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Production of volatile fatty acids through co-digestion of sewage sludge and external organic waste: Effect of substrate proportions and long-term operation

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ABSTRACT

Volatile fatty acids (VFAs) are intermediates of anaerobic digestion with high value and wide range of usage. Co-digestion of sewage sludge and external organic waste (OW) for VFA production can help achieve both resource recovery and ensure sustainable and innovative waste management. In view of this, the effect of substrate proportions on VFA production from co-digestion of primary sewage sludge and OW is studied. Long-term operation in a semi-continuous reactor was performed to assess the resilience of such a system and the VFA-rich effluent was tested for its ability to be used as carbon source for denitrification. Co-digestion was initially carried out in batch reactors with OW proportion of 0%, 25%, 50%, 75%, 100% in terms of COD and scaled up in a semi-continuous reactor operation with 50% OW. In the short-term operation in the batch mode, acetic acid dominated, however, increasing OW fraction resulted in increased valeric and caproic acid production. Moreover, in the long-term semi-continuous operation, caproic acid dominated, accounting for ≈55% of VFAs. The VFA-rich effluent from the semi-continuous reactor achieved the highest denitrification rate as a carbon source when compared with acetic acid and methanol. The results demonstrate that co-fermentation can increase VFA yield and shift products from acetic acid to caproic acid in long-term operation and the VFAs can be used within wastewater treatment plants to close the loop.

1. Introduction

Resource recovery from waste streams is an important contributing factor dealing with the United Nations Sustainable Development Goals 6, 7 and 11. Wastewater treatment process results in the production of sludge, a semi-solid material that needs to be treated before disposal. In the year 2010 alone, about 9 million tons of dry solids of sewage sludge were produced in the European Union (EU) (Mateo-Sagasta et al., 2015). The energy contents of primary (2.0–8.0% total dry solids) and secondary (0.83–1.16% total dry solids) sludge are estimated as 6.4–8.1 and 5.2–6.4 kWh/kg respectively (Nazari et al., 2018). Sewage sludge, therefore, offers an opportunity for energy and material recovery from wastewater treatment plants (WWTPs). Anaerobic digestion is a well-known sludge treatment method that can lead to energy and material recovery from waste.

In order to fulfil the Paris agreement, countries are taking measures to cut down the emission of greenhouse gases. A typical example of such measures is the Swedish new climate policy framework which has the ambitious goal for Sweden to have zero net emissions of greenhouse gases by 2045 and thereafter achieve negative emissions (Ministry of the Environment and Energy, 2018). The end products of anaerobic digestion are mainly methane and carbon dioxide, very well-known greenhouse gases. In view of this, the Stockholm Municipality, for instance, has decided to use electric buses in public transport instead of biogas buses by 2021. This puts high demands on the development of next-generation wastewater treatment technologies to turn these carbon-rich waste streams into new bio-based chemicals and materials instead of biogas.

Volatile fatty acid (VFA) is an intermediate product of anaerobic digestion. Thus, the reaction time of VFA production is much shorter than biogas production. VFA production is currently getting attention due to its higher value and a wider range of usage than biogas (Atasoy et al., 2018; Lee et al., 2014). The prices of VFAs can range from USD 600 to 3,815 /ton depending on the number

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of carbon atoms in the molecular structure, whereas biogas is valued at only USD 150/ton (Calt, 2015). When the acidogenic and methanogenic potential of six different agroindustry residues was determined, it was shown that the production of VFA from the residues can have economic value more than three times higher than if the residues are valorised for methane production (Perimenis et al., 2018). Thus, a total of 304 tonnes of brewery residues has a potential economic value of € 29,250 if used for VFA production, composed mainly of acetic (10–43%), butyric (30–52%) and caproic (0–45%) acid, but only €8,600 if used for methane production. It should be mentioned that the study only considered the value of the final products. In a recent full-scale study, a more comprehensive economic comparison of VFAs and biogas production processes from sewage sludge by anaerobic digestion was done and it showed that VFA had a net profit of 9.12 USD/m^3, while biogas has a net value of only 3.71 USD/m^3 (Liu et al., 2018). Focus on VFA recovery can turn WWTPs to a biorefinery with the possibility of recovering other products such as phosphorus. A recent study showed that recovery of VFA together with phosphorus released during the acidogenic fermentation before biomethanization can increase the potential market value of cellulosic primary sludge from 0.46 €/capital.year up to 1.95 €/capita.year (Crutchik et al., 2018).

Besides sewage sludge, there are other bio-wastes with inherent value, that need to be treated. Even though sewage sludge has an enormous tendency to produce energy and bio-based products, the production rate can be lower due to lower biodegradable organic matter content. Anaerobic co-digestion of sewage sludge with external organic wastes such as food waste, beverage waste, dairy waste, has the potential to enhance resource recovery efficiency from waste streams (Xie et al., 2018). Moreover, in the EU alone, about 88 Mt of food is wasted. There is the potential of 170 Mt of CO_2 emission from the production and disposal of the amount of food that is wasted (Directorate General for Communication, 2017). Therefore, co-digestion of sewage sludge and external organic waste such as food waste can help achieve resource recovery and sustainable waste management, concurrently. It has been shown that anaerobic digesters at most WWTPs are operating below their capacities and therefore co-digestion can be a sustainable option (Nghiem et al., 2017). However, while there are a number of studies on co-digestion of sewage sludge and organic waste for biogas production with a number of full-scale plants, there is limited literature on VFA production through co-digesting of sewage sludge and external organic wastes. This presents an opportunity to maximise resource recovery from waste streams.

The kind of substrate used for VFA production is known to influence not only the amount of VFA produced but also the composition (Atasoy et al., 2018). This is because the degree of acidification and the prevailing metabolic pathways of the fermentation process, are influenced by the properties of the substrate (Chen et al., 2013; Silva et al., 2013). It is therefore important to understand how co-digestion of sewage sludge and external organic waste will influence VFA production and composition. The effect of the proportion of external organic waste on the VFA yield and composition is yet to be studied. In view of this, a comprehensive study of the effect of co-digestion of sewage sludge with different proportions of external organic waste was carried out in the current study.

