Effect of reactor operating conditions on carboxylate production and chain elongation from co-fermented sludge and food waste

Foncillas, Clara Fernando; Varrone, Cristiano

Published in:
Journal of Cleaner Production

DOI (link to publication from Publisher):
10.1016/j.jclepro.2021.126009

Publication date:
2021

Document Version
Accepted author manuscript, peer reviewed version

Citation for published version (APA):
Foncillas, C. F., & Varrone, C. (2021). Effect of reactor operating conditions on carboxylate production and chain elongation from co-fermented sludge and food waste. *Journal of Cleaner Production*, *292*, Article 126009. https://doi.org/10.1016/j.jclepro.2021.126009

Effect of reactor operating conditions on carboxylate production and chain elongation from co-fermented sludge and food waste

C. Fernando-Foncillas, C. Varrone*

Section for Sustainable Biotechnology, Aalborg University Copenhagen, A.C. Meyers Vænge 15, 2450 Copenhagen, Denmark

ABSTRACT

Nowadays, fermentation of organic wastes for the production of carboxylic acids as precursors of higher-value products has attracted significant attention. In this paper, sewage sludge and food waste were co-fermented to produce carboxylic acids and study the subsequent chain elongation process. The Copenhagen waste stream scenario was taken as a case study. Firstly, design of experiments was used to investigate the overall carboxylic acids and hexanoic acid production in batch, as a function of the co-fermentation ratio, substrate to co-culture ratio and initial pH. Statistical optimization increased the overall carboxylic acid titer by 41%, while co-fermentation allowed to increase hexanoate annual production up to 77%. Optimal operating conditions for hexanoic acid were obtained with SS/FW 6.61, S/Xo 6.73 and initial pH 6.83. Furthermore, a continuous fermentation experiment was performed to study the effect of reactor operating conditions. The overall carboxylates titer was 2 times higher, which also favored chain elongation compared to batch mode. An increasing loading rate did not affect the overall carboxylate titer, however the hexanoic acid titer increased by 44%. A maximum titer of 4.9 g/l of hexanoic acid was produced, achieving a productivity of 2.46 $g \cdot l^{-1} \cdot d^{-1}$ of hexanoic acid with a retention time of 2 days and no external electron donor

* Corresponding author.
E-mail address: cva@bio.aau.dk

addition. This would correspond to 610 tons/y of hexanoic acid and 350 tons/y of other carboxylic acids that could be produced, based on the waste availability in Copenhagen.

KEYWORDS

Co-fermentation

Sewage sludge

Food waste

Carboxylic acids

Hexanoic acid

Chain elongation

ABBREVIATIONS

AD	Anaerobic digestion
ANOVA	Analysis of variance
BBD	Box-Behnken design
COD	Chemical oxygen demand
CSTR	Continuous stirred tank reactor
HRT	Hydraulic retention time
MCCA	Medium chain carboxylic acids
OFMSW	Organic fraction of municipal solid waste
OLR	Organic loading rate
SS	Sewage sludge
SRT	Solids retention time
TS	Total solids
VFA	Volatile fatty acids

VS	Volatile solids
WAS	Waste activated sludge

1. Introduction

In the last years, resource recovery from organic wastes has become an important opportunity in order to move towards a more circular economy. One of the strategies to achieve this goal is the (co)fermentation of different organic wastes to produce carboxylic acids, also known as the carboxylate platform (Agler et al., 2011). Due to the immense variety of available organic wastes (in terms of biochemical composition, C/N ratio, recalcitrance/bioavailability, etc), any bioprocess should be adapted to the specific type of waste, in order to become economically profitable. Sewage sludge (SS) is one of the most abundant organic wastes and a potential raw material for resource recovery (Healy et al., 2015). A total production of 464,000 tons dry matter was generated only in Scandinavia in 2018 (Dansk Vand- og Spildevandsforening (DANVA), 2019; Statistics Sweden (SCB), 2020; Statistisk sentralbyrå Norway (SSB), 2020), and 13 million tons are estimated in Europe in 2020 (European Commission, 2008). A common strategy to manage sewage sludge is anaerobic digestion (AD) (Zhou et al., 2020), classic example of resource recovery from wastes. Carboxylic acids, intermediate products during AD, have higher market value than biogas (Kleerebezem et al., 2015). Their production can be optimized through different approaches, such as pretreatment of feedstock, optimization of inoculum (i.e. through adaptation, enrichment and/or bioaugmentation) (Varrone et al, 2018) or the mixture of different organic wastes to balance some of the nutrients (to obtain i.e. a more favorable C/N ratio), reduce possible inhibitory compounds and exploit the synergistic effect during co-fermentation (Marone et al., 2015; Mata-Alvarez et al., 2011; Zhou et al., 2014).

Previous studies have investigated carboxylic acids production from sewage sludge, using primary and/or waste activated sludge (WAS) (Luo et al, 2019). These studies were based on the effect of different parameters such as pH (Ma et al., 2016; Zhao et al., 2018), the addition of biosurfactants (Huang et al., 2015) or pretreatment of the substrate material (Chen et al, 2020; Liu et al., 2016; Liu et al., 2020; Zhang et al., 2019). Even though numerous studies focused on developing efficient pretreatment methods, it is worth noting that sewage sludge is mainly composed of water; therefore, pretreatment of the material by mechanical methods or addition of chemical compounds implies an additional expense to valorize this waste.

The addition of another organic waste to sewage sludge has been investigated to produce i.e. methane or volatile fatty acids (VFA), using different sources, such as the organic fraction of municipal solid waste (OFMSW, also known as food waste) (Feng et al., 2011; Gottardo et al., 2015; Koch et al., 2016), conditioning with agricultural or industrial residues (Zhang et al., 2020) or microalgae (Olsson et al., 2014). Moreover, the co-fermentation of sewage sludge and food waste for VFA production has been statistically optimized, based on different parameters such as pH or co-fermentation ratio (Chen et al., 2013; Hong and Haiyun, 2010; Khan et al., 2016; Wu et al., 2016).

Despite the higher value of VFA compared to biogas, their recovery is still challenging as it consists of a mixture of products with very similar properties (Cabrera-Rodríguez et al., 2017). Therefore, some alternative strategies to further use the produced VFA have been investigated, including for instance PHA production (Kourmentza et al., 2017), use as carbon source for biological nutrient removal during wastewater treatment (F. Liu et al., 2016) or chain elongation to medium chain carboxylic acids (MCCA) (Angenent et al., 2016). During chain elongation, short chain carboxylic acids are elongated to MCCA using ethanol (or eventually lactate) as electron donor. Following the reverse β oxidation pathway, after ethanol or lactate oxidation, acetate is elongated to butyrate, which is further elongated to hexanoate

(Contreras-Davila et al., 2020; Spirito et al., 2014). MCCA, such as hexanoic, heptanoic or octanoic acids (also known as caproic, enanthic and caprylic acids, respectively), have higher market value than VFA (Zacharof and Lovitt, 2013) and might thus represent a valuable alternative. Different complex organic wastes and the influence of some key parameters, as for example pH or temperature (Candry et al, 2020; Cavalcante et al., 2017; De Groof et al., 2019), have been studied during the chain elongation process. Furthermore, hexanoic acid production has been investigated using different organic wastes such as food waste (Nzeteu et al., 2018), diluted yeast fermentation beer (Ge et al., 2015) or switchgrass-derived stillage (Scarborough et al., 2018). However, hexanoic acid production varies depending on the operating conditions (e.g. biomass immobilization, use of a two-stage system, etc.) and substrates used. Other key parameters that can influence the chain elongation process are reactor configuration, organic overloading, the type of inoculum and the electron donor. Additionally, in situ extraction has been studied in order to avoid product toxicity, which results in low concentration of MCCA in the fermentation broth (De Groof et al., 2019). During continuous fermentation, productivity values (without additional electron donor) ranged from 0.04 $g \cdot l^{-1} \cdot d^{-1}$ using sewage sludge to 1.42 $g \cdot l^{-1} \cdot d^{-1}$ with switchgrass-derived stillage (Jankowska et al., 2018; Scarborough et al., 2018). To date, one of the highest hexanoic acid production rates was obtained using a two-stage system and food waste as substrate. Ethanol was added to enhance the process and up to 55.8 $g \cdot l^{-1} \cdot d^{-1}$ of hexanoic acid were produced (Grootscholten et al., 2014).

