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Coupling electrochemical ammonia extraction and cultivation of methane oxidizing bacteria for production of microbial protein

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Abstract

Conventional treatment of residual resources relies on nutrient removal to limit pollution. Recently, nutrient recovery technologies have been proposed as more environmentally and energetically efficient strategies. Nevertheless, the upcycling of recovered resources is typically limited by their quality or purity. Specifically, nitrogen extracted from residual streams, such as anaerobic digestion (AD) effluents and wastewaters, could support microbial protein production. In this context, this study was performed as a proof-of-concept to combine nitrogen recovery via electrochemical reactors with the production of high quality microbial protein via cultivation of methanotrophs. Two types of AD effluents, i.e., cattle manure, organic fraction of municipal solid waste, and urine were tested to investigate the extraction efficiency. The results showed that 31-51% of the nitrogen could be recovered free of trace chemicals from residual streams depending on the substrate and voltage used. Based on the results achieved, higher nitrogen concentration in the residual streams resulted in higher nitrogen flux between anodic and cathodic chambers. Results showed that the extraction process has an energy demand of 9.97 (±0.7) - 14.44 (±1.19) kWh/kg N, depending on the substrate and operating conditions. Furthermore, a mixed-culture of methanotrophic bacteria could grow well with the extracted nitrogen producing a total dry weight of 0.49±0.01 g/L. Produced biomass contained a wide range of essential amino acids making it comparable with conventional protein sources.

Keywords: Nutrient recovery; Electrochemical cells; Microbial protein; Methane oxidizing bacteria
1. Introduction

Food scarcity is becoming one of the most important challenges for the ever-growing world population as a result of limited natural resources and arable lands (Müller et al., 2008; Hertel, 2015). In parallel, the demand for protein has increased on a global scale due to its contribution to healthy aging and diets (Delgado, 2003; Popkin et al., 2012; Rafiee et al., 2016). The increased demand for plant and animal-based protein has exerted considerable pressure on the environment, generating large greenhouse gas emissions, high water consumption, and deforestation (Henchion et al., 2017). In addition, more nitrogen fertilizers, whose production accounts for 1−2% of the worldwide energy consumption (Matassa et al., 2015), are required for crop cultivation to cope with the increased demand of vegetable protein (Maurer et al., 2003). On the other hand, according to Christiaens et al. (Christiaens et al., 2017) and Hulsen et al. (Hülsen et al., 2018) the usage efficiencies of nitrogen fertilizers in plant and meat protein production are as low as 9% and 7% of the total fertilizer consumed, respectively. This is due to the low uptake of N-based fertilizers by protein crops and the subsequent losses through upcycling of protein crops to animal protein (Hülsen et al., 2018). Thereby, there is no doubt that more efficient protein sources are required to replace conventional animal feed and food supplements.

In this context, microbial protein (MP) has shown a great advantage because it can be independent of climate, soil characteristics and available land (Matassa et al., 2016; Hülsen et al., 2018). Moreover, its high protein content and high conversion efficiency of nutrients to biomass make MP capable of closing the protein gap (Verstraete et al., 2016; Khoshnevisan et al., 2019). Methanotrophs, as an example, are a group of microorganisms which exploit methane as their carbon and energy source and assimilate inorganic nitrogen which is stored as protein that has the quality for livestock, fish or human consumption (Matassa et al., 2016). Although MP can be
produced from algae, yeast and other bacteria, methanotrophs contain higher protein than yeast while have more digestible cell walls than algae (Bajpai, 2017; Tsapekos et al., 2019). *Methylococcus capsulatus*, as a methanotroph, has long been used for commercial MP production, albeit the production is dependent on fossil-based feedstocks, i.e., natural gas and synthetic nitrogen (Unibio, 2016). Indeed, the natural gas based MP is currently commercially produced and has gained significant attention (Strong et al., 2015).

The high anthropogenic nitrogen loads which are discharged into water bodies, especially in the form of ammonia, lead to eutrophication and toxicity (Zhao et al., 2018). Thus, nitrogen present as ammonia in wastewater is removed either via traditional nitrification-denitrification processes or emerging technologies such as simultaneous nitrification–denitrification and partial nitritation-Anammox (Sun et al., 2010). Biological nitrogen removal is yet another energy intensive process where the invested energy per g-N removed equals to the energy invested for g-N fixed via Haber-Bosh process (Matassa et al., 2015). In addition, these biological nitrogen removal processes lead to nitrogen losses as nitrogen gas or potent greenhouse gases such as nitrous oxide (Domingo-Félez and Smets, 2019). Therefore, if nitrogen could be recovered from residual sources and used for MP production, the overall anthropogenic nitrogen cycle would be more economical and energy efficient (Christiaens et al., 2017). Up-cycling of the nitrogen available in concentrated streams such as municipal wastewater and anaerobic digestion effluent into protein-rich MP can be highly beneficial for both food security and the environment (Khoshnevisan et al., 2020).

Nitrogen rich residual streams such as manures or municipal wastewater may contain trace chemicals which can inhibit the growth of microorganisms (Rasouli et al., 2018; Duan et al., 2020). Moreover, residual streams normally contain pathogens, antibiotics or heavy metals, which
compromise the quality of the produced MP, making it unsuitable for human or animal consumption. Hence, nutrient extraction methods that prevent proliferation of pathogens or transfer of trace chemicals and heavy metals to the fermenters growing microbes for MP production are needed. A vast number of methods have been investigated and tested to recover nitrogen from aqueous environment including ion exchange (Tada et al., 2005), electrodialysis (Ippersiel et al., 2012), micro and nanofiltration (Zarebska et al., 2015), reverse osmosis (Mondor et al., 2008), and microbial fuel cells (Kuntke et al., 2012). Each method has its own advantages and disadvantages. For instance, ion exchange process is an environmentally friendly method with high treatment capacity, fast kinetics, and low maintenance cost. However, it suffers from calcium sulfate fouling, iron fouling, adsorption of organic matter, organic contamination from the resin, and bacterial and chlorine contamination (Kang et al., 2004; Gupta et al., 2015). As another example, the nanofiltration method faces some unresolved challenges including membrane fouling, poor selection between different solutes, chemical resistance and limited lifetime of membranes (Van der Bruggen et al., 2008). Electrochemical processes have also attracted great interest for ammonia removal and recovery from wastewaters (Porada et al., 2013; Huang et al., 2014). Electrodialysis configuration with selective ion exchange membranes is one of the electrochemical processes with improved efficiency of ammonia recovery (Zhang et al., 2013; Ren et al., 2017).