It has been shown that co-digestion of sewage sludge with food waste can increase VFA yield up to 867.42 mg COD/g VS without external pH adjustment (Wu et al., 2016). In a more recent study, co-digestion of sewage sludge and organic fraction of municipal solid waste (OFMSW) in a continuous operation was investigated and achieved a yield of 288.3 mg VFA/g VS at pH of 9 (Garcia-Aguirre et al., 2019). The above studies on VFA production through co-digestion are usually narrowed to sewage sludge and a specific organic waste in a short-term operation of a maximum of 30 days. To have an insight in the performance of VFA production system when there is a scale-up, long-term operation of semi-continuous reactor for more than 100 days was carried out to investigate the effect of long-term operation on the VFA yield and composition during co-digestion of sewage sludge and external organic waste.

Moreover, waste-derived VFA can be used as a carbon source for biological nutrient removal within WWTPs (Zhang et al., 2016). Using VFA from waste streams as a carbon source is a sustainable option to replace fossil-based carbon sources such as methanol. This will close the loop for WWTPs. Thus, VFA-rich liquid has great potential to be used directly as an external carbon source in biological nutrient removal which can make WWTPs self-sufficient plants in terms of chemical use (Liu et al., 2018). Therefore, in the current study, the VFA produced from co-digestion of sewage sludge and external organic waste is employed as a carbon source for denitrification in comparison with traditional external carbon sources, methanol and acetate.

The main objective of the study was to systematically investigate the impact of substrate ratio of sewage sludge and external organic waste with complex composition on VFA production and application. Specifically, the following research questions were sought to be answered: (1) What is the impact of substrate proportion on VFA yield and composition in a short-term batch reactor at pH 5? (2) How does substrate proportion affect biogas production? (3) What is the effect of long-term semi-continuous operation on the VFA production and composition when there is an upscaling? (4) How could the produced VFA be used in other processes?

2. Materials and methods

2.1. Substrate and inoculum

Primary sewage sludge (PS) and organic wastes (OW) were collected from the Hammarby Sjöstadsverk research facility (Stockholm, Sweden) and Himmerfjärden WWTP (SYVAB, Sweden), respectively. The external OW consisted of alcohol and soda beverage, food, dairy, fruit, fat and oil wastes. At Himmerfjärden WWTP, the OW is ground and hygienised at 71 °C for 61 min. Digested sludge taken from a full-scale anaerobic digestor (Henriksdal WWTP, Stockholm, Sweden) was used as seed sludge. Fresh PS was collected prior to feeding while OW was collected in bulk, aliquoted and frozen. An aliquot of OW was thawed at room temperature prior to feeding. Characteristics of the PS, OW and seed sludge are shown in Table 1.

2.2. Experimental set-up and procedure

2.2.1. Batch tests

Batch experiments were carried out using an automatic methane potential test system (AMPTS II, Bioprocess Control,

Table 1
Characteristics of substrates and seed sludge.

Parameters[a]	Primary sludge	Organic waste	Seed sludge
pH	5.9 ± 0.5	4.1 ± 0.3	7.0
Alkalinity	16 ± 3	144 ± 10	770 ± 95
TS	29 ± 3	54 ± 6	21 ± 1
VS	25 ± 2	48 ± 6	13 ± 0
Total COD	43 ± 10	148 ± 4	24 ± 3
Soluble COD	2.2 ± 0.1	77 ± 11	1.6 ± 0.1
Total VFA	0.8 ± 0.1	2.4 ± 0.1	0.3 ± 0.1
Total Nitrogen	0.6 ± 0.1	2.6 ± 0.2	1.3 ± 0.1

[a] units of parameters are in g/L except pH and alkalinity (mg$CaCO_3$/L).

Sweden) with a biogas measurement unit and glass reactors. The test system consists of fifteen glass bottles with a working volume of 450 mL placed in a water bath to control temperature. The temperature of the reactors was maintained at 35 °C. The produced biogas passes through a CO_2-capturing unit (3 M NaOH with 0.4% Thymolphthalein pH-indicator) to remove CO_2 before being measured by a gas volume measuring device.

The amount of substrate was maintained at initial total chemical oxygen demand (COD) concentration of 15 g/L, whereas the initial amount of seed sludge was set at VS of 7.5 g/L to achieve substrate/inoculum (S/I) ratio of 2 gCOD/gVS because S/I ratio of 2–4 gCOD/gVSS has been found to maximize VFA production (Silva et al., 2013). Reactors' contents were diluted with tap water (left overnight in fume hood) to achieve the desired initial TCOD and VS concentration. Co-digestion was carried out in triplicate with OW proportion of 0%, 25%, 50%, 75%, 100% as total COD. Thus, 0% OW and 100% OW represent mono-digestions of PS and OW, respectively. The pH of the reactors was adjusted to 5 at the beginning of the experiments and was not controlled. Methanogenic activities are known to be inhibited at pH above 8.0 or below 6.0 (Atasoy et al., 2018).

2.2.2. Semi-continuous reactor

Based on the results of the batch tests, there was a scale up to a long-term operation in semi-continuous mode with a total volume of 15 L and a working volume of 10 L volume. The semi-continuous reactor employed in the study was a two walled glass reactor with an agitator driven by brushless DC motor. The reactor was connected to a gas meter (MILLIGASCOUNTER, Type MGC-10, Ritter) as well as methane and carbon dioxide sensors (TDS0068 and TDS0054, Dynament) for biogas measurement and analysis (Fig. 1). A pH sensor (PHEX 112 SE, Prominent) with measurement and control unit (DULCOMETER, Prominent) was installed to automatically control the pH of the reactor. 2 M HCl and 5 M NaOH were used as dosing solutions to maintain the pH to 5 throughout the experimental period. The system was equipped with a temperature probe and heated water was circulated through the two walls of the reactor to keep the temperature of the process at 35 °C. A data acquisition, process control, and data analysis system, DAQFactory (AzeoTech) was used to control and record data online.

