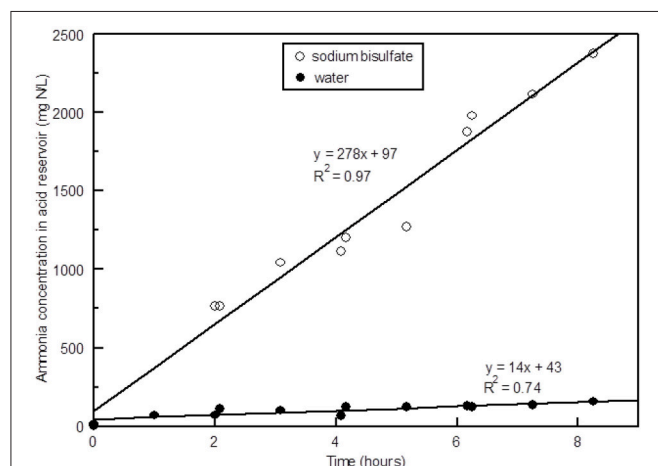


FIGURE 6 | Ammonia (plus ammonium) concentration in the acid scrubber vs. time with NaHSO_4 vs. tap water.

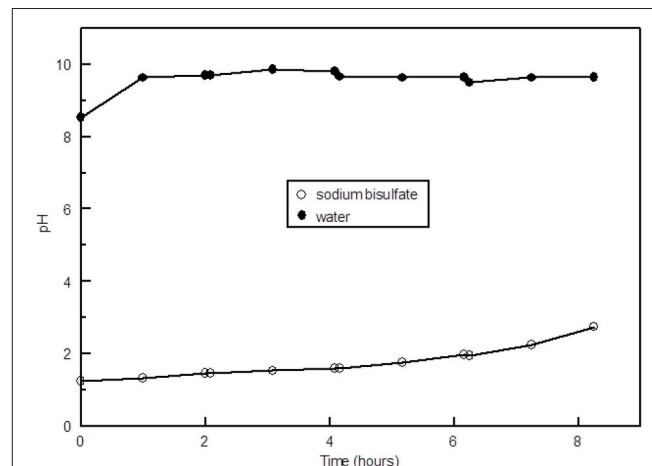


FIGURE 7 | Solution pH vs. time when NaHSO_4 and tap water were used to scrubber NH_3 .

TABLE 1 | Relative NH_3 reduction efficiency and rate of N captured for strong acids, acid salts, calcium chloride and water at two ventilation rates.

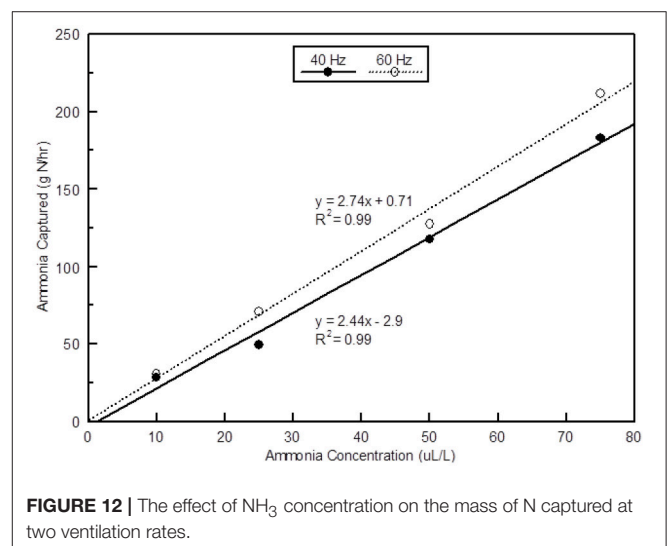
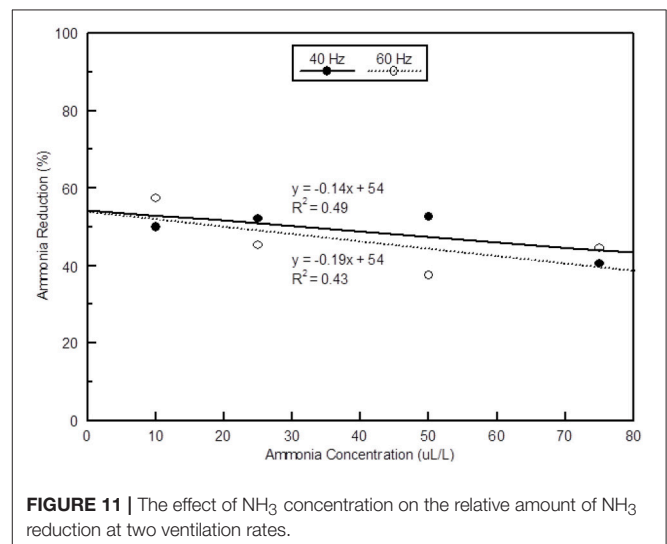
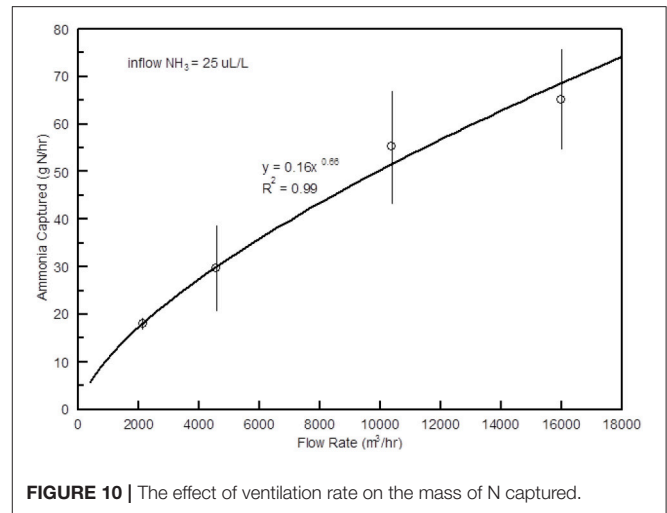
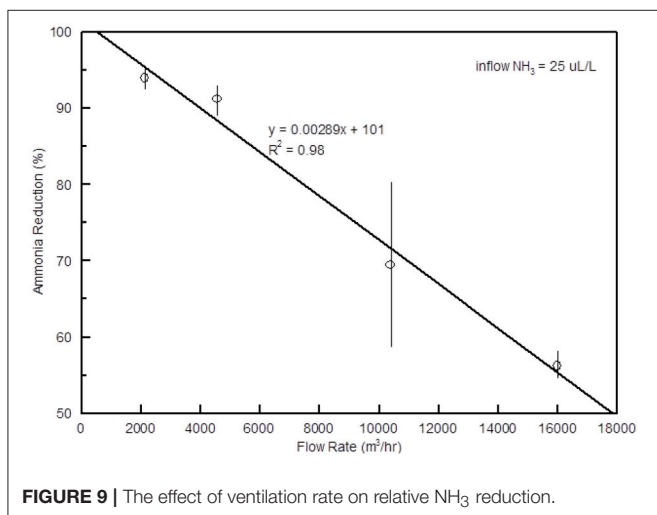
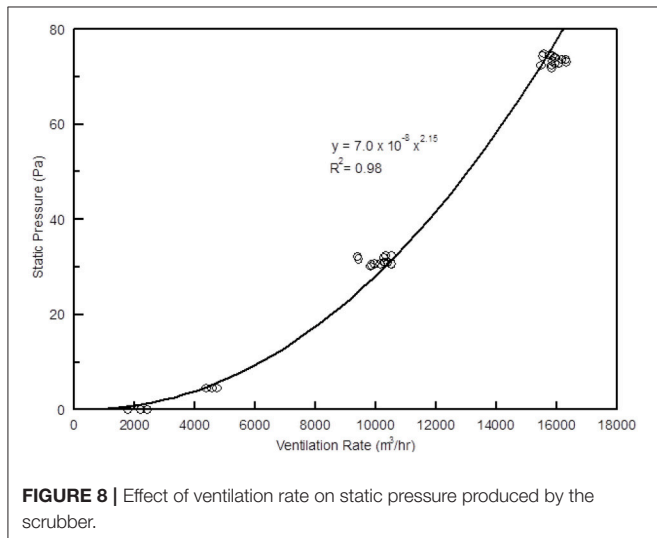
	% NH_3 reduction at 40 Hz	% NH_3 reduction at 60 Hz	Nitrogen captured At 40 Hz (g N h^{-1})	Nitrogen captured At 60 Hz (g N h^{-1})
Sulfuric acid	80 BC	74 BCD	159 ABCD	215 DE
Hydrochloric acid	82 ABC	69 CDE	125 E	154 F
Nitric acid	77 C	67 DE	135 CDE	204 E
Phosphoric acid	72 C	65 E	142 BCDE	244 BCDE
Sodium bisulfate	72 C	70 CDE	133 DE	226 CDE
Potassium bisulfate	81 BC	75 BC	127 E	245 BCDE
Aluminum sulfate	80 BC	72 EDE	177 A	261 ABC
Calcium chloride	13 D	0.5 G	16 F	17 G
Ferric chloride	89 AB	90 A	181 A	287 AB
Aluminum chloride	81 ABC	81 B	166 ABC	298 A
Ferric sulfate	91 A	91 A	170 AB	256 ABCD
Water	23 D	9 F	10 F	—4 G

Values represent the means of three 2-h runs. Means followed by a different letter are significantly different ($p < 0.05$). Fan speeds of 40 and 60 Hz resulted in air flow rates of $10,367$ and $15,981 \text{ m}^3 \text{ h}^{-1}$, respectively.

The efficiency of the scrubber was over 90% when air flow rates were $4,000 \text{ m}^3 \text{ h}^{-1}$ or less with an intake NH_3 concentration of $25 \text{ } \mu\text{L L}^{-1}$, but dropped linearly as the flow rate increased (**Figure 9**). At $10,000 \text{ m}^3 \text{ h}^{-1}$ the average NH_3 reduction was around 70%. Although the efficiency decreased as the flow rate increased, the amount of N captured increased from less than 20 g N h^{-1} at lowest flow rate to near 60 g N h^{-1} at the highest (**Figure 10**).

There was a slight reduction in NH_3 removal efficiency when the intake NH_3 concentration was increased from 10 to $75 \text{ } \mu\text{L L}^{-1}$ (**Figure 11**). The scrubbing efficiencies reported here are similar to the two stage scrubber tested by Manuzon et al. (2007) that had a 64–70% efficiency at NH_3 concentrations of 100 and $30 \text{ } \mu\text{L L}^{-1}$, respectively.

The mass of N captured increased linearly from around 25 g N h^{-1} at $10 \text{ } \mu\text{L L}^{-1}$ to approximately 200 g N h^{-1} at $75 \text{ } \mu\text{L L}^{-1}$ (**Figure 12**). As mentioned earlier, Moore et al. (2011) found average NH_3 levels in poultry houses were much higher during cooler months ($42.5 \text{ } \mu\text{L L}^{-1}$) than in the summer ($11.1 \text{ } \mu\text{L L}^{-1}$),



because during warmer periods of the year the ventilation in the houses is greatly increased to relieve heat stress from the birds. These data indicate that these scrubbers will capture the greatest amount of N during the cooler months of the year when NH₃ concentrations are highest.

Although the ARS Air Scrubber is not nearly as effective as European scrubbers, which have NH₃ removal efficiencies of up to 95%, they can be manufactured for a small fraction of the cost and they will operate under very dusty conditions. However, these scrubbers were designed for minimum vent fans on broiler house, in order to capture the most N per dollar spent (Melse et al., 2006); therefore when birds are older and tunnel ventilation is being used, only a small percentage of the total air volume will be scrubbed.

CONCLUSIONS

A full-scale prototype of the ARS Air Scrubber was constructed and a series of experiments were conducted to evaluate its ability to capture NH₃. Research on the effects of ventilation

rate and NH₃ concentration indicated that as either variable was increased, the efficiency of NH₃ removal decreased somewhat, whereas the mass of N captured increased dramatically. As NH₃ concentrations increased from 10 to 75 $\mu\text{L L}^{-1}$ the amount of N captured increased by almost an order of magnitude (25–200 g N h⁻¹), indicating it is most efficient when NH₃ levels are high, which is typical during cooler months. This technology could potentially capture a significant fraction of N currently lost from poultry and swine facilities as NH₃, which may improve the sustainability of these operations.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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Recovery of Nutrients From Wastewaters Using Microalgae

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In this chapter the relevance of microalgae-based processes for the recovery of nutrients contained in wastewaters is reviewed. The fundamentals of the process are discussed from the biological and engineering standpoints and it is shown that on this type of processes the nutrient recovery capacity is mainly a function of solar radiation availability. If adequately designed and operated up to 450 tCO₂, 25 tN, and 2.5 tP per hectare and per year can be fixed, producing up to 200 t/year of valuable microalgae biomass. The utilization of microalgae-based processes reduces to half the energy consumption of conventional wastewater treatment and allows recovering up to 90% of the nutrients contained into wastewater. Still the technology available (photobioreactors, harvesting, downstream) must be improved to reduce the land requirement and the hydraulic retention time, but current technology is ready to be demonstrated at large scale, so that the first initial facilities based on this technology have been recently developed. Moreover, this technology must be adapted to the different wastewater types, from sewage to manure. The major advantage of microalgae-based processes is the production of large amounts of valuable biomass, useful for the production of biofuels but much more interesting for animal feeding and agriculture uses, thus enhancing the productivity and sustainability of foods production.

Keywords: microalgae, nutrients recovery, wastewater treatment, biomass, bioenergy, biofertilizers

INTRODUCTION

Microalgae have been proposed for a wide range of applications, such as the production of pharmaceuticals and nutraceuticals, the production of biofuels, and depuration processes to carry out the treatment of effluents as flue gases and wastewater (Spolaore et al., 2006; Acién Fernández et al., 2012b). The reasons why microalgae are considered fit for such different applications include (i) its high growth rate, over 1 day⁻¹, (ii) being capable to growth in widely different environments, from warm areas such as the tropic or deserts to cold areas in high mountains and poles, (iii) their valuable biochemical composition, as its biomass is rich in proteins and lipids among others valuable compounds, and (iv) they do not require fertile land or usable water, growing even in wastewaters (Chisti, 2012). When using wastewaters microalgae allows recovering the nutrients contained in those (Olguín, 2012).

Microalgae are photoautotrophic microorganisms, so they use solar energy to reduce inorganic nutrients to organic matter thus producing biomass. Reported biomass productivity of microalgae cultures varies as a function of reactor technology, microalgae strain and environmental conditions, but values ranging from 40 to 150 tn/ha-year (dry matter) have been reported (Benemann, 2003; Chisti, 2012). However, to achieve high productivity values large amounts of nutrients are required.

For instance, to produce 100 tn of microalgae biomass up to 200 of CO₂, 10 tn of N, and 1 tn of P are consumed. Usually CO₂ is supplied as pure food grade CO₂, whereas N is supplied as nitrate salts, and P is supplied as phosphate salts. To supply these nutrients chemical fertilizers are usually utilized. Those are based on fossil energy and have a minimum production cost of around 0.5 €/kg. To reduce this production cost the utilization of flue gases and wastewater as nutrients source is mandatory (Acién et al., 2012a). Moreover, the recovery of nutrients from wastewaters produces an economic benefit that make the process really profitable.

Production of microalgae coupled to the treatment of wastewater has been widely reported, including for largely different effluents as sewage, centrate, manure, etc. (Olguín, 2012; Craggs et al., 2013; Hernández et al., 2013). According to the composition of the effluent the overall process must be adequately designed and operated. Producing microalgae on these effluents allows recovering the N and P contained on these effluents as valuable biomass, thus up to 1 kg of dry microalgae biomass can be produced per m³ of sewage, whereas more than 10 kg of dry microalgae biomass can be produced per m³ of manure. That is the reason why microalgae have been also proposed as a nutrient recovery technology. In this case the nutrient recovery capacity is limited by the microalgae biomass production, the higher the biomass productivity the larger the nutrients recovery capacity, with maximum theoretical values of 10 tN/ha-year and 2 tP/ha-year reported as possible (Acién et al., 2016).

In this chapter the capacity of microalgae to contribute to nutrients recovery processes is revised, in addition to the technologies currently used, the application of these processes to different effluents and finally the potential uses of the released biomass. This is a fast growing field on which numerous research groups and companies are now involved, due to the high potential of microalgae based processes and interesting applications of produced biomass. This field is directly related with the bioeconomy sector and the concept of circular economy, because the aim is to recover and recycle nutrients from wastewaters to make human activities more sustainable, especially wastewater treatment and foods production if the produced biomass is used as biofertilizer or for animal feeding.

RECOVERING NUTRIENTS FROM WASTEWATER USING MICROALGAE

Fundamental of Microalgae/Bacteria Consortia

As microalgae can recover nutrients from wastewaters they have been proposed for wastewater treatment as alternative to conventional technologies such as those based on activated sludge. However, when considering the utilization of microalgae for wastewater treatment it must be bore in mind that what there exists is actually a consortium of microalgae and bacteria (Muñoz et al., 2006). As in these systems no sterile conditions are possible, the consortium that finally prevail in the reactors will be that naturally occurring as a function of the wastewater

composition, environmental conditions, reactor design, and operation conditions. Regardless the biological composition of the consortium a general pattern is assumed (**Figure 1**). Thus, bacteria existing in the culture oxidize the organic matter to inorganic compounds, consuming oxygen in this step, whereas microalgae use the light to uptake the inorganic nutrients that have been released by the bacteria and produce biomass, during this step releasing in turn the oxygen required by the bacteria for the oxidizing step (Muñoz et al., 2006). According to this scheme a “natural” equilibrium between microalgae in bacteria establishes whatever the conditions on the reactor. However, the composition of the consortium in this equilibrium can differ widely depending on the conditions prevailing in the reactor. Thus the relative composition of the consortium determines the relative relevance of the phenomena (oxygen production, CO₂ consumption, nitrogen and phosphorous fixation, etc.), finally occurring in this type of processes (García et al., 2017).

The performance of microalgae-based wastewater treatment processes is mainly a function of light availability in the reactor, moreover it is mainly a function of solar radiation availability (a function of the location, date, and solar hour) and culture depth. Due to the presence of biomass in the culture the light impinging on the reactor surface is attenuated along the culture depth, the average irradiance at which the cells are exposed the culture thus becomes a function of culture depth (Molina Grima et al., 1996). The relevance of this parameter the performance of microalgae-based wastewater treatment has been discussed at great length. General agreement exists about that fixation of nutrients by microalgae, mainly nitrogen and phosphorous, is a direct function of average irradiance. If this is so, then the shorter the culture depth the higher the average irradiance is and thus the higher the nitrogen and phosphorous fixation rate (Olguín, 2012; Cabanelas et al., 2013a; Posadas et al., 2014; Acién et al., 2016). On the other hand, the shorter the culture depth the smaller the volume of the reactor is and this decreases the amount of wastewater that can be treated per reactor surface unit. Thus, an optimum culture depth that balances both bactors must be defined.

Optimizing the water depth in wastewater treatment processes using microalgae is not easy because it is a function of major objective of the facility, such as to produce high quality biomass or to treat larger volume of wastewater as possible. Thus, to maximize the recovery of nutrients and the production of microalgae biomass the culture depth must be short, <0.2 m, whereas to alternatively to maximize the capacity of wastewater treatment the culture depth must be large, more than 0.3 m. It must be highlighted that in an extreme scenario of large culture depth, the light availability could be enough to produce enough oxygen for the oxidation of the organic matter by the bacteria, but in these conditions it is possible that not enough light is available for the microalgae to take up the released inorganic compounds, then nitrification and denitrification would take place in large extension (Sutherland et al., 2014). Concluding, at these extreme conditions nitrogen and phosphorous would be not recovered but released to the atmosphere (N) or precipitated (P) with the sludge, thus approximating to the phenomena taking place in conventional activated sludge systems (Morales-Amaral

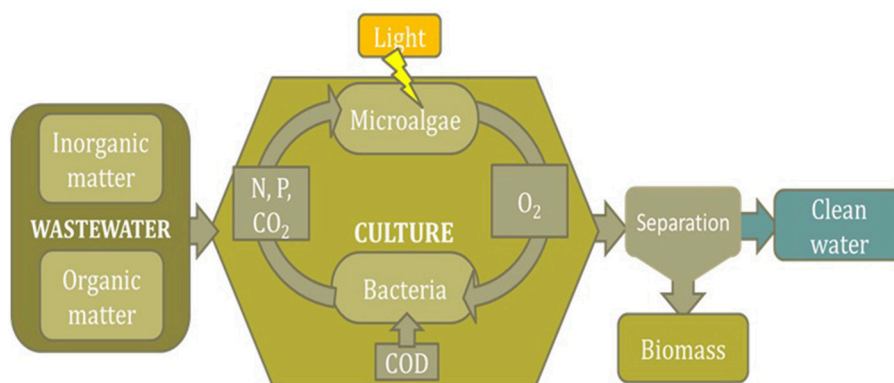


FIGURE 1 | Scheme of the main biological phenomena taking place when using microalgae/bacteria consortia for nutrients recovery from wastewaters.

et al., 2015a). To maximize the efficient recovery of nutrients it is mandatory to reduce the culture depth below 0.2 m. The shorter the culture depth the higher the proportion of nutrients finally fixed as valuable microalgae biomass.