In line with the concept of combining nitrogen removal and microbial protein production proposed in literature (e.g., Matassa et al., 2015), this study attempted to demonstrate the valorization of inorganic nitrogen from residual streams through the cultivation of methane oxidizing bacteria as a proof of concept. Synthetic urine, real male urine, anaerobically digested manure and organic fraction of municipal solid waste (OFMSW) were tested as residual streams
for nitrogen extraction. We investigated i) effect of nitrogen concentration and voltage on the nitrogen extraction efficiency; ii) fate of micro-pollutants from residual streams during the extraction; iii) effect of nitrogen sources on the nitrogen extraction efficiency; and iv) cultivation of methanotrophs in the extracted nitrogen. The last objective was set to demonstrate that micro-pollutants cannot transfer across the membrane, thus producing a clean cultivation media for methanotrophs. Moreover, the cultivation of methanotrophs in the extracted nitrogen allowed investigating whether or not pure medium without supplementary nutrients was enough for successful cultivation of MP.

2. Materials and methods

The set-up employed for the electrochemical extraction processes, substrates tested, and the methods are discussed in details in the following sub-sections.

2.1. Experimental setup

A two chamber electrochemical cell, with an effective volume of 200 cm³ in each chamber, was used for electrochemical extraction of ammonium nitrogen (Figure 1). The reactor consisted of an anodic and cathodic electrode as well as a cation exchange membrane (CMI-7000, Membranes International Inc., Ringwood, US) which has a total exchange capacity of 1.6±0.1 meq/g. The anode and cathode compartments were made from Perspex frames bolted tightly together. A 10 Ω resistor was put in the circuit so that the current could be determined. The anodic electrode was a titanium alloy electrode coated with IrO₂. The anodic alloy meshes had a projected surface area of 16 cm² (dimensions: 4×4 cm; 1 mm thickness; Magneto Special Anodes, The Netherlands). A titanium woven wire mesh was used in cathodic chamber. The projected surface area was 16 cm² with an open area of 64% (William Gregor Limited, London, UK).
The reactor was operated at ambient temperature (22±2 °C) under batch mode. Anodic chamber was filled with the ammonia rich substrates while a 50 mmol sodium bicarbonate solution was poured in the cathodic chamber.

2.2. Effect of nitrogen concentration and voltage on the nitrogen extraction efficiency

The objective of this set of experiments was to scrutinize how different nitrogen concentrations in the aqueous residual streams, as well as the voltage set for the extraction process, would affect the overall extraction efficiency. High and low ammonia concentrations, i.e., 5 vs 12 g N/L, and two different voltages, i.e., 3 vs 3.5 V, were considered while synthetic urine was used as feed for the electrochemical reactor. Summary of alternated parameters for this experiment is presented in Table 1. Synthetic urine was prepared as suggested by Bonvin et al (Bonvin et al., 2015). Total nitrogen from ammonia (TAN) and pH were monitored in the anodic and cathodic compartments. The assays were terminated when no significant TAN extraction in the cathodic chamber was observed in two consecutive measurements (i.e., 48 h).

Table 1. Summary of alternated parameters for each experiment
2.3. Effect of nitrogen sources on the nitrogen extraction efficiency

The main objective of this set of experiment was to compare the ammonia extraction efficiency and thus, analyze which feedstocks are more promising for microbial protein production. Anaerobically digested source-sorted OFMSW, as previously described by Khoshnevisan et al. (2018), was used as a substrate for ammonia extraction. The digestate was collected from a pilot reactor working under mesophilic conditions. Based on the results from some preliminary tests (data not shown), no pretreatment such as filtration or centrifugation were needed on the digestate prior to use as feed for anodic compartment. However, for optimized scaled-up operation, pretreatment before nitrogen recovery may be beneficial especially for fiber-rich substrates. The second substrate was digestate collected from a lab-scale CSTR reactor treating cattle manure under thermophilic condition (Sun et al., 2019). Finally, male human urine was collected and used as third feedstock. The characteristics of the different substrates are shown in Table 2. The experiments were performed in duplicate and each batch assay continued for 48 hours.

Table 2. Characterization of aqueous residual streams tested in the present study for nitrogen recovery

2.4. Transfer of micro-pollutants from residual streams to extracted nitrogen

In the experiment run with male human urine, the anode compartment was spiked with different pharmaceuticals including carbamazepine, ketoprofen, diclofenac, and bezafibrate. The aim of this experiment was to demonstrate the recovery of ammonia free of trace pollutants, thus allowing its use for feed or food production. Real male urine was used as anode feed and the micro-
pollutant drift was monitored. Chemical properties of the studied micro-pollutants as well as their concentrations are shown in the supplementary information (Table S2).

