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**Energy recovery from wastewater microalgae
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potential, continuous reactor operation and
modelling aspects**

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Abstract

A mixture of piggery slurry and algal species (mainly composed of *Nannochloropsis limnetica*), grown in municipal wastewater, were used as substrates for biogas production. Mono- and co-digestion experiments were performed at batch and continuous reactor operation. The mono-digestion of wastewater microalgae led to the highest methane yield (408 ± 34 NmL/gVS). However, for manure-based biogas plants, a 60:40 v/v piggery slurry to wastewater microalgae ratio in terms of organic matter was identified as the most efficient mixture in batch assays (355 ± 27 NmL/gVS). The advantage of co-digestion was also evidenced under continuous reactor operation, which had markedly higher biogas production (23%, $p < 0.05$) compared to the mono-digestion of livestock manure. Moreover, it was demonstrated that the co-digestion process resulted in a more robust process as indicated by lower accumulation of acetate (i.e. presented during mono-digestion of piggery slurry) and propionate (i.e. recorded during mono-digestion of wastewater microalgae). The experimental data were compared with dynamic modelling (BioModel). A new set of biodegradability parameters was estimated and employed to improve the simulations of mono-digestion scenarios. Subsequently, the co-digestion scenario was used for model validation. Results obtained from simulations showed that the co-digestion can lead to relatively high methane productivity and prevent process instabilities.

Keywords

Wastewater microalgae; piggery slurry; anaerobic digestion; biogas; methane; modelling

1. Introduction

Anaerobic digestion (AD) of cultivated land-based biomass and especially, the usage of first generation biofuels (e.g. food crops) is steadily reduced due to the imperative need of producing food for the rising world population. On the other hand, second (e.g. wastes and residues) and third (e.g. algae) generation substrates can efficiently support the strong need for providing organic matter to the increased number of biogas plants [1].

Focusing on the second generation biofuels, the policy makers promote livestock manure as a basic substrate for full-scale applications by granting subsidies [2]. In addition, manures have several advantages for biogas production; they are available in large quantities, have high buffer capacity and can provide the digesters with all the micro- and macro-nutrients needed as well as essential microbes for an efficient AD process [3,4]. Also, biogas plants provide storage capacity for manures during rainy periods before they can be applied as bio-fertilisers, in order to limit nutrients wash-out. Thus, manures are already used as a main organic matter provider to farm-scale and centralised biogas plants. For example, piggery wastewaters are consistently used as feedstock in numerous full-scale AD plants in Europe [5]. However, this particular livestock slurry is not recommended as a sole substrate due to its low volumetric methane potential [6] and increased risk for process failure due to ammonia toxicity [7]. Hence, co-digestion of piggery slurry with substrates that have dissimilar chemical composition is typically followed to overcome the technical challenges [8].

Among alternatives, third generation feedstocks are lately considered as a potential source of biomass. For example, algal biomass has some advantages compared to the alternative first and second generation substrates (e.g. annual growth, high growth rate,

high theoretical methane potential, no need for either herbicides or pesticides) [9,10]. In addition, cultivation of microalgae in industrial wastewaters has an important environmental benefit due to carbon dioxide fixation [11,12].

The integration of the aforementioned substrates in the feedstock of biogas reactors was previously examined in the literature. However, contradictory conclusions were drawn by comparing the outcomes of different studies. For example, Mahdy et al. [13] examined the co-digestion of cattle manure and microalgae under high ammonium levels and a synergistic effect of enriched feedstock was revealed. Based on the results, 80% share of microalgae on organic matter basis was recommended in order to increase the methane output under both biochemical methane potential (BMP) tests and continuous mode experiments. On the contrary, González-Fernández et al. [6] found that the BMP value of swine manure was significantly higher than the corresponding methane yield of microalgae biomass. Based on González-Fernández et al. [6], the hardly degradable cell walls of microalgae acted as a shield protecting from enzymatic hydrolysis; in contrast, swine manure is an easier substrate for anaerobic biodegradation. On this concept, to overcome the limited methanation of algal biomass, the co-digestion with swine manure was examined, and indeed, the methane production was increased due to the co-substrate addition.