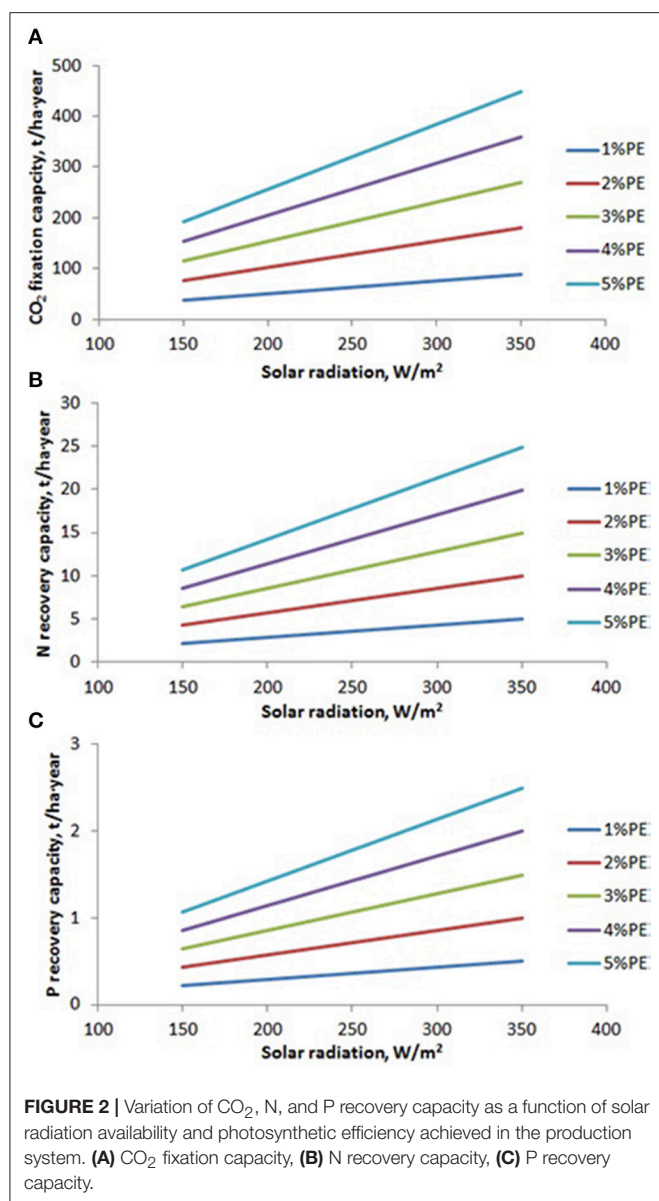
Capacity of Microalgae to Recover Nutrients

The coupling of wastewater treatment and microalgae production was initially proposed as alternative for reducing the microalgae production cost, but today it is considered as an alternative to the utilization of conventional wastewater treatment systems. The main reason for this is that the utilization of microalgae allows recovering nutrients carried by the wastewater, while minimizing the emissions of greenhouse gases and saving energy (Olguín, 2012; Craggs et al., 2013). Conventional technologies require expending large amounts of energy, up to 0.5 kWh/m³, that costs up to 0.2 €/m³ to remove the contaminants from the wastewater and to release clean water. In addition, the treatment cost can be higher if advanced oxidation processes for the removal of nitrogen as Anammox and others are applied, up to 5–8 €/kg (Aqualia, personal communication). Most of the compounds contained into the wastewater are then released to the atmosphere, carbon as CO₂, and nitrogen as N₂, whereas P is precipitated and usually removed with digested sludge. Thus, in the case of FCC Aqualia, one of the largest wastewater treatment company in Europe, this company annually treat up to 500 Mm³ of wastewater, from this treatment dissipating more than 1,000 kt/year of CO₂, 25 kt/year of nitrogen, 5 kt/year of phosphorous. These amounts are largely relevant as shows the fact that they are equivalent to 11 and 26% of the overall nitrogen and phosphorous based fertilizers produced by Fertiberia, the largest fertilizer-producing company in Spain. If the treatment of this large amount of wastewater were done using microalgae it would be possible to produce up to 500 kt/year of microalgae biomass, avoiding the emission of the corresponding amount of CO₂ and other greenhouse gases produced in conventional wastewater treatment plants. Moreover, processes based on microalgae requires much less energy, as little as 0.2 kWh/m³, saving more than a half of the

energy currently spent in conventional wastewater treatment processes.

The capacity of microalgae to growth and to produce biomass is a function of solar radiation availability and photosynthetic efficiency. Daily solar radiation is mainly a function of location and day of the year, ranging from 150 to 350 W/m². Regarding the photosynthetic efficiency, it is a function of how close the culture conditions (temperature, pH, etc.) are to the optimum required by the strain used. Photosynthetic efficiency values reported ranged from maximum of 5% achieved in optimized reactors, normally operated in perfect controlled conditions at laboratory or pilot scale, to minimum of 1% achieved in open reactors and large facilities where the control of culture conditions is very difficult (Tredici et al., 1997; Tredici, 2010). On the basis of solar radiation availability and photosynthetic efficiency, and considering the consumption of nutrients by microalgae per unit mass produced, the nutrients recovery capacity by microalgae-based processes can be estimated (Figure 2). Results shows how the CO₂ fixation capacity ranges from 40 to 200 t/ha-year in locations with low solar radiation, and from 70 to 450 t/ha-year in tropical areas, when the photosynthetic efficiency modifies from 1 to 5%. Regarding N recovery, it ranges from 2 to 10 t/ha-year in locations with low solar radiation, and from 5 to 25 t/ha-year in tropical areas, when the photosynthetic efficiency modifies from 1 to 5%. On the same way, the P recovery range from 0.2 to 1.1 t/ha-year in locations with low solar radiation, and from 0.5 to 2.5 t/ha-year in tropical areas, when the photosynthetic efficiency modifies from 1 to 5% (Figure 2). These are the limit values of nutrients recovery capacity when using microalgae bases processes, and although this has been reported at laboratory and pilot scale, real facilities must still be developed to demonstrate the feasibility of this technology at commercial scale (Posadas et al., 2013; Ledda et al., 2015; Morales-Amaral et al., 2015b; Sepúlveda et al., 2015).

Nutrients recovered by microalgae-based processes can partially replace the production of synthetic N and P based fertilizers. Thus, soluble forms of N and P are produced at large scale worldwide because they are the pillars underpinning food production by agriculture. Regarding nitrogen, the actual production systems use atmospheric nitrogen and large amounts



of microalgae-based treatment processes is a key issue in this field (Acién et al., 2016).

Bottlenecks of Microalgae Based Processes for Nutrients Recovery

In spite of the great advantages of microalgae-based processes for recovering nutrients from wastewater, this technology still has not been extensively used, only a few examples are in operation worldwide. The reason for this is the limited performance of the technology currently available. The microalgae-based wastewater treatment technology still must be improved. The actual technology for microalgae culturing requires long hydraulic residence times, of up to 7–10 days, and extensive surfaces, up to 10 m² per equivalent person (Matamoros et al., 2015). To make this technology more suitable for wastewater depuration at industrial scale these parameters need to be largely optimized, decreasing the hydraulic retention time to <1 day and the required surface to 1 m² per equivalent person. Due to the relevance of wastewater treatment field, large companies focus their efforts on this challenge. In this sense, FCC Aqualia recently inaugurated the first demonstration facility of wastewater treatment based on microalgae using optimized technology requiring <2 days of hydraulic retention time and 2 m² per equivalent person. This facility of 10 ha will be able to process the wastewater from the town of Chiclana in the South of Spain and has been supported by the EU Commission through the ALLGAS project (FCC Aqualia, 2018). Other relevant projects on this field includes SABANA project (www.eu-sabana.eu) focused into the production of biofertilizers and Aquafeed from wastewater using seawater, or AlgaeBioGas project (www.algaebiogas.eu) focused into the treatment of biogas digestate using microalgae. In spite of this successful examples, there is still room for improvement of the technology, and specially it must be demonstrated in different locations and using wastewaters with different compositions. The technology used in this type of processes and the influence of wastewater composition into the performance of the system are analyzed next.

BIOREACTORS AND OVERALL PROCESS

The recovery of nutrients by using microalgae is a process that must be carefully planned and performed. The main steps involved in a microalgae-based process for the recovery of nutrients are: (i) pre-treatment of effluent, (ii) recovery of nutrients and production of biomass in the photobioreactor, (iii) harvesting of biomass, (iv) treatment of used water for recirculation or disposal, and (v) transformation of the biomass into end products. Pre-treatment required is similar to that performed in conventional wastewater treatment plants, so it is necessary to carry out filtration steps in order to minimize the total solids content and maximize the transparency of the wastewater. The photobioreactor and the harvesting are the most relevant steps into the process. Treatment of used water is habitually not necessary if the harvesting step is enough efficient in the removal of solids. Moreover, as microalgae processes remove pathogens and micro-contaminants quite

of energy (10–15 kWh/kgN) to transform it into ammonia and nitrate by the Haber process, which makes this process a large contributor to the global warming effect. Regarding phosphorous, the reservoirs of this nutrient are limited and some reports have been warning for some time about a crash of actual food production system based on phosphorous before the end of this century (Cordell et al., 2009). The process of transforming P-rich rocks into fertilizers also needs huge amounts of energy (5–10 kWh/kgP). To avoid these problems it is necessary to enforce the recovery of nitrogen and phosphorous from wastes and residual streams, and microalgae can naturally carry out these processes (Craggs et al., 1996). Thus microalgae are capable to completely remove N and P contained in wastewater streams, using only using solar energy in the process and producing valuable biomass at the same time. The development

efficiently, no additional UV or ozone treatment are necessary for water disposal or reuse (Matamoros et al., 2015). Finally, the downstream process will be a function of the final uses of the biomass, always related to human uses. The relevance of photobioreactor and harvesting technologies are discussed next.

Bioreactors

The core of the process is the photobioreactor in which the microalgae biomass is produced. Many bibliographic references are already available about the design and operation of various photobioreactors but for wastewater treatment and nutrients recovery mainly open raceway reactors are used (Posten, 2009; Lundquist et al., 2010; Craggs et al., 2012).

Open raceway reactors consist of a low depth carrousel in which the water is recirculated mainly using paddlewheels (Figure 3). They are basically large water reservoirs with low depth to facilitate light penetration and thus increase the biomass productivity. Details about the construction of this type of reactors has been extensively published, although it is still a “hot research field” due to the relevance of this technology not only for wastewater treatment, but also for other microalgae-based processes. Thus, raceway reactors are the most extended microalgae production technology with more than 90% of total worldwide microalgae production being done in those systems. The performance of open raceways for the recovery of nutrients from wastewater has been confirmed but it can be further improved. Major efforts on this field are focused on the reduction of energy consumption by optimizing its fluid-dynamics (Sompech et al., 2012; Chiaramonti et al., 2013; Mendoza et al., 2013a), the improvement of mass transfer capacity (Li et al., 2013; Mendoza et al., 2013b; de Godos et al., 2014), and development of overall performance models (Fernández et al., 2016; Huesemann et al., 2016; Solimeno et al., 2017).

The most relevant facts about these reactors are that the energy consumption must be lower than 10 W/m^3 , and that the culture depth can be modified from 0.4 to 0.1 m. When using for wastewater treatment the energy consumption must be lower as possible, always lower than 5 W/m^3 , and the culture

depth ranges from 0.2 to 0.4 m. Recently FCC Aqualia patented a new design of ©LEAR reactor consuming $<2 \text{ W/m}^3$, which is a really low energy requirement (Figure 3). The main advantages of the raceway reactors are its low cost, below 10 €/m^2 , and its scale up potential. Currently, single units of up to $5,000 \text{ m}^2$ are been used at commercial scale. Another advantage of this technology is its low energy consumption, that makes them particularly suitable for the production of biofuels (Chisti, 2013). The main disadvantages of raceway reactors are related with the poor control of culture conditions and low productivity. The size of a single open raceway reactor is up to $5,000 \text{ m}^2$, but large facilities up to 20 ha exist that have been built by installing multiple reactors.

Harvesting of Biomass

Harvesting of microalgae biomass is a critical step in every microalgae based-process. The main reasons for that are that microalgae cells are small in size ($2\text{--}20 \mu\text{m}$), have a density similar to water and their concentration in the cultures is rather low, ranging from 0.5 to 3.0 g/L . Thus, recovering efficiently and completely the biomass from large culture volumes is a challenge. It has been reported that separation of the biomass from microalgae cultures can sum up to 30% of the total biomass production cost (Grima et al., 2005). When recovering nutrients from wastewaters only low energy-demanding and low-cost technologies can be considered. Fortunately, the wastewater treatment industry has ample experience in the removal of low concentration small solids from large volumes of water and thus the technologies from the wastewater treatment sector are recommendable.

In conventional microalgae-based processes centrifugation is the most extended technology for the recovery of biomass from microalgae cultures. The is technology already available for this application, including some especial equipment being developed from companies as GEA Westfalia. Using conventional self-discharge centrifuges the energy consumption approximates to 1 kWh/m^3 , whereas using nozzle separators it can be reduced to 0.4 kWh/m^3 (GEA communication). For the separation to be energetically positive, the energy consumption of harvesting step must be minimized below 0.1 kWh/m^3 . Considering a biomass concentration of 1 g/L and energy consumption of harvesting step of 1 kWh/m^3 , the specific energy consumption is of 3.6 MJ/kg , close to 20% of the total energy content of produced biomass. When using nozzle separator, the specific energy consumption reduces till 7% but still this energy consumption needs to be reduced. The answer to this necessity has been the development of two steps processes. In these, a first step carries out a low cost preconcentration of the biomass in the culture broth while a second step a more complete dewatering is performed to achieve the final biomass concentration targeted, over 100 g/L . Cheap and low energy demanding technologies proposed for microalgae harvesting includes sedimentation and flotation, including the utilization of coagulants/flocculants to modify the physical properties of microalgae biomass (Udom et al., 2013; Gutiérrez et al., 2015). The utilization of dissolved air flotation is one of most extensively recommended, allowing to pre-concentrate the microalgae biomass up to 40 g/L



FIGURE 3 | Image of raceway reactors operated by FCC Aqualia in ALLGAS project for wastewater treatment and nutrients recovery using microalgae. Left, a regular raceway reactor, Right, the LEAR system.

sludge, releasing clean water free of solids then accomplishing regulation (Bare et al., 1975). Using these technologies, the energy consumption can be greatly reduced below 0.1 kWh/m³, so the specific energy consumption of the harvesting step is reduced to <2% of total energy content of the biomass. Regardless of the harvesting technology finally used, it must be efficient enough to bring the final concentration of biomass in the outlet stream up to code with the regulations.

RECOVERING NUTRIENTS FROM DIFFERENT EFFLUENTS

Composition of Different Effluents

Each effluent is different and its composition and properties can change as a function of time and operational conditions. The mean composition of most relevant effluents is showed in **Table 1**. It can be seen how large differences exist between the (i) effluents from farms, mainly manure and centrate from anaerobic digestion of manure and other residuals, and (ii) the effluents from sewage, including the raw sewage and centrate produced from anaerobic digestion of activated sludge. The composition of other effluents from agroindustry also detailed on this table are in the middle between these two extreme scenarios. The main characteristics of an effluent that need to be taken into account when considering nutrient recovery from these effluents using microalgae are discussed next.

Turbidity

Light penetration is a critical parameter in any microalgae-based process, as it reduces sharply as the turbidity increases due to the solids content of the effluent. Values over than 3,000 NTU, equivalent to 1,000 mg/L, largely reduce the performance of microalgae cultures (Ledda et al., 2015). To solve this problem, a pre-treatment of the effluent is necessary either by filtration, or alternatively by dilution of the effluent with water but in this last case the total volume of effluent to be processed increases, making this alternative not recommendable. It is important to note that the suspended solids are finally hydrolyzed in the microalgae reactor, but this is a slow process and these solids remain a long time, thus to maximize the capacity of the microalgae process it

is recommendable to minimize the presence of suspended solids in the inlet water (Ledda et al., 2016).

Nitrogen

Nitrogen is a major nutrient for microalgae production, but it is normally supplied as nitrate in concentration of 50 mgN/L when preparing artificial culture medium. When using effluents nitrogen is present mainly as ammonia, and at concentrations ranging from 65 to over 9,000 mgN/L. It has been widely reported that ammonia is toxic at concentrations higher than 100 mg/L, but some strains are more tolerant than others and this tolerance is also modulated by the culture conditions (Collos and Harrison, 2014; Morales-Amaral et al., 2015a). It is important to notice that because microalgae reactors are operated in continuous mode, the real concentration of ammonium at which the cells are exposed inside the reactor is not the same as the one of the inlet effluent. Thus, the real concentration inside the culture is a function of the ammonium concentration in the inlet, of the imposed dilution rate and of the biomass productivity as this determines the nutrients removal capacity. It has been demonstrated that even with ammonium inlet concentrations over than 600 mg/L the ammonium concentration into the reactor can be lower than 100 mg/L (Morales-Amaral et al., 2015b; Sepúlveda et al., 2015).

Phosphorous

Phosphorous is the other major nutrient required for microalgae production. In effluents, phosphorous is normally found as phosphate or in organic compounds, but in the microalgae reactors it is always oxidized to phosphate due to the high oxidative conditions prevailing. There are not reports on phosphorous toxicity for microalgae cultures probably because always low concentrations are always provided. The most relevant problem concerning phosphorous management in microalgae reactors is the precipitation of calcium phosphate that happens in alkaline conditions (Morales-Amaral et al., 2015a). To minimize this problem, it is necessary to limit the presence of calcium in the culture medium and to reduce the pH during the operation of the reactor (Posadas et al., 2015). In any case, the precipitation of phosphorous is not a problem if finally it is harvested with the biomass and adequately used in the final application of the biomass.

Chemical Oxygen Demand/Biological Oxygen Demand

The organic matter contained in the effluent is the major contaminant to be removed in whatever wastewater treatment process. Biodegradable compounds are included into the biological oxygen demand (BOD), while chemical oxygen demand (COD) includes the total degradable compounds. Although heterotrophic and even mixotrophic growth of microalgae have been reported, it has been demonstrated only using low molecular weight molecules such as glycerol, glucose, etc., thus it cannot be assumed that microalgae are able to degrade large organic molecules (Cerón García et al., 2000; Ren et al., 2014). This role is performed in microalgae/bacteria consortia by the heterotrophic bacteria, so the higher the concentration

TABLE 1 | Proximate composition of different effluents that can be processed by microalgae to recover nutrients and to produce biomass.

Effluent	COD/BOD, mg/l	N, mg/l	P, mg/l	Total suspended solids, mg/l	Turbidity, NTU
Digestate	9,000/7,000	8,000	400	10,000	30,000
Manure	16,000/12,000	9,000	500	3,000	9,000
Agro-industrial (Breweries)	4,000/3,800	30	10	1,000	3,000
Centrate	300/200	500	12	1,000	3,000
Sewage	700/500	65	11	300	900
Microalgae requirement		50	10	0	0

of organic matter in the wastewater to be treated the higher the population of bacteria in the final biomass produced will be. It is important to note that bacteria prevailing in microalgae/bacteria consortia are not pathogenic, so the competence reduces the presence of *E. coli* and *Clostridium* with respect to that contained into the inlet effluent (Ouali et al., 2015). When using effluents with high concentration of organic matter larger hydraulic retention times must be used to allow the complete degradation of these contaminants. In any case, the rate at which the organic matter is oxidized to inorganic compounds by the heterotrophic bacteria is faster (hours) than the rate it takes to the microalgae to fix the released compounds (days) (Gómez-Serrano et al., 2015).

Recovering Nutrients From Sewage

Sewage is produced by human activities. The total amount of sewage produced is continuously increasing due to the population rise and the improvement of life style. Microalgae can be used for the recovery of nutrients contained in the sewage, but the final process must be properly designed according to the composition of wastewater to be used. Thus, different possible schemes exist. When considering the processes performed in a conventional wastewater treatment plant three main streams are identified: (i) wastewater after primary treatment it containing all the soluble compounds (organic and inorganic), (ii) wastewater after secondary treatment it containing only a minor concentration of inorganic compounds but not organic matter, and (iii) centrate from anaerobic digestion of sludge that contains both organic and inorganic compounds but in reduced state (Figure 4, Table 2).