2.5. Microbial protein (MP) production

The protocol described by Valverde-Pérez et al. (Valverde-Pérez et al., 2020) was adapted to characterize methanotrophic activity using recovered nitrogen. In brief, 250 mL serum bottles were autoclaved and closed with autoclaved teflon caps and aluminum lids. The working volume was set at 100 mL and 3% v/v was inoculum. The inoculum used in this research study was an enriched methanotroph culture grown in an innovative bioreactor with gas supply through hydrophobic hollow fiber membranes (Valverde-Pérez et al., 2018). The 16S rRNA gene sequencing analysis using the Microbial genomics module plug in (QIAGEN) showed that more than 70% of the microbial community can be represented by *Methylophilus sp.* (44%), *Methylomonas sp.* (14%), and *Comamonadaceae sp.* (13%) (Tsapekos et al., 2019). After inoculation, the trapped gas in the headspace was removed and replaced with a mixture of oxygen and methane at a favorable ratio of 2 v/v of oxygen to methane. Theoretically, a mixture of oxygen and methane at a ratio of 1.45 v/v is required to bio-oxidize natural gas (mainly methane) with ammonia and oxygen (Villadsen et al., 2011; Al Taweel et al., 2012). In order to ensure unlimited oxygen supply to fully oxidize the methane, during the batch cultivation of MP, the ratio was kept at 2 v/v for all the experiments. For control experiments, pure methane was used as the carbon source, whilst for the remaining experiments bio-methane was employed. This bio-methane was collected from a lab-scale reactor upgrading synthetic biogas to the bio-methane with a purity higher than 98% (Bassani et al., 2015). Gas in the headspace was periodically removed using a 100 mL syringe and replaced with a fresh mixture of pure oxygen and methane. The gas mixture was injected to the headspace through 0.2 µm glass fiber filter (Sartorius, Germany). For control
experiments, diluted ammonium mineral salt (dAMS) was used as cultivation medium (Supplementary materials, Table S1). Two different strategies for nitrogen valorization were tested: i) recovered nitrogen was pH adjusted and supplemented with trace elements according to the dAMS recipe; and ii) recovered nitrogen was pH adjusted with phosphate buffer and used as cultivation media. In all cases, cultivation media were autoclaved. However, in industrial scale, where the electrochemical extraction unit is combined with the fermenter, the extracted media will be already sterile. Table 3 summarizes the experimental conditions used to grow microbial protein.

**Table 3.** A summary of experimental conditions for microbial protein cultivation

Batches were sampled for gas composition characterization, biomass density and ammonium concentrations. Experiments were run in triplicates at 25 °C and shaken at 150 rpm. Optical density at 600 nm (OD$_{600}$) and gauge pressure inside the serum bottles were used as a measure to determine the end of the experiments. The stable OD$_{600}$ at two consecutive readings as well as the stable gas pressure were appropriate signs showing that the bacterial growth reached stationary phase. Soon after reaching to the stationary phase, the final samples were collected and the biomass was centrifuged and freeze-dried to measure cell dry weight (CDW) and determine the amino acid profile.

**2.6. Sampling, analytical methods and calculations**

OFMSW and manure digestate as well as urine were characterized to determine total solids (TS), volatile solids (VS), total Keijdal nitrogen (TKN), and total ammonia nitrogen (TAN) following the standard methods (APHA., 2017). Regarding the electrolyte, containing extracted nitrogen, only TAN was measured. TKN and TAN were quantified using a Kjeldahl Apparatus (FOSS Foss™ Kjeltec™ 8100).
During the cultivation of MP, biomass growth was tracked using OD at 600 nm (Helios™
Epsilon visible spectrophotometer, Thermo Scientific, USA) and total suspended solids (TSS)
(APHA., 2017). Liquid samples from cultivation medium were taken once serum bottles were
inoculated and sealed, and after reaching stationary phase to measure soluble compounds such as
ammonium nitrogen. Soluble compounds were measured after the filtration of liquid samples with
0.2µm glass fiber filter (Sartorius, Germany). Ammonium, nitrite and nitrate concentrations were
determined colorimetrically by a continuous-flow auto-analyzer (SKALAR San++, Netherlands).

Methane, oxygen, and carbon dioxide contents in the gas phase were monitored using gas
chromatography (GC Trace 1310. Thermo Scientific, USA). Two different columns were used for
different gases. A HP-Plot/Q column (Agilent Technologies, USA, length 15 m, diameter 0.32
mm, film 20 µm) was used for methane and carbon dioxide, whilst HP-Molesieve column (Agilent
Technologies, USA, length 30 m, diameter, 0.53, film 50 µm) was used for oxygen and methane
analyses. Calibration was done by injecting gas mixtures of known concentrations (methane to
carbon dioxide ratios (v/v) of 60/40, 40/30 and 5/5 for the first method; Air to methane ratios (v/v)
of 100/0 and 50/50 for the second method).

Once stationary phase was reached, the broth was centrifuged at 10,000 rpm for 10 min. After
centrifugation, the supernatant was collected and stored for further analysis and the biomass was
freeze-dried to determine cell dry weight (CDW) (Figure S1).

The average fixation rate of ammonium-nitrogen during MP cultivation was calculated as
follows:

\[
\text{NH}_4^+ - \text{N fixation rate (mg/L.day)} = \frac{\text{NH}_4^+ - N_i (mg/L) - \text{NH}_4^+ - N_i (mg/L)}{t (d)}
\]
where “NH$_4^+$-Ni” and “NH$_4^+$-N$_r$” represent the initial and residual ammonium-nitrogen concentration in the cultivation medium, respectively, and “t” stands for cultivation time. The CDW yield on methane and ammonium-nitrogen was calculated as follows:

\[
Y_{CDW/CH_4} \left( \frac{g_{CDW}}{g_{CH_4}} \right) = \frac{CDW (g/L) \times \text{Liquid volume (L)}}{CH_4 \text{ uptake (g)}}
\]

\[
Y_{CDW/NH$_4^+$-N} \left( \frac{g_{CDW}}{g_{NH$_4^+$ - N}} \right) = \frac{CDW (g/L) \times \text{Liquid volume (L)}}{NH$_4^+$ - Ni (mg/L) - NH$_4^+$ - Nr (mg/L)}
\]

The energy consumption by electrochemical reactor during ammonia extraction was calculated as follows:

\[
E [\text{kWh}] = \frac{V[\text{volt}] \times I[\text{amp}] \times t[\text{hours}]}{1000}
\]