It is well known that a universal co-digestion strategy does not exist and the energy output is significantly affected by various parameters such as biochemical substrate characteristics, operational conditions, microbial communities and reactor configurations [14]. Thus, linking these variables with process monitoring (e.g. methane productivity, pH and accumulation of intermediates) can be extremely helpful to optimize substrates utilisation and to identify feedstock mixtures that enhance biogas

production. On this concept, simulation of experimental datasets using dynamic bioconversion modelling can markedly improve the microalgae-based AD performance [15]. Subsequently, the optimum feeding strategy can be defined focusing on both bioenergy yield improvement and avoidance of instabilities, e.g. due to ammonia or volatile fatty acids (VFA) accumulation.

The overall aim of the present study was to optimise the co-digestion process of microalgae and piggery slurry. This was investigated experimentally through mono- and co-digestion tests using both batch and continuously fed biogas reactors. Subsequently, the results from continuous mode experiments were validated using the BioModel [16] and the model simulation outputs were used to point out the most suitable feedstock composition.

2. Materials and methods

2.1. Inoculum and substrate

Well-digested anaerobic sludge was collected from the effluent tank of a centralized biogas plant (Snertinge, Denmark) and used as inoculum. The biogas plant treated animal slurry (approximately two third of pig manure and one third of cattle slurry) under thermophilic conditions. The inoculum was stored for 10 days in a thermophilic incubator in order to deplete the biomethanation from the residual biodegradable organic material.

The piggery slurry was collected from Lemvig biogas plant (Midtjylland, Denmark). After arrival to the laboratory, the slurry was sieved (5 mm) in order to discard large particles. Microalgae, which were grown in municipal wastewater (denoted as wastewater microalgae), were provided from municipality of Kalundborg (Sealand,

Denmark). The algal biomass consisted of mixed algal species (mainly composed of *Nannochloropsis limnetica*). Both substrates were placed at -20 °C for storage and thawed at 4 °C for two days prior to their usage. The major characteristics of inoculum, piggery slurry and wastewater microalgae are presented in Table 1.

Table 1. Characteristics of inoculum, piggery slurry and wastewater microalgae

Characteristics	Inoculum	Piggery slurry	Wastewater microalgae
pH	8.21	8.50	5.87
Total Solids (TS), g/L	21.56 ± 1.08	33.50 ± 1.68	24.60 ± 1.23
Volatile Solids (VS), g/L	19.92 ± 1.00	21.79 ± 1.09	20.95 ± 1.05
Carbohydrates, g/L	16.86 ± 0.84	6.53 ± 0.33	8.19 ± 0.41
Proteins, g/L	2.25 ± 0.11	7.63 ± 0.38	10.00 ± 0.50
Lipids, g/L	0.01 ± 0.00	0.12 ± 0.01	2.63 ± 0.13
Total Kjeldahl Nitrogen (TKN), g/L	2.66 ± 0.13	4.31 ± 0.22	1.78 ± 0.09
Ammonium Nitrogen (NH₄⁺-N), g/L	2.57 ± 0.13	3.09 ± 0.15	0.18 ± 0.01
Total Volatile Fatty acids (TVFA), g/L	0.79 ± 0.04	6.33 ± 0.32	0.13 ± 0.01

2.2. Experimental set up

Methane yield tests were performed to define the methane production of wastewater microalgae and piggery slurry under mono- and co- digestion experiments, following the guidelines of the standard protocol [17]. Regarding the co-digestion experiments, the co-substrates were examined in VS-ratios of 80:20, 60:40, 40:60 and 20:80 in the feedstock. The experimental sets were conducted at thermophilic conditions (53 ± 1 °C) due to the several advantages that this temperature range offers compared to mesophilic [18]. The total and working volume of the batch reactors were 337 and 100 mL,

respectively. In batch reactors the inoculum occupied 80% of the working volume (i.e. 80 mL) and the initial organic load was set at 2 gVS/L. The reactors were flushed with nitrogen gas and sealed with rubber stoppers and aluminium screw caps in order to ensure anaerobic conditions. The biogas production was monitored twice a week in replicate samples. The methane yield was calculated at standard temperature and pressure conditions based on the initial content of organic matter (i.e. NmL/gVS).

