The effect of mixing on fermentation of primary solids, glycerol, and biodiesel waste
Marzieh Ghasemi and Andrew A. Randall

ABSTRACT
In this study, the effect of mixing on volatile fatty acid (VFA) production and composition was investigated through running five identical bench-scale reactors that were filled with primary solid and dosed with either pure glycerol or biodiesel waste. Experimental results revealed that there was an inverse correlation between the mixing intensity and the VFA production. The total VFA production in the un-mixed reactor was \(9,787 \pm 3,601\) mg COD/L, whereas in the reactor mixed at 100 rpm this dropped to \(3,927 \pm 1,175\) mg COD/L, while both types of reactor were dosed with pure glycerol at the beginning of each cycle to reach the initial concentration of \(1,000\) mg/L \((1,217\) mg COD/L). Propionic acid was the dominant VFA in all the reactors except the reactor mixed at 30 rpm. It is hypothesized that low mixing facilitated hydrogen transfer between obligate hydrogen producing acetogens (OHPA) and hydrogen consuming acidogens in these non-methanogenic reactors. Also, in a narrower range of mixing \((0\) or \(7\) rpm), the total VFA production in biodiesel waste-fed reactors was considerably higher than that of pure glycerol-fed reactors.

Key words | acetic acid, biodiesel waste fermentation, glycerol fermentation, prefermentation, propionic acid, volatile fatty acid

INTRODUCTION
The enhanced biological phosphorus removal (EBPR) process has been widely used in wastewater treatment over the last few decades. The success of this process depends on both the wastewater volatile fatty acid (VFA) concentration (Abu-ghararah & Randall 1991) and composition (Chen et al. 2004; Lopez-Vazquez et al. 2009). In earlier studies, acetic acid was introduced as the most effective substrate for EBPR (Smolders et al. 1994); however, further investigations showed that the EBPR efficiency was at its maximum when acetic acid and propionic acid were available at a \((1:1)\) carbon molar ratio (Chen et al. 2004). One way to increase the VFA concentration of the influent is to employ a separate unit process called a prefermenter where the acidification of the influent chemical oxygen demand (COD) takes place (Rößle & Pretorius 2001; Barajas et al. 2002; Gonzalez-Barceló & Gonzalez-Martinez 2006). The operational conditions (e.g. temperature, mixing and pH) have a great impact on prefermenters’ performance (Lee et al. 2014). Mixing is one of the parameters that affect the fermentation process. However, there few studies have been conducted to investigate the effect of mixing on the prefermentation process (Danesh & Oleszkiewicz 1997; Banister & Pretorius 1998). Some studies into the failure of hydrogen producing fermentation (i.e. dark fermentation) have observed that low or poor mixing results in VFA accumulation (Trad et al. 2016). VFA quantity and composition can also be affected by the substrate fermented. Different substrates have been used to increase the VFA concentration of wastewater; however, there is a continuous effort to find more efficient substrates. On the other hand, glycerol has been widely used in different industries, such as cosmetic, pulp and paper and various chemical production, and attracted more attention during the last few decades because of its abundance and low price. Compared with sugars, glycerol has a greater degree of reduction, which makes it possible to produce more reduced chemicals such as succinate, ethanol and propionate through a fermentation process (da Silva et al. 2009). Moreover, with the increase in biodiesel production there is increased interest in finding uses for by-products of the biodiesel manufacturing process.
One of the major by-products is glycerol, which is typically the major component of a mixture of compounds in biodiesel waste. This brought up the idea of using pure glycerol, and more appealingly crude glycerol (the main by-product of biodiesel production), as an external substrate in biological nutrient removal processes. This could change the economics of propionic acid use in wastewater treatment and benefit the biodiesel and waste disposal industries as well. Fermentation of pure glycerol in reactors seeded with pure culture showed promising results in VFA production, specifically in the form of propionic acid (da Silva et al. 2009; Zhang & Yang 2009; Zhu et al. 2010). In a study conducted by Ma et al. (2017), acetic acid and propionic acid were reported to be the main VFAs produced through the co-fermentation of waste activated sludge and glycerol content-waste. However, there are a few studies that investigated the effect of using pure glycerol and crude glycerol in EBPR systems (Guerrero et al. 2012; Coats et al. 2015; Taya et al. 2015).

With all this in mind, the objective of this research was to optimize the mixing intensity in a mixed culture side stream reactor fed with either biodiesel waste or pure glycerol to augment the VFA production from primary solids and yield a composition (e.g. high propionic/acetic acid mix) that is more favorable for EBPR (and possibly denitrification as well).

MATERIALS AND METHODS

This research was conducted in two separate phases. For both phases, fresh primary solids were obtained from the Glendale Wastewater Treatment Facility (Lakeland, Florida) once a week and stored in a walk-in cooler at 4 °C. The primary solids’ characteristics varied in a wide range as they were collected on different weeks. Table 1 shows the feed characteristics in Phase 1 and Phase 2. The solids retention time (SRT) was set at four days, as in the previous study the SRT of four days was reported to be the optimum SRT for glycerol and primary solids co-fermentation (Ghasemi & Randall 2017). The reactors had a total volume of 2 L with active volume of 1.5 L (each reactor had a head space of 0.5 L). Therefore, 375 mL of fermented primary solids from the reactors was replaced with the same amount of fresh primary solids daily. SRT was equal to hydraulic retention time (HRT), since these were fed batch reactors. Mixing was provided by U shape plastic blades with 0.31-inch shaft diameter and 13.8-inch shaft length (Cole-Parmer, Vernon Hills, Illinois) connected to Grainger gear motors (Orlando, Florida) at different rpms. 0 rpm reactors were mixed once a day to waste solids. The tests were run at room temperature (22 °C). The initial pH was not changed and remained uncontrolled during the entire test. The initial pH and final pH of each cycle were recorded and stated in Table 1, Table 2 and Table 3. As is shown in Table 1, there were some soluble COD and VFAs present in the feed, mostly in the form of acetic acid and propionic acid. The initial VFA concentration and soluble COD data had a significant variability because different batches were used each week. Also, analytical variability due to the highly concentrated nature of the samples was significant.