The protocol for micropollutant analysis was adapted from Torresi et al., (Torresi et al., 2019). During batch experiment, at each sampling time, 3 mL of sample was filtered with a syringe filter 0.2 µm and transferred to a glass vial. 0.8 mL acetonitrile were immediately added into the filtered sample to stop further bioactivity. All samples were preserved at -20 °C in a freezer before analysis by HPLC-MS/MS. For analysis, the samples were taken from the freezer and left to reach room temperature. 900 µL of each sample was transferred into a HPLC vial and 100 µL of internal standard solution consisting of mecoprop and atrazine were added afterwards into the HPLC vial. Lastly, 100 µL of sample from the HPLC vial were injected and analyzed by HPLC-MS/MS. Further information regarding HPLC-MS/MS are reported in section 2 in SI. The limit of quantification (LOQ) for targeted micropollutants ranged from 0.05 to 10 µg/L (Table S3) and overall 98% of the theoretical concentration was recovered.
Determination of the amino acid composition was performed on 15 mg of the freeze dried biomass, following the protocol by D’Este and colleagues (D’Este et al., 2017). This amount was placed into a microwave tube and 6 mL of 6N hydrochloric acid were added. Tubes were flushed with nitrogen to prevent oxidative degradation and closed with a cap afterwards. Hydrolysis of the sample took place in a microwave oven (Anton Paar Multiwave 3000, AT) at 500W and 150ºC for 30 min. After that time, hydrochloric acid was removed from the tube by evaporation using the same microwave, at 600W for 20 min. Then, 5 mL of distilled water were added to the pellet and mixed. Samples were filtered by using a 0.2 μm pore size syringe filter and then analyzed through HPLC. For amino acid quantification, a HPLC (Thermo Scientific Dionex UltiMate 3000 UHPLC, USA) equipped with a c18 reversed phase column (Agilent Technologies USA Eclipse Plus C18) and an in-line guard column (Macherey-Nagel Germany EC 4/2 Universal RP) was used. Calibration was done with a commercial amino acid mix (AAS18, Sigma-Aldrich) containing L-Alanine, L-Arginine, L-Aspartic acid, L-Cystine, L-Glutamic acid, Glycine, L-Histidine, L-Isoleucine, L-Leucine, L-Lysine, L-Methionine, L-Phenylalanine, L-Proline, L-Serine, L-Threonine, L-Tyrosine and L-Valine.

3. Results and discussions

3.1. Nitrogen recovery under different nitrogen concentrations and voltage

The first experiment was performed with synthetic urine to investigate how recovery would be affected under different nitrogen levels and voltages. As shown in Figure 2 (B), when the average initial nitrogen concentration in the anodic chamber was 4,920 mg N/L and the voltage was set at 3.5V, almost 60% of the ammonia was recovered in the cathodic compartment after 48 h. When voltage decreased to 3V, only 28.3% of the nitrogen in synthetic urine was recovered. Based on the results, the nitrogen flux at higher voltage was almost 2.7 times greater than that at
lower voltage. The nitrogen flux at the voltage of 3.5 and 3V, was calculated at 0.35 and 0.13 mg N/cm² h, respectively. This shows that the increased voltage accelerated ammonia transfer between two chambers by 63%.

By increasing the initial concentration of ammonia in the anodic chamber, the nitrogen transfer rate was also enhanced. The nitrogen flux under higher ammonia concentration was calculated at 0.51 mg N/cm² h when voltage was set at 3.5V. Under such circumstances, 35% of ammonia was recovered during the first 48 h (Figure 3 (B)). By decreasing the voltage to 3V, nitrogen flux dropped by 48% reaching to 0.27 mg N/cm² h. This reduction in nitrogen transfer resulted in lower total nitrogen recovery. Accordingly, the nitrogen recovery under high ammonia concentration and low voltage was almost 19% of the initial concentration during the first 48 h.

Based on the results achieved herein, both nitrogen concentration and voltage could affect nitrogen transfer between anodic and cathodic chambers. Having considered two voltages tested in this study, the higher voltage for ammonia recovery, the higher nitrogen flux attained. Moreover, the results showed that the nitrogen transfer rate from anodic compartment to cathodic chamber during the first 48 hours was higher for streams with higher ammonia concentration. The nitrogen transfer rate between two compartments would decline by deceased ammonia concentration in the residual stream and decreased voltage.
Figure 2. Nitrogen transfer between anodic and cathodic chambers under low nitrogen concentration; (A) Low voltage - (B) High voltage.

Figure 3. Nitrogen transfer between anodic and cathodic chambers under high nitrogen concentration and; (A) Low voltage - (B) High voltage.

3.2. Use of different residual stream for ammonia recovery

Human urine was used as a residual stream to extract nitrogen by the electrochemical reactor. Initial TKN and pH were 10.33 g N/L and 6.1, respectively. After 48 hours, TKN in the anode chamber dropped by 32%, reaching to 7.02 g N/L. The results obtained demonstrated that 32% of the initial nitrogen transferred to the cathodic compartment during the 48 hours extraction process. As shown in Figure 4, this transfer of nitrogen between two chambers caused a significant pH drop in the anode chamber. Under such conditions, the average nitrogen flux was estimated at 0.55 mg N/cm² h which was in the range achieved with synthetic urine. This showed a huge potential for ammonia recovery from source separated urine.

Figure 4. pH drift and transfer of ammonia nitrogen between anodic and cathodic chambers using male urine as nitrogen source in the anodic chamber

OFMSW-based digestate was another substrate used for nitrogen recovery using the electrochemical reactor. The overview of the results obtained from electrochemical cell after 48-hour run can be seen in Table 4. The results demonstrated that on average 33% of the nitrogen in the OFMSW digestate was recovered in the cathode compartment. The results obtained herein
showed that the average nitrogen flux was 0.145 mg N/cm² h. In a study conducted by Desloover et al., (Desloover et al., 2012), an attempt was made to optimize an electrochemical cell coupled with a stripping column and an absorption column for ammonium recovery from digestate. They reported that the recovery efficiency varied from 38 to 63% depending on the applied current.

**Table 4.** Overview of the results obtained from electrochemical nitrogen extraction from OFMSW, manure digestate, and real urine after 48 hours.

Manure digestate was also tested as the third substrate for nitrogen recovery using the electrochemical cell. Under such circumstances, a recovery efficiency of 50.8% was achieved. The pH in the cathodic compartment increased to 12 while that of anodic chamber dropped to 5.1 after 48 hours. An average nitrogen flux of 0.20 mg N/cm² h was attained when manure-based digestate was used as the substrate for nitrogen recovery.