Comparing the composition of microalgae culture medium with that of different effluents from conventional wastewater treatment plants it is concluded that all of them contain the same components that are also the main elements of the biomass such as carbon, nitrogen and phosphorous (Table 2). However, the total concentration, and in some cases the relative concentration of each one of these compounds is not the same among the different mediums. as it can be seen, when using microalgae to treat wastewater after primary or secondary treatment the nutrient limiting the production of microalgae is N, whereas when centrate from anaerobic digestion of sludge is used the nutrient limiting the production of microalgae is P (Acíén et al., 2016). Also the maximal microalgae biomass concentration

achievable using the different effluents depends on its total nutrients concentration, resulting that centrate from anaerobic digestion is the richest in nutrients allowing to achieve the highest microalgae biomass concentration, up to 1.2 g/L. The second best is wastewater after primary treatment that allows to achieve up to 0.65 g/L of microalgae biomass, and finally the wastewater after secondary treatment is the poorest effluent only being able to achieve a microalgae biomass concentration of 0.2 g/L.

The recovery of nutrients from wastewater after primary treatment is the most extended scenario. This scenario is equivalent to replace conventional activated sludge systems by microalgae-based technologies, so that the process becomes cheaper and less energy-consuming than conventional systems. In this case up to 1 kg of microalgae biomass can be produced per m³ of processed wastewater. Since the water at the end of the process must accomplish the EU regulation, the nitrogen concentration must be lower than 10 mg/L and P concentration must be below 2 mg/L. The entire process must be designed and operated to accomplish these criteria. Some works demonstrated that this is a highly interesting and feasible strategy (Cabanelas et al., 2013a; Craggs et al., 2014).

The recovery of nutrients from wastewaters after secondary treatment has been proposed as alternative to tertiary treatment, thus allowing to reduce the nitrogen and phosphorus concentration in the effluent from conventional wastewater treatment processes (Cabanelas et al., 2013b). The main

TABLE 2 | Composition of microalgae culture medium and effluents from wastewater treatment plants, nutrient limiting the production of microalgae biomass when using each one, and the maximal achievable biomass concentration according to the concentration of the limiting nutrient.

Parameter	Microalgae medium	Primary treatment	Secondary treatment	Anaerobic digestion
Nitrogen, mg/L	114	65	20	511
Phosphorous, mg/L	41	11	10	12
Carbon, mg/L	47	296	82	247
Limiting nutrient	N	N	N	P
Maximal biomass concentration according to limiting nutrient, g/L	1.14	0.65	0.2	1.2

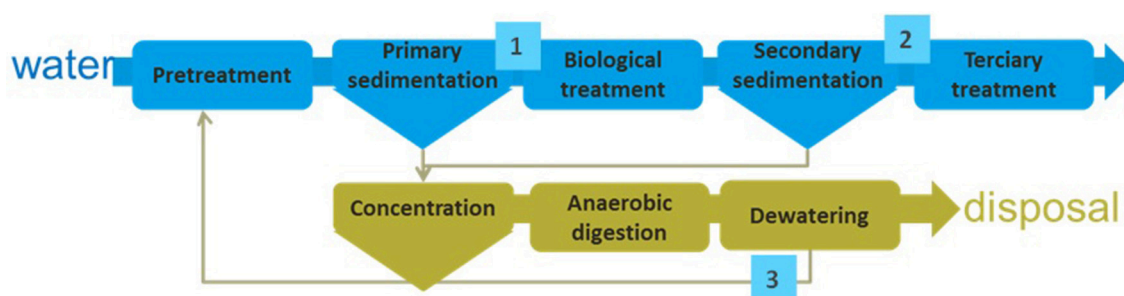


FIGURE 4 | Scheme of a conventional wastewater treatment process. Composition of wastewater at different points inside the wastewater treatment plant is included in Table 2.

challenge in this case is the low concentration of nutrients in treated wastewater that limits the microalgae biomass production capacity. To solve this problem the utilization of membranes to separate the hydraulic and the cellular retention times has been proposed. Using membranes, it is possible to maintain the cellular retention times at the optimum value of 3–4 days, whereas reducing the hydraulic retention time up to 1 day so largely increasing the capacity of wastewater treatment (Marbelia et al., 2014; Gómez-Serrano et al., 2015).

The recovery of nutrients from centrate obtained after anaerobic digestion of activated sludge is a relevant issue. Centrate from anaerobic digestion contains up to 1,000 mgN/L and 30 mgP/L and its removal in wastewater treatment plants entails a high cost and energy consumption. The flow of centrate in wastewater treatment plants is not a negligible issue, as it can be up to 2% of total wastewater flow, which is recirculated to the process increasing the cost and energy consumption of wastewater treatment processes. For a medium size conventional plant processing 50,000 m³/day of wastewater, avoiding the necessity of recirculate and treat this centrate can allow a reduction in the power consumption up to 12,000 kWh/day, with an annual cost reduction of 430 k€/year (FCC Aqualia, personal communication). Microalgae can recover nutrients from this centrate, but it must be added to the photobioreactor carefully to avoid excess of turbidity and ammonium. The dilution of centrate prior to its addition to the photobioreactor is usually done, but to make the process more sustainable the water used for the dilution of the centrate can be recirculated from the effluent (Morales-Amaral et al., 2015a). Due to the high nutrients content of centrate from conventional wastewater treatment plants, it has been proposed also as a suitable single nutrients source to produce marine strains (Ledda et al., 2015; Sepúlveda et al., 2015).

Recovering Nutrients From Manure

Livestock agriculture in Europe has developed into an efficient industry during the last decades, but its future is tarnished by several environmental problems (e.g., water, air, and soil pollution) that stem from the large quantities of wastes produced within several intensively farming regions. The entire manure production in the EU that potentially is available for manure processing is estimated to 1.4 billion tons. Germany is the first producer with up to 202 million tons, whereas Spain produced 117 million tons, and Italy up to 88 million tons per year. Most of the manure is stored and land spread contributing to contamination of water reservoirs and causing emissions to the atmosphere. Alternatively, manure can be processed to change its physical and/or chemical properties as an objective itself, or in order to recover energy from the livestock manure, make the livestock manure more stable, or remove nutrients from the main stream (Ledda et al., 2016). In this scenario farms are obliged to develop and apply specific solutions adapted to the various types of manure produced and the local situation. Different technologies can be applied to manage manure, but no universal solutions exist. Processed and unprocessed manures are usually land spread, but production of these residues overpasses the capacity of the arable land available. Also, only a small fraction of the nutrients presents in the slurry (particularly N

and P) are consumed by plants, causing enormous environmental problems related to leaching, pollution of surface waters, emissions of noxious gases into the atmosphere and surface water eutrophication. Moreover, the Nitrates Directive (1991) establishes action programs to cut down nitrogen pollution and states the limit of 0.170 tonN/ha-year from livestock manure to control pollution, improve water quality, and protect human health.

The application of microalgae-bacteria consortiums allows using solar energy to transform manure nutrients (C, N, P) into valuable biomass that can be used to produce commodities as feed and biofertilizers. The feasibility of this process has been demonstrated at small scale it being reclaimed as a suitable solution for mild climates, allowing to valorize the manure as raw material for the production of valuable compounds instead of as a residual (Godos et al., 2009; Min et al., 2014). The main disadvantage of manure for microalgae production is the excess of ammonium contained on this effluent, that makes necessary to supply this effluent carefully as nutrients source to microalgae cultures (Liao et al., 1995; Min et al., 2014). Recently a demonstration project performed in Spain titled “PURALGA” has demonstrated the feasibility of microalgae based processes for the production of biofertilizers from raw manure. Techno economic analysis of the process show that using microalgae it is possible to treat the manure at cost below 2 €/m³, but even more interesting is that the incomes from biofertilizers produced are much higher than the operation cost of the treatment plant, thus achieving a profitable process instead a of costly process (unpublished data).

APPLICATIONS OF MICROALGAE BIOMASS

When coupling the production of microalgae with the recovery of nutrients from wastewater using the microalgae biomass for human related applications it is not allowed. However, it could be used for energy, animal or agriculture uses. The major factor limiting today the widespread utilization of microalgae biomass on these markets is the enormous amounts of biomass required for these markers, overpassing by several order of magnitude the actual microalgae production capacity of 30 kt/year worldwide (Benemann, 2013). Coupling the recovery of nutrients from wastewaters with the production of microalgae will allow to greatly increase the availability of microalgae biomass for these non-human related applications.

Energy Uses

Energy demand is continuously increasing and the utilization of renewable energy is being enforced by most of the countries in worldwide. Among different alternatives, microalgae have been proposed as highly interesting for the production of biofuels, mainly biodiesel (Chisti, 2007). The reasons for that include the relatively high lipid content of microalgae biomass in addition to its high production capacity per land surface. However, if using clean water and fertilizers the microalgae production cost is too high to enter the energy market. Only

coupling the production of microalgae with nutrients recovery from wastewater the microalgae biomass production cost can be reduced below 1 €/kg, which is the limit for suitable processes (Acién et al., 2017). Moreover, although microalgae can be used to produce biodiesel from the lipid fraction, it sums up no more than 30% of the total biomass which makes necessary to develop biorefinery schemes that can process and use the remaining 70% of the biomass.

Microalgae-based biorefineries have been studied for a long time. Numerous references have been published in this field (Vanthoor-Koopmans et al., 2013; Trivedi et al., 2015; Moreno-Garcia et al., 2017). As a general conclusion it can be summarized that to produce biofuels the best option is to produce biogas. The production of biogas from microalgae biomass allows transforming up to 65% of the total biomass into final product biogas, whereas for bioethanol only a maximum of 40% of the total biomass can be transformed, and focusing on biodiesel the limit decreases to a maximum of 30% of the biomass that can be transformed (Vanthoor-Koopmans et al., 2013). Other biofuels or intermediates as bio-oil among others, give similar results and imply more complex processes. The development of biorefineries involving different steps as oil extraction to produce biodiesel, carbohydrates extraction to produce bioethanol, and finally the waste biomass being used to produce biogas has been also proposed but the complexity of the process is excessive and the losses of raw materials in each step reduce the overall performance of the global process (Ortiz Montoya et al., 2013). Production of biogas from anaerobic digestion is a well-established technology, including the possibility of upgrading the biogas to biomethane for automotive uses (Figure 5). From microalgae biomass more than 200 L of biomethane can be produced per kg of microalgae biomass. Bearing this in mind, only FCC Aqualia wastewater treatment plants would be able to produce up to 100 Mm³ of biomethane if actual conventional wastewater treatment were replaced by microalgae wastewater treatment schemes, this would be enough to fuel 65,000 cars each year on a fully sustainable mode (FCC AQUALIA, personal communication).

Animal Uses

Regarding animal uses, the valuable composition of microalgae biomass is highly attractive for this application (Yaakob et al., 2014). The most traditional use of microalgae for feeding is related with aquaculture. On this field, the microalgae are used for feeding the first stage of larvae and juvenile, then it is replaced by fish-oil and fishmeal because of its lower cost. In the final stages even fish oil and fish meal are replaced by cereals and other raw materials to keep the price of the feed as low as possible (Shah et al., 2018). If microalgae were available at low price and in enough amounts to be competitive in this market it would be widely used because of its superior nutritional valuable and acceptance by the animals. The strains used for aquaculture include marine strains such as *Nannochloropsis*, but also freshwater strains such as *Chlorella*, *Spirulina*, or *Scenedesmus* among others (Muller-Feuga, 2000). Using only microalgae for feeding in aquaculture is not possible due to the enormous size of this market compared to the current microalgae

production capacity, but it has been widely reported that the inclusion of 1–10% of microalgae biomass in the Aquafeed greatly improves the quality of feed and the final health of animals produced (de Cruz et al., 2018). For this market the value of microalgae biomass can be higher than 1 €/kg, which means that since up to 1 kg of microalgae biomass can be produced per m³ of wastewater, a revenue of 1 € can be potentially obtained per m³ of wastewater. Still these figures must be demonstrated in real scale processes.

Concerning animal uses, an equivalent scenario has been proposed. In addition to proteins, carbohydrates and lipids, microalgae contain vitamins, minerals, antioxidants, and other valuable components that can be included in animal diets (Brune et al., 2009). The benefits of incorporating microalgae in animal feeding has been widely reported to be related with the improvement of the health of the animals and the improvement of color and other properties of the end products obtained from these animals (meat, eggs, etc.) (Becker, 2007; Yaakob et al., 2014). Just as in the case of aquaculture, the major limitation to the incorporation of microalgae in animal feeding is the current high price of this raw material and its low availability. If the price were reduced and the availability increased it could be incorporated initially in the feed of young animals and later in the feed for adults. A way to reduce the cost of microalgae biomass for animal feeding is to combine the extraction of lipids for biodiesel with the utilization of protein-rich remaining biomass for animal feeding, thus combining to applications requiring large amounts of biomass (Gatrell et al., 2014).

It is important to mention that for this application it the use of the microalgae biomass is allowed even if produced from wastewaters, as long as it is demonstrated that the biomass is safe, free of pathogens, etc. Moreover, the utilization of microalgae biomass on these markets is the most efficient strategy in terms of sustainability and nutrients recovery, because the nutrients recovered from the wastewater are directly used as feed for animals, avoiding the need to produce plants or vegetables in an intermediate step. For these reasons the development of this scheme is likely to become a reality in the next future.

Agriculture Uses

Regarding the products to be obtained for agricultural uses, the current increasing world food demand and the contamination effect brought about by the increased use of chemical fertilizers to enhance crop yields highlight the importance of products such as biostimulants, biopesticides, and biofertilizers as interesting candidates in sustainable agriculture. These bioproducts are fully biodegradable and non-toxic both to plants and their consumers. As a result, there are no toxicity problems or ecotoxicity or harmful residues. Additionally, these formulations are safe from an operational point of view (Bhardwaj et al., 2014). Microalgae have been reported to be a source of valuable compounds for agriculture uses. Marine algae have been shown to have bactericidal, antifungal, and insecticidal activities (Goud et al., 2007; Hernández-Carlos and Gamboa-Angulo, 2011; Najdenski et al., 2013). Green microalgae genera like *Nannochloropsis*, *Chlorella*, and *Scenedesmus* can produce phytohormones including indol acetic acid (IAA), cytokinins,

gibberellins, abscisic acid (ABA), and/or jasmonic acid as well as other substances with biostimulant activity (Lu and Xu, 2015). On the other hand, some cyanobacteria like *Arthrospira* (Spirulina) are able to produce IAA, cytokinins and/or jasmonic acid, and including are very effective “biopesticide” agents (Tarakhovskaya et al., 2007).

Controlling the microalgae growing in wastewater treatment processes is not easy, but by the modification of the culture conditions such as pH, nutrients supply, and other key parameters it is possible to favor a given type of microalgae and cyanobacteria strain. Regardless of the photosynthetic microorganism produced, it would be rich in proteins in any case and thus rich in valuable amino acids in addition to carbohydrates and lipids. These compounds confer interesting properties to microalgae-based products for agricultural uses. The processing of microalgae biomass to obtain the final product

is a key step. Enzymatic hydrolysis under mild conditions is highly recommendable although other alternatives such as chemical hydrolysis or other downstream processing strategies have been proposed (Romero García et al., 2012). The quality of microalgae-based biofertilizers is largely a function of the downstream processing as well as of the quality of the microalgae biomass used. In this process it is not necessary to use dry biomass, but a cell disruption step is mandatory and the dosage the optimum type of enzymes needs to be chosen carefully (Figure 6). The final separation of solids and stabilization of the product are also key challenges that are kept secret by the companies. This type of microalgae-based products is more frequent in agriculture by the day and will continue to grow due to its demonstrated positive effects in the enhancement of growth and production of plants.

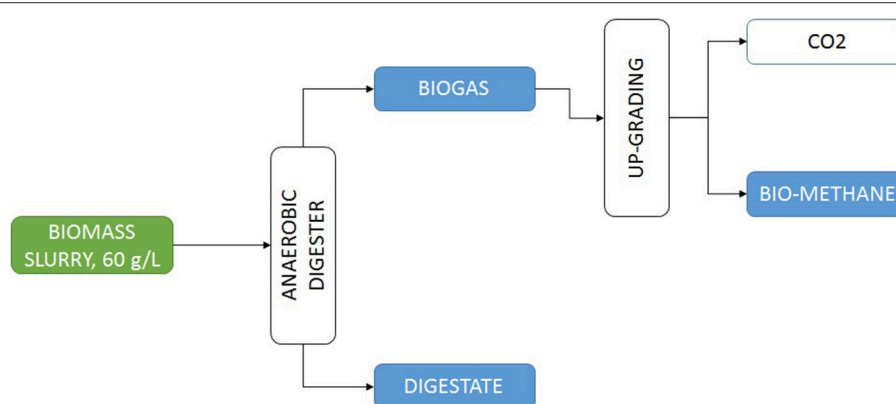


FIGURE 5 | Scheme of a conventional process for the production of biogas from microalgae biomass produced by recovering nutrients from wastewater.

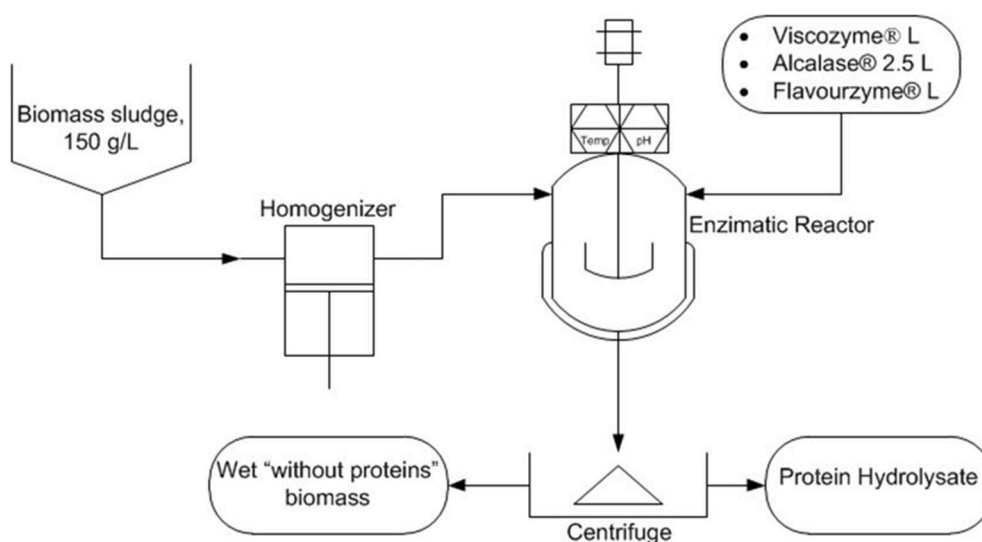


FIGURE 6 | Scheme of the enzymatic hydrolysis process for the production of amino acids concentrates from microalgae biomass produced by recovering nutrients from wastewater.

CONCLUSIONS

The recovery of nutrients from wastewaters using microalgae is a reliable process, which can be applied to different wastewater types from sewage to centrate and manure. If adequately designed and operated up to 450 tCO₂, 25 tN, and 2.5 tP per hectare and per year can be fixed, producing up to 200 t/year of valuable microalgae biomass. To achieve these values, the technology actually used must be improved, taking into account the existence of microalgae/bacteria consortia and their different requirements. To maximize the light utilization is a major challenge on these systems. Coupling microalgae production with wastewater treatment has a large impact in the reduction of treatment cost and the increase of sustainability of wastewater treatment processes, treatment cost reducing to half and sustainability increasing by reduction of energy requirement and greenhouse gases emissions. The major challenge today is to demonstrate this type of processes at large scale, at different conditions and using different wastewater types, different industrial scale projects being in progress. The produced biomass is a valuable resource for different applications. Although the production of biofuels is always attractive, microalgae are much more interesting for chemicals, animal feeding, and agricultural uses. Thus, biodiesel and bioethanol can be produced from microalgae biomass in addition to biogas/biomethane, this last being the most recommendable. Additionally, microalgae biomass can be used for the production of intermediates

for chemical synthesis. However, the recommended use of microalgae biomass is for animal feeding and agriculture. Microalgae contains valuable compounds (proteins, fatty acids, biostimulants, etc.) that allows the enhancement of yield, in addition to improvement of sustainability and economic balance, of foods production processes. There are no doubts that in the next years more microalgae-based processes will be installed coupling the recovery of nutrients with biomass production, thus expanding the relevance of microalgae biotechnology.

