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A comprehensive study of volatile fatty acids production from batch reactor to anaerobic sequencing batch reactor by using cheese processing wastewater

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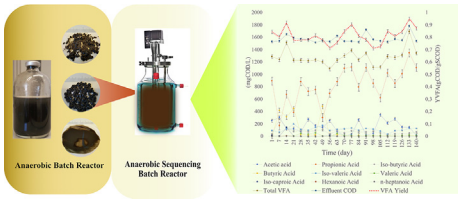
GRAPHICAL ABSTRACT



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ABSTRACT

Volatile fatty acids (VFAs) has great potential for closed-loop production in dairy industries *via* resource recovery from waste-streams. In the current study, the transition of VFA production from batch reactor to anaerobic sequencing batch reactor (ASBR) by using cheese industry wastewater under alkali pH was evaluated with respect to seed sludge structure, microbial diversity and reactor type. The transition from the batch reactor to the ASBR demonstrated that the maximum VFA production yield (g COD/g SCOD) was comparable in two reactors (batch: 0.97; ASBR: 0.94), whereas, the dominant acid type was different (batch: 49% lactic acid; ASBR: 80% propionic acid). There was a significant correlation between the productions of butyric acid with *Gracilibacteraceae* and *Desulfovibrionaceae*; propionic acid with *Desulfovibrionaceae* and *Synergistaceae*; lactic acid with *Pseudomonadaceae* and *Rhodocyclaceae*. The high VFA production efficiency can be achieved by long term reactor operation, which enables the shift from industrial waste-streams to biorefineries.

1. Introduction

According to Food and Agriculture Organization of the United Nations (FAO), the dairy products consumption per capita were 22.2 kg for the developed countries and 10.6 kg for the developing countries (FAO and OECD, 2018) in 2017. Moreover, the consumption of dairy products is estimated to increase by 22% by 2027 (FAO and OECD, 2018). Approximately 2,250,000 m^3 wastewater is generated per day from dairy industries all over the world and it will continue to rise (FAO and OECD, 2018; Hansen, 2012). From the circular economy

Abbreviations: ASBR, Anaerobic sequencing batch reactor; BMP, Biomethane production; COD, Chemical oxygen demand; F/M, Food microorganism ratio; HRT, Hydraulic retention time; LCA, Life cycle assessment; OLR, Organic loading rates; PC, Principal components; PCA, Principal Component Analysis; PCR, Polymerase chain reaction; SCOD, Soluble chemical oxygen demand; SD, Slurry sludge; SL, Large granular seed sludge; SRT, Sludge retention time; SS, Small granular sludge; TCOD, Total chemical oxygen demand; TS, Total Solids; UASB, Up-flow anaerobic sludge blanket reactors; VFA, Volatile fatty acids; VS, Volatile Solids; VSS, Volatile Suspended Solids; WPGMA, Weighted Pair Group Method with Arithmetic Mean

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perspective, the dairy industry wastewater is one of the most valuable waste-streams because of its rich carbohydrate, protein and fat content (Britz and van Schalkwyk, 2006) as well as its large amount of production.

The conventional wastewater treatment plants are evolving to self-sufficient next generation wastewater treatment plants, which aim to recover all valuable sources from waste-streams instead of disposing of them (Puyol et al., 2017). Nowadays, resource recovery studies focus on using fermentation of waste streams to obtain bio-based compounds such as biofuels, biogas, bioplastics, biohydrogen, biodiesel and VFA. VFA are particularly important because they can be used not only as a carbon source for the production of other bio-based compounds but also as a raw material in pharmaceutical, food and chemical industries (Atasoy et al., 2018). Besides their wide range of application area, VFA also has a high market price/demand, low greenhouse gas emission during production and easy transportation/storage(Kleerebezem et al., 2015; Zhou et al., 2017).

VFA production under various operational conditions by using different reactor types and substrates is widely reported. Most of these studies tested the VFA production efficiency under acidogenic conditions, while a few showed that alkali pH conditions can improve the VFA production yield (Atasoy et al., 2019a; Dahiya and Mohan, 2019; Jankowska et al., 2018). Similarly, our previous study demonstrated that strong alkali conditions (pH 10) maximized the VFA production efficiency compared to when pH was 8 (Atasoy et al., 2019b). However, studies on the effect of alkali conditions on VFA production as well as the microbial community structure underlying this process are scarce. In addition to the understanding of the effect of pH on VFA production, improved knowledge and experience of long term reactor operation for VFA production regarding different reactor types are required.

In this study, the VFA production efficiency, fatty acid composition and underlying bacterial community structure were evaluated during the transition process from the batch reactor to the ASBR. As stated previously, one of the most valuable industrial waste-stream comes from dairy industries. Therefore, the cheese production wastewater was selected as a substrate to investigate the potential VFA production efficiency. The main aim of this study was to evaluate how the reactor type and reactor volume affect the VFA production efficiency and acid composition. The results represent valuable outputs for future transition studies from the batch reactor to other reactor types. In line with these aims, firstly, cheese production wastewater was fermented by three different inocula (small and large granular sludge and anaerobic digestion sludge in slurry form) under alkali pH in anaerobic batch reactors. During the fermentation, bacterial community and fermentation process were investigated. Then, the batch reactor was transitioned to ASBR using the most effective sludge, and the VFA production efficiency and composition, as well as the cycle analysis, were conducted to observe acid type alteration.

2. Material and methods

2.1. Inoculum and substrate

In this study, three inocula with two different physical structures (granular and slurry) were used. These are small granular seed sludge (SS; ≈1.5 mm with 44% TS) and 30% VS), large granular seed sludge (SL; ≈3.5 mm with 43% TS and 30%VS) and anaerobic digester seed sludge (SD; slurry form with 19% TS and 12%VS). The characteristics of the anaerobic digester seed sludge were given in a previous study (Atasoy et al., 2019b). In ASBR, the large granular seed sludge (SL) was used because of its high VFA production efficiency in the batch reactors. The granular sludge samples were collected from two different UASB at Hammarby Sjöstadsverk Pilot Plant, Stockholm, Sweden. Anaerobic digestion sludge was collected from Henriksdal Wastewater Treatment Plant, Stockholm, Sweden. They were stored at +4 °C until the set-up of the experiments. Before setting up the retentions, inocula were incubated with growth medium (OECD 311).