The focus of previous studies has been typically set on the improvement of hexanoic acid production by addition of electron donor or the optimization of reactor configuration. However, the use of sewage sludge as substrate and the co-fermentation of two (waste) substrates are not well studied (Wu et al., 2021). Therefore, there is a lack of knowledge regarding the effect of substrates co-fermentation on chain elongation. The current paper focuses on carboxylate production followed by chain elongation to hexanoic acid from co-fermentation of two organic wastes, namely sewage sludge and food waste. The addition of food waste to largely abundant sewage sludge, which was the base organic waste, expected to enhance its valorization. The study was based on the real waste scenario of the Copenhagen area; for that reason the experiments were applied to the availability and waste streams collected in the region. In Copenhagen, collection of food waste is followed by pretreatment and storage on industrial scale, which may enhance the fermentation experiments. Firstly, design of experiments was used in order to study the effect of 3 key parameters on the overall batch production of carboxylic acids (2 to 6 carbon atoms) and hexanoic acid. Then, continuous fermentation of the two organic wastes was performed to evaluate the impact of hydraulic retention time (HRT) and organic loading rate (OLR) on the overall production and distribution of the different carboxylic acids. Particular emphasis was set on the chain elongation process and hexanoic acid production.

2. Materials and Methods

2.1. Organic wastes and co-culture

Two different waste streams were used as substrate, namely sewage sludge and food waste. In addition, digested sludge was used as co-culture in order to provide the reactors with a microbial consortium adapted to anaerobic fermentation of sewage sludge. Municipal sewage sludge was obtained from BIOFOS, the largest wastewater treatment plant in Denmark. The sewage sludge consisted of a mixture of primary and secondary sludge, also known as waste activated sludge. Samples of food waste were collected from the waste receiving plant of HCS A/S Transport & Spedition in Glostrup, Denmark. This organic waste consists of a mixture of organic waste from households (80%), leftovers from restaurants and expired food from supermarkets (20%). The food waste was pretreated at HCS with an industrial hammer mill and mixed with rain water to generate a pulp. Copenhagen area was estimated to generate around 600,000 tons of sewage sludge (wet weight) per year, while 57,000 tons of food waste were calculated to be produced every year (BIOFOS, 2017; Ebdrup and Mortensen, 2019). Samples of digested sludge from the AD digester, adapted to sewage sludge and used as co-culture, were also provided by BIOFOS. The main characteristics of these samples are presented in Table 1.

2.2. Experimental design and statistical analysis for batch experiments

A Box-Behnken design (BBD) for three variables, each at three levels, was applied to design the experiments and create the model. The experimental design was applied to minimize the number of experiments needed to optimize the desired response. The software Design Expert (version 11.1.2.0, Stat-Ease Inc., US) was used to design the experiment, which required 42 runs: 12 different conditions with triplicates and six replicates at the center point. Preliminary experiments with the three variables were used to define the range of each variable (Figure S1). The three variables were: SS/FW (ratio of sewage sludge to food waste, based on volatile solids (VS) content), S/Xo (substrate to co-culture ratio, based on VS) and initial pH. A fixed working volume of co-culture was selected. As a consequence, the organic load of each bottle varied according to the different SS/FW and S/Xo ratios: firstly, the volatile solids load of the substrate was calculated according to the S/Xo (substrate to co-culture ratio); then, the organic load of food waste and sewage sludge were calculated according to the SS/FW ratio. Finally, the set working volume was reached by addition of distilled water.

The overall net titer of carboxylic acids ranging from C2 to C6, expressed in g/l, was set as response. The net titer was calculated by subtracting the initial amount of the carboxylates in the bottle (at time zero) to the final amount, as well as the amount from the control experiments. The design space was defined as presented in Table 2. The experimental design with the design parameters are presented in Table S1.

The duration of the experiments (days of incubation) was chosen based on observations from preliminary batch tests. The regression analysis of the experimental data was performed using analysis of variance (ANOVA). In addition, the fitting of the polynomial model was expressed by the coefficient of determination R^2. The fitted polynomial equation was expressed as three-dimensional surface plots to visualize the effects of the different variables on the design range. The "Point prediction" and "Confirmation" tools in Design Expert were used to validate the model. For this purpose, an additional batch test was set up in triplicate as described in section 2.3.

2.3. Batch experiments

42 identical bottles with a volume of 1125 ml were operated in batch mode according to the experimental design presented in Table S1. In addition, three different control conditions were set in triplicate (one for each level of the variable "initial pH") resulting in a total of 51 bottles. After addition of the correct amount of substrate and co-culture, the final volume was adjusted to 400 ml with distilled water. The batch bottles were adjusted to the corresponding initial pH by addition of 3M HCl or 3M NaOH, flushed with N_2 gas and sealed with a rubber stopper and metal cap. Batch tests were run for 9 days at 37 °C and samples for overall carboxylic acids (C2 to C6 compounds), CH_4 and H_2 were taken daily.

2.4. Continuous experiments

Based on the knowledge gained from the statistical analysis, a continuous experiment was performed in order to evaluate the effect of the operating conditions on the carboxylic acids and hexanoic acid production, as well as to study the long-term stability of the process. For the continuous experiment, performed in duplicate, a continuous stirred tank reactor (CSTR) was used with a volume of 3 liters. The CSTR was first run with 4 days HRT until reaching steady state (based on preliminary experiments, Figure S2), from day 0 to 25, and then decreased to 2 days HRT, from day 25 to 45. The objective was to evaluate different HRT and simple pretreatment of the material to homogenize the feed and reduce the risk of tube clogging. For that purpose, the feed was pretreated by homogenization with a kitchen blender for 20 seconds.

The SS/FW ratio of the substrate was kept constant during the whole experiment, the initial pH was 9, and the temperature was kept constant at 37 °C, using a heating jacket connected to a water bath. Total solids (TS), VS and chemical oxygen demand (COD) in the feed were analyzed regularly. A liquid sample was taken directly from the reactor every second day and analyzed for carboxylic acids content (i.e. acetic, propionic, butyric, iso-butyric, valeric, iso-valeric, hexanoic and heptanoic acids). For that purpose, the liquid sample was centrifuged at 10,000 rpm and 4 °C for 20 min. The supernatant was then filtered using a 0.45µm filter. The overall net titer was calculated by subtracting the concentration in the feed to the concentration in the reactor.

2.5. Analytical methods

All the samples were chemically characterized in terms of pH, TS, VS, carboxylic acids content and COD. TS and VS were analyzed according to standard methods (APHA, 2005). COD was determined by spectrophotometric analysis using the kits LCK 514 and LCK 914 (Hach-Lange). The concentration of total carboxylic acids and ethanol was determined using a gas chromatograph (Shimadzu GC-2010 Plus) equipped with an Agilent 19095F-123 capillary column (30 m length and 0.53 mm i.d.) followed by a flame ionization detector (FID). The carrier gas was nitrogen at 30 ml/min. The temperatures of the injector and the detector were 240 and 240 °C, respectively. Lactic acid concentration was analyzed by HPLC on a Dionex Ultimate 3000-LC system with an Aminex HPX-87H column coupled to a refractive index detector (RID). As mobile phase H_2SO_4 (4 mmol/l) was used. Methane and hydrogen production was analyzed using a gas chromatograph (SRI GC model 310) equipped with a

Porapak Q column (182.88 cm length and 2.1 mm i.d.) followed by thermal conductivity detector (TCD). Nitrogen was used as carrier gas.

3. Results

3.1. Optimization of batch experiments through Box-Behnken design

3.1.1. Overall carboxylic acids

Multiple regression analyses were applied to the experimental data, and a quadratic model with a second order polynomial equation was fitted. In addition, the experimental data was treated by square root transformation with the constant k = 0.003, as suggested by Design Expert and presented in Eq. 1. The influence of the different variables on the response is also presented in Eq. 1: the linear effect of all variables A (SS/FW), B (S/Xo) and C (Initial pH) was positive, while the quadratic effect was negative for all of them. In addition, variable B had the highest linear and quadratic effect compared to the other variables, suggesting a higher effect of the S/Xo on the response.

$$sqrt(Y + 0.003) = -0.11738 + 0.01142A + 0.19563B + 0.13980C - 0.00010AB - 0.00098AC + 0.02097BC - 0.00009A^2 - 0.02134B^2 - 0.011725C^2 \quad \text{(Eq. 1)}$$

The experimental results were further analyzed by ANOVA (Table S2). The quadratic model was significant (with an F-value of 369.75 and low probability value $p<0.0001$), while the lack of fit was not significant ($p = 0.5917$). The determination coefficient R^2 was 0.9905, which indicated that 99% of the variability could be explained by the model (Table S3). The predicted and actual values of this response are presented in Table S4. Figure 1 presents the response surface plots for the combination of the different factors used during the experiment. More specifically, Figure 1A presents the effects of variables A and B when the variable C is in the optimal level. The overall titer increased when SS/FW decreased (i.e. higher food waste load) and S/Xo increased. Figure 1B confirms the influence of S/Xo and pH on the overall titer, as presented in Eq. 1. Likewise, the response was maximized with increasing pH and decreasing SS/FW (Figure 1C). According to the model, the highest predicted response of 3.55 g/l was achieved with a SS/FW value of 16.8, S/Xo of 7.68 and initial pH 8.92, which corresponds to a 41% increase compared to the ratio of real waste availability (SS/FW = 30/70). However, when SS/FW ratio had a value around 50/50 or higher, the overall titer decreased (the sub-optimal area ranged between SS/FW 0/100 and 50/50). Therefore, the titer decreased as the sewage sludge load increased (Figures 1A and C). In order to facilitate comparison with other studies in literature, the yield results for the different conditions are presented in Table S7. In this study, the highest experimental yield in batch (250 mg/gVS) was achieved with a SS/FW value of 0, S/Xo of 3.25 and initial pH 9.