AUTHOR CONTRIBUTIONS

FA is the leader of the research group performing this research in the last years. CG-S is the researcher in charge of most of the experimental work performed. JF-S is co-leader of the group and responsible of modeling and data analysis.

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Recovery of Protein Concentrates From Microalgal Biomass Grown in Manure for Fish Feed and Valorization of the By-Products Through Anaerobic Digestion

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The recovery of proteins from microalgae is gaining special attention for animal feed applications especially for fish feed, as the costs of aquaculture feeds represent between 40 and 70% of the costs of the fish produced. Besides, the use of pig manure to produce microalgal biomass could contribute to manure bioremediation and therefore to reduce the environmental impacts of its storage. The objective of this study was to recover protein concentrates from microalgal biomass grown in pig manure, paying especial attention to the quality of the extracted proteins that can be used as feed source for fish, and to the amino acids composition, essential amino acids content and availability. Methane potential of the by-products obtained after protein recovery was also determined in order to fully apply the biorefinery concept to valorize the resulting biomass. Results showed a maximum protein recovery of $54.5 \pm 3.2\%$ from initial microalgal biomass. Protein content in the protein concentrate accounted for 84.5 g protein per 100 g biomass in total solids (TS) basis. In the case of the amino acids profile from protein concentrate, essential amino acids accounted for 47.5 g 100 g amino acids⁻¹ in TS basis. After protein recovery, the resulting by-products named spent and liquid fraction were anaerobically digested in batch assays, obtaining a maximum methane production of 181 mL CH₄ g VS⁻¹_{added}.

Keywords: microalgae, protein extraction, amino acids, fish feed, nutrient recovery, pig manure, by-products, anaerobic digestion

INTRODUCTION

The costs of aquaculture feeds represent 40–70% of the costs of the fish produced (FAO, 2014), where the fishmeal is incorporated as main source of protein. The increasing demand of fishmeal for aquaculture feeds has resulted in the overexploitation of fisheries with the subsequent price increase (FAO, 2014). Soybean, rapeseed, gluten meal or wheat meal and other protein-rich terrestrial plants have been introduced into the fish diets as sustainable alternative source of protein, but the presence of anti-nutritional factors (ANFs) in the digestive tract have shown that it affects to the bioavailability of the protein, has evidenced the need to find a suitable and sustainable alternative for aquaculture feed (Collins, 2014).

In order to satisfy this demand, the utilization of microalgae as an alternative to fishmeal is being widely studied in different fish species (Olvera-Novoa et al., 1998; Patterson and Gatlin, 2013; Kiron et al., 2016; Sørensen et al., 2016). Most microalgae are rich in fiber, mineral salts, trace-elements, vitamins, polyunsaturated fatty acids, chlorophylls and essential amino acids (EAA), fulfilling requirements of most commercial fish feed and avoiding supplementation of vitamins and amino acids (Vizcaino et al., 2014). In addition, microalgae present a low concentration of ANFs like phytic acid, tannins, glucosinolates, saponins, isoflavones, among others (Collins, 2014). In this manner, the partial substitution of fishmeal proteins by microalgal proteins may trigger positive effects in fish metabolism, evidencing that they are a suitable fish feed (Dallaire et al., 2007).

However, there are some aspects that must be addressed in order to make microalgae a suitable source for fish feed. Microalgal growth requires high amounts of nutrients, mainly nitrogen (N) and phosphorous (P). The supply of P is limited and N production is energy intensive and contributes to global warming. One alternative to synthetic culture media is to use agro-industrial wastewater, which usually presents high N and P concentration (Riaño et al., 2012; Chisti, 2013; Hernández et al., 2013). Specifically, the use of microalgal for pig manure (PM) bioremediation has been widely studied, reaching high microalgal productivities in the range of 9–55 g volatile suspended solids $\text{m}^{-2} \text{d}^{-1}$ (Lundquist et al., 2010; Masojídek et al., 2011; Hernández et al., 2016) and removing organic matter, nutrients, pathogen and emerging pollutants (de Godos et al., 2009; Posadas et al., 2017; García et al., 2018).

On the contrary, few studies have been focused on the valorization of macromolecular components (proteins, carbohydrates and lipids) of the microalgae when biomass comes from wastewater treatment. In this context, microalgae biomass grown using PM is usually rich in ash (Kebede-Westhead et al., 2006). This ash content decreases feed digestibility, which affects to the optimal health and development of the fish. In this sense, protein extraction from microalgal biomass could be a suitable tool to increase the protein content and to eliminate ash from the resulting protein concentrate during the extraction process, increasing digestibility of some essential amino acids as histidine (Hys) and lysine (Lys) and decreasing presence of ANFs. However, the high-energy demand required for microalgae cell disruption and the extraction of proteins makes difficult the use of microalgal biomass as fish feed in a cost-effective way. The protein extraction method comprises microalgal cell disruption and the subsequent solubilization, separation, precipitation, and extraction of the proteins. The efficiency of this method is highly affected by the microalgal cell wall composition and the amino acid nature of the proteins. Cell disruption must be carried out by a pretreatment able to release intracellular proteins. Several mechanical, chemical, and biological (i.e., enzymatic) methods have been studied for cell disruption (Möllers et al., 2014; Hernández et al., 2015). Despite of high temperatures may degrade proteins, chemical methods have previously shown to be optimal to break down microalgal cell wall as a previous step for saccharification (Hernández et al., 2015), but they also can lead to the formation of amino acid complexes (Maillard

reactions), which limit the availability of amino acids (Boisen et al., 2000). Furthermore, alternative mechanical methods such as tangential and ultrafiltration of proteins obtaining extraction efficiencies up to 76% of the solubilized proteins (Ursu et al., 2014) have also been studied, but they were discarded due to the high costs. Low-cost mechanical methods are still generally preferred for large-scale applications aimed to obtain high added value products (Günerken et al., 2015; Hernández et al., 2015). In this context, more conservative methods like sonication or high pressure cell disruption have been studied, reporting high protein solubilization (from 52.3 to 73.0%) of the total initial proteins in the microalga using pH 11 in *Nannochloropsis* sp. (Gerde et al., 2013), pH 12 in *Chlorella vulgaris* (Ursu et al., 2014) and pH 5.7 in *Haematococcus pluvialis* (Ba et al., 2016). These studies evidence the key role of the pretreatment method and the pH used to solubilize proteins despite of the microalga used. Furthermore, the isoelectric point of the microalgal proteins must be determined to precipitate proteins efficiently for further extraction.

The use of by-products, from microalgal protein extraction, for anaerobic digestion presents many advantages as a consequence of the pretreatment applied such as cell wall rupture, solubilization of macromolecular components (carbohydrates and lipids), increase of biodegradability, improvement of the carbon/nitrogen (C/N) ratio, among others that enhance CH_4 production (Ehimen et al., 2011). To the best of our knowledge, little effort has been made to investigate the valorization of residues from microalgal protein extraction.

The objective of this study was to obtain protein concentrates from microalgae biomass, maximizing protein solubilization, separation, precipitation and extraction. The amino acid composition and its suitability, as well as proportion and availability of the extracted proteins as feed source for fish were determined. Finally, and in order to valorize the resulting by-products obtained after protein extraction and to fulfill the biorefinery concept, biological methane potential of different mixtures of the by-products obtained after protein extraction was investigated.

METHODS

Microalgae Production

Microalgae biomass was obtained in lyophilized form from the Food Innovation and Sustainability Center (Almería, Spain). Biomass was cultured outdoors using a thin layer photobioreactor with a total working volume of 1,200 L, and a surface of 33 m^2 (del Mar Morales-Amaral et al., 2015). The photobioreactor was fed with 10% diluted centrifuged PM at a hydraulic retention time of 0.3 days. The volume of the photobioreactor was checked daily and water lost due to evaporation was fully replenished with treated PM. Biomass productivity was 17.1 ± 4.7 g volatile suspended solids $\text{m}^{-2} \text{d}^{-1}$. The culture was mainly dominated by *Scenedesmus almeriensis*. Other species such as *Oscillatoria*, *Scenedesmus*, *Chlorella*, and *Nitzschia* also appeared in less proportion in the culture medium. Finally, the biomass was centrifuged (Watermaster centrifuge, GEA, Westphalia, Germany). Therefore, the microalgal paste

was lyophilized at -18°C with a freeze-dryer-cryodo (Telstar, Barcelona, Spain) for further use. Lipid, carbohydrate and protein contents of the lyophilized biomass were determined (Table 1).

Protein Concentrates

Pretreatments for Protein Solubilization

Lyophilized biomass was suspended in distilled water in a ratio 1:20 (weight weight $^{-1}$) by agitation for 24 h at 4°C . Different pretreatments were tested, obtaining seven different trials (Figure 1). Trial 1 was carried out without cell wall rupture process, while in the rest of trials different physical pretreatments were applied to the microalgae solution. In the case of Trial 2, a thermal pretreatment was applied. Thus, samples were processed in an autoclave (Selecta, Presoclave II, Spain) at 120°C for 15 min. In the case of Trials 3, 4, 5, 6, and 7, biomass was pretreated by sonication (Vibracell W-75043; Sonics and Materials, EEUU). Sonication pretreatment was performed with lyophilized microalgal biomass suspended in 100 mL of distilled water to set a final concentration of 50 g VS L^{-1} . The operational conditions of the probe were optimized and operated as follows: constant frequency of 20 Hz and the ultrasonic energy was applied in continuous (non-pulsed) mode with constant amplitude of 70%. Samples were kept on ice cold to maintain temperature between 4 and 7°C during the sonication. The sonication procedure was repeated three times for 1 min each, followed by a settling step of 10 min. After the physical pretreatment, the microalgae solution was subjected to an alkaline treatment by adjusting the pH with NaOH 2.5 N for all the trials. The alkaline treatment for Trials 1, 2, and 3 was performed in distilled water (i.e., 100 mL) and carried out for 120 min, pH = 12 at 150 rpm and 45°C . For Trials 4, 5, 6, and 7 a double alkaline treatment was carried out after sonication. More specifically, both alkaline treatments were performed at the same conditions (120 min, 150 rpm, and 45°C) followed by a centrifugation step (15 min, 15,006 g, $4-7^{\circ}\text{C}$). Double amount of distilled water (i.e., 100 mL + 100 mL) was added for the double alkaline treatments, thus the supernatant from the first alkaline treatment (i.e., 100 mL) was collected and the pellet was resuspended in another 100 mL of distilled water. The pH for each double alkaline treatment was adjusted as follows: Trial 4 (1° pH = 12; 2° pH = 12), Trial 5 (1° pH = 13; 2° pH = 13), Trial 6 (1° pH = 13; 2° pH = 11.5), and Trial 7 (1° pH = 11.5;

2° pH = 13). After the alkaline treatments, a centrifugation step (Avanti J-30I Centrifuge, Beckman Coulter, USA) was performed (15 min, 15,006 g, $4-7^{\circ}\text{C}$) obtaining two fractions, namely spent (SP) and primary supernatant (containing the solubilized proteins).

Protein Extraction

Protein precipitation was optimized adjusting pH with HCl in a range of 3.3–4.5, obtaining the higher protein recovery at pH 3.5 (data not shown). Thus, the solutions corresponding to the primary supernatants were adjusted to pH 3.5 in every trial. After that, the acidic supernatants were centrifuged at 15,006 g for 15 min at $4-7^{\circ}\text{C}$ (Avanti J-30I Centrifuge, Beckman Coulter, USA). Two fractions were obtained for each trial: a protein concentrate (PC) and a supernatant or liquid fraction (LF). The same experimental procedure was followed for all the trials.

Protein recovery was calculated according to Equation (1):

$$\text{Protein recovery (\%)} = 100 * (\text{TKN}_{\text{PC fraction}} * \text{FW}_{\text{PC fraction}}) / (\text{TKN}_{\text{IB}}^{-1} * \text{FW}_{\text{IB}}^{-1}) * 100 \quad (1)$$

Where the subscript IB correspond to initial biomass, TKN corresponds to total Kjeldhal nitrogen and FW corresponds to fresh weight.

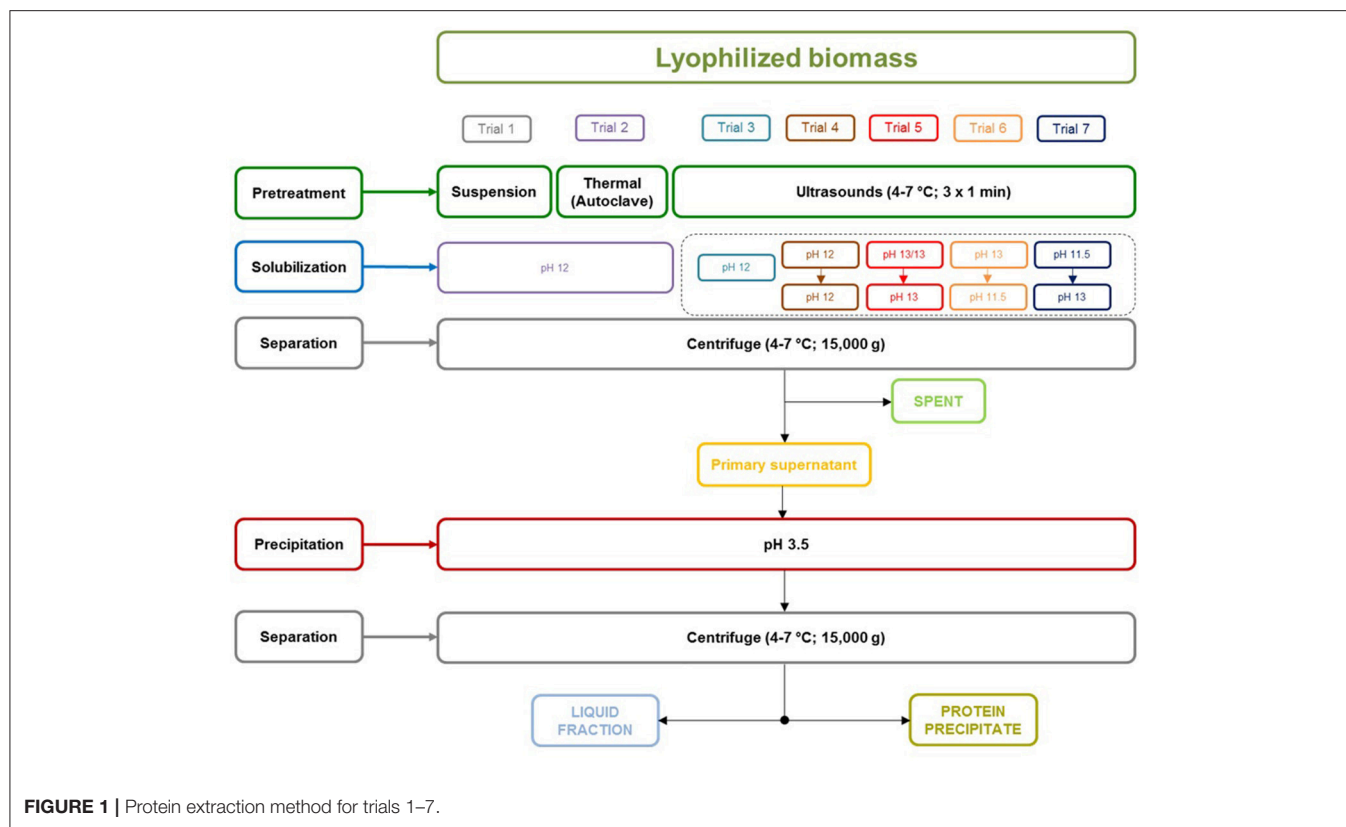
Anaerobic Digestion

Anaerobic biodegradability assays were carried out at $37.1 \pm 0.6^{\circ}\text{C}$ for 46 days in 0.57 L bottles. Quantities were calculated to reach a final volume of 0.30 L, allowing a headspace of 0.27 L for biogas accumulation. The bottles were closed with a septum and the headspace was flushed with N_2 for 5 min to ensure anaerobic conditions. The biogas production was determined by measuring the overpressure in the headspace with time frequency (Colleran et al., 1992). Constant agitation was provided by a shaker at 50 rpm. Anaerobic sludge was collected at the municipal wastewater treatment plant of Valladolid (Spain) and used as inoculum. Anaerobic sludge presented a total solids (TS) and volatile solids (VS) concentration of 26.6 ± 0.9 and $14.7 \pm 0.1 \text{ g L}^{-1}$, respectively. The batch assays were filled in a substrate/inoculum (S_0/X_0) ratio of 1.0 (based on VS); this ratio was selected according to Santamaría-Fernández et al. (in press). For these assays, two kinds of by-products obtained

TABLE 1 | Macromolecular and ionic characterization on total solid basis of microalgal biomass grown in diluted pig manure.

Microalgal biomass	Proteins	Carbohydrates	Lipids	Ash						
Macromolecular composition (% of TS*)	56.6 (0.2)	17.3 (0.1)	9.1 (0.2)	15.4 (0.2)						
Ash composition (mg/kg of ash)	PO_4^{-3}	SO_4^{-2}	Cl^{-}	F^{-}	K^{+}	Mg^{+2}	Cu^{+2}	Na^{+}	Zn^{+2}	Ca^{+2}
	7,800 (60)	500 (40)	1,400 (110)	190 (15)	8,100 (790)	5,110 (460)	1,660 (130)	1,390 (140)	800 (60)	48,200 (5,780)

Analyses were carried out in duplicate. Standard deviation is shown in brackets. *TS, Total solids.



from protein extraction were used, namely SP and LF. Two different assays were carried out. Buffered assays using 12 g L⁻¹ of NaHCO₃ (3.6 grams in each sample) were performed in M1–M5 trials, while in N1–N5 trials samples were not buffered. More specifically, the following five different mixtures in terms of the percentage of VS of LF used in relation with percentage of VS of SP used (%VS_{LF} %VS_{SP}⁻¹) were studied using the ratios: (100/0) for M1 and N1, (75/25) for M2 and N2, (50/50) for M3 and N3, (25/75) for M4 and N4 and (0/100) for M5 and N5. For the determination of endogenous methane production, blanks containing only anaerobic sludge were also tested. After that, the pH was adjusted to 7.7 using HCl at the beginning of the experiment. All the experiments were carried out in triplicate and the results were expressed as means.

Analytical Procedures

Macromolecular Composition of Microalgal Biomass

Lipids were extracted using chloroform-methanol 2:1 (volume volume⁻¹) as solvent, following the method proposed by Kochert (1978). Once the extraction was completed, the lipid concentration was determined by gravimetric analysis at 45°C until constant weight. The concentration of carbohydrates was determined by using an adaptation of Sluiter et al. (2008) protocol, to determine carbohydrates from lignocellulosic biomass by using phenol-sulfuric acid method (Dubois et al., 1956). For protein determination, the specific N-Protein conversion factor (5.62) was calculated using the amino acid composition of the biomass by Ultra-Performance Liquid

Chromatography and TKN. Thus, true protein content in the samples was determined by multiplying TKN content by the calculated conversion factor. The calculation of this conversion factor based on the amino acid profile is a key aspect, since protein and other constituents of microalgae contain nitrogen (i.e., nucleic acids, amines, glucosamides, and cell wall materials). In this manner, this calculation overestimates protein content (crude protein) and must be adapted by using an optimized conversion factor to estimate true protein (Becker, 2007). This author evidenced that the content of non-protein nitrogen amounts reached to 12% in the microalga *Scenedesmus*, which is the main microalga of the biomass used during this study.

Chemical Characterization and Amino Acid Analysis of By-Products

Lyophilized biomass, PC fractions, SP fractions, and LF fractions were analyzed for TS, VS, TKN, total alkalinity, and partial alkalinity (PA) in duplicates were measured following (APHA, 2005). Intermediate alkalinity (IA) was calculated as the difference between total alkalinity and partial alkalinity. Amino acid composition in lyophilized microalgae, SP fractions, and PC fractions was analyzed. Samples were stored at 4°C, and were homogenized prior to analysis. Firstly, an acid hydrolysis by using HCl 6N for 24 h was carried out in order to break down proteins into amino acids. Then, the acid volume of the resulting hydrolysis was reduced by vacuum evaporation and extracts were re-suspended in distilled water. The samples were then ready for

analysis. After that, Ultra-Performance Liquid Chromatography AccQ Tag Ultra C₁₈, 2.1 × 100 mm column (Waters, Milford MA) and a gradient system with the mobile phase consisting of buffer A (0.5 mM TDFHA) and buffer B (0.5 mM TFHA in acetonitrile; 100%) at a flow rate of 700 µL min⁻¹ (split less) was performed at 37°C. One minute prior to the next sample injection the flow was set to 700 µL min⁻¹. The injected volume was 1 µL. Run-to-run time was 30 min.