In both the batch reactors and ASBR, the real cheese production wastewater was used as a substrate, which was collected from a cheese production industry (Stockholm, Sweden). The wastewater comprised of 20,000 ± 60 mg COD/L, 200 mg/L total nitrogen, 18 mg/L total phosphate and 11 mg/L orthophosphate. The VFA composition of the substrate was 3.11 ± 0.24 mg COD/L acetic acid, 5.70 ± 0.21 mg COD/L propionic acid, 0.29 ± 0.13 mg COD/L *iso*-butyric acid, 1.60 ± 0.34 mg COD/L butyric acid, 0.73 ± 0.45 mg COD/L *iso*-valeric acid, 1.45 ± 0.15 mg COD/L valeric acid, 0.68 ± 0.18 mg COD/L *iso*-caproic acid, 0.53 ± 0.13 mg COD/L caproic acid and 0.27 ± 0.03 mg COD/L n-heptanoic acid. In addition, the substrate contained 0.22 ± 0.08 mg COD/L lactic acid.

The growth medium, vitamin, and trace element solutions were prepared according to OECD 311 (OECD, 2003). The medium and the trace element solution were autoclaved separately for 30 min. at 121 °C. Also, the vitamin solution was filtered through a membrane filter with a 0.22 μm pore size for sterilization.

2.2. Experimental design of anaerobic batch reactors

Anaerobic batch reactors were operated using the three inocula for VFA production under initial pH 10. Twelve experimental bottles (100-mL working volume) was set-up in triplicate (total 36 bottles), flushed with nitrogen gas for 15 min to provide anaerobic conditions and the pH of reactors was adjusted to 10 by using 1 M NaOH and 1 M HCl. The reactors were operated for 1, 5, 10, and 15 days with 120 rpm mixing at 35 °C. The volatile solids content (VS) in each reactor was adjusted to 2000 mg/L. The COD concentration in the reactors was adjusted to 3000 mg COD/L.

2.3. Experimental design of anaerobic sequencing batch reactor

The AMPTS II System (Bioprocess Control, Sweden) was used for ASBR. The reactors were operated by cycling through a sequence of four phases in a single reaction vessel. One cycle was 24 h, which included 10 min for feeding, 23 h for reaction, 45 min for settling and 5 min for decanting. The reactors had 2000 mL total volume with 1400 mL active volume. Approximately 30% of the active volume discharged per cycle. The reactors were flushed with nitrogen gas during and after feeding to provide anaerobic conditions.

The reactors were inoculated by large granular seed sludge (2000 mg VSS/L), which had the maximum VFA production efficiency in the batch reactors. The system was mixed continuously at 120 rpm under 35 °C and pH 10 ± 0.5, which was adjusted using 2 M NaOH.

The operation of the ASBRs included a start-up period of 200 days for acclimation and steady-state with a sequence of four different OLR. The steady-state conditions were reached based on influent and effluent COD concentrations. The reactors were operated with OLR of 600 mg COD/L *day with 0.3F/M ratio for the first 20 days. Then, it increased to 1800 mg COD/L *day with 0.6F/M ratio in a stepwise manner. The SRT was 35 days which calculated based on the VSS loss during decanting. The HRT was 3.5 days.

The cycle analysis was conducted on day 14 after the steady-state conditions were established with F/M ratio 0.6. In total 26 samples were taken each hour during one cycle (24 h) as well as before (−1st h) and after feeding (0th h).

2.4. Analytical methods

Total COD, soluble COD, organic acids, VFA compositions, and pH were monitored in both influent and effluent in anaerobic batch reactors and ASBR. The COD equivalent of each VFA was calculated to validate the mass balances derived. In addition, 0.348 g COD/g acid for formic acid, 1.067 g COD/g acid for acetic acid, 1.514 g COD/g acid for propionic acid, 1.818 g COD/g acid for *iso*-butyric and butyric acids,

2.039 g COD/g acid for *iso*-valeric and valeric acids, 2.207 g COD/g acid for *iso*-caproic and caproic acids, 1.07 g COD/g acid for lactic acid, were used for the conversion of each acids COD equivalents.

The SCOD/TCOD, organic acids, Total Nitrogen and Phosphorus were measured using LCK 514 COD (100 – 2000 mg/L), organic acids LCK 365 Organic Acids (50 – 2500 mg/L), LCK 238 Total Nitrogen (5 – 40 mg/L TN), LCK 348 Phosphate (orto + total) (0.5 – 5 mg /LPO_4 - P) (Hach Lange, US) cuvette tests by Hach Lange DR 3900 spectrophotometer. Also, TS and VS of the sludge were measured according to the Standard Methods (APHA, 2012).

The concentration and composition of VFA (formic, acetic, butyric, propionic, valeric, isovaleric, caproic and heptanoic acids) in the effluents were analyzed by gas chromatography (GC 6890, Agilent) with a flame ionization detector as described in a previous study (Atasoy et al., 2019b). Additionally, lactic acid was measured by Hach Lange DR 3900 spectrophotometer as described before (Borshchevskaya et al., 2016). Formic, acetic, butyric, propionic, valeric, isovaleric, caproic and heptanoic acids were measured as VFA composition; lactic, acetic, butyric and propionic acids were represented separately as target VFAs of this study. For this reason, formic, valeric, isovaleric, caproic and heptanoic acids were given as “other acids” in the VFA composition results.

In ASBR, biomethane production was monitored during operation. During and after the feeding of the reactors, the reactors flushed with nitrogen gas to enable anaerobic conditions.

2.5. Carbon mass balance

The carbon mass balance was derived using the ASBR results in the cycle analysis. Mass balance was calculated using substrate and biomass mg COD eq. as inputs and total VFAs (mg COD eq.) soluble and particulate COD and methane (mg COD eq.) as outputs as described by Cetecioglu et al., 2015.

2.6. Bacterial community analysis

Bacterial diversity in the batch reactors was determined by next-generation sequencing of the 16S rRNA gene. The procedure for DNA extraction and PCR were explained previously (Atasoy et al., 2019b).

Pooled sequencing libraries were sequenced using the Illumina MiSeq platform by Science for Life Laboratory, the National Genomics Infrastructure, NGI (Sweden). QIIME 2 was used to merge and quality filter the sequence data as well as to assign taxonomies at 97% similarity cut-off value (Caporaso et al., 2011). Raw sequence data is available at NCBI with a project no of PRJNA539860.