3.1.2. Hexanoic acid

The experimental data were also used to study the hexanoic acid production, in order to determine the influence of the three variables on chain elongation. As previously described for the overall titer, a quadratic model and second order polynomial equation were also fitted to the hexanoic acid titers. Moreover, the experimental data was treated by square root transformation with the constant k = 0.001, as suggested by Design Expert and presented in Eq. 2.

$$sqrt(Y + 0.001) = +0.3279 - 0.0798A + 0.1072B + 0.0349C - 0.0878AB + 0.0564AC + 0.0118BC - 0.0824A^2 - 0.1419B^2 - 0.0111C^2 \qquad \text{(Eq. 2)}$$

The linear effect of variable A (SS/FW) was negative on the titer, while variables B (S/Xo) and C (initial pH) had a positive impact. On the other hand, all variables had a negative quadratic effect. Variable B had the highest effect on the hexanoic acid titer, as previously observed for the overall titer. The ANOVA analysis of the experimental results is presented in Table S5. The quadratic model was significant (with an F-value of 23.72 and low probability value p<0.0001), and the lack of fit was not significant (p = 0.1576). The determination coefficient R^2 was 0.89, which indicated that 89% of the variability could be explained by the model (Table S6). The predicted and actual values of this response are presented in Table S4. Response surface plots for the influence of the different factors are presented in Figure 2. The hexanoic acid titer increased as the SS/FW ratio decreased and S/Xo increased (Figure 2A), as occurred for the overall titer in section 3.1.1. However, when S/Xo was higher than 7.3, the hexanoic acid titer started decreasing, in contrast with the model of the overall titer. In addition, a neutral pH value favored the response when variable A was in the optimal level (Figure 2B). The combination of variables SS/FW and pH also indicated a higher titer as SS/FW decreased (Figure 2C). According to Figures 2A and 2B, a higher organic load (S/Xo) also increased the hexanoic acid production (as presented in Eq. 2), with a minimum load of 4 (g VS substrate per g VS co-culture). In the current study, the highest predicted response of 0.155 g/l was achieved with a SS/FW value of 6.61, S/Xo of 6.73 and initial pH 6.83. This corresponds to a 34% increase compared to the ratio of real waste availability. In the optimized conditions, hexanoic acid represented 6.2% of the overall carboxylic acids.

3.2. Continuous co-fermentation

For continuous operation, the combination of the variables was chosen in order to maximize the overall carboxylic acids. The CSTR was run with a SS/FW ratio of 20/80, high start-up organic load (S/Xo) and initial pH 9 (based on the optimized co-fermentation ratio from batch experiments). Preliminary experiments showed that with a HRT of 6 days, the methanogenic bacteria were enriched and the content of carboxylic acids was reduced, favoring methane production (Figure S2). For this reason, HRT > 4 days was excluded from further testing. The final OLR differed for each HRT, resulting in about 15 gVS/l/day for 4 days HRT and 30 gVS/l/day for 2 days HRT. The initial pH of 9 decreased quickly (and remained constant) oscillating between 5.5 and 5 after the first 4 days, without any chemical addition. The main results of the continuous experiment are shown below (Figure 3).

3.2.1. Effect of HRT and OLR

Figure 3 presents the individual and overall carboxylic acid net titer for compounds ranging from 2 to 7 carbon atoms. During the first 8 days, the overall net titer reached a maximum of 11 g/l and then decreased to about 7.7 g/l once stabilized (until day 25). However, the titer predicted by the batch model in comparable conditions (in terms of initial pH, SS/FW and S/Xo) was lower, 3.5 g/l. After 25 days of operation, the HRT was decreased from 4 to 2 days, and then run during 20 days. Since the substrate was the same during the whole experiment, the decrease in HRT implied an increase in OLR, from 15 gVS/l/day to 30 gVS/l/day. The titer of carboxylic acids remained stable around 7.7 g/l both with 4 and 2 days HRT, indicating no change despite the OLR increase. Additionally, the overall yield also remained stable around 130 mg/gVS (Figure S3). The increase in OLR had higher influence on the hexanoic acid production; from day 9 to 25, the average hexanoic acid titer was 3.4 g/l, while after decreasing the HRT it reached 4.9 g/l, thus leading to an increase of 44% and a maximum productivity of 2.46 $g \cdot l^{-1} \cdot d^{-1}$. Ethanol was spiked in day 39 (as indicated by the arrow in Figure 3) to investigate its effect on the hexanoic acid production, but no significant effect was observed on the long term. Nevertheless, from day 39 to 41, butyric and hexanoic acid titers increased by 14 and 12%, respectively.

4. Discussion

4.1. Influence of selected variables during batch fermentation

4.1.1. Overall carboxylic acids

The model created with the experimental data for carboxylic acids production showed that the response (overall titer in g/l) increased as variable A (SS/FW ratio) decreased (i.e. higher food waste load) and variables B (S/Xo) and C (initial pH) increased. A high S/Xo value implied that the organic load was high, which could inhibit methanogenic activity due to carboxylic acids accumulation (González-Fernández and García-Encina, 2009; Shahbaz et al., 2019). In addition, the decrease in sewage sludge load in co-fermentation increased the titer, as expected, due to the recalcitrant nature of this substrate and its low VS content (Koch et al., 2016). A high organic load, in combination with high initial pH, increases the overall titer since the carboxylic acids are accumulated in the dissociated form (due to the alkaline pH), thus reducing the inhibition to the microorganisms. Previous studies analyzed the influence of different variables in the VFA yield, utilizing similar substrates, but only taking into account compounds from 2 to 5 carbon atoms and working in semi continuous mode instead of batch mode (Chen et al., 2013; Hong and Haiyun, 2010; Shahbaz et al., 2019).

On the other hand, the influence of pH on VFA production has been extensively studied (Jankowska et al., 2017; Jiang et al., 2013; Ma et al., 2016; Zhang et al., 2009). In addition, co-fermentation of WAS with food waste was also studied in batch mode and different pH, showing positive effect of alkaline pH compared to neutral pH (Feng et al., 2011).

4.1.2. Hexanoic acid

As presented in section 3.1.2, increasing food waste load (i.e. decreasing SS/FW), together with increasing S/Xo ratio and initial pH, increased the hexanoic acid titer. However, the titer decreased when S/Xo was higher than 7.3. Previous studies suggested that a S/Xo ratio of 5 favors hexanoic acid production, while increasing it to 10 inhibits it (Coma et al., 2017). The increasing food waste load (implying a decrease in the SS/FW ratio) may have favored chain elongation to hexanoic acid due to the ethanol and lactic acid content in food waste (Table 1), which can be used as electron donors during chain elongation. In addition, controlling the pH for hexanoic acid production implies a compromise between primary fermentation and chain elongation, since the optimal conditions differ for each process. While alkaline pH favors VFA fermentation (Feng et al., 2011; Ma et al., 2016), chain elongation is less thermodynamically favorable under these conditions and higher productions were reported at neutral pH (Liang and Wan, 2015). The highest predicted titer for batch tests in this study was 0.155 g/l, in agreement with previous studies under similar conditions (i.e. complex substrate and no additional electron donor), which reported hexanoic acid titers ranging from 0.02 to 0.12 g/l (in batch mode) within 3 to 5 fermentation days (Jankowska et al., 2015; Weimer et al., 2015). It is worth noting that the influence of these variables and different operating conditions on hexanoic acid production are not well studied in literature, especially for continuous operation and co-fermentation of sewage sludge with food waste. Therefore, a continuous experiment was performed (see section 3.2).

4.1.3. Co-fermentation as waste management strategy

Design of experiments for the statistical optimization of key process parameters can be used as a decision-making tool in order to study different waste management scenarios (Marone et al., 2015). Considering that food waste was the limiting substrate (compared to the huge volumes of sludge available), the highest predicted titer of 3.55 g carboxylates/l would allow to obtain 396 tons of carboxylic acids per year, by valorizing all available food waste together with 9% of the available sludge (which corresponds to 17% of the total available substrates). Additionally, 772 tons of carboxylic acids could be produced by valorizing the remaining sludge alone under the best conditions, which would correspond in total to 1168 tons of carboxylic acids (Scenario A, Table S8). On the other hand, if these waste streams were valorized separately, 203 tons of carboxylic acids could be produced every year only from food waste and 849 tons from sludge, achieving in total 1052 tons of carboxylates (Scenario B, Table S8). Therefore, co-fermentation of substrates in the optimized condition integrated with the mono fermentation of the remaining sludge would increase the carboxylate production by 11%. Furthermore, the SS/FW ratio corresponding to the real waste availability in Copenhagen area (SS/FW 70/30) would produce a total of 1650 tons of per year, according to the model. This apparent contradiction can be easily explained when considering the overall valorization / fermentation strategy of multiple wastes, when the availability of a feedstock stream represents the limiting factor (as further elaborated below).