In order to characterize free amino acids, the hydrolysis step using HCl was not performed. Thus, only free amino acid contained on the PC fraction were determined. True protein content was obtained after summing all the amino acids present in samples.

Ash Characterization

In order to determine ash composition (Table 1), different chromatographic methods were performed. The ionic chromatography Dionex ICS-2000 (Sunnyvale, CA, USA) was used for the separation and suppressed conductivity detection of the following anion: fluorides, chlorides, sulfates, and phosphates. Instrument control and data acquisition were performed using Chromeleon[®] 6.60 software. ICS-2000 instrument was used in conjunction with Dionex AS autosampler to enable simultaneous separation of anions. Separation was performed on a column IonPac AS19 (250 mm, 4 mm ID) used with a Dionex AG19 guard column (50 mm, 4 mm ID), coupled to a Dionex ASRS Ultra II 4 mm suppressor. Hydroxide eluent gradients were generated using the Dionex EluGen II EG50 KOH cartridge and ultrapure water (Milipore Corporation). The optimized hydroxide eluent gradient was: 10 mM isocratic for 0–20 min and a gradient of 10–45 mM for 20–25 min.

Different metals were determined by flame atomic absorption spectroscopy (AAS) in VARIAN AA240FS, Atomic Absorption Spectrophotometer, following the method proposed by Xie et al. (2008).

Biogas Composition and Total Volatile Fatty Acids Concentration in Anaerobic Digestion Assays

Biogas composition was analyzed using a gas chromatograph (Agilent 7890A) with a thermal conductivity detector, separated by a HP-Plot column (30 m 0.53 mm 40 µm) followed by a HP-Molesieve column (30 m 0.53 mm 50 µm). Helium (7 mL min⁻¹) was used as the carrier gas. The injection port temperature was set at 250°C and the detector temperature was 200°C. The temperature of the oven was set at 40°C for 4 min and after that increased to 115°C for 1 min and 45 s. Individual volatile fatty acids were determined using a gas chromatograph (Agilent 7890A) equipped with an Teknokroma TRB-FFAP column of 30 m length and 0.25 mm followed by a flame ionization detector. The carrier gas was helium (1 mL min⁻¹). The temperature of the detector and the injector was 280°C. The temperature of the oven was set at 100°C for 4 min, then increased to 155°C for 2 min and thereafter increased to 210°C. Furthermore, the concentration of total volatile fatty acids (VFA) were determined by the sum of individual volatile fatty acids. VFA concentration was converted

to COD concentration by using the following conversion factors: 1.07 for acetic acid, 1.51 for propionic acid, 1.82 for butyric and isobutyric acid, 2.04 for valeric and isovaleric acid, 2.21 for hexanoic acid, and 2.34 for heptanoic acid according to Yuan et al. (2011).

RESULTS

Initial Biomass Composition

Macromolecular characterization of biomass is presented in Table 1. Microalgae biomass contained 57, 17, and 9% of proteins, carbohydrates and lipids, respectively, on a total solid basis. Ash content accounted for 15% of the total solid basis. The ash composition in the microalgae cells is presented in Table 1, being especially remarkable the high presence of Ca⁺² (48,200 mg kg⁻¹), K⁺ (8,100 mg kg⁻¹), PO₄⁻³ (7,800 mg kg⁻¹), and heavy metals like Cu⁺² and Zn⁺² (1,660 and 800 mg kg⁻¹, respectively).

Protein Concentrates From Microalgae: Optimization of Solubilization and Precipitation Methods

Figure 2 presents true protein recovery for trials 1 to 7, calculated as the percentage of recovered protein in the concentrates in relation to the protein content in the initial biomass (Equation 1). Protein recovery for Trial 1 was very low (9.1 ± 0.1%), showing the need of a pretreatment to breakdown cell walls as a previous step for protein precipitation. The application of a thermal pretreatment in Trial 2 and ultrasound in Trial 3 increased protein recovery to 21.5 ± 1.3% and to 18.4 ± 0.5%, respectively.

The combination of an ultrasound pretreatment and a double alkaline solubilization and separation (Trials 4, 5, 6, and 7) resulted in up to 3-fold higher protein recovery yields, when compared to simple alkaline extraction pretreated (Trial 3). More specifically, the highest protein recovery yield was achieved in Trial 5 after two consecutive alkaline treatments at pH 13 (i.e., 54.5 ± 3.2%).

Protein Quality of the Protein Concentrates and Suitability as Fish Feed

Mass balances in terms of TS, VS, ash, and protein content were performed for Trial 5 (Table 2). This trial was selected due to the highest protein recovery that was achieved during the protein extraction process. The biomass loss during the extraction process accounted for 1.7% on VS basis (Table 2). After protein extraction, 54.5% of the initial protein was recovered in the PC. This product showed a protein concentration of 84.5 g protein per 100 g TS. Moreover, this PC fraction presented very low ash content, since 87.6% of the ashes from the initial biomass were found in the by-products. More specifically, SP and LF accounted for 38.3 and 49.3% of the total amount of ash from the initial biomass.

As it has been previously described, the highest protein recovery (54.5%) was reached when applying ultrasounds followed by a double alkaline treatment at pH 13 (Trial 5). Protein concentrate from Trial 5 was evaluated as a

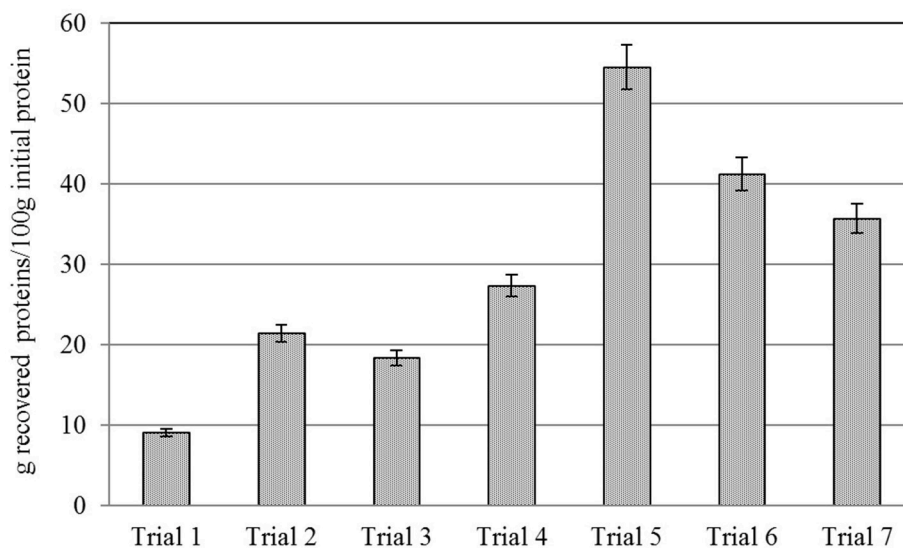


FIGURE 2 | True protein recovery* for Trials 1–7.

TABLE 2 | Mass balances of total solids, volatile solids, ash and protein for Trial 5 using 100 g of biomass (in total solid basis).

Trial 5*	Biomass (g/100 g TS)	PC (g/100 g TS)	SP (g/100 g TS)	LF (g/100 g TS)	Loss (g/100 g TS)
Total solids	100.0 (0.0)	33.8 (0.4)	53.2 (0.6)	13.0 (0.3)	0.0 (0.0)
Volatile solids	84.6 (0.1)	31.0 (0.4)	47.3 (0.4)	4.6 (0.1)	1.7 (0.1)
Ash	15.4 (0.2)	1.9 (0.1)	5.9 (0.1)	7.6 (0.2)	0.0 (0.0)
Protein	53.5 (0.3)	29.2 (1.7)	13.6 (0.2)	0.4 (0.0)	10.3 (0.1)

Analyses were carried out in duplicate. Standard deviation is shown in brackets. *TS, Total solids; PC, Protein concentrate; SP, Spent; LF, Liquid fraction.

possible supplement for rainbow trout feed in terms of content, proportion and availability of amino acids. The amino acid profile in terms of grams of amino acid per 100 g of protein is presented in **Table 3**. Amino acid profile for the initial microalgae biomass was very similar when compared to amino acid profile of the protein concentrate. Only small differences in phenylalanine (Phe) and Lys content were detected. The content of EAA accounted for 48% of the total amino acids concentration in PC and free amino acids reached 6.5%.

Anaerobic Digestion of By-Products Obtained After Protein Extraction

The feasibility of the two by-products obtained after protein extraction, namely SP and LF, as sole and combined substrates for anaerobic digestion was evaluated. Both substrates presented different pH values (3.7 ± 0.1 for LF and 12.9 ± 0.3 for SP) and VS contents (4 g VS L^{-1} for LF and 143 g VS L^{-1} for SP). Concerning SP fraction, protein content accounted for 25.4 g protein per 100 g TS, while negligible protein content was found in LF. The concentration of carbohydrates in LF was 3-fold higher than in SP, while lipid content in SP was 1.5-fold higher than in LF. Both substrates were co-digested in five different assays to evaluate the influence of LF/SP mixture ratio in terms of methane yield.

Figure 3 shows the accumulated methane production of the different mixtures of LF and SP in (**Figure 3A**) buffered (M1–M5) and (**Figure 3B**) unbuffered (N1–N5) assays. The highest methane yields were achieved by M1 and M2 (181 ± 3.5 and $160 \pm 22.3 \text{ mL CH}_4 \text{ g VS}_{\text{added}}^{-1}$), corresponding to the highest contents of LF in buffered assays. The highest total COD removals were also reached in M1 and M2, accounting for 40.8 and 40.2%, respectively and also the highest VS removals achieving 30.1 and 32.9%, respectively. The methane production in N1–N5 was remarkably lower than in buffered assays with methane productions lower than $97 \text{ mL CH}_4 \text{ g VS}_{\text{added}}^{-1}$ in all cases.

DISCUSSION

Protein Recovery: Optimization of the Solubilization and Precipitation Methods

Characterization of biomass from **Table 1** is similar than that previously reported by Becker (2007) for the same microalga (*Scenedesmus*). The concentration of ash accounted for 15.4% of the total dry weight, which may be considered as high compared to previous results reported by Sterner (1993) and Becker (2007) who accounted from 3.8 to 17% of ash content in the same microalga. As it is shown in **Table 1**, the concentration

TABLE 3 | Amino acid composition of microalgal biomass and protein concentrate fraction (grams of amino acids correspond to average values \pm standard deviation).

Amino acid composition	Initial biomass (g/100 g protein)	PC* fraction (g/100 g protein)	Free amino acid in PC* fraction (g/100 g protein)	Rainbow trout requirements of amino ^a acids (g/100 g protein)
ESENTIAL AMINO ACIDS				
Arg	6.3 \pm 0.3	6.4 \pm 0.2	0.5 \pm 0.0	3.5
His	1.8 \pm 0.1	2.2 \pm 0.1	0.0 \pm 0.0	1.6
Ile	3.9 \pm 0.2	4.5 \pm 0.2	0.4 \pm 0.0	2.4
Leu	8.4 \pm 0.4	8.8 \pm 0.3	0.3 \pm 0.0	4.4
Lys	8.4 \pm 0.2	5.2 \pm 0.2	0.5 \pm 0.0	5.3
Met	1.6 \pm 0.1	1.5 \pm 0.1	0.5 \pm 0.0	1.8
Phe	4.6 \pm 0.2	5.4 \pm 0.2	0.0 \pm 0.0	3.1
Trp	n.d.**	1.4 \pm 0.1	0.1 \pm 0.0	0.5
Thr	5.4 \pm 0.2	5.7 \pm 0.2	0.4 \pm 0.0	3.4
Val	6.4 \pm 0.4	6.4 \pm 0.2	0.4 \pm 0.0	3.2
NON-ESSENTIAL AMINO ACIDS				
Ala	8.8 \pm 0.4	8.0 \pm 0.3	0.3 \pm 0.0	n.e.***
Asp	10.6 \pm 0.5	12.0 \pm 0.4	0.4 \pm 0.0	n.e.
Cys	0.4 \pm 0.0	1.2 \pm 0.0	0.6 \pm 0.0	n.e.
Glu	12.3 \pm 0.5	11.5 \pm 0.4	0.5 \pm 0.0	n.e.
Gly	6.7 \pm 0.3	6.3 \pm 0.2	0.2 \pm 0.0	n.e.
Pro	5.0 \pm 0.2	5.5 \pm 0.2	0.4 \pm 0.0	n.e.
Ser	5.3 \pm 0.2	4.8 \pm 0.2	0.4 \pm 0.0	n.e.
Tyr	4.1 \pm 0.3	3.2 \pm 0.1	0.6 \pm 0.0	n.e.

^aBlanco-Cachafeiro, 1995.

*PC, Protein concentrate.

**n.d., not determined.

***n.e., not essential.

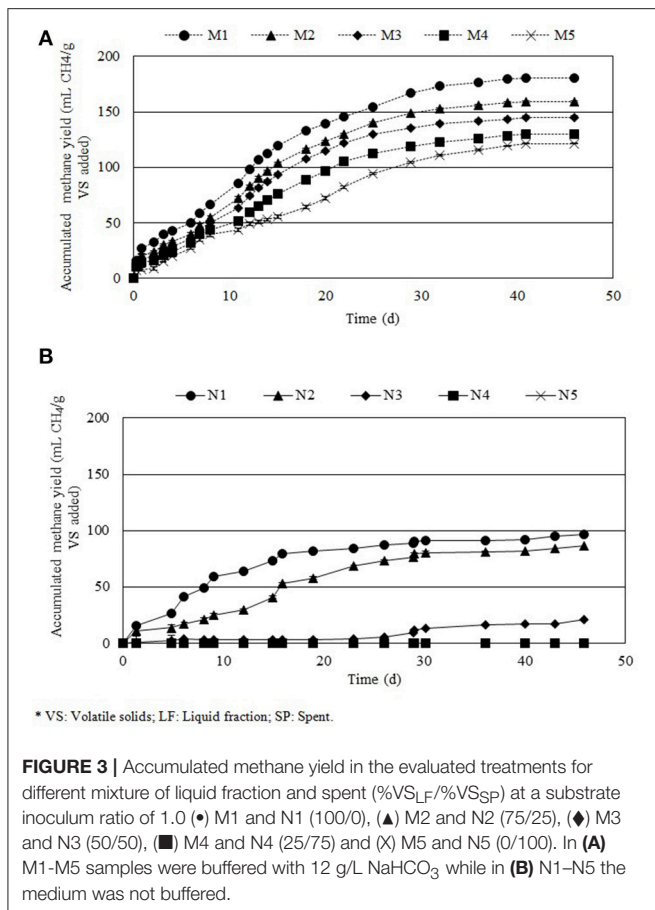
of Cu^{+2} and Zn^{+2} in the biomass was considerably high. Also, high amounts of Ca^{+2} , Cl^- , K^+ , Mg^{+2} , Na^+ Ca^{+2} were determined, probably due to the high salinity of the PM to balance the osmotic potential and to maintain the turgor pressure (Bisson and Kirst, 1995), increasing thus the ash content.

The results obtained for the alkaline treatment without any physical pretreatment (Trial 1) evidenced the need of a pretreatment to breakdown microalgal cell wall in order to release intracellular proteins for further protein precipitation (Figure 2). These results are in agreement with Miranda et al. (2012), who reported the need of a pretreatment in *Scenedesmus* to break down its rigid cell wall. In this context, the use of ultrasounds as a pretreatment previous to protein extraction resulted in a remarkable recovery increase (Trial 3) in comparison with Trial 1. These differences may be attributed to the cell wall breakdown caused by ultrasounds and subsequent solubilization of proteins into the medium. Furthermore, the combination of two consecutive alkaline extractions, especially when using a pH of 13 (Trial 5) resulted in further enhancement of protein recovery. However, despite of the combination of two alkaline extractions enhances protein recovery; the identification of an optimal pH to solubilize proteins resulted to be the most important parameter to maximize protein recovery. In this manner, Trial 4 recovered only 27.4% of the proteins when using pH 12, while Trial 5

recovered 54.5% of the initial protein content. Hence, when pH is not optimized, an important amount of proteins may be joined to cellular structures inside the cell and they may not be recovered.

In contrast to consecutive extraction, during autoclave pretreatment and further alkaline treatment (Trial 2) an important amount of proteins were degraded (close to 20%), probably due to the amino acid hydrolysis during autoclave pretreatment. This hypothesis is in agreement with Papadopoulos et al. (1986), who also evidenced the degradation effects of thermal pretreatments of feather meal on proteins and amino acids.

Mass balances in terms of TS, VS, ash, and protein content were performed under the optimal extraction conditions (Trial 5) for PC and by-products. It is important to note the high protein concentration recovered despite of the thick cell wall of this microalga (Miranda et al., 2012). The maximum protein recovery was 54.5% of initial protein content (Table 2), achieving a final protein concentration in the PC of 845 g protein kg TS^{-1} of PC. These data demonstrate the effectiveness of the treatment. The results presented herein showed higher protein recoveries than those previously reported by Gerde et al. (2013), who optimized the isolation of proteins from *Nannochloropsis* biomass obtaining protein recoveries close to 30 g protein per 100 g microalga and with protein concentrations close to 57%.



Protein Quality of the Protein Concentrate and Suitability as Fish Feed

The use of PC obtained from the optimized protein extraction (Trial 5) may be an interesting source of proteins for aquaculture. Table 3 reveals the high nutritional aptitude of this biomass due to its high content in EAA. Additionally, the amino acid profile in PC did not change during the protein extraction process, thus degradation of proteins or amino acids did not take place. The requirements of the rainbow trout fit well with those present in the PC (Blanco-Cachafeiro, 1995); being thus a balanced protein source that could be used for fish feed, partially replacing the use of amino acid supplements. Tryptophan (Trp) was determined in the PC fraction and in the free amino acids present in PC fraction accounting 1.4 g per 100 g proteins.

The key amino acids included in fish feed diet in terms of interest and costs are Lys and methionine (Met), which are EAA and they are usually supplemented since the fish requirements are higher than its content in traditional biomass. However, when protein content comes from microalgal biomass extraction, Lys and Met may be found as free amino acids, increasing digestibility and diminishing its supplementation. According to Fleurence (1999), the use of algae in fish diets improves body weight gain and increases the triglyceride and protein deposition in muscle. Overall, the use of protein concentrates from microalgal biomass for fish feed like rainbow trout may be an interesting

alternative to substitute the proteins of traditional ingredients like fish and vegetable meals, due to high protein content, high EAA, important amount of Lys and Met, free amino acids and low ash content. Furthermore, the low pH values reached during protein extraction could inhibit the growth of different enterobacterium (Engberg et al., 2009), proving a protective effect against pathogenic enterobacterium in fish as *Yersinia ruckerii* and *Edwardsiella* spp.

Results presented by Vizcaino et al. (2014), showed that one of the main advantages on the use of microalgal biomass compared to plants is the low content in ANFs, therefore no negative effects on growth or on digestion enzymes activity is expected. In this sense, in a preliminary assay in rainbow trout with increasing concentrations of *Scenedesmus* spp. (Larrán-García et al., 2017) up to 10% in feed formulation, negative effects in growth rates were not observed. Thus, similar results would be expected using PC, where amino acid digestibility is higher, proteins are more accessible to fish enzymes and ash content is remarkably lower (from 15.4 to 1.9%).

Hence, according to the results previously reported, PC fraction has an ideal nutritional quality due to: (i) high protein concentration, (ii) the amino acid profile fits well with rainbow trout requirements, and (iii) ash content is lower than 5%. Also, the increase in Phe in the PC fraction (from 4.6 to 5.4%) and in free Lys (from 0 to 0.5%) could diminish amino acid supplementation, making therefore, this biomass a suitable candidate source to fulfill fish feed requirements.