The semi-continuous experiment was carried out with a feed of PS:OW ratio of 50%:50% in terms of COD. At the set-up, seed sludge was added together with the feed in such a way to achieve a substrate/inoculum ratio of 2 gCOD/gVS. The reactor was allowed to run for 7 days for acclimation. Subsequently, the reactor was run in a semi-continuous mode with a retention time of 7 days by withdrawing and feeding three times a week. The 7-day retention time was chosen based on the results of the batch study. The semi-continuous reactor was operated for 115 days.

2.3. Denitrification test

VFA-rich effluent from the semi-continuous reactor was tested for its suitability as a carbon source for denitrification in comparison with acetate and methanol. Manometric tracking method was used for the denitrification tests. The tests were done using airtight glass bottles with a working volume of around 320 mL, and two top openings, sealed by rubber septa and kept in place by a plastic lid. The bottles were connected with a manometric measuring device, featured with a data logger. The test temperature was maintained at 25 °C with the help of a water bath. All tests were performed with activated sludge from a membrane bioreactor pilot plant situated at the Hammarby Sjöstadsverk research facility as inoculum. The tests were performed at a carbon-nitrogen (C/N) ratio of 4.5 with an initial nitrate concentration of 28.5 mg NO_3-N/L. All experiments were conducted in triplicate.

2.4. Analytical methods

Total solids (TS), volatile solids (VS) and volatile suspended solids (VSS) were determined according to Standard Methods (APHA, 2005). Chemical oxygen demand (COD), Total nitrogen and alkalinity were analysed with respective cuvette test kits

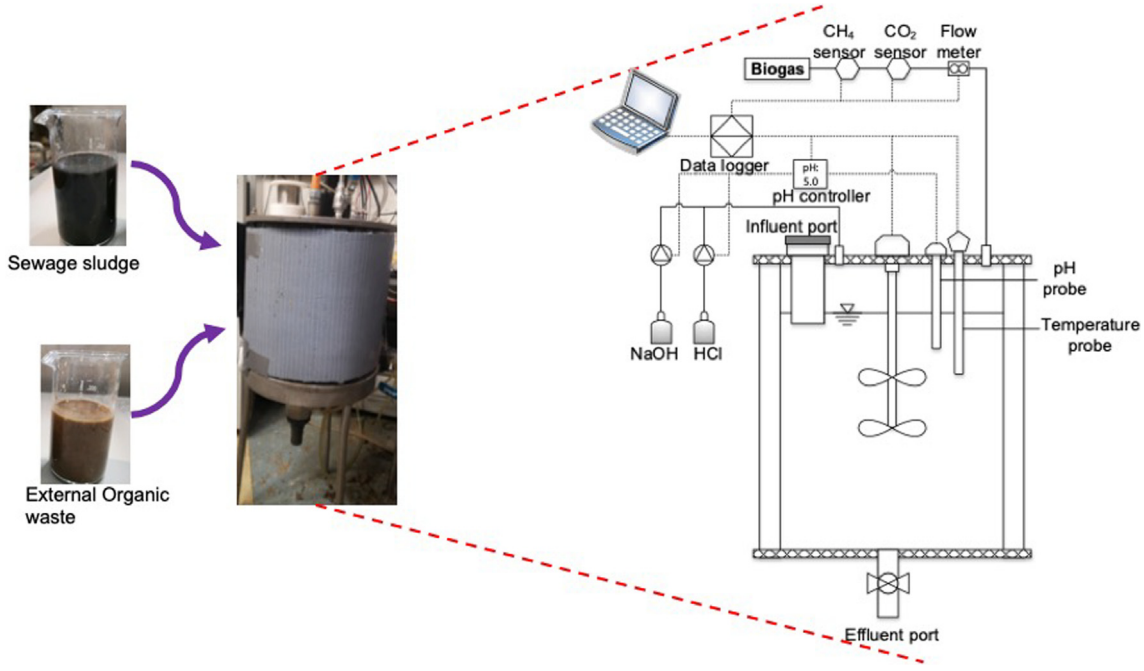

Fig. 1. Photo and schematic diagram of the semi-continuous reactor.

(WTW, Germany) and a spectrophotometer (PhotoLab 6600 UV Vis, WTW, Germany).

The VFA compositions and concentrations were analysed by gas chromatography (Intuvo 9000 GC System, Agilent) equipped with a Flame Ionization Detector (FID) and a capillary column (DB-WAX Ultra Inert, 30 m × 250 μm × 0.25 μm, Agilent 122-7032UI). The temperature of the FID detector was set at 280 °C with helium as the carrier gas at a flow rate of 2 mL/min. The oven started with a temperature of 70 °C and an equilibrium time of 1 min and a ramp rate of 10 °C/min and a maximum of 200 °C. The peaks corresponding to each VFA components were identified using mixed VFA standard solution (Volatile Free Acid Mix, CRM46975 Supelco) and individual pure VFA solution to match each pick. Concentration was calculated from the area of peak based on calibration with standards using Agilent OpenLab Software. The VFA concentration was expressed in COD units using stoichiometric COD factors of 1.0667 for acetic acid, 1.512 for propionic acid, 1.813 for butyric acid, 1.813 for isobutyric acid, 2.036 for valeric acid, 2.036 for isovaleric acid, 2.207 for caproic acid, and 2.207 for isocaproic acid.

2.5. Statistical analysis

For the batch scale experiments, PAST 3 (PAST 3.20, University of Oslo, Norway) was used to conduct a two-way ANOVA to test the statistical difference of the VFA yields between experiments conducted with different OW proportions and between retention times. PAST 3 was also used to conduct a T-test to determine if there was a statistical difference between the total influent carbon and total effluent carbon during the semi-continuous experiment.