The continuous mode operation was evaluated in three continuously stirred tank reactor (CSTRs), denoted as R1, R2 and R3. The first reactor (R1) was fed exclusively with piggery slurry while wastewater microalgae was the sole substrate of R2. The third reactor (R3) was co-digesting piggery slurry and wastewater microalgae in a VS-ratio of 60:40. As it will be further discussed, this mixture ratio was chosen on basis of the results from the batch assay. The reactors had a total and working volume of 1000 and 750 mL, respectively. The hydraulic retention time (HRT) was set at 15 days by feeding the CSTRs once a day with 50 mL using peristaltic pumps. The organic loading rate (OLR) was 1.4 gVS/L/d during the whole experimental period. The influent bottles were equipped with magnetic stirring devices and turned on 15 minutes before the feeding in order to homogenize the entering substrates. In addition, the CSTRs were equipped with a magnet and placed on the top of stirring devices to ensure the homogeneity of working volume and avoid the formation of dead zones. Thermophilic conditions (53 ± 1 °C) were achieved by covering the CSTRs with electrical thermal jackets. The temperature was monitored using a submerged probe into the CSTRs' working volume. The biogas production was quantified daily by the water displacement method using calibrated gas meters and gas counters [19]. The biogas composition, pH and VFA accumulation were analysed twice a week. All measurements were conducted

in triplicate samples.

2.3. Modelling and simulation approach

2.3.1. BioModel description

The dynamic model (BioModel) developed by Angelidaki et al. [20] was used to investigate the mono and co-digestion scenarios in this work. The BioModel distinguishes three enzymatic pathways: 1) hydrolysis of undissolved carbohydrates, 2) hydrolysis of undissolved proteins, and 3) hydrolysis of undissolved lipids, and eight bacterial groups: (1) glucose-fermenting acidogens, (2) lipolytic bacteria, (3) LCFA-degrading acetogens, (4) amino acid-degrading acidogens, (5) propionate, (6) butyrate, (7) valerate-degrading acetogens, and finally (8) aceticlastic methanogens. The hydrolytic steps are modelled with first order kinetics whilst acidogenic, acetogenic and aceticlastic pathways are modelled with Monod growth kinetic, respectively. Furthermore, BioModel includes VFA inhibition of the hydrolytic steps, acetate inhibition of the acetogenic steps, free ammonia inhibition of the methanogenic step, and pH and LCFA inhibition of all biological steps. A detailed description of the equations and main pathways for anaerobic degradation of organic matter used in the BioModel is illustrated in Fig. S1.1 (Supplementary material).

Organization and processing of parameters defining substrates, pump and flow rates, metabolic steps and chemical components, as well as the collection of model output variables was set up as described by Kovalovszki et al. [16] and integration of the model ordinary differential equation system was performed using the MATLAB's ode15s solver. Numerical optimization of selected parameter values was performed with the trust-region reflective algorithm of MATLAB's lsqcurvefit function, by fitting the

experimental data with the simulation results and solving a nonlinear least squares minimization problem. In addition, the 95% confidence intervals of the estimated parameters were defined using the `nlparci` function built in MATLAB and applying nonlinear regression.

2.3.2. BioModel simulation, calibration and validation

As starting point, the set of general parameters determined by Kovalovszki et al. [16] together with the input conditions (substrate composition, process operation variables) were used to simulate the mono-digestion of piggery slurry and wastewater microalgae. This set of general parameters has been tested to describe satisfactorily various co-digestion scenarios [16,21]. The biogas and methane productivities of both mono-digestion scenarios were used to perform model calibration so that BioModel predictions could be improved.

In a first attempt, parameters related to biodegradability and hydrolysis rate of carbohydrates, proteins and lipids were considered for model calibration based on 1) the extent of biodegradability depends on substrate organic fraction composition and 2) hydrolysis pathway is the rate limiting step during AD of organic residues [22]. Nevertheless, the estimation of the hydrolysis kinetic parameters did not show a significant improvement in the model calibration compared to biodegradability parameters. Hence, the latter ones were finally used for the model calibration of the mono-digestion scenarios. The parameter values were estimated numerically, considering the experimental biogas and methane productivities (Table 2). The rest of BioModel parameters were not modified.