2.7. Calculation methods of VFA production yield and hydrolysis rate

The VFA production efficiency (Y_{VFA}) (Eq. (1)) was calculated as the ratio of total VFA concentration to SCOD concentration (Jankowska et al., 2017). The hydrolysis yield (η_h) (Eq. (2)) was calculated as the ratio of effluent SCOD concentration to the initial SCOD concentration of the substrate.

$$Y_{VFA} = \frac{VFA_{pro}}{sCOD_{con}} \tag{1}$$

$$\eta_h = \frac{sCOD_{out}}{sCOD_{in}} \tag{2}$$

where VFA_{pro} is the total VFA production (g COD/L), $SCOD_{con}$ is the consumed SCOD concentration (g COD/L), $SCOD_{out}$ is the effluent soluble COD concentration (g COD/L) and $SCOD_{in}$ (g COD/L) is the initial soluble COD equivalent of the substrate.

In addition, the average acid composition for each inoculum was evaluated by Eq. (3) and the weighted analysis based WPGMA (Weighted Pair Group Method with Arithmetic Mean) (David, 2017) was calculated by Eq. (4) to compare effects of each inoculum on both total VFA production and acid composition.

$$\text{Caverage} = \frac{(CatRT1/1) + (CatRT5/5) + (CatRT10/10) + (CatRT15/15)}{4} \tag{3}$$

$$\text{PWPGMA} = \frac{(P_tatRT1/1) + (P_tatRT5/5) + (P_tatRT10/10) + (P_tatRT15/15)}{4} \tag{4}$$

where average $C_{average}$ is the average of each acid type concentration (g COD/L) for each inoculum, RT is the retention time, P_t is the percentage each acid type from all inoculum (%).

2.8. Statistical analysis

The PCA was applied to the bacterial diversity data to discriminate the samples according to the operational conditions. ANOVA was used to evaluate microbial community variation from the seed to the fermentation stage under each retention time. Pearson’s correlation analysis was conducted to identify the relationship between the bacterial diversity (the first two principal components) and VFA composition (acetic, butyric, propionic acids, total VFAs which includes “other” acids and lactic acid concentrations). All analysis was conducted by using IBM SPSS Statistics, Version 25.0.

3. Results and discussion

3.1. VFA production and composition in anaerobic batch reactors

In the anaerobic batch reactors, three different inocula with two dissimilar physical sludge structures were used, and their effects on VFA production and composition were evaluated. The VFA production results as concentration (mg COD/L) and production yield (Y_{VFA}) (g COD/g SCOD) for each inoculum were given in Fig. 1. Additionally, the VFA production efficiency as production yield (Y_{VFA}) and hydrolysis yield (%), as well as the pH drop (from 10), were summarized in Table 1. The highest VFA production in the reactors with large granular sludge (SL), slurry sludge (SD) and small granular sludge (SS) were 1993 ± 58 mg COD/L, 1140 ± 67 mg COD/L and 1056 ± 120 mg COD/L, respectively. Also, the average daily VFA productions were 370 ± 105 mg COD/L, 337 ± 116 mg COD/L and 303 ± 108 mg COD/L by SL, SD and SS, respectively. In line with the VFA production results, the production yield was also maximum for SL with 0.97 g VFA/g SCOD. The VFA production yields for SD and SS were 0.38 g VFA/g SCOD and 0.36 g VFA/g SCOD, respectively.

The results indicated that the VFA production efficiency changed by the inoculum type. In line with the VFA production, the highest VFA production yield was 0.97 (g COD/g SCOD) for SL. In a study by Jankowska et al. (2017), the highest VFA production yield was observed as 0.71 (g COD/g SCOD) using slurry sludge as inoculum under the same operational conditions and substrate type with our study. Additionally, our previous study using a model substrate under pH 10 showed that SL gives the highest VFA production yield (Atasoy et al., 2019b). Despite granular seed sludges have been used for biogas production commonly (Gaur and Suthar, 2017; Logar et al., 2017) because of its several advantages such as higher production efficiency with lower energy requirement for mixing, faster sludge precipitation and easier operation (Cakmakci et al., 2015), it is a new approach to use it for VFA production (Tamis et al., 2015). Our results demonstrate that using a granular sludge is a promising approach to obtain high VFA production efficiencies.

In addition to the VFA production, the composition of VFA was also evaluated for each inoculum (Fig. 2.a) using Eq.3. The results showed that the dominant acid type was lactic acid in each inoculum set (SS:58 ± 9%; SL:44 ± 7%; SD:41 ± 12%), even though the

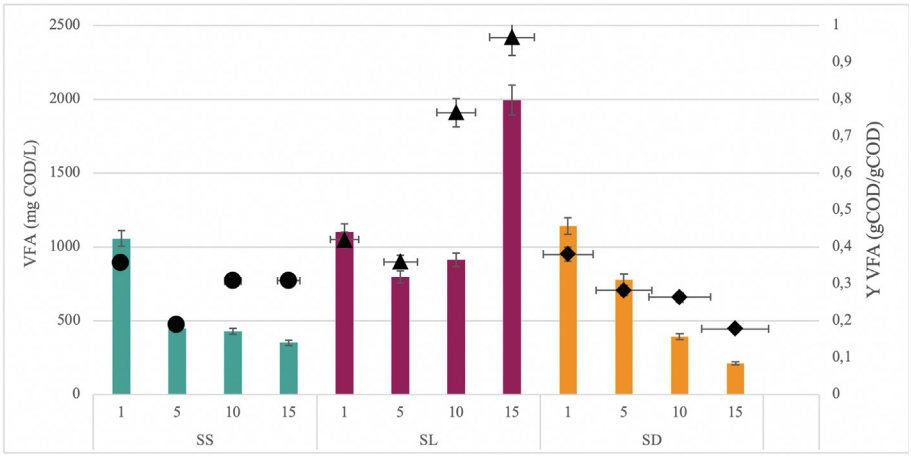

Fig. 1. VFA Production Results as concentration and production yield for each inoculum (●: SS; ▲: SL; ◆: SD) during retention time at anaerobic batch reactors.

Table 1
Process Efficiency for VFA production and Hydrolysis at anaerobic batch reactors.