3. Results and discussion

3.1. Batch tests

3.1.1. Effect of substrates proportions on VFA production during co-digestionwit

Batch tests were done with different percentages of OW proportion up to retention time of 20 days to study the effect of OW proportion on the VFA production during co-digestion of sewage sludge and organic waste under acidic condition. Increasing the proportion of OW content in the substrate resulted in an increase in the VFA produced. Substrate without OW produced only 3052 mg COD/L of VFA at day 8 and reached maximum VFA concentration of 4106 mg COD/L only after day 18. However, for 25%, 50% and 75% of OW the VFA concentrations at day 8 were 4134, 4533, 4841 mg COD/L, respectively, while mono-digestion of OW resulted in VFA concentration of 5270 mg COD/L (Fig. 2A). The increase in VFA production with an increase in the percentage of OW can be attributed to the increase in the biodegradable organics which were readily available for utilization by acid-producing bacteria. It has been shown that co-digestion enhances the solubilization of hard-biodegradable organics which can improve VFA productivity (Wu et al., 2016). There was a slight decrease in VFA concentration at day 10 particularly for the co-digestions (i.e. 25%, 50%, 75%) and began to rise again till day 18. This rise and fall in VFA concentration can be probably due to different fractions of organics with different degrees of biodegradability of the substrates. Thus, relatively easily biodegradable fractions led to an initial rise of VFA concentration, methanogenesis reduced the VFA concentration, later relatively slowly biodegradable fractions are converted to VFA. This was not the case in mono-digestion of PS, where the substrate was more uniform.

In all experiments, the pH initially increased from 5 to about 5.5 for 0%-75% and 5.3 for 100% OW in day 1 when the VFA production was low. The aforementioned slight increase in pH during batch fermentation can be due to the release of ammonia and other alkaline substances that are produced through biodegradation of nitrogenous organic matter (Fang et al., 2019). The initial increase in pH was minimal for the mono digestion of OW in comparison with the other experiments probably due to the relatively higher buffer capacity of OW due to higher alkalinity than PS (see Table 1). The pH decreased again at day 3 to 4.9, 4.9, 5, 5.1 for 0%, 25%, 50%, and 75% respectively, while the pH of 100% OW rather increased to 5.4. The decreasing trend in the change of pH with an increase in OW proportion can be attributed to the fact that the alkalinity of OW was much higher than PS and therefore the production of acid didn't have much influence on the pH (Labatut and Pronto, 2018). Nonetheless, from day 15 the pH began to increase resulting in the reduction of VFA concentrations.

The VFA yield which took into consideration the initial VFA concentrations and the VS of the reactors were used to better understand the concurrent effect of retention time and PS:OW ratio on the actual VFA production. VFA yield in all the different ratios increased sharply up to day 8 and stayed fairly constant (Fig. 3). VFA yield of 250, 301, 301, 291 and 305 mg COD/gVS were achieved for 0%, 25%, 50%, 75% and 100% of OW at day 8, respectively. The results suggest that the addition of 25% and 50% of OW led to about 20% increase in the VFA yield which was only 5 mg COD/g VS lower than mono-digestion of OW (100%). Thus, co-digestion sped up the VFA production and increase the yield. The VFA yield obtained for the current co-digestion (25–75% OW) was similar to what was obtained in another study of co-digestion of sewage sludge and OFMSW but at pH 9 (288.3 mg VFA/g VS) even though the study was conducted at a higher temperature of 55° C (Garcia-Aguirre et al., 2019). However, the yield values obtained were lower than a study by Wu et al., (2016) with pH range (5.2–6.4), similar to the current study, where a VFA yield of up to 867.42 mg COD/g VS was obtained. The higher VFA yield than this study could be due to the fact that, in that study, a higher organic loading of 9 VS/L-d was used and experiments were carried out under a higher temperature of 40 °C. In the current study in the batch reactor, the organic loading at day 8 is estimated as only 1.2–1.5 VS/L-d and the temperature was set at 35 °C.

The VFA yield began to decrease after retention days 18 and 15 for experiments with 0% and 25–100%, respectively. The decrease can be attributed to an accumulation of acids that reduce microbial activity and/or conversion of organic acids to methane (Arslan et al., 2017). A two-way ANOVA was conducted to determine if there was a statistical difference in the VFA yields between the different OW proportions and between the retention times. The results show that statistically there was a significant difference in the VFA yields between the different OW proportion and between the retention times with p values of 0.009244 and 7.02 $x10^{-14}$, respectively.

3.1.2. Effect of substrates proportions on VFA composition during co-digestion

The VFA composition showed that acetic acid accounted for a relatively higher percentage of the VFAs with propionic acid as the second abundant VFA component accounting for 25 ± 3% in all retention times without any much difference among the different PS:OW ratios. While the percentage of acetic acid remained fairly constant (45 ± 4%) throughout the study period for 0% OW experiments, there was a decrease in the acetic acid percentages from 59 to 38%, 67 to 35, 74 to 25% and 88 to 20% for 25, 50, 75 and 100% OW experiments from day 0 to 20, respectively (Fig. 2C). The decrease in acetic acid percentages led to a corresponding increase trend of VFAs with higher numbers of carbons in their molecular structure, particularly, valeric and caproic acids. Valeric acid increased from 0 to 11%, 0.2–13%, 0.1–19%, 0.1 to 22%; and caproic acid increased from 1.3 to 7%, 1.6 to 9%, 2 to 11%, 2 to