The set of optimized biodegradability parameters from mono-digestion scenarios

199 were then used for simulation of the co-digestion scenario piggery slurry (60% VS) and
200 wastewater microalgae (40% VS); and based on the quality of the prediction, these
201 parameters could be fitted again considering the experimental data set of the
202 corresponding co-digestion scenario.

Table 2. Estimated parameters for mono-digestion and co-digestion scenarios

		Mono-digestion					
<i>Biodegradability parameter</i>		Piggery slurry	95% CI	Wastewater microalgae	95% CI	Co-digestion	95% CI
$Y_{carb, sol}$ ($g_{carb, sol}/g_{carb, is}$) [§]	Yield of soluble carbohydrates	0.3600	[0.2914, 0.4285]	0.7030*	-	0.5784	[0.5118, 0.6450]
$Y_{prot, sol}$ ($g_{prot, sol}/g_{prot, is}$) [†]	Yield of soluble proteins	0.4922	[0.2546, 0.7298]	0.7508*	-	0.4922	[0.2546, 0.7298]
$Y_{lip, sol}$ ($g_{lip, sol}/g_{lip, is}$) [‡]	Yield of soluble lipids	0.5000*	-	0.6323	[0.5145, 0.7501]	0.6323	[0.5145, 0.7501]

*original parameter in the BioModel[16]

§Biodegradability of carbohydrates

†Biodegradability of proteins

‡Biodegradability of lipids

2.4. Analytical methods

Measurements of pH, total solids (TS), volatile solids (VS), ammonium nitrogen ($\text{NH}_4\text{-N}$) and total Kjeldahl nitrogen (TKN) were conducted based on the guidelines of Standard Methods [22]. The crude protein content was calculated by multiplying the organic nitrogen by the factor of 6.25 [23]. Structural carbohydrates were detected and quantified using high performance liquid chromatography (HPLC) based on Alvarado-Morales et al. [24]. Lipid extraction was carried out according to Marinho et al. [25]. Gas chromatography (Shimadzu GC-2010, Japan) was used to determine the VFA composition based on Kougiass et al. [26]. The C:N ratios of piggery slurry and wastewater microalgae were calculated using the elementary chemical composition. The methane content in the headspace of batch assays was measured by gas chromatography (Shimadzu GC-8A, Japan) as previously described [27]. For the continuous mode experiment, the biogas composition was determined with a gas chromatograph (Mikrolab, Aarhus A/S, Denmark), equipped with a thermal conductivity detector (TCD). All the determinations were performed in triplicate.

2.5. Statistical analysis

OriginPro 9.0.0 SR2 software (OriginLab Corporation, USA) was used for data analysis. Descriptive statistics were conducted for all variables and standard deviations were calculated. For comparison of significant differences among the methane yield of batch assays, as well as experimental data obtained during CSTR monitoring, analysis of variance (ANOVA) was carried out followed by Tukey Post-Hoc Analysis ($p < 0.05$).

2.6. Synergy or antagonism

To reveal potential synergistic or antagonistic effects occurred during the AD process, the following Equation was used:

$$n = \frac{\text{Practical yield}}{\text{Predicted yield}} \quad (1)$$

The “Practical yield” is the experimentally measured methane yield for each co-digestion mixture. The “Predicted yield” is the calculated methane yield based on the values obtained by the mono-digestion tests. The result of n shows:

- $n > 1$, synergistic effect
- $n = 1$, no effect
- $n < 1$, antagonistic effect