Neither urine nor digestate as substrates for nitrogen recovery were stirred in the anode chamber. While the former is a homogeneous substrate, the latter contains particles and fibers. This could be one of the reasons a lower extraction efficiency and nitrogen flux were attained when digestate was used for extraction process. Moreover, urine has a higher electrical conductivity than digestate which could be regarded as another reason for its higher extraction efficiency and nitrogen flux (Marickar, 2010; Alburquerque et al., 2012).

Here, an attempt was made to exploit the considerable potential for extraction of nitrogen from aqueous residual streams. The electrochemical ammonium recovery through membranes is one of the key components to the successful application of the whole process. In general, the main challenge associated with the membrane is the fouling problem upon the substrate applied. Since the electrochemical cell is abiotic and contains only two chambers, the fouling issue could be
tackled by simple maintenance methods such as chemical or physical cleaning. For practical application, electrode materials with a large surface such as graphite fiber felt could be employed to increase the circuit current and further the transportation of ammonium ions. Future research is required to optimize the extraction process considering each individual substrate.

3.3. Fate of micro-pollutants during electrochemical extraction of nitrogen

Pharmaceuticals are typically removed from wastewater by sorption to solids or biologically degraded (Ramin et al., 2017). Bacterial content in urine is low and thus the fate of the pharmaceuticals will depend on their sorption capacity on reactor materials, including electrodes, reactor walls, and ion exchange membrane, or its transfer across the membrane to the cathodic chamber. Despite the different trends in concentrations in the anode compartment, none of the pharmaceuticals migrated through the CEM, demonstrating its applicability for producing high purity ammonia effluents suitable for further high value valorization. By the end of the experiment no trace element was observed in the cathodic chamber. Bezafibrate concentration decreased over time in the anode chamber, likely due to sorption. Furthermore, ketoprofen and diclofenac disappeared from the anode compartment whiting the first 11 hours. However, carbamazepine was stable during the experiment, as it is not typically adsorbed nor biologically degraded during water treatment (Plósz et al., 2012).

3.4. MP grown in extracted nitrogen

The extracted nitrogen from OFMSW digestate was supplemented with required trace elements and nutrients according to dAMS recipe and then used as growing medium for SCP cultivation. The bacterial growth curves can be seen in Figure S2. Methanotrophic bacteria could grow well in both standard medium and extracted nitrogen supplemented with trace elements.
However, when the mixed-culture was cultivated in the pH-adjusted extracted nitrogen without being supplemented with trace elements, no growth was observed (data not shown). The maximum OD of 0.84±0.01 was achieved for MP cultivation using extracted nitrogen. This OD corresponded to a total dry weight of 0.49±0.01 g/L. The growth rate (h⁻¹) was calculated for the exponential growth phase. The growth rate for extracted nitrogen and standard medium was calculated at 0.53 and 0.54 (d⁻¹), respectively. The growth rate achieved herein showed that methane oxidizing bacteria (MOB) could grow at the same rate, marking extracted nitrogen a proper medium for MOB cultivation.

The methane consumption was monitored during the experiment so that the yield on methane (Y_{MP/CH4}) was calculated. The results showed that Y_{MP/CH4} was 0.88±0.05 and 0.71±0.01 g-CDW/g-CH₄ for extracted nitrogen and standard medium, respectively. A wide range of yields has been reported, varying from 0.15 to 1.78 g-CDW/g-CH₄ depending on the methanotrophic strain used and cultivation conditions (Park et al., 1991; Shah et al., 1996; AlSayed et al., 2018). Theoretical biomass yield on methane under no energy loss, high realistic, and low realistic conditions are reportedly 1.21, 1.01, and 0.93 g-CDW/g-CH₄ (Skadborg., 2018). However, it is worthy to mention that such values have been calculated for methanotrophic bacteria and not enrichments. Y_{MP/CH4} of 1.03 g-CDW/g-CH₄ was reported by Harwood and Pirt (1972) who evaluated the growth of Methylococcus capsulatus. Rostkowski et al. (2013) reported that the yield on methane for two strains of methanotrophs, i.e., Methylosinus trichosporium and Methylocystis parvus, varied from 0.51 to 1.05 g-CDW/g-CH₄ depending on the nitrogen source and oxygen partial pressure. In another study led by Henard et al. (2018), methane yields of three strains of methanotrophs, including Methylomicrobium alcalophilum 20ZR, Methylosinus trichosporium
OB3b and *Methylococcus capsulatus* Bath were calculated at 1.03, 0.78, and 0.85 g-CDW/g-CH₄, respectively.

Having grown the mixed-culture in dAMS and extracted nitrogen, no significant difference was observed in terms of nitrogen fixation rate. The fixation rate was estimated at 0.37±0.002 and 0.36±0.01 mg N/L h for serum bottles contained dAMS and extracted nitrogen, respectively.

Besides $Y_{\text{MP/CH}_4}$, yield on ammonium ($Y_{\text{MP/NH}_4}$) was also calculated. $Y_{\text{MP/NH}_4}$ for control and extracted nitrogen was calculated at 12.13±0.13 and 9.74±0.45 g-CDW/g N. Comparable, but still lower yield on ammonium is obtained when growing methanotrophs in extracted nitrogen. Lower yields on ammonium suggest higher nitrogen assimilation and thus higher protein content. Therefore, methanotrophs grown in extracted nitrogen and bio-methane had higher protein content compared to the culture grown in the dAMS medium.

**Figure 5.** Amino acid profile of the methanotroph mixed-culture grown in extracted nitrogen

The amino acid analysis profile showed that the produced biomass covered a wide range of essential amino acids (Figure 5). The total profile showed that aspartic acid, glutamic acid, glycine, and lysine constituted more than 50% of the total profile, marking the produced biomass comparable with other traditional protein sources (Bajpai, 2017; Henchion et al., 2017). These results achieved are in line with the findings of other researchers who introduce aspartic acid and glutamic acid as the most abundant amino acids in methanotrophs (Rasouli et al., 2018). Having compared the amino acid profile of the produced biomass with that of fishmeal and soybean, methanotrophs could be a suitable substitute for conventional plant-based protein sources in the
livestock and fish feeds. The obtained results demonstrated that the nitrogen extracted from waste streams can be converted into high quality protein.