In the case of chain elongation, the optimized co-fermentation ratio would produce 12 tons/y of hexanoic acid, but valorizing only 3% of the available sludge (and only 12% of the total available waste). The remaining sludge could produce 33 additional tons/y of hexanoic acid, thus achieving 45 tons/y in total (Scenario A, Table S8). On the other hand, mono fermentation of these waste streams could produce up to 43 tons of hexanoic acid per year. In other words, the optimal co-fermentation ratio would only produce 5% more hexanoic acid than mono fermentation, because only 3% of the sludge would be co-fermented, while the remaining 97% would be fermented separately. It is worth noting that the real waste availability (SS/FW 70/30) would produce 76 tons of hexanoic acid per year.

Clearly, in a real life scenario it would be necessary to design a process that valorizes all the available waste, instead of focusing only on optimizing titers (neglecting the real substrate availability), as often done in lab-scale studies. When designing a feasible waste management system, availability of waste is of particular importance. In fact, assuming that there is no limiting substrate, the optimal co-fermentation ratio would clearly produce more carboxylic acids than the real co-fermentation ratio. However, the Copenhagen real case scenario would produce 41% more carboxylic acids and 69% more hexanoic acid per year than the optimized co-fermentation (Scenario A, Table S8). This is due to the higher amount of material treated in co-fermentation; in fact, while the real ratio would co-ferment 100% of the available material, the optimal carboxylate and hexanoate conditions would only co-ferment 17% and 12% of the available material, respectively. On the other hand, downstream processing costs (strictly related to titers) cannot be neglected either, so an integrated assessment would be necessary.

4.2. Continuous carboxylate production and chain elongation

During continuous co-fermentation, the shorter HRT was used as a strategy to reduce the methanogenic activity and to maximize the acidogenesis. Thus, preliminary inactivation of methanogens was unnecessary and the addition of chemicals to regulate pH could be avoided. The higher OLR, compared to an anaerobic digester focused on biogas production, was another strategy used to decrease methane production. In this way, carboxylic acids are expected to accumulate in the reactor, since methanogenic microorganisms cannot consume them as quickly as they are produced (Yuan and Zhu, 2016).

According to (Cheah et al., 2019), the continuous fermentation of food waste under alkaline conditions (pH 10) did not show a significant increase in the VFA yield compared to acidic pH (6). In addition, previous studies found the overall VFA production by co-fermentation of these wastes optimal with a self-regulating pH range between 5.2 and 6.4 (Wu et al., 2016). In the current study, the addition of chemicals to maintain a constant pH during continuous fermentation was discarded in order to avoid additional expenses. This is of particular importance when developing a robust process that could treat up to 657,000 tons/year of waste streams, only considering the Copenhagen region (BIOFOS, 2017; Ebdrup and Mortensen, 2019).

4.2.1. Organic overloading favors chain elongation

As described in section 2.4, the substrate of the continuous experiment was homogenized in order to facilitate reactor operations such as pumping of waste materials. Notably, the overall titer did not change when decreasing from 4 to 2 days HRT, despite the OLR increase, indicating no microbial inhibition due to organic overloading. Nevertheless, the increase in OLR had higher influence on the hexanoic acid production, in good agreement literature (De Groof et al., 2019). The enhancement of chain elongation after increasing the OLR could be due to an adaptation of the microbial population.

During chain elongation, ethanol is typically used as electron donor enabling the carbon incorporation. In fact, previous studies have focused on the addition of ethanol or the use of different substrates to enhance the hexanoic acid production (Duber et al., 2018; Grootscholten et al., 2013). In the current study, the food waste was partially pre-fermented before collection (due to the treatment at the company), thus containing some ethanol and lactic acid. This pre-fermentation could in principle explain the increase in hexanoic acid production as the food waste load increased. However, the ethanol concentration in the reactor was very similar to the concentration in the feed (Figure 3), suggesting that another chain elongation pathway could have been dominant in our reactor.

In order to verify this hypothesis, ethanol was spiked (after reaching steady state) in day 39, but no significant effect on hexanoic acid was observed on the long term. In previous studies, ethanol addition enhanced chain elongation of shorter carboxylic acids to hexanoic acid by 39% (Grootscholten et al., 2013; Roghair et al., 2018). However, in these studies, the biomass was immobilized in the reactor in order to optimize the process, while in the current study there was no such immobilization system. On the other hand, the study by Cavalcante and colleagues suggested that lactic acid can also be used as electron donor for chain elongation (Cavalcante et al., 2017). Interestingly, in the current study, lactate concentration from the feed (resulting from the pretreatment of the material in the company) showed a high consumption in the reactor (Figure 3) and was positively correlated to the hexanoic acid production (Spearman correlation = 0.8), potentially indicating its involvement in the process. This could in principle explain why the ethanol spiking did not show significant effects.

Furthermore, previous studies investigated lactic acid production coupled to chain elongation, using food waste as substrate (Contreras-Dávila et al, 2020). The successful consecutive lactate formation and chain elongation avoided the addition of external electron donor for hexanoic acid production, decreasing the chemicals input. Additionally, the self-regulated pH around 5.5 may have enhanced the chain elongation to hexanoic acid, using lactic acid as electron donor as suggested in previous studies. Candry and colleagues (2020), for instance, studied the influence of pH on the competition for lactic acid between chain elongating and propionic acid producing microorganisms. As a result, the microbial populations shifted depending on the pH, favoring chain elongation to hexanoic acid at mildly acidic pH. Thus, further experiments are needed to clarify the underlying mechanism of chain elongation in the current study, for instance by spiking with lactate and performing a parallel composition analysis of the microbial composition.

4.2.2. Chain elongation as strategy for waste management

Organic overloading has been previously studied as a strategy to enhance the chain elongation process, both at the start-up phase of the reactor (substrate to co-culture ratio, S/Xo) or as increasing OLR during reactor operation (De Groof et al., 2019). This is coherent with our findings, in which increasing OLR (and decreased HRT) led to an enhanced chain elongation. Nevertheless, a higher OLR when using a complex feedstock may not always correspond to a higher production of MCCA, since the feedstock may not be easily biodegraded (especially without any pretreatment). Moreover, the type of reactor, pumping system and the retention time used during fermentation depend on the type of substrate. In the current paper, the focus was set on the valorization of sewage sludge and food waste, two abundant organic wastes. The mixture of these two substrates allowed the use of a CSTR, a simple type of reactor, in which the HRT and solid retention time (SRT) have the same length. In order to compare hexanoic acid production with similar studies, the titer (g/l), productivity ($g \cdot l^{-1} \cdot d^{-1}$) and yield (mg/gVS) are presented in Table 3, both in grams and in COD equivalents. All the studies presented in Table 3 were performed without addition of external electron donor, and depended solely on the composition of the complex waste for the production of MCCA. The HRT in Table 3 varied from 1 to 6 days, and in all cases a CSTR was used for the experiment.

The different composition of the substrates in literature (from grass silage to sewage sludge), together with the varying OLR, resulted in productivities ranging from 0.01 up to 2.46 $g \cdot l^{-1} \cdot d^{-1}$. In the current study, a maximum productivity of 2.46 $g \cdot l^{-1} \cdot d^{-1}$ was achieved with a HRT of 2 days, among the highest obtained from co-fermentation of these types of wastes, without additional electron donor, to the authors' knowledge. The suitability of food waste as substrate for MCCA production could be explained by its pretreatment in the collection plant, where its size was reduced and it was mixed with rain water to produce a pulp. This pretreatment probably enhanced the hydrolysis step, which accelerated the fermentation and allowed the chain elongation with a short HRT of 2 days. As a result, up to 610 tons of hexanoic acid could be produced every year with this reactor configuration (2 days HRT), valorizing 124,500 tons of waste in Copenhagen area (19% of the yearly total). Additionally, 350 tons of other carboxylic acids (acetate, butyrate, valerate and heptanoate) would also be produced at the same time.

As previously mentioned, most of the prior studies focused on optimizing the chain elongation process by addition of electron donor or specific reactor configurations. However, the effect of the different reactor operating conditions has been barely compared, and even less the co-fermentation of different complex waste streams. Clearly, the HRT is another key parameter since it is directly related to the OLR and affects the size of the reactor, and thereby the economic feasibility of the waste management process.