3. Results and discussion

3.1. AD experiments

3.1.1. Batch assays

Methane yield tests at a specific organic load were performed in order to: 1) determine the methane potential of both piggery slurry and wastewater microalgae; 2) define possible synergistic effect during the co-digestion process (Fig. 1). The lowest methane yield was measured for the mono-digestion of piggery slurry (255 ± 65 NmL/gVS). Indeed, this type of wastewater typically leads to low methane production mainly due to its high ammonia content. It has been previously reported that the containing free ammonia diffuses into microbial cells and cause stress conditions[23]. Regarding co-digestion, the partial replacement of animal slurry with wastewater microalgae enhanced the biomethanation under all enriched feedstocks. Especially, the highest methane yield was succeeded when the share of piggery slurry in the feeding

mixture was 60% in terms of organic matter (355 ± 27 NmL/gVS), revealing a synergistic effect on the methane productivity equal to 12%. Overall, the results from methane yield tests indicated that the co-digestion poses the ability to increase the low methane yield of slurry's mono-digestion. In order to validate this result, the co-digestion effect was investigated in continuously fed reactors so as to monitor the process in long-term operation. More specifically, both substrates' mono-digestion and the co-digestion mixture (60:40) with the highest methane yield were examined using CSTRs.

Fig. 1. Methane development plotted against time for the AD of piggery slurry and wastewater microalgae. The co-digestion mixtures represent the share of organic matter between the two substrates (piggery slurry: wastewater microalgae).

3.1.2. Continuous reactor operation

To further evaluate the mono- and co-digestion of piggery slurry and wastewater microalgae, three CSTRs fed with different feedstocks were monitored until steady state conditions were established (i.e. stable biogas production with lower than 10% daily variation for a period at least 10 days) [31]. R1 treated piggery slurry as sole substrate and was associated with the lowest bioenergy output, which is in agreement with the results from methane yield experiments (Fig. 2).

Fig. 2. Methane yield in mono-digestion of piggery slurry (R1), wastewater microalgae (R2) and the co-digestion at 60:40 contribution of organic matter (R3).

In fact, a higher dilution could have revealed the ultimate BMP value as the inoculum would dilute the ammonia to non-inhibitory levels. However, this was not the aim of the present study as the methane yield test was focused on specific conditions. On the contrary, the usage of wastewater microalgae led to significantly higher ($p < 0.05$) bioenergy output (408 ± 34 NmL/gVS) compared to the animal slurry. The augmented methane output can be attributed to the increased carbohydrates which are relatively easy to be anaerobically degraded [28] and to the higher lipids content which have high theoretical methane potential [29]. The obtained methane yield of wastewater microalgae is within the literature values for both pure *Nannochloropsis* sp. (340-414 NmL/gVS) and wastewater consortia (303-347 NmL/gVS) [30].

Despite the limited performance, stable biomethanation was recorded after two HRTs. Before start-up, the CSTRs were filled with effluent from a biogas plant that was fed with swine manure as major substrate. Therefore, the AD microbiome was adapted to the examined substrate and thus, it should have been able to process efficiently the nitrogenous substrate [32].

However, it was previously shown that this type of waste frequently leads to

inhibited steady state conditions which is a “pseudo steady-state” often seen with ammonia rich substrates [33]. In accordance, limited process performance was revealed during the current experiment through the low methane production and the accumulation of VFA (Fig. 3f). Free ammonia (NH_3) is considered as the inhibiting form of ammonia ($\text{NH}_3 + \text{NH}_4^+$) which is increasing along pH and temperature. However, the pH values which directly affect the free ammonia concentration were relatively stable, indicating that an alleviation from ammonia toxicity did not occur [34].

Fig. 3. Comparison between experimental values (open circles) and data obtained from BioModel simulations estimated parameters (straight lines) for the mono-digestion of piggery slurry (R1). Dashed lines indicate simulations of post-experimental period with estimated parameters.

The AD of wastewater microalgae as sole substrate was examined in R2. Increased biomethanation was established and at steady state conditions the methane yield reached $313 \pm 13 \text{ NmL/gVS}$, corresponding to approximately 77% of the expected energy output based on methane yield tests. Similarly, Mahdy et al. [13] found that the mono-digestion of microalgae was associated with comparable methane production (approximately 320 NmL/gVS) under continuous mode operation. Despite the markedly

high methane production, an undesired increasing trend of propionate accumulation was observed at the end of the present experimental research (Fig. 4d). Accordingly, the concentration of propionate is considered as a state indicator for AD process imbalance [35]. Propionate is persistent for AD and accumulation in long duration can lead to pH decrease and subsequently, to potential process failure which is a major bottleneck from an industrial point of view.