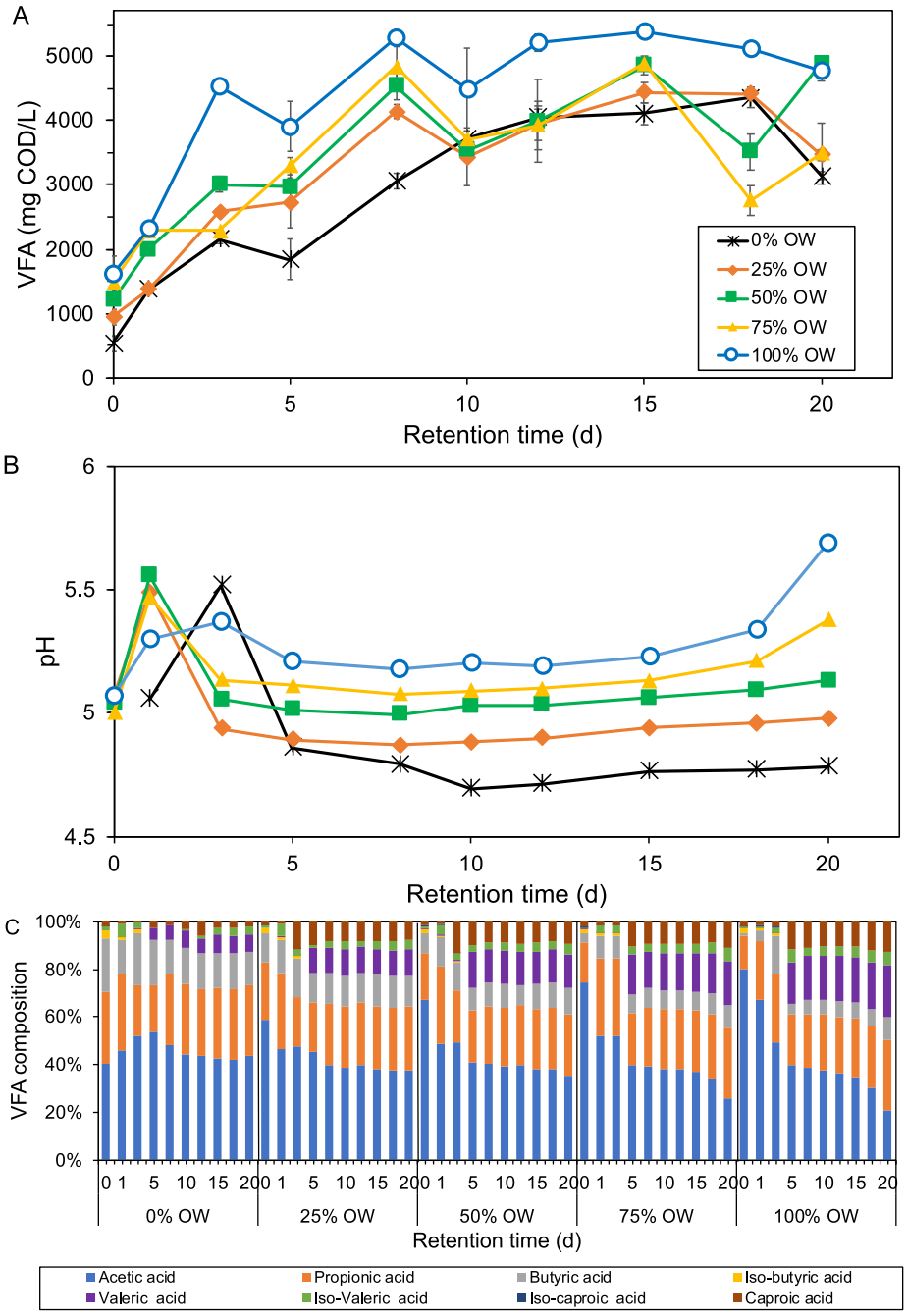

Fig. 2. (A) VFA production, (B) pH and (C) composition of VFA as a function of retention time during the batch experiments with different OW proportions.

13% for 25, 50, 75 and 100% OW experiments from day 0 to 20, respectively. The increase in valeric acid percentage with an increase in the OW proportions can be attributed to the increase in protein content of the OW substrate which is evident from the total nitrogen concentration (Table 1). A similar study has recorded protein-rich substrates can produce VFA with up to 25% valeric acid composition (Shen et al., 2017). Higher molecular weight VFAs such as valeric acid are associated with Stickland fermentation of protein through either reductive deamination of single amino acids or by oxidation–reduction between pairs of amino acids (Parawira et al., 2004). Moreover, the reduction of acetic acid percentages and consequent increase in caproic acid and valeric acid proportions can also be attributed to conversion between the VFAs through β-oxidation followed by isomerization (Parawira et al., 2004). The results, therefore, demonstrate that co-fermentation of sewage sludge and organic waste does not only increase the VFA yield but it also enables product shift from acetic acid to valeric and caproic acid which are more valuable products.

3.1.3. Effects of substrates proportions on biogas production

The cumulative volume of biogas was up to 175 NmL CH_4 for the O% OW experiment and only 73.4 for 100% OW at a retention time of 7 days (Fig. 4). Thus, generally, there was a decrease in the cumulative volume of biogas with an increase in the percentage of OW in the substrate. It can be inferred from the results that PS has a higher tendency to form biogas even at acidic pH of 5 where methanogenic activities are minimal. This can be attributed to the composition of VFA formed by the PS substrate. Shin et al.,

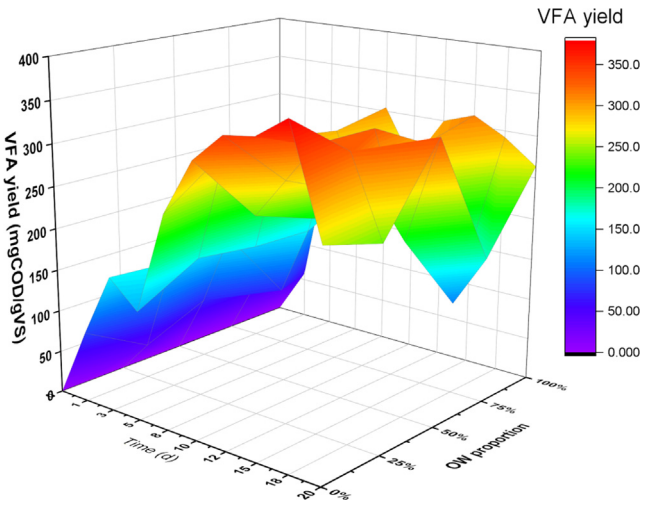

Fig. 3. VFA yield as a function of time and OW proportion during the batch experiments with different OW proportions.

2001 have shown that different kinds of VFA resulted in different biogas yield. Moreover, the relatively higher VFA formed with the addition of OW could have resulted in the inhibition of biogas production in the reactors with higher percentages of OW (Jiang et al., 2018). The current results show that the co-digestion of sewage sludge and organic waste presents a good opportunity to shift production from biogas to VFA which has a more economical value.