3.5. Future perspective

The number of centralized and decentralized biogas plants has been drastically increased all over the world, as it is a suitable technology for the production of energy carrier from renewable resources while achieving multiple environmental benefits (Rehl and Müller, 2011). The digestate which remains after anaerobic digestion of waste streams is rich in readily available macro- and micro-nutrients which makes it a proper substitute for chemical fertilizers (Li et al., 2017). However, the recent studies showed that oversupply of digestate, especially in regions with intensive livestock farming or biogas plants, contributes towards global warming, acidification, and eutrophication (Styles et al., 2018). Besides the over-application of digestate in areas adjacent to biogas plants, the improper distribution of digestate and its application at improper time increase the possibility of ammonia volatilization and eutrophication of nearby water (Nkoa, 2014).

Extraction of nitrogen from anaerobically digested organic wastes for microbial protein production would be a valuable approach to simultaneously close the protein gap and decrease the environmental pollutions. However, nitrogen removal and recovery via electrochemical cells is an energy demand process. Desloover et al., (2012) observed that the electrical demand for nitrogen recovery from digestate was 13 kWh/kg N. Luther et al., (2015) employed electrochemical cell to extract ammonia from human urine. Their results showed that ammonia could be electrochemically extracted at an energy demand of approximately 12 kWh/kg N. In another study (Christiaens et al., 2017), it was reported that the net energy which was required for nitrogen recovery from real urine was calculated as 13.9 kWh/kg N. Table 5 summarizes some of the previously conducted studies on nitrogen recovery from aqueous waste. The energy demand for nitrogen recovery from
OFMSW and manure digestate was calculated at 14.44 (±1.19) and 9.97 (±0.7) kWh/kg N, respectively. Although the energy demands calculated herein were in the range of those reported in the literature, such an energy demand may be regarded as a serious obstacle for upscaling the nutrient recovery from waste streams due to the increased production cost. Novel hybrid systems consisting of a submersible microbial desalination cell and anaerobic digesters could be a promising approach counteracting ammonia inhibition during anaerobic digestion with simultaneous in situ ammonia recovery and electricity production (Zhang and Angelidaki, 2015). Such novel systems would help simultaneously minimize the energy demand and increase the profitability of the system by producing more methane.

Table 5. A comparison between previously conducted studies on nitrogen recovery from aqueous waste

According to Mizuta et al., (Mizuta and Shimada, 2010), the energy demand of a conventional wastewater treatment plant without sludge incineration ranges from 0.30 to 1.89 kWh/m³, while nitrogen removal in these systems comes at high energy costs of 4.4 to 11.1 kWh/kg N (Maurer et al., 2003; Christiaens et al., 2017). Moreover, the production of nitrogen fertilizers via the conventional Haber-Bosch process costs 10.3 to 12.5 kWh/kg N (Maurer et al., 2003). Such comparisons show that nitrogen recovery from waste streams via electrochemical systems and its further use for microbial protein production could be a major competitor to the conventional anthropogenic nitrogen cycle, consisting biological nitrogen removal process and chemical nitrogen fertilizer production. To scale-up MP production via valorization of nitrogen from aqueous residual streams, a techno-economic assessment should be performed with a biorefinery approach. Under a biorefinery approach, all by-products would have an implementation with contributions to economic aspects of the process (Khoshnevisan and Angelidaki, 2018). For
instance, hydrogen gas is produced during the electrochemical extraction process. The produced hydrogen would have several industrial/microbiological applications including microbial protein production via hydrogen oxidizing bacteria (Dou et al., 2019) or biological biogas upgrading (Angelidaki et al., 2018). Under such circumstances, the total production cost is decreased and the profitability is increased.

While urine constitutes only around 1% v/v of the volume of the residential wastewaters, the global output of nitrogen through human urine is anticipated to be almost 30 Mt/y (Rose et al., 2015). Urine contains 60-90% nitrogen, mostly bound as urea which can be hydrolyzed to ammonium during storage (Beler-Baykal et al., 2011). Having considered that nutrient recovery would be more energetically efficient than removing the nutrients, source separation of urine can be regarded as a new paradigm for nutrient recovery from waste streams.

Another major concern that hinders the production and use of microbial protein, which is grown in residual streams, relates to its quality and safety and regulative issues. Although the results obtained in the present study demonstrate that nitrogen can be safely recovered and upcycled to protein with feed grade quality, further research is required to deeply investigate the application of MP as healthy feed supplement while trying to standardize the feed quality and go through its legal aspects.

4. Conclusion

The present research showed that coupling the treatment of aqueous residual streams via electrochemical nitrogen recovery with microbial protein production could have a considerable potential for alleviating food scarcity. Digested manure and organic fraction of municipal solid waste as well as urine were successfully tested as nitrogen rich substrates for electrochemical
extraction of ammonia. Higher ammonia concentration and higher voltage would significantly improve nitrogen recovery. The energy cost for extracting nitrogen from above mentioned waste streams varied from 9.97 (±0.7) to 14.44 (±1.19) kWh/kg N making the extraction process comparable with traditional nitrogen removal processes. Furthermore, the avoidance of chemical fertilizer production and the possibility of using hybrid systems for nitrogen extraction can further improve the profitability of MP production compared to conventional anthropogenic nitrogen cycle. Based on the results achieved herein, micro-pollutants did not transfer from residual streams to the extracted nitrogen, demonstrating the applicability of electrodialysis for producing high purity ammonia suitable for further high value valorization. The extracted nitrogen was successfully tested for single cell protein production containing a wide range of essential amino acids. Further research is needed to optimize the extraction efficiency for each specific substrate as well as sustainability assessment under a biorefinery approach.