The current study showed the potential use of food waste and sludge as substrates for stable hexanoic acid (and overall carboxylic acids) production, with a robust process and simple reactor configuration. The pH was not regulated and no external addition of electron donor was necessary (therefore no additional expenses were generated), while reaching among the highest productivities reported in literature within comparable process conditions. On the other hand, more advanced systems using i.e. immobilized biomass and addition of external carbon and electron source reached significant improvements. Clearly, different reactor configurations could be studied to further optimize the chain elongation process. Although the current study demonstrated the better performance of CSTR compared to a batch system, other semi-continuous operating modes could also be investigated. However, these type of wastes are generated in enormous amounts so a cheap and robust bioprocess should be developed in order to exploit the waste valorization. It would also be necessary to take into consideration the potential downstream technologies that could be applied to the produced carboxylates, both short and medium chain compounds. In fact, previous studies suggested higher affinity for medium chain carboxylates (Fernando-Foncillas et al., 2021; Rebecchi et al., 2016). Additionally, a techno-economic assessment should be performed in order to evaluate the feasibility of the process.

5. Conclusions

In this study, the effect of sewage sludge and food waste co-fermentation ratio (together with the reactor type and operating conditions) on carboxylic acid production and chain elongation were evaluated. During the batch experiment, the overall carboxylic acid titer was highly dependent on the initial pH and co-fermentation ratio, increasing with alkaline pH and decreasing sludge ratio. Chain elongation was rather limited, compared to CSTR operations. However, co-fermentation proved to be a valid waste management strategy, increasing hexanoate annual production up to 77%, compared to mono-fermentation. During continuous experiments, the increase in OLR improved the hexanoic acid titer by 44%. A maximum hexanoic acid titer of 4.9 g/l and productivity of 2.46 $g \cdot l^{-1} \cdot d^{-1}$ were achieved with 2 days HRT and no external electron donor addition, one of the highest with this reactor configuration and types of wastes. This would correspond to 610 tons of hexanoic acid and 350 tons of other carboxylic acids that could be produced, based on the waste availability in Copenhagen.

ACKNOWLEDGEMENTS

This study was carried out as part of the COWI Fonden Project A092762-001 Wastewater and Organic Waste Treatment Facilities – Net Green Energy, Nutrients and Bioproducts Producers. The authors would like to thank BIOFOS and HCS A/S Transport & Spedition for providing the samples.

References

Agler, M.T., Wrenn, B.A., Zinder, S.H., Angenent, L.T., 2011. Waste to bioproduct conversion with undefined mixed cultures: the carboxylate platform. Trends Biotechnol. 29, 70–78. https://doi.org/10.1016/j.tibtech.2010.11.006

Angenent, L.T., Richter, H., Buckel, W., Spirito, C.M., Steinbusch, K.J.J., Plugge, C.M., Strik, D.P.B.T.B., Grootscholten, T.I.M., Buisman, C.J.N., Hamelers, H.V.M., 2016. Chain elongation with reactor microbiomes: Open-culture biotechnology to produce biochemicals. Environ. Sci. Technol. 50, 2796–2810. https://doi.org/10.1021/acs.est.5b04847

APHA, 2005. Standard Methods for the Examination of Water and Wastewater, 21st edition. American Public Health Association, American Water Works Association and Water Environment Federation, Washington DC.

BIOFOS, 2017. 2016 Årsberetning. URL https://www.danva.dk/media/2762/16613-biofos_a-rsberetning_web_final.pdf

Cabrera-Rodríguez, C.I., Moreno-González, M., de Weerd, F.A., Viswanathan, V., van der Wielen, L.A.M., Straathof, A.J.J., 2017. Esters production via carboxylates from anaerobic paper mill wastewater treatment. Bioresour. Technol. 237, 186–192. https://doi.org/10.1016/j.biortech.2017.02.030

Candry, P., Radic, L., Favere, J., Carvajal-Arroyo, J. M., Rabaey, K., Ganigué, R., 2020. Mildly acidic pH selects for chain elongation to caproic acid over alternative pathways during lactic acid fermentation. Water Res. 186, 116396. https://doi.org/10.1016/j.watres.2020.116396

Cavalcante, W. de A., Leitão, R.C., Gehring, T.A., Angenent, L.T., Santaella, S.T., 2017. Anaerobic fermentation for n-caproic acid production: A review. Process Biochem. 54, 106–119. https://doi.org/10.1016/j.procbio.2016.12.024

Cheah, Y.K., Vidal-Antich, C., Dosta, J., Mata-Álvarez, J., 2019. Volatile fatty acid production from mesophilic acidogenic fermentation of organic fraction of municipal solid waste and food waste under acidic and alkaline pH. Environ. Sci. Pollut. Res. 26, 35509–35522. https://doi.org/10.1007/s11356-019-05394-6

Chen, Y., Luo, J., Yan, Y., Feng, L., 2013. Enhanced production of short-chain fatty acid by co-fermentation of waste activated sludge and kitchen waste under alkaline conditions and its application to microbial fuel cells. Appl. Energy 102, 1197–1204. https://doi.org/10.1016/j.apenergy.2012.06.056

Chen, S., Tao, Z., Yao, F., Wu, B., He, L., Hou, K., Pi, Z., Fu, J., Yin, H., Huang, Q., Liu, Y., Wang, D., Li, X., Yang, Q., 2020. Enhanced anaerobic co-digestion of waste activated sludge and food waste by sulfidated microscale zerovalent iron: Insights in direct interspecies electron transfer mechanism. Bioresour. Technol. 316, 123901. https://doi.org/10.1016/j.biortech.2020.123901

Coma, M., Martinez-Hernandez, E., Abeln, F., Raikova, S., Donnelly, J., Arnot, T.C., Allen, M.J., Hong, D.D., Chuck, C.J., 2017. Organic waste as a sustainable feedstock for platform chemicals. Faraday Discuss. 202, 175–195. https://doi.org/10.1039/C7FD00070G

Contreras-Dávila, C. A., Carrión, V. J., Vonk, V. R., Buisman, C. N.J., Strik, D. P.B.T.B., 2020. Consecutive lactate formation and chain elongation to reduce exogenous chemicals input in repeated-batch food waste fermentation. Water Res. 169, 115215. https://doi.org/10.1016/j.watres.2019.115215

Dansk Vand- og Spildevandsforening (DANVA), 2019. Water in Figures, DANVA statistics & Benchmarking. URL https://www.danva.dk/media/6355/2019_water-in-figures_web.pdf

Dareioti, M.A., Kornaros, M., 2015. Anaerobic mesophilic co-digestion of ensiled sorghum, cheese whey and liquid cow manure in a two-stage CSTR system: Effect of hydraulic retention time. Bioresour. Technol. 175, 553–562. https://doi.org/https://doi.org/10.1016/j.biortech.2014.10.102

De Groof, V., Coma, M., Arnot, T., Leak, D.J., Lanham, A.B., 2019. Medium chain carboxylic acids from complex organic feedstocks by mixed culture fermentation. Molecules 24, 1–32. https://doi.org/10.3390/molecules24030398

Duber, A., Jaroszynski, L., Zagrodnik, R., Chwialkowska, J., Juzwa, W., Ciesielski, S., Oleskowicz-Popiel, P., 2018. Exploiting the real wastewater potential for resource recovery - n-caproate production from acid whey. Green Chem. 20, 3790–3803. https://doi.org/10.1039/c8gc01759j

Ebdrup, M., Mortensen, S.V., 2019. Affald KBH Lejlighed 2019. URL https://kk.sites.itera.dk/apps/kk_pub2/index.asp?mode=detalje&id=1949

European Commission, 2008. Environmental, economic and social impacts of the use of sewage sludge on land - Final report - Part I: Overview report.