Fig. 4. Comparison between experimental values (open squares) and data obtained from BioModel simulations with estimated parameters (straight lines) for the mono-digestion of wastewater microalgae (R2). Dashed lines indicate simulations of post-experimental period with estimated parameters.

Furthermore, wastewater microalgae cannot be widely considered as a main feedstock for full-scale applications, because of their low substrate availability and also, proximity to the biogas plants is not always ensured for this type of feedstock. In contrast, large amounts of livestock wastewater are easily available within the agricultural sector [36]. In this context, the co-digestion with livestock slurry is a rather more realistic strategy for full-scale implementation. Moreover, the buffer capacity of the system can be increased and the microbiome be enriched with more diverse members that could positively alleviate the aforementioned weaknesses and

consequently, boost the AD performance.

The results from the co-digestion reactor (R3) showed negligible accumulation of propionate or other intermediate compounds in contrast to R1 and R2, during the whole experimental period. In addition, after two HRTs the total VFAs were at low levels, indicating a well-functioning process [37]. Similarly to batch assays, the continuous mode operation of co-digestion led to higher bioenergy production (216 ± 17 NmL/gVS) than the mono-digestion of piggery slurry (176 ± 18 NmL/gVS). Specifically, the methane yield was significantly higher (23%, $p < 0.05$) than the corresponding one from the degradation of the nitrogenous substrate. In addition, the achieved methane yield during continuous reactor operation was approximately 68% of the ultimate value based on batch assays. Compared to livestock's slurry mono-digestion, the pH was slightly lower and thus, the free ammonia concentration had decreased values at steady conditions (<0.5 g-N/L). Additionally, it was previously concluded that substrates with increased lipid content, like the wastewater microalgae, could alleviate the digester from the toxicity of nitrogenous co-substrate [20]. Hence, process instabilities were avoided and the positive effect of utilizing dissimilar substrates was revealed.

3.2. Simulation results

3.2.1. Model simulation and calibration

As shown in Fig. 3 and 4, the BioModel with the default parameters exhibited a good performance in describing the experimental trend of both mono-digestion scenarios. However, with the new set of parameters, the experimental trend is better captured during steady state period, particularly for the pH, biogas and methane flow

rates. The set of estimated parameters was used to perform further simulations in order to investigate the process performance of the three scenarios after the experimental period (50 days).

For the case of piggery slurry mono-digestion (R1), biogas and methane productivities were well captured by the model (straight black and red lines) during the entire experimental period (Fig. 3a-b). However, biogas productivities were slightly underestimated between days 15-30. In addition, acetic acid concentrations were underestimated between days 25-35, though after this the model predicts the experimental trend. The model predicted a low intermediates' concentration trend during the start-up period, to subsequently increase, leading to the accumulation of VFAs (Fig. 3d-f). Eventually VFA concentration stabilized, reaching a steady state (blue dash line) value of 2.02 g/L (Fig. 3f). This is explained by simulation results, where a slight increase in free ammonia concentration is observed during the adaptation period reaching a maximum at 0.85 g-N/L (Fig. 5a), which can slow down the methanogenic activity leading to VFA accumulation [38], although not being a critical cause of process failure. In parallel, the pH level was also changed to more alkaline conditions (i.e. from 8.07 to 8.26), shifting the equilibrium from NH_4^+ towards NH_3 formation [39]. However, due to the buffer capacity of the system this effect is counteracted and thus, free ammonia concentration decreased to 0.80 g-N/L leading to a stable performance of the reactor in the long-term operation (Fig. 5a). The pH also became stable at 8.32; a value that is consistent with the experimental trend from day 32 and onward (Fig. 3c).