It was noted that the production of biogas stopped after day 7 for 0% and 25% OW, while biogas production for the reactor with 50% OW substrate rose steadily with time. The production of biogas for reactors with 0% and 25% OW substrate stopped due to the fact that the pH in the reactors has reduced to 4.7 and 4.9 respectively. At such low pHs, methanogenic activities might have been completely inhibited. On the other hand, the production of methane by reactors with 75% and 100% OW reactors increased steadily with time until after day 17 where there was a steep rise in biogas production. The sharp increase in methane production can be attributed to rise in pH (Fig. 2B) which might have led to an increase methanogenic activities thereby decreasing VFA concentration. However, the rise in methane was only steady for 0%, 25% and 50% after day 17 even though there was a decrease in VFA concentration, particularly for 25% and 50% OW experimental sets. It could be that other content of biogas which was not measured in the study such as hydrogen was formed instead of methane.

3.2. Long-term operation of the semi-continuous reactor

3.2.1. VFA production and composition

Semi-continuous experiment was run for 115 days to understand the performance of the VFA production system in the long-term operation when there is a scale-up. The VFA concentrations from the semi-continuous experiments were higher than those of the batch experiments because the initial total COD of the batch experiments was set at only 15 g/L to ensure uniformity for the various substrate ratios. However, with the semi-continuous experiment, the total COD concentration of the feed was about ≈75 g/L. The total VFA production increased initially steeply up to 14287 mg COD/L on day 16 (Fig. 5A). The VFA production began to drop after day 16 to 6450 mg COD/L at day 23 probably due to an accumulation of VFA which might have resulted in inhibition (Roume et al., 2016). Accumulation of VFA in fermentation reactors with mixed cultures is known to have an adverse effect on the process due to the fact that there is inhibitory effect of the VFAs and their consumption in internal conversion reactions (Arslan et al., 2017). Undissociated VFAs can be transported through microbial cell membranes and cause acidification of the cell interior. Acidification of the cell interior has a potential inhibition of enzymatic processes and imposes a metabolic burden through the use of the ATP-dependent ATPase enzyme to remove the excess protons. Acidification of microbial cell interior can also cause cell turgor and disruption of key amino acid pools (Jarboe et al., 2013). It was shown in a study that the production rate of VFAs was affected by accumulating undissociated VFAs which inhibited the microbial species (Ge et al., 2015). This explains the decrease in VFA production after day 16.

After day 23, the concentration of VFA was in the range of 4805 to 7696 with an average value of 6772 ± 1007 mg COD/L until day 51 when there was a rise in the VFA production to about 11000 mg COD/L. The VFA reached a maximum of 17242 mg COD/L on day 113. The rise in VFA concentration from day 51 can be attributed to the adaptation of the acid-producing microbes and/or increase in the dominance of species which are more tolerant to VFA toxicity. Andersen et al., 2017 found out that in continuous fermentation of biorefinery thin stillage, the production of VFA which was declining initially recovered owing to an increase in the dominance of species with high tolerance to VFA toxicity. It seems the changes in the VFA concentration didn't change much the volatile solids. The VS initial increase from about 21 g/L and reach a stable value after day 10 with an average of 32.3 ± 6.8 g/L (Fig. 5B).

The various components of VFA showed that initially acetic acid, propionic acid, butyric acid and valeric acid were the dominant species. Caproic acid kept rising and became the dominant

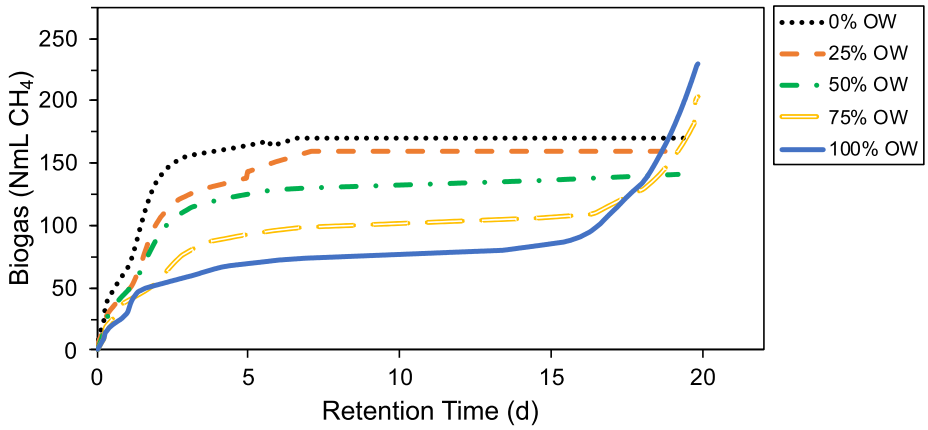

Fig. 4. Cumulative biogas production for batch experiments with different OW proportions.