Seed Sludge	RT (day)	ΔpH (–)	ηh (%)	Y_{VFA} (gCOD/gSCOD)
SS	1	2.88	0.99	0.36
	5	2.89	0.80	0.19
	10	2.93	0.46	0.31
	15	2.57	0.38	0.31
SL	1	2.78	0.87	0.42
	5	2.76	0.74	0.36
	10	2.91	0.40	0.76
	15	2.64	0.69	0.97
SD	1	2.84	1.00	0.38
	5	2.88	0.92	0.28
	10	2.88	0.50	0.26
	15	2.64	0.40	0.18

distribution of acid types varied by each inoculum. Besides, the average concentration of each acid type was calculated based on inoculum type to evaluate inoculum effects on VFA composition as explained in the Eq.4. The results were represented in Fig. 2.a, which showed that acetic acid production was 62 ± 11% in SD, while it was 26 ± 7% and 11 ± 4% in SS and SL, respectively. This was followed by propionic acid (40 ± 9% in SD, 38 ± 16% in SL and 21 ± 8% in SS) and butyric acid production (70 ± 4% in SL, 17 ± 8% in SS and 12 ± 2% in SD). On the other hand, each inoculum produced similar amounts of lactic acid (37 ± 9% in SS, 34 ± 7% in SL and 29 ± 4% in SD), other acids (38 ± 17% in SD, 37 ± 12% in SS and 25 ± 4%in SL) and total VFA (38 ± 14% in SD, 37 ± 19% in SL and 30 ± 12% in SS). Interestingly, despite the initial pH was adjusted to 10, the effluent pH was decreased to 7 ± 0.5 in each reactor (Table 1).

Contrary to previous reports, which indicated that the inoculum type did not affect VFA composition using a model substrate (glucose) (Atasoy et al., 2019b), the results of the current study and the previous study which used milk processing wastewater as substrate (Atasoy et al., 2019a) showed that the inoculum type affected the VFA composition when a complex substrate was used. In light of current results, the desired acid composition might be produced by selecting the inoculum type.

The VFA composition showed that lactic acid was the most dominant acid group in all inoculum sets (Fig. 2.b). However, Jankowska et al. (2018) showed that propionic acid (~30%), Bengtsson et al. (2008) revealed that acetic acid (43%) and Domingos et al. (2018) indicated that caproic acid (33%) were the dominant acid types during cheese whey fermentation. Although the substrate was cheese whey in those studies, the production process and product type affected the wastewater characteristics significantly (Britz and van Schalkwyk, 2006). Therefore, these results can be interpreted as the characteristics of our substrate affected the VFA composition.

3.2. Bacterial community structure in anaerobic batch reactors

Sequence analysis of the 16S rRNA genes from the samples showed that the most abundant families were *Porphyromonadaceae* (18.5 ± 6%), *Clostridiaceae* (14 ± 9%), *unassigned Bacteroidales* (11.6 ± 4%) and *Peptostreptococcaceae* (6.5 ± 3%; Fig. 3.a). The bacterial community results revealed that three family members (*Porphyromonadaceae*, *Clostridiaceae* and *unassigned Bacteroidales)* constituted more than 50% of the bacterial community, nevertheless, their amount varied significantly with the retention time. These are the most

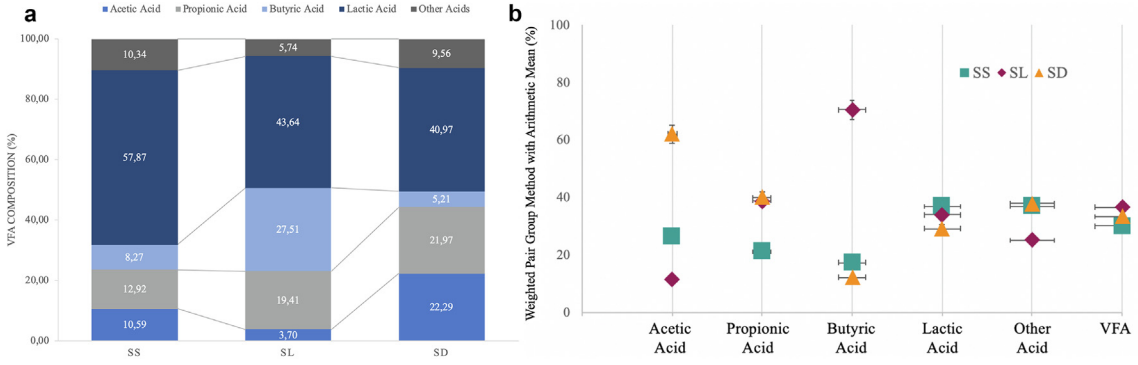

Fig. 2. (a) Average VFA composition for each inoculum, (b) The weighted pair group method with arithmetic mean for each acid type and total VFA regarding each inoculum.