Anaerobic Digestion of By-Products Obtained After Protein Extraction

The feasibility of using the resulting by-products (SP and LF) as sole and combined substrates for anaerobic digestion was evaluated. The use of these substrates is interesting due to SP and LF are complementary in terms of pH and volume of resulting by-product. The highest methane yield was obtained for M1 (180.7 mL CH₄ VS_{added}⁻¹) for buffered samples and N1 (96.5 mL CH₄ VS_{added}⁻¹) for unbuffered samples. In order to study the biodegradability of the different mixtures, theoretical methane yields were calculated. The theoretical methane yield of the substrates was calculated based on their macromolecular composition in terms of lipids, carbohydrates and proteins. According to Sialve et al. (2009) each gram of lipids, carbohydrates and proteins results in 1.014, 0.415, and 0.521 mL of CH₄, respectively. According to the theoretical productivity of CH₄ for M1–M5, the highest production would occur on M1 (343 mL CH₄ g VS_{added}⁻¹), while the lowest would take place on M5 (305 mL CH₄ g VS_{added}⁻¹). Hence, differences between methane yields in all assays should be small. However, after anaerobic digestion of buffered by-products, experimental methane yields differed between 47 and 60% from the theoretical ones (i.e., in the case of M1, theoretical methane production was 343 mL CH₄ g VS_{added}⁻¹ and the experimental yield resulted in 181 mL CH₄ g VS_{added}⁻¹, resulting in a productivity reduction of 47%). Moreover, when the anaerobic digestion media was not buffered (Figure 3B; N1–N5), methane yields ranged between 96.5 and 0 mL CH₄ g VS_{added}⁻¹, differences between theoretical

and experimental methane yields differed between 71.3 and 100% from the theoretical ones. The highest experimental methane yield (96.5 mL CH₄ g VS_{added}⁻¹) corresponds to the assay with the highest content of LF when the medium was buffered (M1), as can be seen in **Figure 3**. Methane yield decreased concomitantly with the decrease of LF in the mixture. This result evidenced the high biodegradability of LF, which is mainly composed by solubilized monosaccharides, in comparison with SP, which is mainly composed by proteins and lipids inside unbroken cellular structures and hindering thus the access to anaerobic bacteria. Results from **Table 4** revealed a direct correlation between methane yield and VS removal and total COD removal for all the assays performed. Hernández et al. (2014) obtained similar methane yields (203 mL CH₄ g VS_{added}⁻¹) using the same microalga (*Scenedesmus*) than those observed in the present work, when producing biogas from the resulting by-products after lipid extraction through a mechanical pretreatment (supercritical extraction).

In unbuffered assays (N1–N5), methane production was considerably lower than in buffered assays (M1–M5). These differences may be attributed to a partial inhibition of the anaerobic digestion in the unbuffered assays. Buffered assays were performed to determine the methane potential of each mixture. **Table 4** presents initial and final values of pH, NH₄⁺-N, VFA, VS, and totalCOD concentration before and after anaerobic digestion for buffered and unbuffered assays. All final pH values oscillated from 6.9 to 7.9, being compatible with normal development of microorganisms. Final ammonium concentrations (**Table 4**) were below inhibitory threshold levels (from 1,700 to 5,000 mg NH₄⁺-N L⁻¹; Stams et al., 2003) reaching the maximum concentration in M1 (409.5 mg NH₄⁺-N L⁻¹). Under these ammonia concentrations, the biogas production is not affected and acetate-utilizing methanogenic Archaea, hydrogen-utilizing methanogens and syntrophic bacteria are not

influenced (Zeng et al., 2010). Previous anaerobic digestion, the VFA concentration was lower than inhibitory threshold (from 1,000 to 6,000 mg COD L⁻¹ (Siegert and Banks, 2005), ranging from 52.6 to 349.2 mg COD L⁻¹ for all trials performed. After anaerobic digestion, the VFA concentration was also lower than inhibitory values ranging from 253 to 540 mg COD/L for buffered assays and from 40 to 215 mg COD L⁻¹ for unbuffered assays.

The IA and the PA were determined previous and after anaerobic digestion. In PA titration from original sample to pH 5.75 corresponds to bicarbonate alkalinity. In IA alkalinity, titration from pH 5.75 to 4.3 approximates to VFA alkalinity. Successful anaerobic digestion depends on both maintenance of adequate bicarbonate buffering and avoidance of excessive VFA concentrations, hence when the IA:PA ratio ranges from 0.1 to 0.35 it is considered well-operated digesters (Ripley et al., 1986). In the buffered trials, IA:PA ratio ranged from 0.17 to 0.29 while in unbuffered trials IA:PA ratio it ranged from 1.4 to 0.78. Thus, in unbuffered trials a partial inhibition of the process probably took place, especially in N4 and N5, where anaerobic digestion was not even initiated (**Figure 3**). The percentage of methane content in biogas in N1–N5 varied from 64 to 7%, decreasing when SP content increases, demonstrating that high SP content in the mixture affected negatively to the anaerobic digestion, when assays were not buffered. In this context, the results presented herein, evidenced the need to buffer the culture medium to stabilize the process.

In this context, the optimization of protein extraction from *Scenedesmus* allows not only to higher protein recovery, but also it could increase the biodegradability of the resultant biomass due to a higher cell wall break down as previously reported by Hernández et al. (2014). The use of NaHCO₃ in M1–M5 samples decreased the IA:PA ratio to values lower than 0.3, resulting in higher methane productions. From an economical point of view, the use of NaHCO₃ incurs in high cost, decreasing profitability

TABLE 4 | Evolution of pH, NH₄⁺-N, total volatile fatty acids, volatile solids and total chemical oxygen demand previous and after anaerobic digestion process of (A) buffered (M1–M5) and (B) unbuffered samples (N1–N5).

		pH		NH ₄ ⁺ -N (mg/L)		VFA* (mg COD/L)		VS* (g/L)		TCOD* (mg/L)	
		Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
(A)											
Buffered	M1	7.7 (0.1)	7.8 (0.0)	186 (1.3)	410 (18.5)	156 (34)	541 (30)	2.3 (0.2)	1.6 (0.2)	6,550 (293)	3,880 (82)
	M2	7.7 (0.0)	7.9 (0.1)	172 (2.6)	359 (1.5)	107 (5.4)	347 (7.4)	2.3 (0.1)	1.5 (0.2)	6,730 (373)	4,025 (65)
	M3	7.8 (0.1)	7.9 (0.0)	168 (1.3)	355 (5.5)	78 (3.2)	375 (66.1)	2.4 (0.2)	1.8 (0.3)	6,970 (493)	4,220 (521)
	M4	7.7 (0.1)	7.9 (0.1)	161 (1.3)	286 (3.0)	56 (2.7)	253 (36.2)	2.2 (0.1)	1.7 (0.2)	7,360 (347)	4,450 (456)
	M5	7.7 (0.1)	7.9 (0.0)	154 (3.3)	267 (18.0)	53 (3.5)	281 (36.9)	2.3 (0.2)	1.8 (0.4)	7,550 (155)	4,860 (668)
(B)											
Unbuffered	N1	6.9 (0.0)	6.9 (0.1)	214 (0.2)	366 (10.2)	349 (74.3)	54 (2.6)	2.2 (0.3)	1.7 (0.2)	6,040 (469)	4,160 (118)
	N2	7.0 (0.1)	7.0 (0.0)	196 (4.0)	338 (6.4)	204 (8.7)	40 (1.7)	2.2 (0.3)	1.8 (0.3)	6,730 (312)	4,710 (74)
	N3	7.1 (0.0)	7.4 (0.0)	196 (1.1)	315 (7.5)	185 (5.8)	182 (12.9)	2.5 (0.4)	1.9 (0.2)	6,970 (117)	4,910 (87)
	N4	7.8 (0.1)	7.0 (0.1)	147 (1.6)	291 (3.6)	151 (41.4)	133 (25.1)	2.3 (0.2)	1.9 (0.3)	7,860 (115)	5,620 (217)
	N5	8.8 (0.2)	7.2 (0.1)	258 (1.3)	268 (4.8)	78 (2.7)	215 (30.2)	2.4 (0.3)	2.1 (0.3)	8,170 (125)	6,140 (222)

Different mixtures were studied (%VS_{LF}/%VS_{SP}): M1 (100/0), M2 (75/25), M3 (50/50), M4 (25/75), and M5 (0/100). Standard deviation is shown in brackets. *VFA, Total volatile fatty acids; VS, Volatile solids; TCOD, Total chemical oxygen demand.

of the anaerobic digestion process that can hardly be performed in a pilot plant. In this context, the production of methane from samples N1 and N2 results more interesting, and therefore a co-substrate should be added in order to buffer the process.

FUTURE DIRECTIONS

To ensure the successful implementation of the protein extraction process for fish feed and further anaerobic digestion, the following parameters must be optimized: (i) the use of different microalgal species must be carried out to confirm the results obtained herein, (ii) the protein extraction should be performed in a pilot-scale size, (iii) different diets should be tested using intact microalgae and protein concentrates extracted from microalgae as main protein source in trials with different fish species, (iv) a techno-economical evaluation of protein extraction and anaerobic digestion process compared to the use of intact microalgae as protein source should be performed.

CONCLUSIONS

It is possible to recover protein concentrates from microalgae biomass obtained after pig manure treatment. The highest protein recovery was $54.5 \pm 3.2\%$, obtained after a sonication pretreatment, followed by solubilization by double alkaline treatment and a precipitation step at pH 3.5. Protein concentration in the protein concentrate reached 845 g protein

per 1,000 g biomass (TS), being an optimal biomass in terms of essential amino acids, resulting in a suitable protein source for fish feed. Methane yields up to 181 mL CH₄ g VS_{added}⁻¹ were obtained using 100% the liquid fraction obtained after protein extraction as substrate. The higher LF content in the anaerobic digestion, the higher methane yield. Therefore, anaerobic digestion of the obtained by-products is only feasible if the anaerobic media is buffered. Therefore, the use of PC for fish feed may be a suitable source of proteins for fish feed due to: high protein content, high EAA, low ash, absence of ANF and high amino acid digestibility.

AUTHOR CONTRIBUTIONS

DH performed the experimental set up and wrote the manuscript. BR and MG-G performed the experimental design and contributed to the revision of the discussion. BM-S contributed to discussion and the revision of the manuscript. CT-A and AL-G gave feedback in aquaculture and protein supplementation for fish feeding.

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Research Trends on Nutrient Management From Digestates Assessed Using a Bibliometric Approach

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Anaerobic digestion is often applied for biological conversion and valorization of organic waste, waste water and other biomass sources as renewable energy and biofuel in the form of biomethane. Composition of the material remaining after digestion, or digestate, is highly dependent on processed feedstocks. This by-product is usually rich in nutrients such as nitrogen and phosphorus, so it is potentially reusable as fertilizer or nutritive broth in agricultural systems. Alternatively, the digestate may need post-treatment based on nutrient removal or recovery strategies. The use of life-cycle assessment tools is becoming popular to analyze nutrient handling scenarios. This study reviews, through a bibliometric-based approach, the research outputs and global trends in the area of knowledge of nutrient management from digestates in the last 30 years, 2017 included. Documentary production followed an upward trend, with a relative productivity in the last 3 years greater than 37% of the total number of appeared publications. China, USA and Spain were the three most prolific countries. The particular interest in nutrient management alternatives and its evolution were identified. Trends for promoting sustainability include low environmental impact, holistic agro-energy solutions, reduced consumption of resources during digestate processing, and circular economy scenarios based on concepts such as (bio)refinery and recovery of valuable and marketable products.

Keywords: biogas digester effluent, nitrogen, phosphorus, soil application, treatment, nutrient removal, nutrient recovery, life-cycle assessment

INTRODUCTION

Anaerobic digestion (AD) is an environmental biotechnology which is increasingly applied for the energetic valorization of organic waste(water) streams and other biomass sources (Appels et al., 2011; Wang et al., 2013). Owing to this technology meet targets such as production of renewable energy and mitigation of climate change, it helps fostering transition from dependence on fossil fuels to other more sustainable energy-producing scenarios. Positive energy balance, stabilization of organic matter, potential for inactivating pathogenic microorganisms, reduction of sludge handling requirements, robustness of the process, and mitigation of greenhouse gas (GHG) emissions are some of the factors that explain current attention to this technology. Resulting products from the AD process are biogas and digestate.

The biogas which is formed in AD (60–70% vol. of methane, CH₄) has a high calorific value, and it can be used for the generation of heat and electricity. Additionally, if upgraded to biomethane, then it can be injected into the natural gas grid or used as transportation fuel (Pöschl et al., 2010). Co-digestion based on blending different raw organic materials from agricultural, industrial, or urban sources is frequently applied to boost biogas production (Mata-Alvarez et al., 2014). In addition, there is a growing concern in upgrading conventional operative energy-consuming wastewater treatment plants (WWTPs), which mostly depend on the aerobic biodegradation of the organic carbon (C), by moving towards anaerobic technologies, including AD, that may allow energy neutrality, or even net energy recovery (Scherson and Criddle, 2014).

The neologism “digestate” is increasingly used to refer to the digested effluent produced in anaerobic digesters (Magrí et al., 2017). Composition and quality of this by-product is strongly dependent on the processed feedstocks and applied treatment conditions (Makádi et al., 2012; Fuchs and Drosch, 2013). Thus, to assure that quality and safety are preserved, presence in digestate of undesired materials and pollutants of physical, chemical or biological nature must be prevented. Digestate produced using agricultural, agro-industrial and food processing feedstocks is usually a high-quality product which can be used advantageously as fertilizer. A robust and stable AD process has a positive impact on the quality of the digestate because, in some measure, is capable to degrade many of the undesired compounds and contaminants eventually supplied with the feedstock (Al Seadi and Lukehurst, 2012). The dry matter content will determine handling of the digestate as a solid or as a liquid stream. The total nitrogen (N) and phosphorus (P) contents are not affected by the digestion process although it favors their mineralization (Mehta and Batstone, 2013).

Typical challenges for waste management in agroecosystems are the improvement on nutrient availability in soil cycling; the development of technologies for nutrient reuse; the mitigation of contaminants and improvement of food safety; the mitigation of environmental emissions; and the enhancement of soil health and function (Bernal, 2017). The implementation of sustainable agro-energy systems integrating bioenergy and crops production is also attracting increasing attention (Siegmeier et al., 2015). Digestate application to agricultural soils as a nutrient source according to local regulations is interesting (Makádi et al., 2012; Möller and Müller, 2012; Nkoa, 2014) from both the economic and environmental perspectives owing to the implicit replacement of mineral fertilizers. However, factors such as transport needs, water content, and presence of heavy metals, organic micropollutants or pathogens may hinder this handling strategy (Ghafoori and Flynn, 2007; Nkoa, 2014). Beyond soil application, therefore, additional treatment of the digestate may be required to improve the capability of transporting valuable constituents, to protect human health and to prevent negative impacts on the receiving agricultural ecosystems, water bodies and atmosphere (Sheets et al., 2015). Storage, land application or post-treatment under inappropriate conditions may lead to nutrient leaching and runoff (Zhu et al., 2009) as well as gaseous

N emission as ammonia (NH₃) and nitrous oxide (N₂O)—the latter being a powerful GHG (Amon et al., 2006; Massara et al., 2017).

Solid-liquid separation of the digestates is frequently implemented and provides two different mass fractions that can be handled independently (Fuchs and Drosch, 2013; Tambone et al., 2017). The solid fraction -which usually contains a large amount of fibers and P- can be transported longer distances in order to be used as slow release fertilizer owing to the diminution in the water content, or undergo further treatment (e.g., composting, drying, etc.) to produce added-value products (Rehl and Müller, 2011; Sheets et al., 2015). Otherwise, the liquid fraction -also named reject water, supernatant, or centrate, among other names, depending on the context and separation technology applied, and which usually contains the larger part of N and potassium- can be used for the fertigation of nearby arable land, or post-processed in accordance with its typical low C:N ratio applying nutrient removal or nutrient recovery alternatives (Malamis et al., 2014; Mehta et al., 2015; Monlau et al., 2015; Sheets et al., 2015; Vaneckhaute et al., 2017; Monfet et al., 2018). N-removal involves the conversion of ammonium (NH₄⁺) to nitrogen gas (N₂), an innocuous gas which is released to the atmosphere. Mostly, this group encompasses biological treatments including both conventional strategies based on nitrification-heterotrophic denitrification (NDN) and advanced strategies such as partial nitrification-anaerobic ammonium oxidation (PN-anammox) (Malamis et al., 2014). Recovery consists on producing new material flows which subsequently can be reused -e.g., as agricultural fertilizer or nutrient broth-. Concentration by vacuum evaporation, NH₃ stripping and absorption, biological accumulation by prokaryotic organisms and algae, membrane filtration, and phosphate precipitation (e.g., as struvite) are some particular processes that belong to this group (Mehta et al., 2015; Vaneckhaute et al., 2017). The latter approach enables closing the nutrient cycle, which sounds always attractive in relation to sustainability. Nonetheless, other aspects like influent strength, resource consumption, process efficiency, environmental impact, local market prices, current legislation and national policies must be regarded to assess the final feasibility of these technologies (Rehl and Müller, 2011; Magrí et al., 2013; Rodriguez-Garcia et al., 2014; Batstone et al., 2015).

In many disciplines of science and engineering, research outputs generated by researchers and scholars worldwide are mostly disseminated by publishing papers in specialized journals. The impact of these publications into the research community is frequently assessed through bibliometric analysis. This method also enables the quantitative review of scientific productivity and the identification of trends on research (Moed et al., 2005). Bibliometrics has been applied in particular in the field of environmental science and engineering. This is the case, for instance, in topics such as AD (Wang et al., 2013), solid waste (Yang et al., 2013), upflow anaerobic sludge blanket -UASB- technology (Zhang et al., 2014), life-cycle assessment (Hou et al., 2015), ammonia oxidation (Zheng et al., 2017a), and nutrient management from digestates (Magrí et al., 2017), among others. The mapping of tendencies at the intersection of two research

topics has also been considered; e.g., algae and bioenergy (Konur, 2011), organic farming and bioenergy (Siegmeier and Möller, 2013), or wastewater and energy (Zheng et al., 2017b).

As an update of the work published by Magrí et al. (2017), the author addresses the bibliometric assessment of the scientific development and global trends in nutrient (N and P) management from digestates during the last three decades. Thus, the above referred study, originally focused on the joint analysis of publications and patents, has been revised. The new analysis is exclusively focused on publications, the multi-term topic search is enhanced, the time span is extended from 20 years (from 1995 to 2014) to 30 years (from 1988 to 2017), and new review comments are finally provided.

METHODS

Data Source

Publications were joined on-line via the Science Citation Index Expanded (SCIE) accessed through the Web of Science Core Collection -Clarivate Analytics, United States of America (USA)-. This is the most frequently used database for the analysis of scientific publications in journals. In 2016, SCIE indexed 8,879 journals across 177 subject categories. The time span considered for this study was the last three decades, from 1988 to 2017 included (retrieved data was updated on February 1st, 2018). The search was carried out within the fields: *title*, *abstract*, *author-provided keywords*, and *keywords plus*. Nonetheless, on some occasions, particularly for the publications appeared before 1990, such fields in the database are partly empty. This fact may have limited the number of publications finally retrieved in those early years. Search for publications was conducted after revision of the multi-term topic search (TS) used by Magrí et al. (2017):

$$TS = TS1 \text{ AND } TS2$$

where TS1 aims to identify the by-product on which the study is focused. It contains different terms that may be used to mention such by-product; i.e., short name (e.g., digestate, biogas residue, reject water, etc.), or a descriptive explanation by linking several words describing the process, or site of production, and the material treated (e.g., anaerobically treated manure, biogas digester effluent, etc.),

$$TS1 = [\text{"*digestate*"} \text{ OR } \text{"reject* *water*"} \text{ OR } \text{"biogas effluent*"} \text{ OR } \text{"biogas residue*"} \text{ OR } \text{"biogas slurr*"} \text{ OR } \text{"anaerobic liquor*"} \text{ OR } \text{"anaerobic supernatant*"} \text{ OR } ((\text{"digestion"} \text{ NEAR/3 } (\text{"anaerobic"} \text{ OR } \text{"biogas"} \text{ OR } \text{"*methane*"})) \text{ OR } \text{"digested"} \text{ OR } \text{"*digester*"} \text{ OR } \text{"*digestor*"} \text{ OR } ((\text{"treat*"} \text{ OR } \text{"*reactor*"})) \text{ NEAR/3 } \text{"anaerobic*"})) \text{ AND } (\text{"biogas"} \text{ OR } \text{"*methane"} \text{ OR } \text{"*fuel*"})) \text{ AND } ((\text{"digestion"} \text{ OR } \text{"digested"} \text{ OR } \text{"*digester*"} \text{ OR } \text{"*digestor*"} \text{ OR } \text{"treat*"} \text{ OR } \text{"*reactor*"})) \text{ NEAR/3 } (\text{"*waste*"} \text{ OR } \text{"*water*"} \text{ OR } \text{"sludge*"} \text{ OR } \text{"manure*"} \text{ OR } \text{"dung*"} \text{ OR } \text{"effluent*"} \text{ OR } \text{"*slurr*"} \text{ OR } \text{"residu*"} \text{ OR } \text{"biosolid*"} \text{ OR } \text{"leachate*"} \text{ OR } \text{"supernatant*"} \text{ OR } \text{"liquor*"} \text{ OR } \text{"*stream*"} \text{ OR } \text{"centrate*"}))]]$$

whereas TS2 aims to identify the nutrients (e.g., nitrogen, phosphorus, etc.) to be handled and other fertilization-related topics.

$$TS2 = [\text{"nitrogen*"} \text{ OR } \text{"ammoni*"} \text{ OR } \text{"phosph*"} \text{ OR } \text{"nutrient*"} \text{ OR } \text{"ferti*"} \text{ OR } \text{"soil*"} \text{ OR } \text{"land"}]$$

Different authors may refer to the same concept using different terms, and chaining multiple words. In addition, the wide spectrum of this field of research, including many management and treatment alternatives, not only applying to digestates, hindered the definition of a simpler TS.