Feng, L., Yan, Y., Chen, Y., 2011. Co-fermentation of waste activated sludge with food waste for short-chain fatty acids production: effect of pH at ambient temperature. Front. Environ. Sci. Eng. China 5, 623–632. https://doi.org/10.1007/s11783-011-0334-2

Fernando-Foncillas, C., Cabrera-Rodríguez, C.I., Caparrós-Salvador, F., Varrone, C., Straathof, A.J.J., 2021. Highly selective recovery of medium chain carboxylates from co-fermented organic wastes using anion exchange with carbon dioxide expanded methanol desorption. Bioresour. Technol. 319, 124178. https://doi.org/10.1016/j.biortech.2020.124178

Ge, S., Usack, J.G., Spirito, C.M., Angenent, L.T., 2015. Long-term n-caproic acid production from yeast-fermentation beer in an anaerobic bioreactor with continuous product extraction. Environ. Sci. Technol. 49, 8012–8021. https://doi.org/10.1021/acs.est.5b00238

González-Fernández, C., García-Encina, P.A., 2009. Impact of substrate to inoculum ratio in anaerobic digestion of swine slurry. Biomass and Bioenergy 33, 1065–1069. https://doi.org/10.1016/j.biombioe.2009.03.008

Gottardo, M., Micolucci, F., Mattioli, A., Faggian, S., Cavinato, C., Pavan, P., 2015. Hydrogen and methane production from biowaste and sewage sludge by two phases anaerobic codigestion. Chem. Eng. Trans. 43, 379–384. https://doi.org/10.3303/CET1543064

Grootscholten, T.I.M., Steinbusch, K.J.J., Hamelers, H.V.M., Buisman, C.J.N., 2013. Chain elongation of acetate and ethanol in an upflow anaerobic filter for high rate MCFA production. Bioresour. Technol. 135, 440–445. https://doi.org/10.1016/j.biortech.2012.10.165

Grootscholten, T.I.M., Strik, D.P.B.T.B., Steinbusch, K.J.J., Buisman, C.J.N., Hamelers, H.V.M., 2014. Two-stage medium chain fatty acid (MCFA) production from municipal solid waste and ethanol. Appl. Energy 116, 223–229. https://doi.org/10.1016/j.apenergy.2013.11.061

Healy, M.G., Clarke, R., Peyton, D., Cummins, E., Moynihan, E.L., Martins, A., Béraud, P., Fenton, O., 2015. Resource recovery from sewage sludge, in: Stamatelatou, K., Tsagarakis, K.P. (Eds.), Sewage Treatment Plants: Economic Evaluation of Innovative

Technologies for Energy Efficiency. IWA Publishing, pp. 139–162. https://doi.org/10.2166/9781780405025

Hong, C., Haiyun, W., 2010. Optimization of volatile fatty acid production with co-substrate of food wastes and dewatered excess sludge using response surface methodology. Bioresour. Technol. 101, 5487–5493. https://doi.org/10.1016/j.biortech.2010.02.013

Huang, X., Shen, C., Liu, J., Lu, L., 2015. Improved volatile fatty acid production during waste activated sludge anaerobic fermentation by different bio-surfactants. Chem. Eng. J. 264, 280–290. https://doi.org/10.1016/j.cej.2014.11.078

Jankowska, E., Chwialkowska, J., Stodolny, M., Oleskowicz-Popiel, P., 2017. Volatile fatty acids production during mixed culture fermentation – The impact of substrate complexity and pH. Chem. Eng. J. 326, 901–910. https://doi.org/10.1016/j.cej.2017.06.021

Jankowska, E., Chwiałkowska, J., Stodolny, M., Oleskowicz-Popiel, P., 2015. Effect of pH and retention time on volatile fatty acids production during mixed culture fermentation. Bioresour. Technol. 190, 274–280. https://doi.org/10.1016/j.biortech.2015.04.096

Jankowska, E., Duber, A., Chwialkowska, J., Stodolny, M., Oleskowicz-Popiel, P., 2018. Conversion of organic waste into volatile fatty acids – The influence of process operating parameters. Chem. Eng. J. 345, 395–403. https://doi.org/10.1016/j.cej.2018.03.180

Jiang, J., Zhang, Y., Li, K., Wang, Q., Gong, C., Li, M., 2013. Volatile fatty acids production from food waste: Effects of pH, temperature, and organic loading rate. Bioresour. Technol. 143, 525–530. https://doi.org/10.1016/j.biortech.2013.06.025

Khan, M.A., Ngo, H.H., Guo, W.S., Liu, Y., Nghiem, L.D., Hai, F.I., Deng, L.J., Wang, J., Wu, Y., 2016. Optimization of process parameters for production of volatile fatty acid, biohydrogen and methane from anaerobic digestion. Bioresour. Technol. 219, 738–748. https://doi.org/10.1016/j.biortech.2016.08.073

Kleerebezem, R., Joosse, B., Rozendal, R., Van Loosdrecht, M.C.M., 2015. Anaerobic digestion without biogas? Rev. Environ. Sci. Biotechnol. 14, 787–801. https://doi.org/10.1007/s11157-015-9374-6

Koch, K., Plabst, M., Schmidt, A., Helmreich, B., Drewes, J.E., 2016. Co-digestion of food waste in a municipal wastewater treatment plant: Comparison of batch tests and full-scale experiences. Waste Manag. 47, 28–33. https://doi.org/10.1016/j.wasman.2015.04.022

Kourmentza, C., Plácido, J., Venetsaneas, N., Burniol-Figols, A., Varrone, C., Gavala, H.N., Reis, M.A.M., 2017. Recent advances and challenges towards sustainable polyhydroxyalkanoate (PHA) production. Bioengineering 4, 55. https://doi.org/10.3390/bioengineering4020055

Liang, S., Wan, C., 2015. Carboxylic acid production from brewer's spent grain via mixed culture fermentation. Bioresour. Technol. 182, 179–183. https://doi.org/10.1016/j.biortech.2015.01.082

Liu, F., Tian, Y., Ding, Y., Li, Z., 2016. The use of fermentation liquid of wastewater primary sedimentation sludge as supplemental carbon source for denitrification based on enhanced anaerobic fermentation. Bioresour. Technol. 219, 6–13. https://doi.org/10.1016/j.biortech.2016.07.030

Liu, H., Xiao, H., Yin, B., Zu, Y., Liu, He, Fu, B., Ma, H., 2016. Enhanced volatile fatty acid production by a modified biological pretreatment in anaerobic fermentation of waste activated sludge. Chem. Eng. J. 284, 194–201. https://doi.org/10.1016/j.cej.2015.08.121

Liu, X., Du, M., Yang, J., Wu, Y., Xu, Q., Wang, D., Yang, Q., Yang, G., Li, X., 2020. Sulfite serving as a pretreatment method for alkaline fermentation to enhance short-chain fatty acid production from waste activated sludge. Chem. Eng. J. 385, 123991. https://doi.org/10.1016/j.cej.2019.123991

Luo, K., Pang, Y., Yang, Q., Wang, D., Li, X., Lei, M., Huang, Q., 2019. A critical review of volatile fatty acids produced from waste activated sludge: enhanced strategies and its applications. Environ. Sci. Pollut. Res. 26, 13984–13998. https://doi.org/10.1007/s11356-019-04798-8

Ma, H., Chen, X., Liu, He, Liu, Hongbo, Fu, B., 2016. Improved volatile fatty acids anaerobic production from waste activated sludge by pH regulation: Alkaline or neutral pH? Waste Manag. 48, 397–403. https://doi.org/10.1016/j.wasman.2015.11.029

Marone, A., Varrone, C., Fiocchetti, F., Giussani, B., Izzo, G., Mentuccia, L., Rosa, S., Signorini, A., 2015. Optimization of substrate composition for biohydrogen production from buffalo slurry co-fermented with cheese whey and crude glycerol, using microbial mixed culture. Int. J. Hydrogen Energy 40, 209–218. https://doi.org/10.1016/j.ijhydene.2014.11.008

Mata-Alvarez, J., Dosta, J., Macé, S., Astals, S., 2011. Codigestion of solid wastes: A review of its uses and perspectives including modeling. Crit. Rev. Biotechnol. 31, 99–111.

https://doi.org/10.3109/07388551.2010.525496

Nzeteu, C.O., Trego, A.C., Abram, F., O'Flaherty, V., 2018. Reproducible, high-yielding, biological caproate production from food waste using a single-phase anaerobic reactor system. Biotechnol. Biofuels 11, 1–14. https://doi.org/10.1186/s13068-018-1101-4

Olsson, J., Feng, X.M., Ascue, J., Gentili, F.G., Shabiimam, M.A., Nehrenheim, E., Thorin, E., 2014. Co-digestion of cultivated microalgae and sewage sludge from municipal waste water treatment. Bioresour. Technol. 171, 203–210. https://doi.org/10.1016/j.biortech.2014.08.069

Pakarinen, O., Kaparaju, P., Rintala, J., 2011. The effect of organic loading rate and retention time on hydrogen production from a methanogenic CSTR. Bioresour. Technol. 102, 8952–8957. https://doi.org/10.1016/j.biortech.2011.07.020

Rebecchi, S., Pinelli, D., Bertin, L., Zama, F., Fava, F., Frascari, D., 2016. Volatile fatty acids recovery from the effluent of an acidogenic digestion process fed with grape pomace by adsorption on ion exchange resins. Chem. Eng. J. 306, 629–639. https://doi.org/10.1016/j.cej.2016.07.101

Roghair, M., Liu, Y., Strik, D.P.B.T.B., Weusthuis, R.A., Bruins, M.E., Buisman, C.J.N., 2018. Development of an effective chain elongation process from acidified food waste and ethanol into n-caproate. Front. Bioeng. Biotechnol. 6, 1–11. https://doi.org/10.3389/fbioe.2018.00050