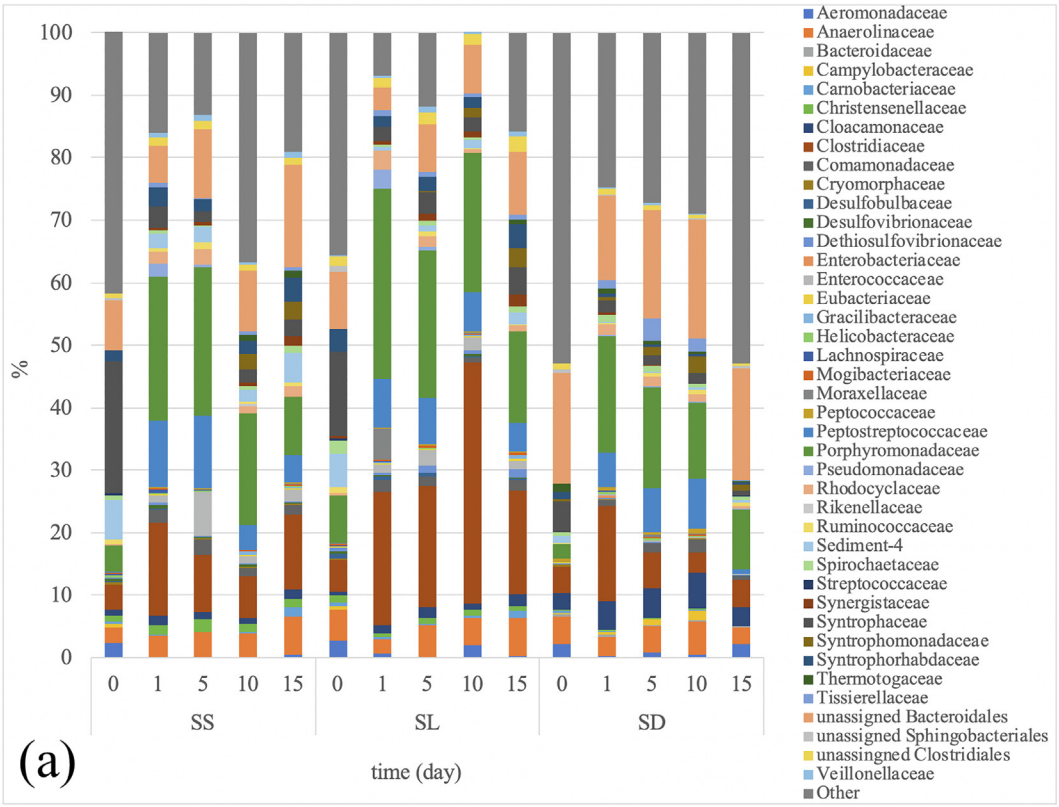

Fig. 3. (a) The bacterial community relative abundance of family level for each inoculum; (b) Heat maps of relative abundance phylum and family for each set which shows the color scale varies from low abundance (white: 0%) to high abundance (red: 100%). In Krona graph, the bacterial community composition from phylum to genus level for SL seed in the beginning was represented. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

common taxa found in anaerobic digesters treating sewage sludge (Braz et al., 2019). Similarly, a previous study investigating the bacterial community in milk processing wastewater fermentation for VFA production demonstrated that the reactors were dominated by Porphyromonadaceae (41.5 ± 12%), Clostridiaceae (13 ± 5%); unassigned Bacteroidales (5.3 ± 3.3%), Streptococcaceae (4.2 ± 2.3%) and Peptostreptococcaceae (4 ± 1.5%) regardless of the inoculum type and retention time (Atasoy et al., 2019a). Therefore, the current study showed that despite the dominant bacterial community was similar, the relative abundance of dominant species depends on substrate type.

The relative abundances of different taxa in the batch reactors at phylum and family levels were represented by a heat map, showing the effects of different inocula and retention time on the bacterial community composition (Fig. 3.b). *Bacteroidetes* (30 ± 6%, average of all sets) and *Firmicutes* (25 ± 11%, average of all sets) were the most abundant phyla in all sets. Additionally, *Chlorobi* (9 ± 2%, average of SS and SL) and *Caldithrix* (11 ± 5%, average of SS and SL) were also abundant in granular sludge sets (SS and SL), while *Acidobacteria* (4 ± 2% in SD) and *Planctomycetes* (3.7 ± 1% in SD) were abundant in slurry sludge sets (SD), which demonstrates the effect of sludge structure type on the bacterial community.

The most dominant family member in all sets was *Porphyromonadaceae* from the *Bacteroidetes* phylum. Their relative abundance increased significantly in comparison to the seed sludge responsible for the propionic acid production. The relative abundance of *Porphyromonadaceae* increased in all sets from the seed to during retention time. As Rosenberg et al. (2014) stated the growth of *Bacteroidetes* is enhanced by protein hydrolysates which might be a reason for their dominance in the bacterial community in this study.

The second most dominant family member was *Clostridiaceae*, which belong to the phylum of *Firmicutes*. In the highest butyric acid production set, the relative abundance of *Clostridiaceae,* which are responsible for mainly butyric acid production (Jha et al., 2014; Rosenberg et al., 2014) increased from seed sludge to fermentation. As previously stated (Atasoy et al., 2019b; Ma et al., 2017), *Clostridiaceae* are the predominant family member in reactors producing VFA.

The relative abundance of *Syntrophaceae,* which degrade VFA in association with methanogens (Westerholm and Schnürer, 2019) decreased during fermentation possibly due to the operational conditions in our experiments, which were not suitable for the growth of methanogens.

The variations in the bacterial community structures in the batch reactors with different retention times (1, 5, 10 and 15 days) were compared with the bacterial community in the seed sludge (day 0) to determine how the bacterial community shifted during the fermentation process. The standardized coefficients indicated that the bacterial communities at day 1 and 5 were not affected in any of the sets by the seed sludge (before fermentation) microbial composition. Nevertheless, in the SD set, the bacterial composition at 10 and 15 days was affected by the microbial composition of the seed sludge.

To reveal the link between bacterial community composition and the bioreactor performance in terms of VFA production and composition, the first two principal components (PC1 and PC2) of bacterial relative abundance data in the family level were correlated with concentrations of acetic acid, butyric acid, propionic acid, lactic acid, other acids and total VFA production. Although there was no significant correlation between the bacterial diversity at the family level and performances of the reactors in terms of VFA production, the bacterial community was negatively correlated (-0.628) with acetic acid concentration at $p < 0.05$. The results of PCA also supported that the retention time affected the bacterial diversity in granular sludge sets (SS and SL) but not in SD.