As noticed after the experimental period, the trend of both methane and biogas shows a slight decrease over time whilst VFA concentration shows the opposite trend

(Fig. 3a, b, d-f). Therefore, in order to investigate whether or not the accumulation of VFA might have a detrimental effect in biogas and methane production over time, a simulation was performed until the system reached steady state (data not shown). Total VFA and TAN steady state concentrations corresponded to 3.85 g/L and 2624 mg-N/L, respectively. This TAN concentration is within the inhibitory range (1500-7000 mg/L)[40]. On the other hand, biogas and methane productivities decreased to 0.147 and 0.131 NL/L/d, respectively. This might be an indication that in the long-term operation, the system could reach the so called “inhibited steady state [33]”.

Fig. 5. Simulation results for free ammonia composition: a) mono-digestion of piggery slurry, b) mono-digestion of wastewater microalgae and c) co-digestion of wastewater microalgae and piggery slurry.

Likewise, for the mono-digestion of wastewater microalgae (R2) biogas and methane productivities were also well captured by the updated model (straight lines) as observed in Fig. 4a-b; although the model could not capture the experimental trend between days 21-27 for biogas productivity. VFA process dynamics were slightly overestimated (Fig. 4d-f) and specifically, the observed trend in the accumulation of propionate starting from day 40 could not be captured by the model. In spite of the rapid

increase in propionate concentration at day 40, both biogas and methane productivities were kept stable until the end of the experimental period. It is known that the accumulated intermediates could lead to reactor acidification and process failure in long-term operation [41]. However, simulation results after the experimental period revealed that no further intermediate accumulation took place, keeping VFA below the levels considered harmful for process stability [42]. In addition, process simulation results showed a decrease in free ammonia concentration from 0.72 to 0.24 g-N/L over the complete experimental period (Fig. 5b). This observation is consistent with the drop in pH exhibited by both experimental results and model simulations. Along with the aforementioned, simulation results for the post-experimental period show a stable long-term operation of the system.

3.2.2. Simulation of co-digestion scenario and model validation

Results of the co-digestion experiment were used for model validation. At this point, the biodegradability parameter of carbohydrates was fitted, whilst the protein and lipid biodegradability parameters were the same as for the mono-digestion scenarios (Table 2). Biogas and methane productivities were predicted quite well (Fig. 6a-b) and only a slight overestimation in the case of methane was observed. As depicted in Fig. 6d, dynamics for propionate were well predicted during the adaptation period until day 32. Afterwards, the simulated concentration of propionate remained stable (0.12 g/L) in contrast to the experimental trend (dropping from 0.11 to 0.02 g/L). In the case of acetate, process dynamics were not accurately captured during the start-up process, but were adequately predicted from day 35 and onward, where the estimated concentrations were in agreement with the experimental trend.

In general, the levels of VFA during the whole experimental period were far below inhibitory thresholds to cause potential process failure. This indicates stable digester performance and good synergistic activities of acidogenic and methanogenic microorganisms [43], in contrast to the mono-digestion of piggery slurry (R1). Furthermore, simulation results after the experimental period showed that the long-term co-digestion of piggery slurry and wastewater microalgae did not promote the accumulation of VFA. Similarly to the low VFA accumulation, simulation results also revealed that free ammonia concentration was below critical levels (<1.1 g-N/L) for inhibition or toxicity problems in the AD system [44,45]. Lastly, the findings also showed the advantage in performing co-digestion of dissimilar substrates, in order to avoid process failure.

Fig. 6. Model validation for the co-digestion of wastewater microalgae and piggery slurry: simulation (straight lines) vs experimental data (open triangles).

4. Conclusions

The present study demonstrated that the anaerobic digestion of wastewater microalgae can lead to relatively high methane production. The benefit of using wastewater microalgae in the feedstock of an anaerobic digester was revealed in batch

and continuous mode operation. In addition, the positive effect of using two dissimilar, yet complementary waste streams for biogas production was clearly shown. Specifically, the partial replacement of piggery slurry with wastewater microalgae by 40% in terms of organic matter in the influent feedstock improved the bioenergy production by 23%. Moreover, the applicability of the BioModel to efficiently simulate both mono- and co-digestion strategies was revealed. In addition, the simulations indicated that the long-term co-digestion of second and third generation substrates is associated with high gas productivity and absence of inhibition risks due to ammonia or volatile fatty acid accumulation.

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