Acknowledgements

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Benyamin Khoshnevisan: conceptualization, investigation, data curation, visualization and writing; Mark Dodds: conceptualization, investigation; Panagiotis Tsapekos: conceptualization; Elena Torresi: conceptualization and data curation; Barth F. Smets: conceptualization and supervision; Irini Angelidaki: conceptualization; Yifeng Zhang: conceptualization, data curation and supervision; Borja Valverde-Pérez: conceptualization, data curation, visualization and supervision. All coauthors contributed to review and editing of the manuscript.
References:


Delgado, C.L., 2003. Rising consumption of meat and milk in developing countries has created a new food revolution. The Journal of nutrition 133, 3907S-3910S.


Skadborg., M.M., 2018. Tailoring the macromolecular composition of methanotrophic bacteria cultivated in bubble-free continuous-flow reactors Department of environmental engineering. Technical University of Denmark, Denmark.


Van der Bruggen, B., Mänttäri, M., Nyström, M., 2008. Drawbacks of applying nanofiltration and how to avoid them: a review. Separation and purification technology 63, 251-263.


Figure 1. Electrochemical cell used for ammonium extraction: left anodic chamber filled with nitrogen rich waste streams and right cathodic chamber filled with bicarbonate media.
Figure 2. Nitrogen transfer between anodic and cathodic chambers under low nitrogen concentration; (A) Low voltage - (B) High voltage.

Figure 3. Nitrogen transfer between anodic and cathodic chambers under high nitrogen concentration and; (A) Low voltage - (B) High voltage.
Figure 4. pH drift and transfer of ammonia nitrogen between anodic and cathodic chambers using male urine as nitrogen source in the anodic chamber

Figure 5. Amino acid profile of the methanotroph mixed-culture grown in extracted nitrogen
Tables:

Table 1. Summary of alternated parameters for each experiment

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Objective</th>
<th>Source of Nitrogen</th>
<th>Total nitrogen</th>
<th>Voltage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Effect of nitrogen concentration and voltage on extraction efficiency</td>
<td>Synthetic urine</td>
<td>5 and 12 g N/L</td>
<td>3 V</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.5 V</td>
</tr>
<tr>
<td>2</td>
<td>Transfer of micro-pollutants during electrochemical extraction</td>
<td>Real male urine</td>
<td>10.33 g N/L</td>
<td>3.5 V</td>
</tr>
<tr>
<td>3</td>
<td>Effect of nitrogen sources from different residual streams on extraction efficiency</td>
<td>Manure-AD effluent, OFMSW-AD effluent</td>
<td>Varying based on the feed</td>
<td>3.8</td>
</tr>
</tbody>
</table>

Table 2. Characterization of aqueous residual streams tested in the present study for nitrogen recovery

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>OFMSW digestate</th>
<th>Manure digestate</th>
<th>Urine</th>
</tr>
</thead>
<tbody>
<tr>
<td>TS</td>
<td>g/kg</td>
<td>3.93</td>
<td>6.52</td>
<td>-</td>
</tr>
<tr>
<td>VS</td>
<td>g/kg</td>
<td>3.14</td>
<td>5.26</td>
<td>-</td>
</tr>
<tr>
<td>pH</td>
<td>-</td>
<td>7.3</td>
<td>8.1</td>
<td>6.1</td>
</tr>
<tr>
<td>TAN</td>
<td>g NH₄-N/L</td>
<td>2.5</td>
<td>2.32</td>
<td>0.68</td>
</tr>
<tr>
<td>TKN</td>
<td>g N/L</td>
<td>3.5</td>
<td>3.06</td>
<td>10.33</td>
</tr>
</tbody>
</table>

TS = Total Solid; VS = Volatile Solid; TAN = Total Ammonium Nitrogen; TKN = Total Kjeldahl Nitrogen
Table 3. A summary of experimental conditions for microbial protein cultivation.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Nitrogen source</th>
<th>Methane source</th>
<th>Trace elements</th>
<th>pH adjustment</th>
<th>O₂:CH₄ ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>NH₄Cl</td>
<td>Chemical CH₄</td>
<td>According to diluted ammonium mineral salt</td>
<td>+</td>
<td>2 v/v</td>
</tr>
<tr>
<td>Assay 1</td>
<td>Extracted N</td>
<td>Bio CH₄ (98% purity)</td>
<td>According to diluted ammonium mineral salt</td>
<td>+</td>
<td>2 v/v</td>
</tr>
<tr>
<td>Assay 2</td>
<td>Extracted N</td>
<td>Bio CH₄ (98% purity)</td>
<td>-</td>
<td>+</td>
<td>2 v/v</td>
</tr>
</tbody>
</table>

Table 4. Overview of the results obtained from electrochemical nitrogen extraction from OFMSW, manure digestate, and real urine after 48 hours.

<table>
<thead>
<tr>
<th>Item</th>
<th>Nitrogen recovery (%)</th>
<th>STD</th>
<th>Nitrogen flux (mg N/cm² h)</th>
<th>STD</th>
</tr>
</thead>
<tbody>
<tr>
<td>OFMSW digestate</td>
<td>33.57</td>
<td>0.64</td>
<td>0.15</td>
<td>0.01</td>
</tr>
<tr>
<td>Manure digestate</td>
<td>50.78</td>
<td>0.53</td>
<td>0.2</td>
<td>0.01</td>
</tr>
<tr>
<td>Real male urine</td>
<td>31.73</td>
<td>25.25</td>
<td>0.55</td>
<td>0.44</td>
</tr>
</tbody>
</table>
### Table 5. A comparison between previously conducted studies on nitrogen recovery from aqueous waste.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Cell type</th>
<th>Influent</th>
<th>N flux (mg N/cm² h)</th>
<th>Current density (A/m²)</th>
<th>Energy input (kWh/kg N)</th>
<th>Recovery efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Desloover et al., 2015)</td>
<td>EC</td>
<td>Anaerobic digestate</td>
<td>0.196</td>
<td>10</td>
<td>17</td>
<td>28</td>
</tr>
<tr>
<td>(Luther et al., 2015)</td>
<td>EC, and absorption tower</td>
<td>Synthetic Urine</td>
<td>2.08</td>
<td>50</td>
<td>12</td>
<td>57</td>
</tr>
<tr>
<td>(Kuntke et al., 2012)</td>
<td>MFC</td>
<td>Real Urine</td>
<td>0.014</td>
<td>0.5</td>
<td>-0.96</td>
<td>42</td>
</tr>
<tr>
<td>(Arredondo et al., 2017)</td>
<td>EC</td>
<td>Real Urine</td>
<td>1.25</td>
<td>50</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(Tarpeh et al., 2018)</td>
<td>EC</td>
<td>Real Urine</td>
<td>7.125</td>
<td>100</td>
<td>8.5</td>
<td>50</td>
</tr>
</tbody>
</table>