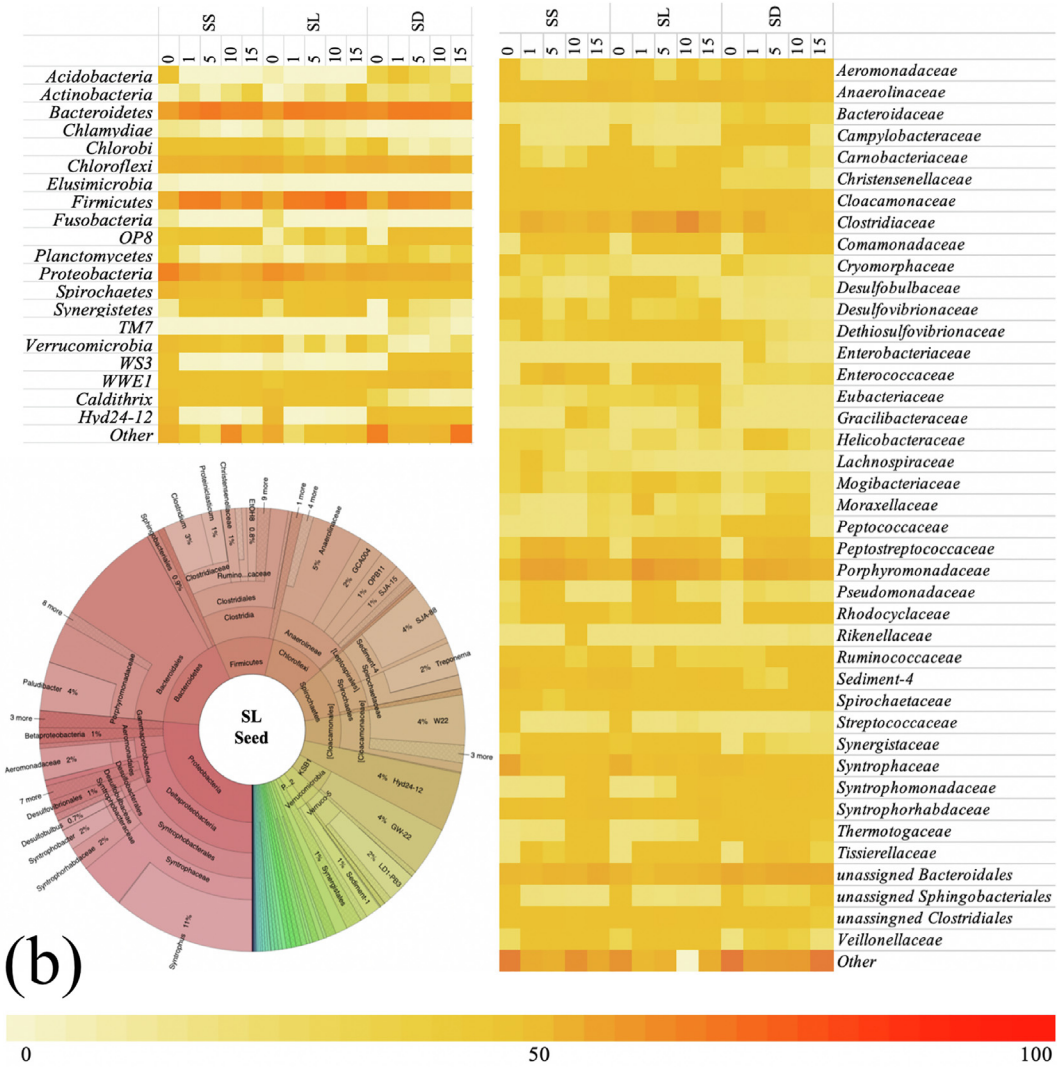

Fig. 3. (*continued*)

Further analysis was carried out to reveal the correlation between the family-level bacterial diversity and the concentrations of different VFA. The results showed that there was no significant correlation between acetic acid and a specific family member. However, there were high correlations between *Desulfovibrionaceae* with butyric acid (0.742) and propionic acid (0.782); *Gracilibacteraceae* with butyric acid (0.936) at $p < 0.01$ and propionic acid (0.693); *Synergistaceae* with butyric acid (0.730) and propionic acid (0.608); *Syntrophaceae* with butyric acid (0.623) and propionic acid (0.636) at $p < 0.05$. In addition, there was a significant correlation between other acids (lactic acid) with *Anaerolinaceae* (−0.584), *Pseudomonadaceae* (0.589) and *Rhodocyclaceae* (0.594) at $p < 0.05$. The correlation analysis confirmed that *Desulfovibrionaceae* and *Syntrophaceae* from the phylum *Proteobacteria* were mostly responsible for propionic acid production (Cai et al., 2016; Yi et al., 2016) while *Gracilibacteraceae* from the phylum *Firmicutes* produced butyric acid (Rosenberg et al., 2014). Also, *Anaerolinaceae* from the phylum *Chloroflexi*, negatively affected lactic acid production as was found previously (Atasoy et al., 2019b; Bi et al., 2016)

3.3. VFA production and composition in ASBR

The VFA production and composition at ASBR in steady-state conditions were represented in Fig. 4. The VFA production reached up to 1691 mg COD/L, whereas the average VFA production was 1278 ± 34 mg COD/L. The average VFA production yield (Y_{VFA}) was 0.81 ± 0.06 g VFA/g SCOD and the maximum yield was 0.94 g VFA/g SCOD. The VFA types and the production efficiency in terms of concentration and yield were fluctuated by retention time.

Despite ASBR is one of the most common reactor types for biogas production, their application for VFA production is relatively new. In our study, the highest VFA production was obtained as 1691 mg COD/L (concentration) and 0.94 g COD/g SCOD (VFA production yield). Nonetheless, the average VFA production during the reactor operation was 1278 ± 34 mg COD/L, as well as the average Y_{VFA}, was 0.81 ± 0.06 g VFA/g SCOD. Calero et al. (2018) evaluated the performance of ASBR on VFA production under different pH (5, 5.5, 6) for different OLR (from 3 to 12 g COD/L) by using cheese whey. In their study, the maximum VFA production (6777 ± 86 mg COD/L) was achieved with 87% acidification rate and 0.89 mg COD/mg COD as VFA production yield. In another study, conducted to investigate the effect of different OLR (5 – 40 g COD/L) on VFA production by using an acidogenic ASBR and pretreated olive mill wastewater under pH 5 (Yarimtepe et al., 2017). Their results indicated that the maximum VFA production (27 g COD/L) was achieved by 20 g COD/L OLR with 68% acidification rate. Also, VFA production by ASBR under different pH (4.5, 5, 5.5) using glucose as a model substrate was evaluated by Tamis et al. (2015). In their study, the highest Y_{VFA} was obtained as 0.66 ± 0.02 g COD/g COD at pH 4.5 (Tamis et al., 2015). Overall, these studies showed that ASBR for VFA production was conducted