Scarborough, M.J., Lynch, G., Dickson, M., McGee, M., Donohue, T.J., Noguera, D.R., 2018. Increasing the economic value of lignocellulosic stillage through medium-chain fatty acid production. Biotechnol. Biofuels 11, 1–17. https://doi.org/10.1186/s13068-018-1193-x

Shahbaz, M., Ammar, M., Zou, D., Korai, R.M., Li, X.J., 2019. An insight into the anaerobic co-digestion of municipal solid waste and food waste: Influence of co-substrate mixture ratio and substrate to inoculum ratio on biogas production. Appl. Biochem. Biotechnol. 187, 1356–1370. https://doi.org/10.1007/s12010-018-2891-3

Spirito, C.M., Richter, H., Rabaey, K., Stams, A.J.M., Angenent, L.T., 2014. Chain elongation in anaerobic reactor microbiomes to recover resources from waste. Curr. Opin. Biotechnol. 27, 115–122. https://doi.org/10.1016/j.copbio.2014.01.003

Statistics Sweden (SCB), 2020. Production and use of sewage sludge from municipal waste water treatment plants by country and use category. Every other year 2014 - 2018. URL http://www.statistikdatabasen.scb.se/pxweb/en/ssd/START__MI__MI0106/MI0106T03/ (accessed 9.16.20).

Statistisk sentralbyrå Norway (SSB), 2020. Discharges and treatment of municipal waste water 05279: Disposal of sewage slugde (C) 2002 - 2018. URL https://www.ssb.no/en/statbank/table/05279 (accessed 9.16.20).

Varrone et al., 2018

Weimer, P.J., Nerdahl, M., Brandl, D.J., 2015. Production of medium-chain volatile fatty acids by mixed ruminal microorganisms is enhanced by ethanol in co-culture with *Clostridium kluyveri*. Bioresour. Technol. 175, 97–101. https://doi.org/10.1016/j.biortech.2014.10.054

Wu, Q.L., Guo, W.Q., Zheng, H.S., Luo, H.C., Feng, X.C., Yin, R.L., Ren, N.Q., 2016. Enhancement of volatile fatty acid production by co-fermentation of food waste and excess sludge without pH control: The mechanism and microbial community analyses. Bioresour. Technol. 216, 653–660. https://doi.org/10.1016/j.biortech.2016.06.006

Wu, S-L., Luo, G.,Sun, J., Wei, W., Song, L., Ni, B-J., 2021. Medium chain fatty acids production from anaerobic fermentation of waste activated sludge. J. Clean. Prod. 279, 123482. https://doi.org/10.1016/j.jclepro.2020.123482

Yuan, H., Zhu, N., 2016. Progress in inhibition mechanisms and process control of intermediates and by-products in sewage sludge anaerobic digestion. Renew. Sustain. Energy Rev. 58, 429–438. https://doi.org/10.1016/j.rser.2015.12.261

Zacharof, M.-P., Lovitt, R.W., 2013. Complex effluent streams as a potential source of volatile fatty acids. Waste and Biomass Valorization 4, 557–581. https://doi.org/10.1007/s12649-013-9202-6

Zhang, D., Jiang, H., Chang, J., Sun, J., Tu, W., Wang, H., 2019. Effect of thermal hydrolysis pretreatment on volatile fatty acids production in sludge acidification and subsequent polyhydroxyalkanoates production. Bioresour. Technol. 279, 92–100. https://doi.org/10.1016/j.biortech.2019.01.077

Zhang, P., Chen, Y., Zhou, Q., 2009. Waste activated sludge hydrolysis and short-chain fatty acids accumulation under mesophilic and thermophilic conditions: Effect of pH. Water Res. 43, 3735–3742. https://doi.org/10.1016/j.watres.2009.05.036

Zhang, Y., Sun, R., Varrone, C., Wei, Y., Shyryn, A., Zhou, A., Zhang, J., 2020. Enhanced acetogenesis of waste activated sludge by conditioning with processed organic wastes in co-fermentation: Kinetics, performance and microbial response. Energies 13, 3630. https://doi.org/10.3390/en13143630

Zhao, J., Wang, D., Liu, Y., Ngo, H.H., Guo, W., Yang, Q., Li, X., 2018. Novel stepwise pH control strategy to improve short chain fatty acid production from sludge anaerobic fermentation. Bioresour. Technol. 249, 431–438. https://doi.org/10.1016/j.biortech.2017.10.050

Zhou, A., Du, J., Varrone, C., Wang, Y., Wang, A., Liu, W., 2014. VFAs bioproduction from waste activated sludge by coupling pretreatments with *Agaricus bisporus* substrates conditioning. Process Biochem. 49, 283–289. https://doi.org/10.1016/j.procbio.2013.11.005

Zhou, A., Liu, H., Varrone, C., Shyryn, A., Defemur, Z., Wang, S., Liu, W., Yue, X., 2020. New insight into waste activated sludge acetogenesis triggered by coupling sulfite/ferrate oxidation with sulfate reduction-mediated syntrophic consortia. Chem. Eng. J. 400. https://doi.org/10.1016/j.cej.2020.125885

FIGURE AND TABLE CAPTIONS

Table 1. Main characteristics of sewage sludge, food waste and co-culture.

Table 2. Levels and experimental range of the variables in the design experiments.

Table 3. Summary of hexanoic acid production from complex wastes without additional electron donor.

Figure 1. 3D response surface for overall titer. (A) Effect of S/Xo and SS/FW; (B) Effect of pH and SS/FW; (C) Effect of pH and S/Xo.

Figure 2. 3D response surface for hexanoic acid titer. (A) Effect of S/Xo and SS/FW; (B) Effect of pH and SS/FW; (C) Effect of pH and S/Xo.

Figure 3. Individual and overall carboxylic acid, ethanol and lactate titers during continuous operation.

TABLES AND FIGURES

Table 1

Parameter	Sewage sludge	Food waste	Co-culture
pH	6.2 ± 0.4	4.6 ± 0.4	6.9 ± 0.3
Total solids (TS) (g/l)	33.05 ± 1.11	138.39 ± 1.79	16.37 ± 0.06
Volatile solids (VS) (g/l)	26.55 ± 0.92	125.77 ± 1.11	10.38 ± 0.08
Total chemical oxygen demand (tCOD) (g/l)	53.20 ± 3.68	248.50 ± 0.71	16.45 ± 0.78
Total VFA (g/l)	1.53 ± 0.01	2.76 ± 0.22	0.14 ± 0.05
Ethanol (g/l)	0.00 ± 0.00	6.77 ± 0.11	0.00 ± 0.00
Lactic acid (g/l)	0.00 ± 0.00	12.61 ± 0.15	0.00 ± 0.00

Table 2

Variable	Name	Units	Coded levels		
			-1	**0**	**1**
A	SS/FW	% VS	0.00	50.00	100.00
B	S/Xo	gVS/gVS	0.50	4.00	8.00
C	Initial pH		4.50	6.75	9.00

Table 3

Substrate	HRT (days)	OLR (g·l^{-1}·d^{-1})		Titer (g/l)		Productivity (g·l^{-1}·d^{-1})		Yield (mg/g)		Inhibition methanogens	Reference
		VS	COD	VS	COD	VS	COD	VS	COD		
Grass silage	6	10.0	-	3.90	8.58	0.65	1.43	65.0	-	High OLR	(Pakarinen et al., 2011)
Switchgrass-derived stillage	6	-	22.6	8.50	18.70	1.42	3.12	-	137.9	-	(Scarborough et al., 2018)
Ensiled sorghum + cheese way + cow manure	5	11.6	19.27	1.50*	3.30	0.30	0.66	26.0	34.3	Short HRT	(Dareioti and Kornaros, 2015)
Cheese whey	4	12.8	18.0	0.10	0.23	0.03	0.06	2.0	3.2	Initial pH 5.2	(Jankowska et al., 2018)
Sewage sludge mixture	4	8.2	20.0	0.05	0.10	0.01	0.02	1.4	1.3	Initial pH 5.2	(Jankowska et al., 2018)
Food waste + sewage sludge	4	15.0	27.0	3.91	8.60	0.98	2.15	64.0	79.6	High OLR	This study
Ensiled sorghum + cheese way + cow manure	3	17.2	28.6	1.00*	2.20	0.33	0.73	19.4	25.6	Short HRT	(Dareioti and Kornaros, 2015)
Food waste + sewage sludge	2	30.0	54.0	4.91	10.80	2.46	5.40	81.8	100.0	High OLR	This study
Cheese whey	1	51.0	71.9	0.04	0.08	0.04	0.08	0.7	1.1	Initial pH 5.2	(Jankowska et al., 2018)
Sewage sludge mixture	1	32.9	79.9	0.04	0.08	0.04	0.08	1.1	1	Initial pH 5.2	(Jankowska et al., 2018)

* Estimated values from Figures.