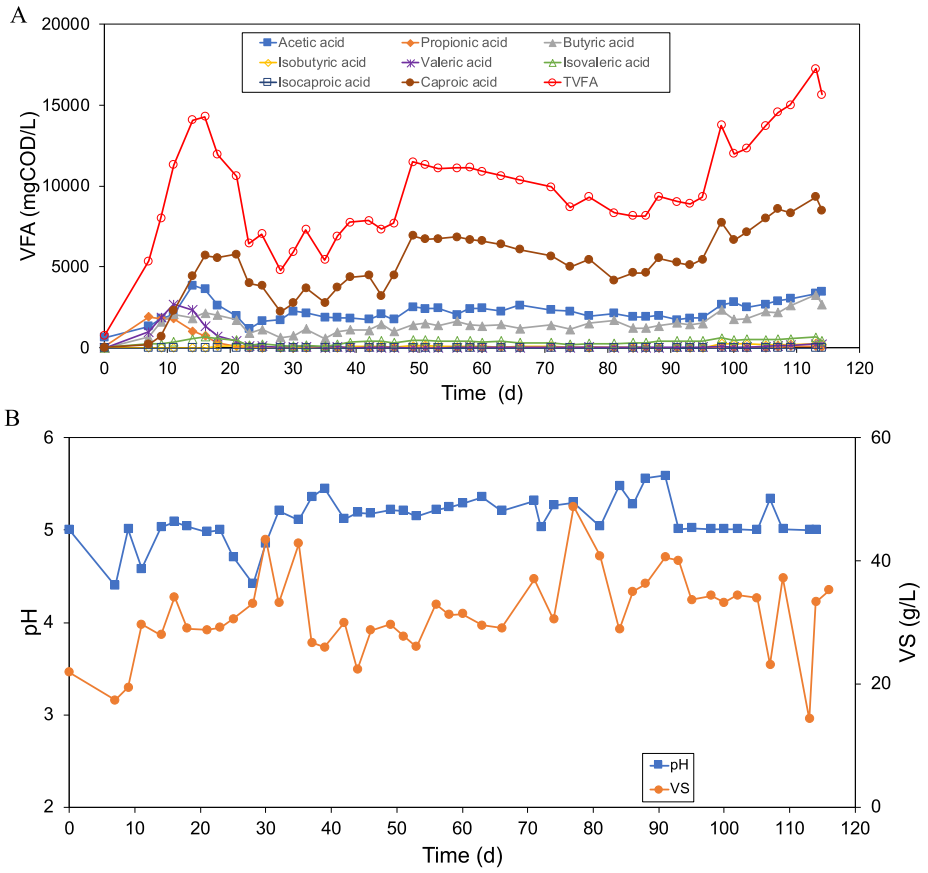

Fig. 5. (A) VFA production with the distribution of various VFA components; (B) pH and VS of the reactor during the semi-continuous operation.

species on day 14. Caproic acid reached a percentage of about 55% on day 23 and remain fairly constant as the dominant VFA throughout the operation with a maximum concentration of 9334 mg COD/L. The result of the current study is an interesting one since caproic acid has now become the focus of many bioproduction because it can be used in diverse applications including antimicrobial animal feed additives and jet fuel precursors (Angenent et al., 2016). Caproic acid is produced through chain elongation of short-chain VFA such as acetic acid and butyric acid through β-oxidation whereby some species can gain energy by increasing the chain length of VFA with reductive substrates such as ethanol and lactic acid (Agler et al., 2012; Wu et al., 2018). The percentages of individual VFAs in the semi-continuous reactor were different from the batch scale experiment where the percentage of caproic acid was only 9% at retention 8 d for 50% OW experiment. The external organic waste consisted of alcohol and soda beverage wastes and therefore the possible presence of ethanol. The higher concentrations of caproic acid observed in the semi-continuous reactor can be attributed to the fact that the continual feeding resulted in the constant availability of ethanol which could act as an electron donor for caproic acid formation from acetic acid and hence the lower acetic acid concentration (Steinbusch et al., 2011). However, in the batch experiments, ethanol could have been limited and therefore acetic acid concentration remained higher. This shows that the mode of reactor operation can influence the VFA composition.

3.2.2. Biogas production and carbon balance

Fig. 6A and B show the biogas flow including methane and carbon dioxide flow rates. At the initial stage until day 23 when the VFA production was high, the biogas production was very low. It has been shown in a similar study that an acidogenic environment helps to maintain low biogas production (Garcia-Aguirre et al., 2019). Although the pH was set at acidic pH of 5 ± 0.3 at which methanogenic activity was supposed to be limited, there was still some biogas production. It has been reported that CH_4 is produced in fermentation systems with a pH range between 5.0 and 9.0 (Liu et al., 2012). Agler et al. (2012) discovered that the simultaneous production of caproic acid and methanogenesis is feasible when methane is produced only from hydrogen and carbon dioxide and not from acetic acid. However, the high VFA production rate helped to maintain low methane values. The methane content of the produced biogas, after day 46 when production was fairly constant, was 15 ± 5% while CO_2 content was 71 ± 11%. These sum up to a total of 96% indicating that other biogas contents that were not measured particularly hydrogen were very low. Hydrogen production in fermentation systems depends on many factors including VFA concentration, pH, substrate type, temperature, etc. (Jankowska et al., 2017; Kothari et al., 2017).

Carbon balance was calculated from the influent TCOD, methane and effluent TCOD. There was a good balance between total influent carbon and effluent carbon in terms of COD (Fig. 6C). Statistical analysis using a T-test was done to determine if there was a statistical difference between the influent carbon and effluent carbon. The T-test showed that there was no statistical difference between the influent carbon and effluent carbon at 5% significant level with a p-value of 0.11787. The carbon mass balance result confirms that there was little loss of carbon through other contents of the produced biogas which was not measured. Moreover, the carbon balance shows that methane accounted for only a small percentage (with an average of 3%) of the total effluent carbon during the period of stable biogas production (from day 46).