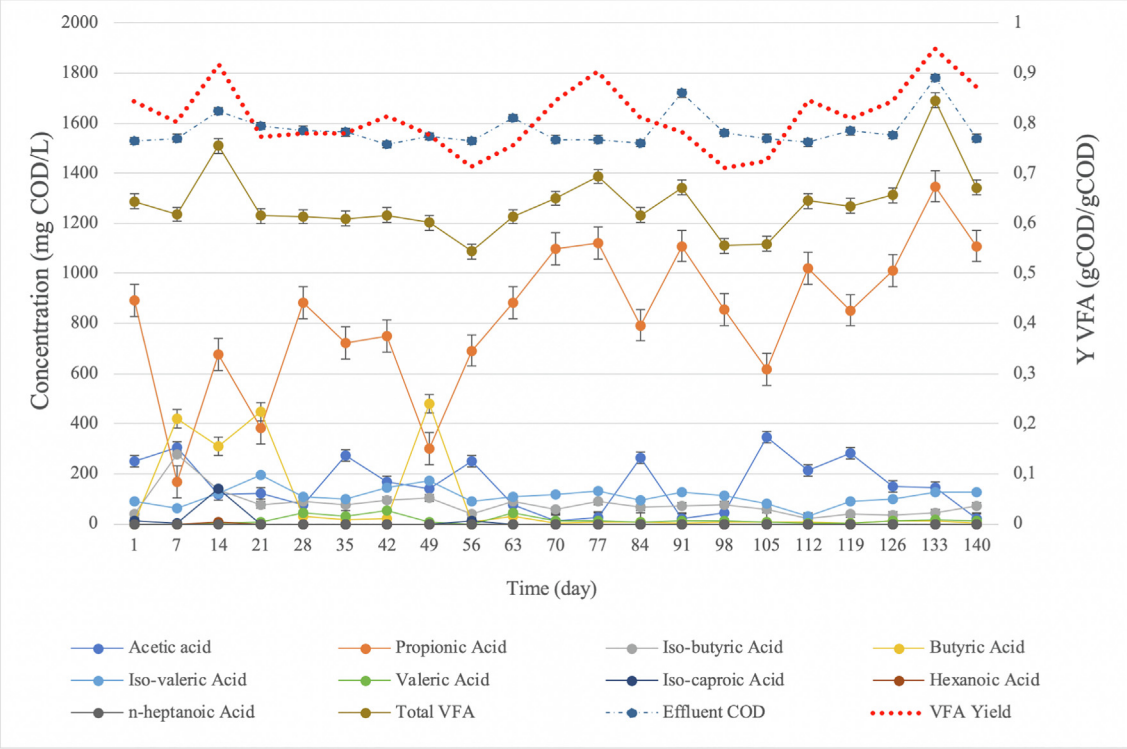

Fig. 4. VFA production and composition in ASBR.

under acidic pH by using different substrates with various OLR. Apart from the substrate, inoculum type and operational conditions, the positive effects of either granular seed sludge structure or alkali pH might be a reason for higher VFA production yields of the current study.

The VFA production yield was low in the batch reactors compared to ASBR because of difficulties to keep the process stability (Zaiat et al., 2001). Nevertheless, our results showed that in ASBR the VFA production yield was slightly similar with the batch reactors though the same operational condition and inoculum.

Despite the changes in each acid concentration by retention time, the dominant acid type in ASBR was mainly propionic acid during operation, unlike the anaerobic batch reactors. Nevertheless, the highest and lowest acid concentration was from 11 to 347 mg COD/L for acetic acid; 167 to 1347 mg COD/L for propionic acid, 18 to 278 mg COD/L for *iso*-butyric acid, 3 to 480 mg COD/L for butyric acid, 30 to 196 mg COD/L for *iso*-valeric acid and 0 to 54 mg COD/L for valeric acid, respectively in ASBR. Additionally, the lactic acid production was not observed during the operation. The VFA composition varied significantly between the two reactor types. The difference might have arisen mainly from either pH difference in the reactors or the reactor type. It is also worth noting that the reactor was operated under pH 10 (10 ± 1.7), therefore biomethane production was not observed during the operation.

3.4. Cycle analysis in ASBR

To further investigate the acid type shift in ASBR, VFA composition was monitored during a cycle (24 h). The acid composition (mg COD/L) and biomethane production (NmL) results showed that the VFA composition was mainly composed of propionic (in average 70% of the VFA mixture) and acetic acids (in average 10% of the VFA mixture) production, nevertheless, the acid types were varied during a cycle (Fig. 5). The propionic acid concentration in the reactor reduced from 1542 mg COD/L to 1052 mg COD/L in 1 h after the feeding step. After the second hour of operation, the propionic acid concentration reached its maximum at 1769 mg COD/L. Afterwards, it was stable for 20 h, then it decreased sharply to 1097 mg COD/L.

The VFA composition was almost similar during the first two h before feeding. Though the composition changed slightly in the second hour of the cycle, it remained stable for 18 h. In the 19th h, propionic acid decreased (from 57% to 37%), acetic acid (from 3% to 33%) and caproic acid (from 0.2% to 24%) increased. Valeric (from 0.1% to 7%) and butyric (from 0.1% to 3%) acids also slightly increased (Fig. 5). However, the composition of the mixture was almost similar before feeding (−1st h) and the end of the cycle (24th h). Overall, the results showed that the highest VFA production was observed in the first 2 h of the cycle.

Beside the VFA composition, the biomethane production was also observed during the cycle, which showed that there was almost no biomethane production (Fig. 5) after feeding.

The cycle analysis was conducted in ASBR to observe the VFA composition shift during a cycle. Nevertheless, the results indicated that propionic acid was the dominant acid type during a cycle and the VFA composition did not change significantly.

The results of the cycle analysis stated that the major changes in VFA composition and the highest VFA production were obtained in the first 2 h of the cycle. For these reasons, the same VFA production might be reached in shorter retention time and in smaller reactor volume, which enables to decrease of operation and/or installation costs.

3.5. Effects of pH and retention time on VFA production composition

The pH affects VFA composition more than VFA production efficiency (Atasoy et al., 2019b). In this study, as stated previously, the VFA production yield did not change dramatically both in batch reactors and ASBR, while the VFA composition changed significantly. Therefore, the difference between VFA composition in the batch reactor and ASBR might be caused by the pH change during fermentation. Similarly, in previous studies, the pH affected the VFA composition during glucose fermentation: at alkali conditions (pH 8 and 10) butyric acid was dominant, at acidic conditions acetic acid was dominant, whereas at neutral pH a mix of acetic and butyric acids was dominant