References:
Supporting Information

Coupling electrochemical ammonia extraction and cultivation of methane oxidizing bacteria for safe production of microbial protein

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### Table S1. dAMS Media recipe (28 mg NH₄-N/L)

<table>
<thead>
<tr>
<th>Items</th>
<th>Chemicals</th>
<th>Amount</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stock A</td>
<td>MgSO₄ x 7H₂O</td>
<td>10 g</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CaCl₂ x 2H₂O</td>
<td>1.5 g</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NH₄Cl</td>
<td>5 g</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Distilled water</td>
<td>1 L</td>
<td></td>
</tr>
<tr>
<td>FeNaEDTA Stock</td>
<td>FeNaEDTA</td>
<td>0.5 g</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Distilled water</td>
<td>100 ml</td>
<td></td>
</tr>
<tr>
<td>Na₂HPO₄ Stock</td>
<td>Na₂HPO₄ x 12H₂O</td>
<td>71.7 g</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Distilled water</td>
<td>1 L</td>
<td></td>
</tr>
<tr>
<td>KH₂PO₄ Stock</td>
<td>KH₂PO₄</td>
<td>27.2 g</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Distilled water</td>
<td>1 L</td>
<td></td>
</tr>
<tr>
<td>Trace solution</td>
<td>Na₂EDTA x 2H₂O</td>
<td>0.5 g</td>
<td></td>
</tr>
<tr>
<td></td>
<td>FeSO₄ x 7H₂O</td>
<td>0.2 g</td>
<td></td>
</tr>
<tr>
<td></td>
<td>H₃BO₃</td>
<td>0.03 g</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CoCl₂ x 6H₂O</td>
<td>0.02 g</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ZnSO₄ x 7H₂O</td>
<td>0.01 g</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MnCl₂ x 4H₂O</td>
<td>0.003 g</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Na₂MoO₄ x 2H₂O</td>
<td>0.003 g</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NiCl₂ x 6H₂O</td>
<td>0.002 g</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CuSO₄ x 5H₂O</td>
<td>0.025 g</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Distilled water</td>
<td>1 L</td>
<td></td>
</tr>
<tr>
<td>dAMS</td>
<td>Stock A</td>
<td>20 ml</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Distilled water</td>
<td>800 ml</td>
<td></td>
</tr>
<tr>
<td></td>
<td>FeNaEDTA Stock</td>
<td>1 ml</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Trace solution</td>
<td>1 ml</td>
<td></td>
</tr>
</tbody>
</table>
Dissolve and bring to 1L

<table>
<thead>
<tr>
<th>Sterilization</th>
<th>Autoclave for 20 minutes, then cool down to 50°C-60°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH adjustment (6.8)</td>
<td>KH₂PO₄ Stock 5 ml Using sterilized filter</td>
</tr>
<tr>
<td></td>
<td>Na₂HPO₄ Stock 5 ml Using sterilized filter</td>
</tr>
</tbody>
</table>
Table S2. Chemical properties of the studied micro-pollutants.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Formula</th>
<th>CAS number</th>
<th>Supplier</th>
<th>Structure</th>
<th>pKa</th>
<th>log Kow</th>
<th>Initial concentration (µg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diclofenac</td>
<td>C_{14}H_{11}Cl_{2}NO_{2}</td>
<td>15307-86-5</td>
<td>Sigma-Aldrich</td>
<td><img src="image" alt="Diclofenac Structure" /></td>
<td>4.1</td>
<td>4.51</td>
<td>21.9</td>
</tr>
<tr>
<td>Ketoprofen</td>
<td>C_{16}H_{14}O_{3}</td>
<td>22071-15-4</td>
<td>Sigma-Aldrich</td>
<td><img src="image" alt="Ketoprofen Structure" /></td>
<td>4.4</td>
<td>3.12</td>
<td>23.8</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>C_{15}H_{12}N_{2}O</td>
<td>298-46-4</td>
<td>Sigma-Aldrich</td>
<td><img src="image" alt="Carbamazepine Structure" /></td>
<td>6.1</td>
<td>2.45</td>
<td>3.3</td>
</tr>
<tr>
<td>Bezafibrate</td>
<td>C_{19}H_{20}ClNO_{4}</td>
<td>41859-67-0</td>
<td>Sigma-Aldrich</td>
<td><img src="image" alt="Bezafibrate Structure" /></td>
<td>3.6</td>
<td>4.25</td>
<td>83.6</td>
</tr>
</tbody>
</table>
**Table S3.** Level of quantification for the studied micro-pollutants.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>LOQ (µg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Astrazine (Internal standard, positive)</td>
<td>0.58</td>
</tr>
<tr>
<td>Bezafibrate</td>
<td>0.28</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>0.43</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>0.05</td>
</tr>
<tr>
<td>Ketoprofen</td>
<td>1.4</td>
</tr>
<tr>
<td>Mecoprop (Internal standard, negative)</td>
<td>0.23</td>
</tr>
</tbody>
</table>

LOQ with 100 µL of injection volume
Figure S1. The process scheme for MP production. A) Extracted nitrogen from OFMSW digestate inoculated with MOBs. B) Microorganism at stationary phase. C) Freeze-dried biomass.
Figure S2. Optical density of cultivated mixed-culture; Control contained dAMS and SCP contained extracted nitrogen supplemented with trace elements and other nutrients.