Refined Data

Only documents reported as articles were selected for conducting this study; reviews, meeting abstracts, notes, book chapters and other types of publications were discarded. Documents simultaneously indexed as articles and proceedings papers were, thus, accepted. The list of pre-selected articles included 5,677 items (93.6% of the total number of retrieved publications). The main indexed contents of these articles were saved to a Microsoft Excel file. Subsequently, such list was refined manually. Only those items that fitted the main goal were kept. Such screening consisted in reading the title and when needed, the abstract, to confirm the selection of each article. This resulted in a total of 2,314 articles (40.8% of the original number of articles), which have been cited 40,157 times and have reached an *h*-index of 82 by the end of the year 2017. Thus, the refining process was essential owing to the heterogeneity in the scope of the automatically retrieved articles, and although it is time-consuming, requires specific criteria and can introduce some subjectivity. The subsequent analysis of the retained articles was useful for quantifying annual productivity, as well as for identifying dominant SCIE subject categories and indexed journals, most productive countries, and relative interest in certain research topics and their development. The contribution of a country to an article was considered by the affiliation of at least one author. Articles published by authors from England, Scotland, Northern Ireland and Wales were grouped under the United Kingdom (UK). Articles published by authors from Hong Kong were grouped under China. Results of the analysis are presented graphically or in tables, and they are referred to the whole study period (1988–2017) and to ten 3-year partial periods, which allow smoothing yearly fluctuations.

RESULTS

Number of Published Articles

In the first 15-year period analyzed in this study (from 1988 to 2002), the number of published articles per year was low and did not experience significant changes, with record counts ranging from 0 to 32 (**Figure 1**). Thus, in the 90s, published articles averaged 16 items per year. Yet, in the next years, the publication rate raised sharply from 38 articles in 2003 up to a maximum of 313 articles in 2017. In order to compare the evolution of the annual number of published articles dealing with nutrient management from digestates with the overall number of

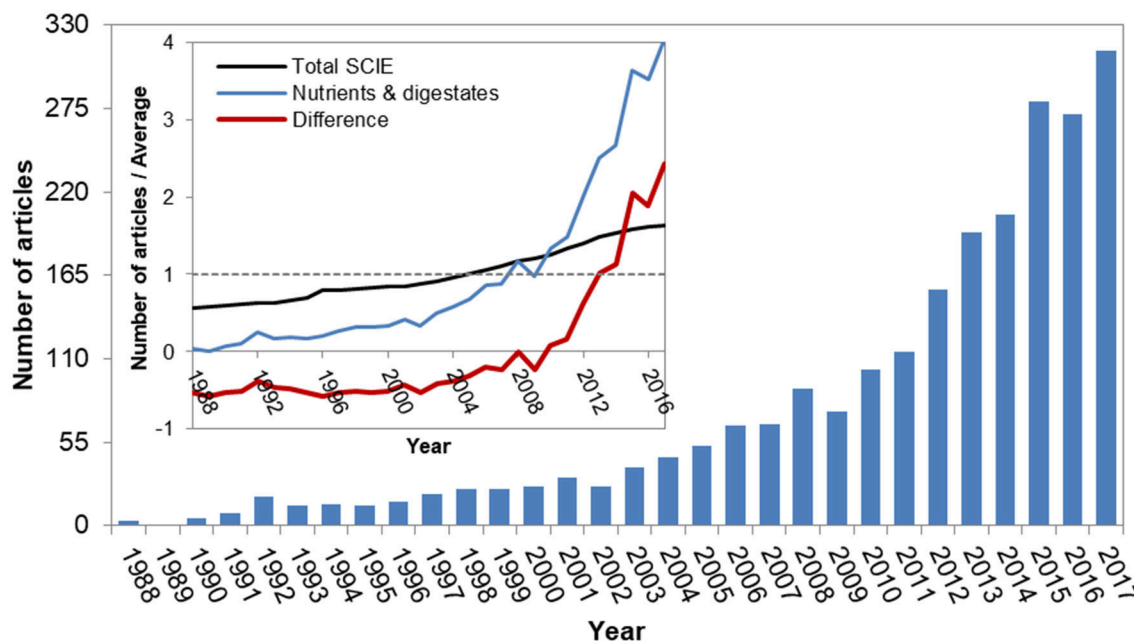


FIGURE 1 | Yearly number of published articles concerning nutrient management from digestates in the period 1988–2017.

articles indexed in SCIE, both time series were standardized by dividing the corresponding number of articles and the 30-year average (**Figure 1**). This analysis shown that, in recent years, the rising trend in the annual number of published papers focused on nutrient management from digestates has been more significant than in the overall number of articles, particularly after 2010. In relative terms, articles published in the last three years (from 2015 to 2017) accounted for 37.3% of the total documentary production.

Subject Categories and Scientific Journals

In the whole study period, articles dealing with nutrient management from digestates fell into 58 different SCIE subject categories, and were published in 351 indexed journals. The 10 most frequent categories are shown in **Figure 2**. The dominant category was “Environmental Sciences” with 1,105 articles (47.8%), followed by “Engineering, Environmental” and “Water Resources” with 717 (31.0%) and 478 (20.7%) articles, respectively. The categories “Engineering, Chemical” (50.2%) and “Environmental Sciences” (37.1%) were dominant in the most recent 3-year period relative to the total number of articles appeared in the period 1988–2017.

The 10 dominant journals are shown in **Figure 3**. Such journals published a total of 897 papers, which is 38.8% of the analyzed items. *Water Science and Technology* included the most with 251 articles (10.8%), subsequently followed by *Bioresource Technology* and *Water Research* with 209 (9.0%) and 92 (4.0%) articles, respectively. Seven of the top 10 journals are currently published by Elsevier. *Water Science and Technology* was also the most prolific journal in the early years with 32 articles published from 1988 to 1997 (28.6% of the total number of articles appeared

in that decade). The journals *Science of the Total Environment* (81.1%) and *Journal of Cleaner Production* (71.1%) published the most number of articles in the last 3-year period relative to the total number of publications in the full period analyzed. In view of the results, only 48 journals (13.7%) published 10 or more articles in the topic under analysis from 1988 to 2017.

Productivity by Country

Authors with affiliation in 84 different countries contributed to the publication of articles dealing with nutrient management from digestates. The 10 most prolific countries were six in Europe, two in North America and two in Asia (**Figure 4**). Documentary production of such countries accounted for 69.5% of the total number of articles. China was the most productive country with 340 articles (14.7%), followed by USA (330 articles, 14.3%) and Spain (218 articles, 9.4%). The USA was the most prolific country in the early years (27 articles during 1988–1997; 24.1% of the total number of articles appeared in that decade). On the other hand, China (54.4%) and Italy (49.7%) published the most articles in the last 3-year period relative to the total number of publications in the full period analyzed. The most productive institutions regarding the total number of articles were mainly European. The list of institutions was headed by Ghent University in Belgium (50 articles), the Swedish University of Agricultural Sciences (45 articles) and Aarhus University in Denmark (44 articles). The first non-European institution was China Agricultural University which ranked 5th (37 articles).

Research Topics and Trends

Articles selected for conducting this study were analyzed to identify main research topics and associated trends. **Figure 5A**

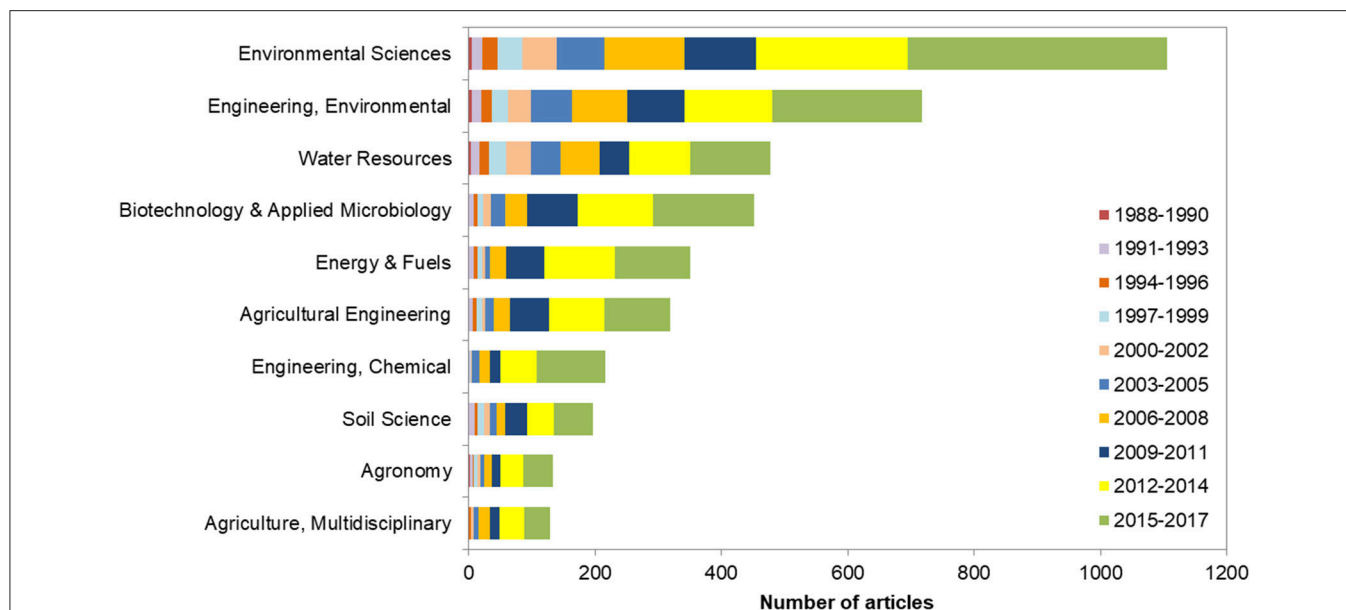


FIGURE 2 | Top 10 dominant SCIE subject categories based on the number of published articles concerning nutrient management from digestates in the period 1988–2017.

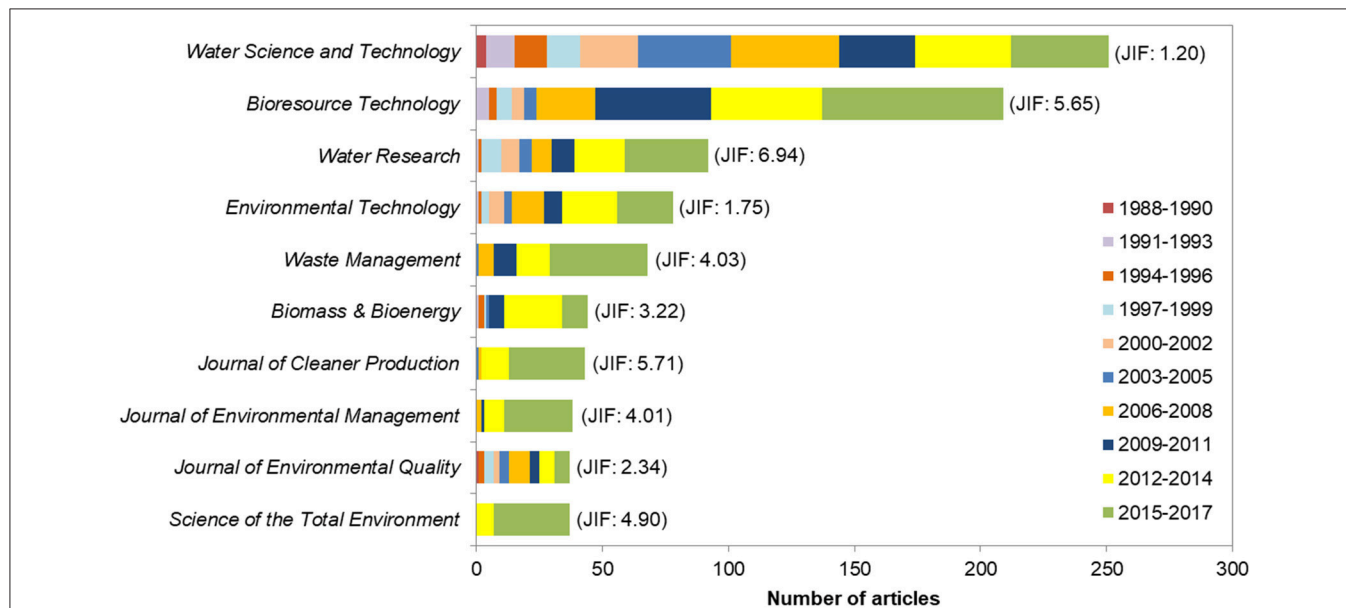
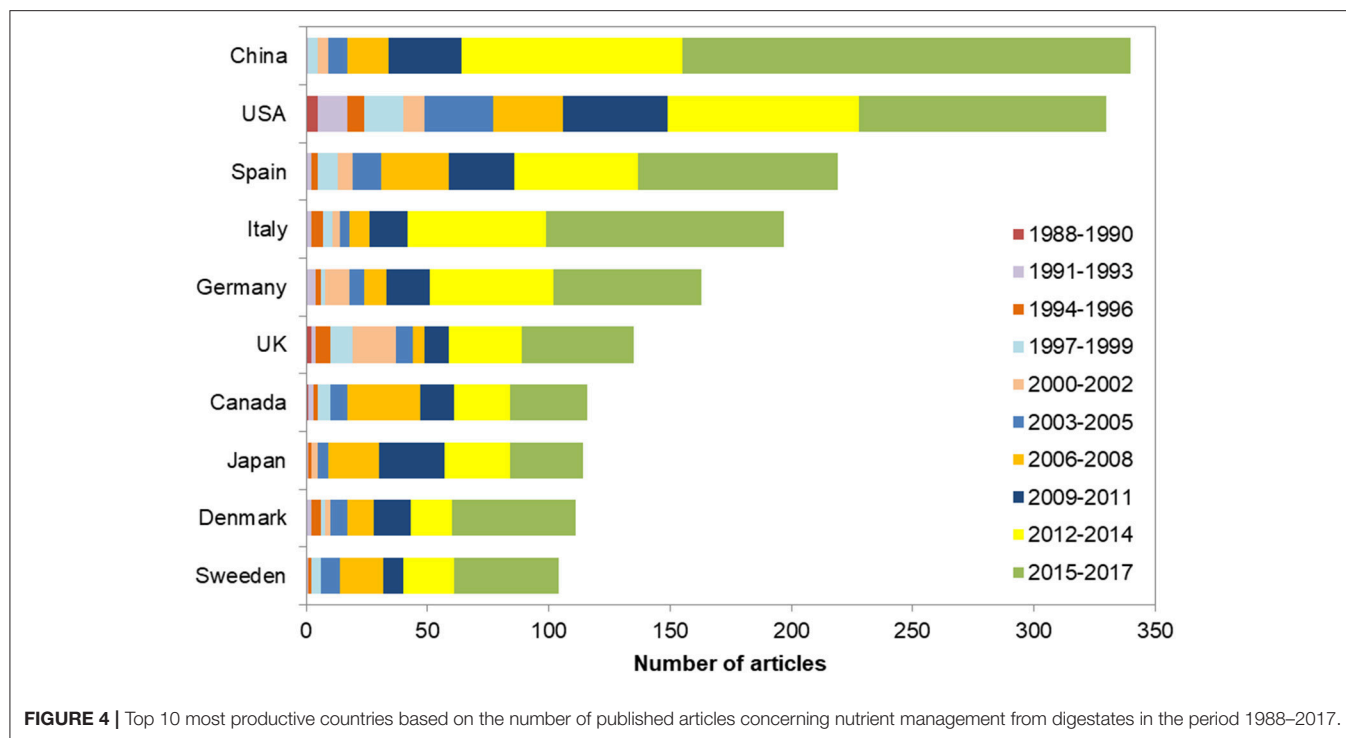


FIGURE 3 | Top 10 dominant journals based on the number of published articles concerning nutrient management from digestates in the period 1988–2017. JIF, Journal Impact Factor (year 2016).

shows the number of items focused on the management of a given nutrient (N and P) according to the publication fields *title* and *author-provided keywords*. Supposedly, such fields contain the most significant information that the authors want to convey the readers, as well as show tendencies in research activity (Li et al., 2009). In **Figure 5A**, “nutrients” refers to unspecific mentions, “nitrogen” includes specific mentions to the chemical element N and other related compounds (e.g., ammonium, ammonia,

struvite, nitrous oxide, nitrite, and nitrate), and “phosphorus” includes specific mentions to the chemical element P and other related compounds (e.g., phosphate, apatite, and struvite). In all cases, there is an increasing trend regarding the number of articles, although “nitrogen” has clearly aroused the greatest interest. Recently, general references to “nutrients” have become more frequent than particular references to “phosphorus.” On the other hand, **Figure 5B** shows the number of publications



dealing with main research topics such as “crop nutrition & fertilization,” “nutrient removal technologies,” “nutrient recovery technologies,” “gaseous emissions,” and “environmental & life-cycle assessment”. The lack of standardization in language may hinder this analysis since some terms are used vaguely; i.e., different authors use the same word to express different things, or use either synonyms or different terminology to define the same concept. Overall, research outputs were mostly linked to “crop nutrition & fertilization,” as well as to the characterization of “nutrient recovery technologies.” Despite the number of published articles dealing with “nutrient removal technologies” start growing significantly in the period 2003–2005, its trend was clearly surpassed in recent years by that of the “nutrient recovery technologies.” Additionally, conceptual studies, addressing “environmental & life-cycle assessment,” are attracting increasing attention since the period 2003–2005. In fact, both research topics “nutrient recovery technologies” and “environmental & life-cycle assessment” held the highest grow in the last three years relative to the total number of articles in the whole period (47%).

Particular trends in research of technologies targeting nutrient removal and nutrient recovery from digestates were also assessed (Figure 6). Removal related topics are shown in Figure 6A, where “nitrification” appears as the most frequent addressed issue. Research on “anammox” was found to start later than research on “heterotrophic denitrification,” but then the first grew faster, particularly in the period 2006–2011. The number of articles appeared in the subsequent years was similar for both N-removal strategies. Research addressed on biological P-removal was much lower. On the other hand, recovery related

topics are shown in Figure 6B. Albeit the highest number of published articles was attained by the topics “algae cultivation” and “phosphate precipitation,” the corresponding publication patterns were significantly different. Thus, while the number of articles focused on the precipitation of phosphates increased quite steadily in the whole study period, the appearance of articles focused on the cultivation of algae raised sharply in the last years.

Finally, a citation impact assessment was performed. This analysis considered all articles, and also partial groups of articles, in accordance with the research topic and time interval (Table 1). The main topics taken into account were the same that those already identified in Figure 5B; this is “crop nutrition & fertilization,” “nutrient removal technologies” (in particular those based on “heterotrophic denitrification” and “anammox”), “nutrient recovery technologies” (in particular those based on “algae cultivation,” “(vermi)composting,” “membrane filtration,” and “phosphate precipitation”), “gaseous emissions,” and “environmental & life-cycle assessment.” The citation rate concerns the average number of cites per article and per year. Citations were grouped in 2-year periods after publication. The global citation rate (i.e., concerning all articles) averaged 2.63 whereas the particular citation rates (i.e., concerning all topics and time periods) ranged from 0.00 to 7.25. The sub-topic “anammox” gathered the highest citation rate for the whole study period (4.55), followed by “environmental & life-cycle assessment” (4.34) and “algae cultivation” (4.24).