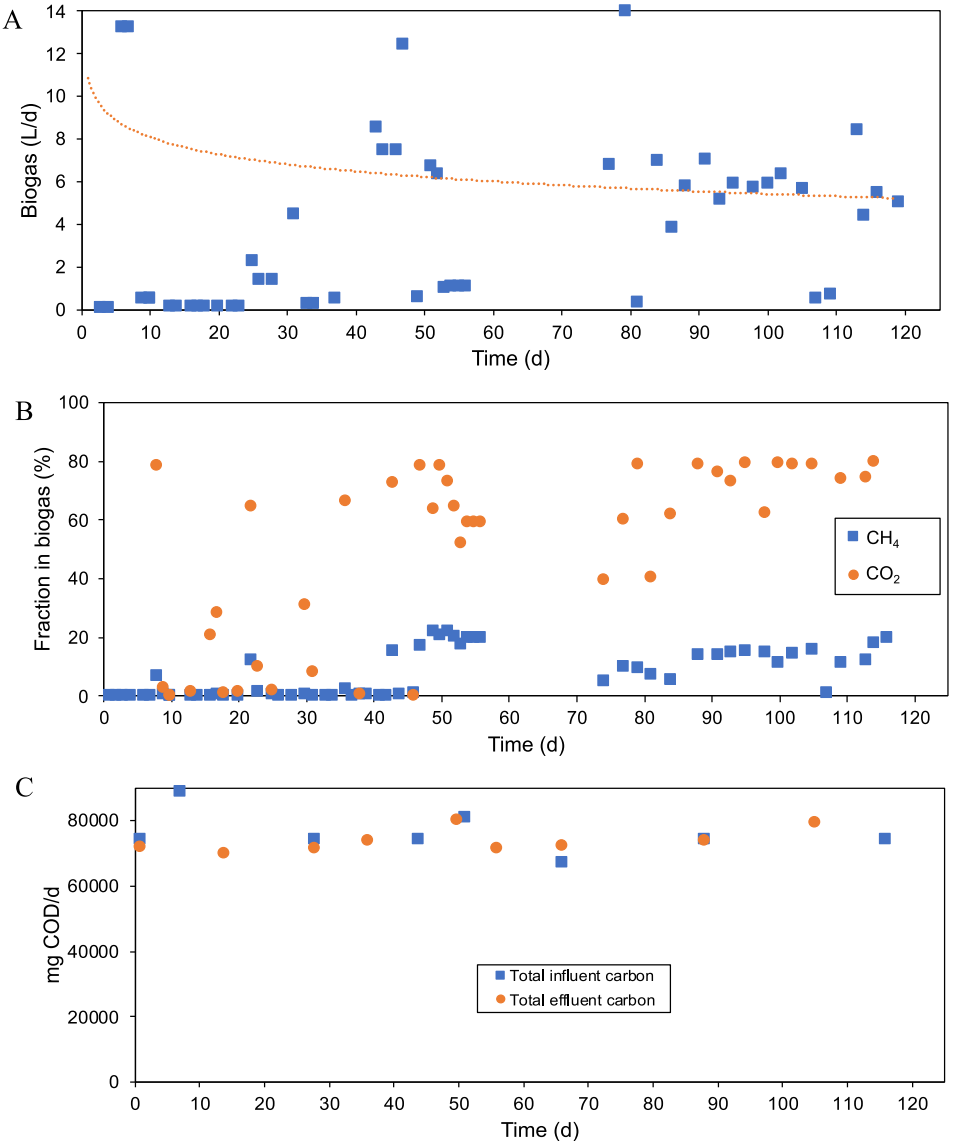

Fig. 6. (A) Biogas production (the dotted line is trendline); (B) percentage of CH_4 and CO_2 and C) Carbon balance obtained for the semi-continuous reactor.

Meanwhile, total VFA achieved a maximum value of 24631 mg COD/d which is about 33% of the total effluent carbon. This means about 67% of carbon could not be utilise and hydrolytic pretreatment could have led to a higher percentage of the VFA. Pretreatment methods such as hydrothermal, alkaline are known to increase the solubility of organic compounds which can be used readily by acidogenic microorganisms for VFA production (Moretto et al., 2019). It has been shown that hydrothermal pretreatment of food waste and sewage sludge can increase VFA yield by 45.8% and 44.6%, respectively, through the promotion of hydrolysis and acidogenesis of the organic substrates (Yu et al., 2018; Zhang et al., 2019).

3.3. Application of VFA for denitrification

To demonstrate that apart from the high value of the produced VFAs due to the high content of caproic acid, the VFA-rich effluent has the potential to be used directly as an external carbon source in the biological nutrient removal process, the VFA-rich liquid from the semi-continuous reactor was used for denitrification. The VFA-rich liquid was taken on day 100 and tested for its denitrification potential in comparison with other conventional carbon sources for denitrification, specifically acetate and methanol. The result shows that the VFA-rich liquid achieved the highest specific denitrification among the three carbon sources. The VFA-rich liquid achieved a specific denitrification rate of 12.6 ± 0.7 mg NO_x-N/(g VSS h) while the specific denitrification rate of acetate was

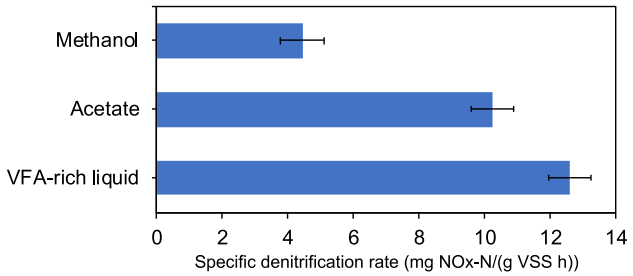

Fig. 7. Specific denitrification rate achieved by the fermentation broth from semi-continuous reactors in comparison with acetate and methanol. (error bars represent the standard deviation of triplicates).

10 ± 0.6 mg NOx-N/(g VSS h) (Fig. 7). Methanol achieved a specific denitrification rate of less than 5 ± 1.1 mg NOx-N/(g VSS h), i.e. specific denitrification rate of the VFA-rich liquid was almost 3 times higher than that of methanol. The low specific denitrification rate for methanol as a carbon source can be explained by the fact methanol can be used directly only by methanol-assimilating bacteria while acetate and VFA-rich broth are easily biodegradable (Liu et al., 2016). The protein in a substrate is hydrolysed into amino acids during an anaerobic digestion process (Angelidaki et al., 1999). One other advantage of VFA-rich broth from fermented sludge as carbon source which could have resulted in higher denitrification rate than acetate is the fact that VFA-rich broths are known to contain denitrifier toxicity reduction compounds such as amino acids. A study has shown that Sulphur-containing amino acid called cysteine helps to reduce toxicity caused by the generation of reactive nitrogen species of denitrifiers during the biological nutrient removal process (Su et al., 2016). This helps to increase the biological nutrient removal performance.

4. Conclusion

The study sought to investigate the effect of substrate proportions and long-term operation in a semi-continuous reactor on VFA production from co-digestion of sewage sludge and organic waste. Acetic acid dominated during the short-term operation in batch mode. Increasing organic waste fraction increased yield and percentages of valeric and caproic acids. Up-scaling to a reactor with long-term semi-continuous operation resulted in a shift of dominant VFA from short-chain acetic acid to more valuable higher carbon caproic acid. The application of the VFA-rich liquid for denitrification resulted in the highest specific rate in comparison with acetate and methanol.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.wasman.2020.05.027.

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