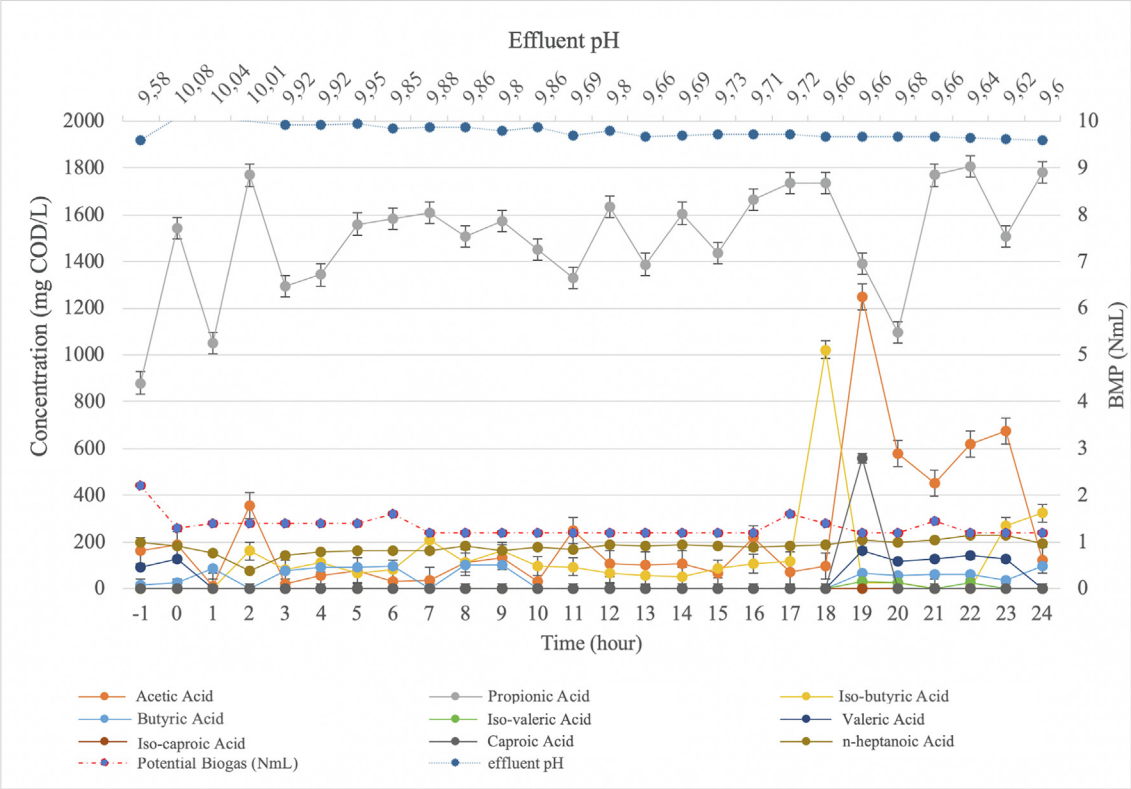

Fig. 5. The cycle analysis of ASBR on day 14 based on the VFA composition and BMP.

(Atasoy et al., 2019b). This finding is supported by Begum et al. (2018), who indicated that acetic acid was the dominant acid type at pH 5.5 while butyric acid was the dominant one at pH 8–10. Also, Chen et al. (2017) showed that the stepwise pH increment from 7 to 9 resulted in a shift in VFA composition, which changed from mixed to the acetic acid dominance. Overall results demonstrate that the VFA composition may be affected by pH and/or substrate type.

VFA production efficiency is directly related to the retention time because it allows sufficient contact time of microorganisms with the substrate (Worwag and Kwarciak-Kozłowska, 2019). From the retention time perspective of VFA production, the highest VFA production was obtained at 15th day in the batch set, whereas almost the same production efficiency was obtained by ASBR at HRT 3.5 days. Similarly, Ziganshin et al., (Ziganshin et al., 2016) investigated the effect of HRT on the microbial activity at ASBR. They stated that the shortening of HRT (from 6 days to 1.5 days) affected the microbial community structure which resulted in higher production efficiency.

3.6. Future aspects in terms of economical and practical production of VFA

Bio-based VFA production from waste-stream is a new approach in resource recovery, therefore, bio-based VFA has not been in the market yet. Moreover, the economical and practical evaluation of VFA production from waste-streams is scarce. In one of these studies, Woo and Kim (2019) showed that to achieve 99% VFA mixture recovery and 99.5% product purity, $0.53 is required for the separation and purification of one kg of VFA. Based on the assumption that the VFA price is $2.70/kg with 3% solvent loss, the application of the scale-up process is profitable despite the utility cost. Besides, their life cycle analysis revealed that the process has 70% fewer eco-scores than comparable bio-production processes. Khoshnevisan et al. (2020) evaluated different biorefinery platform scenarios (six different combinations of biogas production, combined heat and power system, biofertilizer, biomethane production, succinic acid production and lactic acid production) from the environmental life cycle assessment perspective (Khoshnevisan et al., 2020). Based on their assessments, succinic acid and lactic acid production scenarios would achieve −73 and −173 kg $CO_{2,\ eq}$/t biopulp as environmental benefits, respectively (Khoshnevisan et al., 2020). In the direction of these results, which present the positive environmental impacts of organic acids production from waste-streams, the sustainability assessment of fermentative VFA production must be carried out using different tools such as LCA, energy and exergy methods (Rosen, 2018).

According to the report of European Market for Bio-based Chemicals, just 0.3% of the platform chemicals (VFAs, lactic acids, ethanol, etc.) demand is supplied by bio-based production in EU, while, the annual platform chemicals consumption in EU is 60,791 kt (Spekreijse et al., 2019). Nevertheless, to keep global warming below 2 °C as described in the Paris Agreement (UNFCCC, 2015), petrol-based processes must shift to bio-based processes to be sustainable and environmentally friendly. Venkata Mohan et al. (2019) emphasized that the concept of "waste biorefineries" has a significant role in the circular bio-economy approach, which enables shift the source from fossil feedstock to waste feedstocks in line with the several bio-based products. Also, they suggested that implementing of waste biorefineries concept must be supported by new policies and regulations to achieve proper infrastructure of sustainable bio-based production (Venkata Mohan et al., 2019). Our results indicate a high potential to produce a higher purity of one-type VFA by manipulating either the operational conditions or the bacterial community.

4. Conclusions

The main result of the transition process was the VFA production yield which was 0.97 (g COD/ g SCOD) in the batch reactor, whereas, it was 0.94 in the ASBR. The effects of transition of the anaerobic batch reactor to ASBR on VFA production and composition showed that the VFA production efficiency can be enhanced in long-term reactor

operation by either optimizing operational conditions and/or using a bio-augmentation technique with specific microbial species. In this way, the industrial waste-streams can be used as a carbon source for desired bioproducts output *via* VFA production.

CRediT authorship contribution statement

Merve Atasoy: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing - original draft, Visualization. **Ozge Eyice:** Software, Writing - review & editing. **Zeynep Cetecioglu:** Conceptualization, Writing - review & editing, Supervision.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biortech.2020.123529.

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