None of the 10-top articles date back earlier than 1997 nor later than 2014. These articles have been highly cited, averaging from 14.4 to 79.3 times per year (the total number of cites received per article from the year of publication to the end of 2017

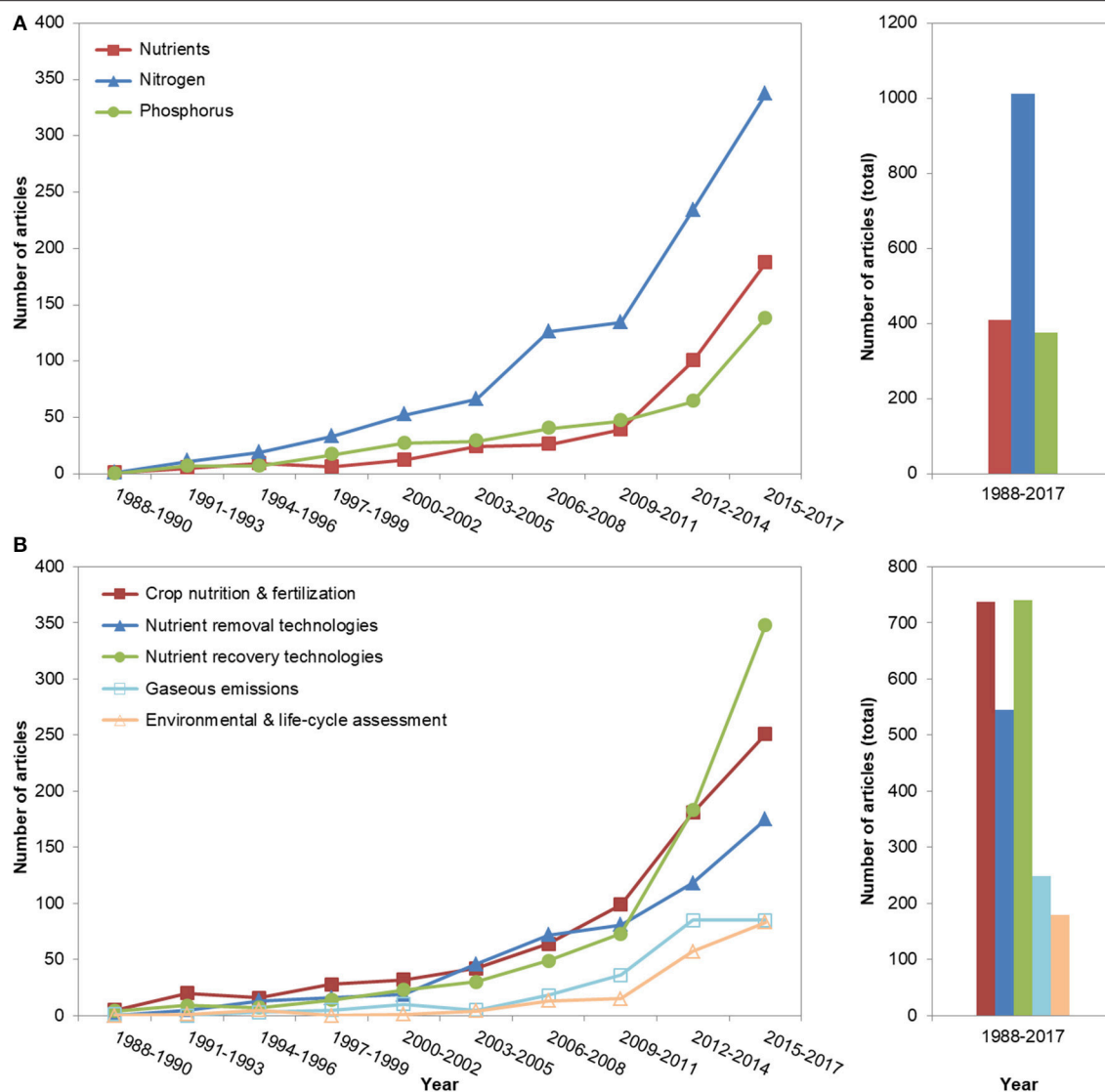


FIGURE 5 | Number of published articles concerning nutrient management from digestates in the period 1988–2017 grouped in 3-year partial periods. **(A)** Trends for assessed nutrients. **(B)** Trends for main research topics.

was from 235 to 469). Five of these articles appeared in the journal *Water Research*, and eight were authored by individuals with affiliation in European institutions. Even though the research topics of these top articles differed, N-removal applying the anammox process was the most recurrent topic (not less than five articles) with 3,185 cites in total (53.2% of the total number of cites counted).

DISCUSSION

The advance of knowledge in the field of nutrient management from digestates has been analyzed using bibliometric tools. A growing interest in this research topic -as main subject or sharing protagonism with the application of the AD process for producing renewable energy and biofuel in the form of

biomethane- was confirmed in terms of annual number of published articles. Thus, 37% of the articles considered in this study appeared in the last three years. Such upward trend in written productivity was also pointed out by Wang et al. (2013) in a bibliometric study concerning research on AD for methane production. As likely reasons for the growing interest in AD, these authors referred to the emergence of a fossil energy crisis, the rise in the price of energy, and the general enhancement of social awareness, among others. Current energy policies are another factor leading in this direction by promoting the use of renewable resources for the production of biofuels. China, USA and Spain were identified as the three countries with higher numbers in published articles addressing nutrient management from digestates. A fast growing rate was particularly spotted for China in recent years -54.4% of the articles were published in

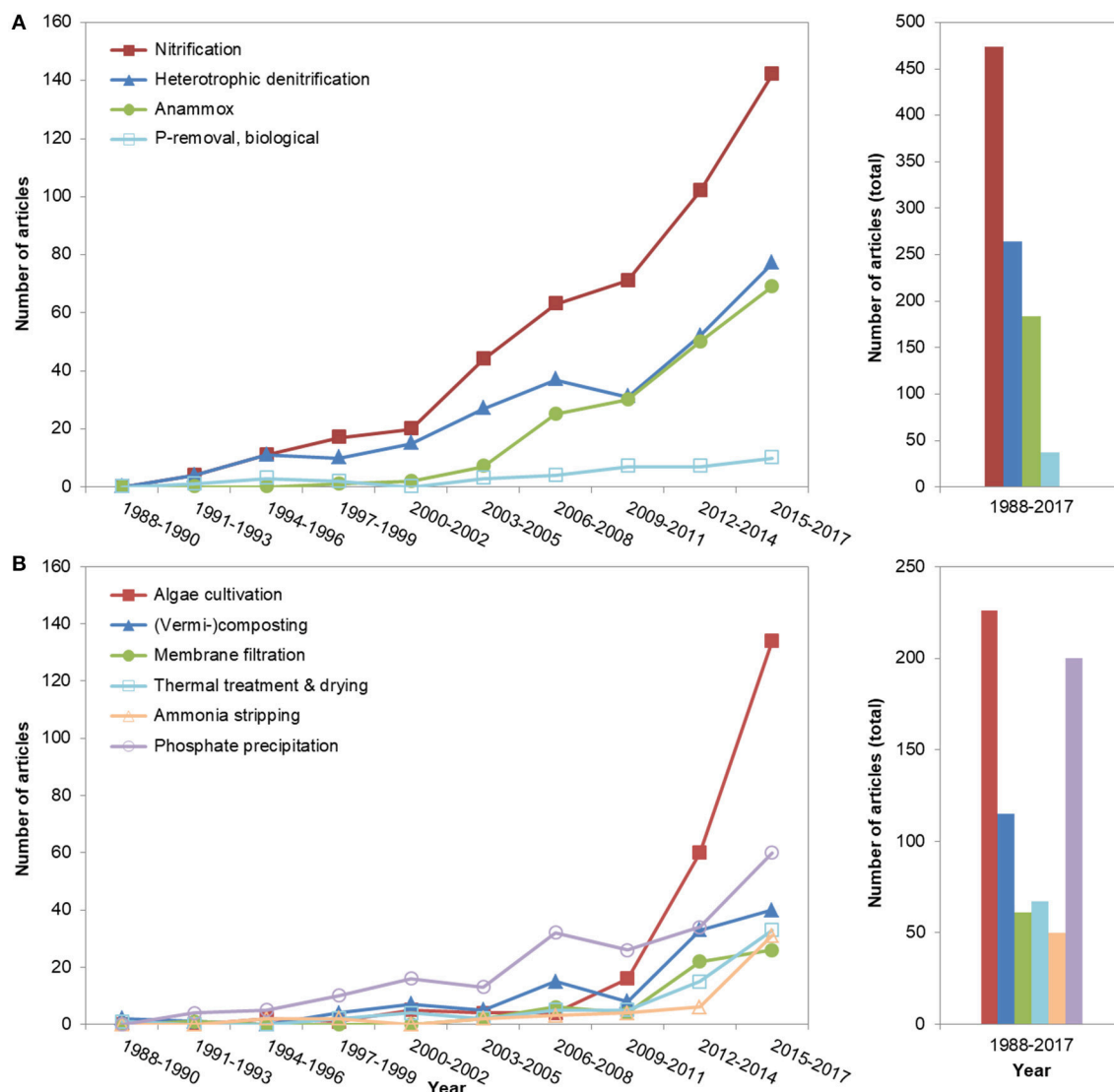


FIGURE 6 | Number of published articles concerning nutrient management from digestates in the period 1988–2017 grouped in 3-year partial periods. **(A)** Trends for technologies aiming to nutrient removal. **(B)** Trends for technologies aiming to nutrient recovery.

the last three years-, similarly as it has already been reported in other fields of chemical engineering (Fu et al., 2014). However, the most active institutions, and also the most cited articles, were still primarily European. Spain occupied the third position of the productivity ranking by country. Such good position may result quite surprising since Spain is not even member of the G7, and has lower population, gross domestic product, and renewable energy and environmental policies development, than other countries. In Europe, other countries such as Germany, UK or Italy produce larger amounts of biogas than Spain, and currently, primary energy production from biogas in this country is not growing. Accordingly, its ranking as European biogas producer has dropped from 7th in 2011 to 10th in 2016 (EurObserv'ER, 2012, 2017). The high rating for Spain regarding publication of scientific articles in nutrient management from digestates

was already identified by Magrí et al. (2017). Nonetheless, the important increase in the number of published articles for the last years may involve fast changes in the rankings; e.g. China -which ranked 3rd in 2014 according to Magrí et al. (2017)- bypassed USA as the most productive country in only 3 years.

The reuse of digestate as fertilizer or soil improver has generated increasing scientific interest in the past decades (Hons et al., 1993; Möller and Müller, 2012; Nkoa, 2014). Questions regarding the effect of digestate application on nutrient mineralization, nutrient uptake, plant growth, phytotoxicity, and possible water pollution according to the land use, kind of soil, and loading rate have frequently been addressed (738 items in **Figure 5B**, accounting for 32% of the total number of articles). Other studies have focused on the monitoring

TABLE 1 | Citation rates for published articles concerning nutrient management from digestates in the period 1988–2017.

Research topic ^a	Full period ^{b,c}		Partial periods									
	1988–2017		1988–1990	1991–1993	1994–1996	1997–1999	2000–2002	2003–2005	2006–2008	2009–2011	2012–2014	2015–2017
All articles	2.63		1.00	0.72	0.85	1.01	1.26	1.49	1.95	2.84	3.35	3.49
Crop nutrition & fertilization	1.87		0.90	0.60	1.28	0.80	0.73	1.13	1.63	2.02	2.42	2.41
Nutrient removal technologies	2.75		0.00	1.10	0.73	1.19	1.97	1.60	2.26	3.48	3.42	3.13
- Heterotrophic denitrification	1.99		0.00	0.75	1.05	0.90	1.50	1.41	1.58	1.69	3.13	2.59
- Anammox	4.55		0.00	0.00	0.00	7.00	7.25	2.50	3.94	4.88	5.28	3.66
Nutrient recovery technologies	3.16		1.13	0.72	0.79	1.00	1.54	1.35	1.78	3.43	3.83	3.96
- Algae cultivation	4.24		0.00	0.00	0.50	0.00	0.80	2.13	2.38	4.88	4.82	4.25
- (Vermi)composting	2.33		0.50	1.00	0.00	1.00	0.57	2.50	1.77	3.63	3.06	2.03
- Membrane filtration	3.26		0.00	0.00	0.00	0.00	0.00	1.25	1.58	1.88	5.02	2.20
- Phosphate precipitation	2.25		0.00	1.38	1.30	1.55	1.88	1.85	1.44	2.67	2.94	3.57
Gaseous emissions	3.32		0.50	0.00	1.00	1.70	0.70	2.80	3.56	4.15	3.63	2.76
Environmental & life-cycle assessment	4.34		0.00	0.00	0.20	0.00	0.50	2.00	2.23	4.53	4.73	5.65

Results were grouped in 3-year partial periods as in **Figures 5, 6**.

^aResearch topics taken into account were: (i) "crop nutrition & fertilization" (including experimental developments concerning digestate characterization, soil amendment, agricultural fertilization, etc.), (ii) "nutrient removal technologies" (based on "heterotrophic denitrification"; "anammox"; etc.), (iii) "nutrient recovery technologies" (based on "algae cultivation"; "vermi)composting"; "membrane filtration"; "phosphate precipitation"; etc.), (iv) "gaseous emissions"; and (v) "environmental & life-cycle assessment".

^bValues correspond to average number of cites per article and per year taking into account the 2 years following publication (the publication year is not considered). Those articles published after 2015 were not included in calculations.

^cAverage number of total citations per article in the full period 1988–2017 (equivalent to cites per article after publication), if reading the research topics in descending order, were 17.4 (all articles), 14.2, 20.6, 15.8, 29.9 (anammox), 16.5, 14.9, 15.3, 16.7, 21.5, 22.1, and 18.9 (environmental & life-cycle assessment).

of potential environmental impacts such as the emission of GHGs (i.e., CO₂, CH₄, and N₂O) and other atmospheric pollutants (e.g., NH₃) during storage and after land spreading of digestate (Amon et al., 2006). Acidification before an eventual solid-liquid separation has been shown as a good emission abatement practice when managing digested livestock slurries (Regueiro et al., 2016). Occurrence and fate of soil pollutants such as pathogens, heavy metals, pesticides, and hormones, which can be harmful if entering in the human food chain, has also been assessed (Govasmark et al., 2011; Rodríguez-Navas et al., 2013; Bonetta et al., 2014). Digestate handling strategies have been analyzed taking into account current legislation, nutrient management planning, local agricultural constraints, and particularities such as continuous digestate production but seasonal nutrient needs in cropping systems, among others (Flotats et al., 2009). Research studies dealing with the use of fresh digestate as source of nutrients have appeared mainly linked to agricultural systems but also to some alternative areas such as hydroponics (Krishnasamy et al., 2012), forestry (Bardule et al., 2018), and soil reclamation (García-Sánchez et al., 2015). Finally, the characterization of livestock-biogas-fish farming systems, which imply integration of crop production, vegetable cultivation, livestock breeding and/or fish culturing, has been addressed in developing countries, particularly in Asia (Wu et al., 2014). Limitations in the use of fresh digestate as source of nutrients in agriculture and other similar areas have motivated the quest for processing technologies.

Research in biological nutrient removal technologies applied to anaerobic supernatants was started in the early 90's. Both intensive (based on the use of bioreactors) and extensive (including wetlands) systems have been considered (Monfet et al., 2018). "Nitrification" was the most active topic because to achieve biological N-removal, nitrification is necessary for both conventional strategies based on heterotrophic denitrification and advanced strategies based on anammox. Besides, the aerobic treatment based on nitrification can seek the transformation of N volatile species to non-volatile species and be applied in nutrient recovery strategies (Botheju et al., 2010). The energy required for aeration to complete nitrification and the organic carbon required in heterotrophic denitrification are two of the main drawbacks of the NDN technologies (Siegrist et al., 2008). In this regard, significant reductions in the requirements of energy and organic carbon have been demonstrated when the NDN process is optimized through the nitrite route (Malamis et al., 2014). Alternatively to classical N-removal, the anammox process was first reported in mid-90s by Mulder et al. (1995) and research in this field is known to speed up after the year 2000 (Magrí et al., 2013; Zhang and Liu, 2014). Hence, over the last 15 years, many technologies based on applying PN-anammox related processes have been developed and characterized in detail, and several of them are currently being implemented at full-scale (mostly addressing the sidestream treatment of reject water generated when dewatering anaerobically digested sewage sludge). In this regard, more than 100 full-scale PN-anammox installations are currently running worldwide (Lackner et al., 2014), and the number of new plants

is increasing rapidly. Lately, mainstream wastewater treatment through energy efficient processes like anammox has attracted interest and has been posed as precedence for innovation and development by the water industry (Vela et al., 2015). The emission of N₂O in N-removal systems owing to the activity of both nitrifying and denitrifying microorganisms has often been addressed in research studies, as summarized by Massara et al. (2017). Other non-biological methods to achieve nutrient removal from digestate have also been reported but in a lesser extend (Fernandes et al., 2017).

The recovery of nutrients from digestates has undergone accelerated development in recent years. Research has frequently focused on the separated handling of solid and liquid fractions instead of the integrated processing of the digestate. Multiple technological alternatives have been considered as reviewed elsewhere (Fytli and Zabaniotou, 2008; Fuchs and Drosch, 2013; Sheets et al., 2015; Vaneeckhaute et al., 2017; Monfet et al., 2018) with significant particularities between N and P (Desmidt et al., 2015; Zarebska et al., 2015). Physicochemical methods usually target the production of high quality, nutrient-rich concentrates that can be placed on the market. Besides, some of these methods may help to prevent problems in AD systems such as, for instance, microbial inhibition by ammonia accumulation within the digester (Nie et al., 2015) and the formation of struvite scale deposits (Le Corre et al., 2009). Research experiences at lab-, pilot- and full-scale have been reported for technologies such as (i) precipitation / crystallization of magnesium and calcium phosphates, (ii) pressure-driven membrane filtration involving microfiltration, ultrafiltration, nanofiltration and/or reverse osmosis, (iii) NH₃ stripping followed by absorption under acidic conditions, (iv) adsorption and ion exchange, and (v) thermal treatment, among others. Biological methods have also been assessed; this includes, among others, (vermi)composting treatments applied to solid material fractions (Hanc and Vasak, 2015; Magrí and Teira-Esmatges, 2015; Zeng et al., 2016) and algae cultivation techniques applied to liquid streams (Cai et al., 2013; Monlau et al., 2015). Composting as post-treatment of the digestate forces the aerobic biodegradation of remaining organic matter, favoring the stability and maturity of the final product. An appropriate rise of temperature during the process will lead to the drying of the material and to the elimination of potential pathogens (Teglia et al., 2011). Blend of the digestate with other materials may be convenient according to its moisture content and C/N ratio. Interest in algae has grown rapidly in recent years, and technical feasibility of many applications has been demonstrated, allowing integrated biofuel production, carbon dioxide mitigation, and nutrient recovery from wastewater streams. The transformation of algae in biogas, followed by the use of the digestate for algae cultivation, enables an interesting closed-loop approach for the production of bioenergy (Prajapati et al., 2014). Owing to the change in perception of WWTPs as resource recovery facilities, conventional mathematical models started to be updated including new processes, technologies and plant layouts (Fernández-Arévalo et al., 2017). Future research in nutrient recovery should focus on innovative technologies not yet consolidated at full-scale (Bakx et al., 2009; Desmidt et al., 2015; Zarebska et al., 2015) engaging with the emerging concept of

(bio)refinery which can be based on physicochemical (Dube et al., 2016), biological (Matassa et al., 2015), or bioelectrochemical processes (Hou et al., 2017), as well as on further verify and improve the characteristics and marketing value of digestates and other derived products toward agricultural and industrial end-users (Dahlin et al., 2015). As pointed out by Monlau et al. (2015), the (bio)refinery concept draws new challenges to be considered, and thus, research on sustainable digestate valorization through (bio)refinery is called to face significant development in the next years. Pilot-scale experiences will be needed to assess the real benefits in terms of energy balance, environmental impact, and economy, and to better decide on the integration of processes from an industrial point of view.

Finally, environmental and life-cycle assessment of integrated agro-energy systems (De Meester et al., 2012; Van Stappen et al., 2016) and digestate treatment technologies (Rehl and Müller, 2011; Rodriguez-Garcia et al., 2014; Vázquez-Rowe et al., 2015) is attracting increasing attention. To move toward sustainability, it will be needed the implementation of integral solutions, a reduced consumption of resources, and circular economy strategies, among other aspects, at the same time that minimizing the affectations on the environment (van Loosdrecht and Brdjanovic, 2014; Molina-Moreno et al., 2017).

CONCLUSIONS

The bibliometric analysis of the articles published in the last three decades (from 1988 to 2017) in the area of knowledge of nutrient management from digestates led to the following main conclusions:

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- The annual publication rate has followed an upward trend, particularly in recent years. Only in the last three years has appeared ~37% of the total production.
- China, USA, and Spain were the three most prolific countries regarding number of articles published. The most productive institutions were primarily European.
- Topic of the articles was mostly connected to 'crop nutrition & fertilization' and the characterization of 'nutrient recovery technologies'. Despite the number of published articles dealing with 'nutrient removal technologies' start growing significantly in the 2000s, its trend was clearly bypassed in recent years by that of the 'nutrient recovery technologies'. Conceptual studies, focusing on 'environmental & life-cycle assessment', are attracting growing attention since the period 2003–2005.
- Trends for promoting sustainability when managing nutrients from anaerobic digestates include low environmental impact, low uptake of resources, integral agro-energy solutions, and circular economy approaches based on the recovery of valuable and marketable products.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and approved it for publication.

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