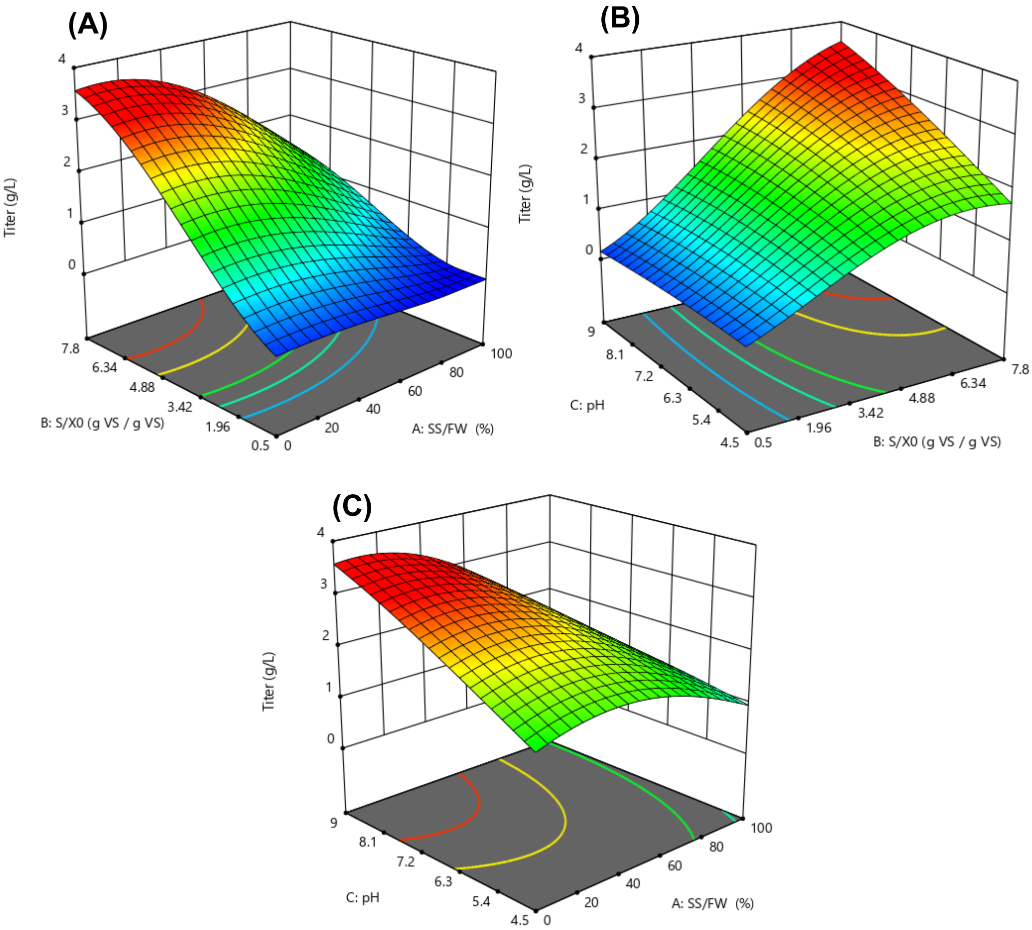

(A)
Titer (g/L)
B: S/X0 (g VS / g VS)
A: SS/FW (%)
(B)
Titer (g/L)
C: pH
B: S/X0 (g VS / g VS)
(C)
Titer (g/L)
C: pH
A: SS/FW (%)

Figure 1

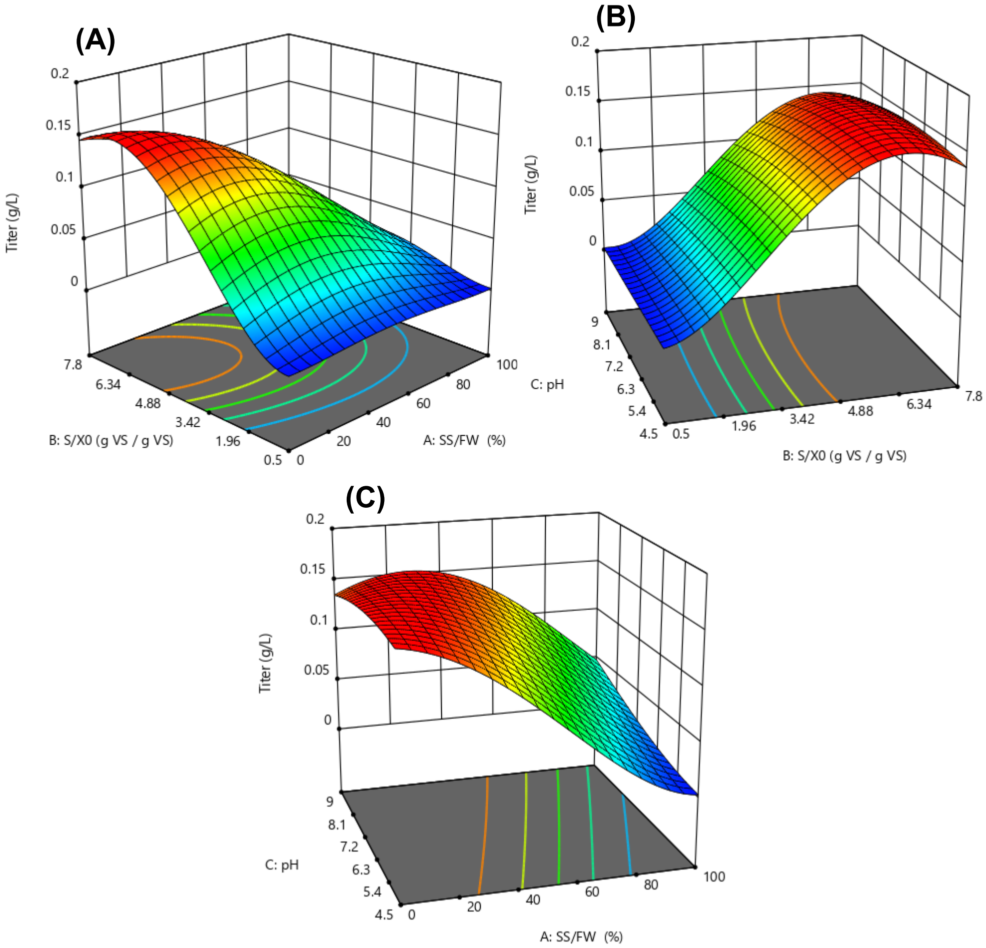
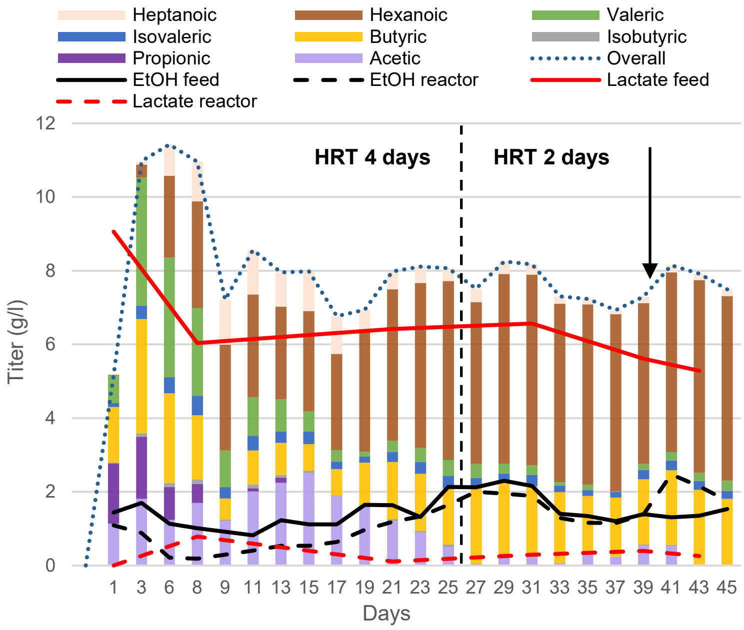

(A)
Titer (g/L)
0.2
0.15
0.1
0.05
0
7.8
6.34
4.88
3.42
1.96
0.5
B: S/X0 (g VS / g VS)
0
20
40
60
80
100
A: SS/FW (%)
C: pH
(B)
Titer (g/L)
0.2
0.15
0.1
0.05
0
9
8.1
7.2
6.3
5.4
4.5
0.5
1.96
3.42
4.88
6.34
7.8
B: S/X0 (g VS / g VS)
(C)
Titer (g/L)
0.2
0.15
0.1
0.05
0
9
8.1
7.2
6.3
5.4
4.5
C: pH
0
20
40
60
80
100
A: SS/FW (%)

Figure 2



797

798 Figure 3

799