Phase 1

In the first phase, five reactors with liquid volume of 1,500 mL were operated under consistent environmental and process conditions with mixing energy as the sole experimental variable ranging from 0 to 100 rpm. The reactor conditions are described in Table 2. After running the reactors for three weeks to reach steady state conditions, sampling was started and conducted twice a week (on two successive days) for five weeks. Reactors were dosed with 1,000 mg/L (1,217 mg/L as COD) pure glycerol daily. The initial glycerol concentration was estimated by conducting a preliminary test in serum bottles for design and analysis of the prefermenter reactor experiments. The serum bottle experiments only informed us of how the glycerol was biochemically fermented by a given population, but because of their short-term nature they did not account for selective pressures and/or acclimation. However, since the biochemical transformations are a major component of the selective pressures in prefermenters, the serum bottle experiments allowed us to have some idea of what operational conditions would likely have an impact on glycerol fermentation. The optimum initial glycerol concentration was determined by adding 100 mL diluted primary solids.

Table 1 | Feed characteristics (non-diluted primary solids in phase 1 and diluted primary solids in phase 2)

<table>
<thead>
<tr>
<th>Phase #</th>
<th>pH</th>
<th>TCOD (mg/L)</th>
<th>SCOD (mg/L)</th>
<th>VSS (mg/L)</th>
<th>TSS (mg/L)</th>
<th>VFA (mg COD/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase 1</td>
<td>4.2–5.5</td>
<td>36,000–52,000</td>
<td>1,800–2,700</td>
<td>10,000–22,000</td>
<td>12,000–33,000</td>
<td>2,300–8,000</td>
</tr>
<tr>
<td>Phase 2</td>
<td>4–6.1</td>
<td>13,300–24,000</td>
<td>850–1,300</td>
<td>5,600–6,500</td>
<td>6,600–11,000</td>
<td>270–1,000</td>
</tr>
</tbody>
</table>

SCOD, soluble chemical oxygen demand; TCOD, total chemical oxygen demand; TSS, total suspended solids; VSS, volatile suspended solids.
solids (50 mL primary solids was diluted with 50 mL waste-water) into 120 mL serum bottles (with 20 mL head space) and sealed using aluminum crimped caps equipped with butyl rubber septa to avoid oxygen exposure during fermentation. Serum bottles were then dosed with pure glycerol at different initial dosages (0 mg/L, 100 mg/L, 500 mg/L, 1,000 mg/L and 2,000 mg/L) using syringes. Initial pH was not changed, and tests were run at room temperature (22°C) for 24 h. Experimental results revealed that the glycerol addition increased propionic acid production during the fermentation process. The maximum average HPr/HAc in pure-glycerol-dosed samples was 1:1 (mmoles-C), which was achieved from samples with an initial glycerol concentration of 1,000 mg/L. Therefore, side stream prefermenters were dosed with 1,000 mg/L glycerol in Phase 1. The primary sludge was collected weekly from the Glen-dale Wastewater Treatment Facility (Lakeland, Florida) once a week and stored in a walk-in cooler at 4°C. Therefore, the concentration of primary solids (total suspended solids, volatile suspended solids) differed from one week to another. The concentration of the primary sludge was not adjusted to a predefined target value.

### Phase 2

In the second phase of the research, the suitability of biodiesel waste and pure glycerol was studied at greater concentrations, while the quantity of primary solids was roughly reduced to half of what they were in Phase 1. Mixing was set at the two optimum rates from Phase 1, i.e. 0 rpm and 7 rpm. Two reactors were dosed with pure glycerol at 5,274 mg COD/L, more than four times higher than Phase 1 at 1,217 mg COD/L. Two additional reactors were dosed with biodiesel waste at a dosage of 8,450 mg COD/L, and one reactor worked as a control with no glycerol or biodiesel waste addition. The experimental and operational conditions of Phase 2 are stated in Table 3. To decrease the fermented primary solids fermentation contribution to VFA production and evaluate the external substrate (glycerol or biodiesel waste) the substrate's initial concentration was increased to 4,333 mg/L in the reactor volume (i.e. an addition of 6,500 mg per cycle), while the primary solids concentration was lowered by diluting it with raw influent received from the Iron Bridge Water Reclamation Facility (Oviedo, Florida) at a ratio of 1:1 (V/V). The glycerol concentration in the biodiesel waste received from SAKAL LLC (Panama City, Panama) was approximately 20% on average, lower than what was expected in a typical biodiesel waste, which is about 56-60% (Bodik et al. 2009). However, COD analysis showed that the total chemical oxygen demand (TCOD) of biodiesel waste was 1.95 mg-COD/mg-biodiesel waste, which is greater than that of pure glycerol (1.217 mg COD/mg glycerol).

### Analytical methods

VFA analysis was conducted by gas chromatography using a Nukul capillary column (30 m × 0.25 mm I.D. × 0.25 μm;...
Supelco, St Louis), which was installed on a Shimadzu GC-14A gas chromatograph (Shimadzu, Columbia, Maryland) equipped with a flame ionization (FID) detector. The initial column temperature was 110 °C, and it increased instantly to 190 °C by a ramp of 5 °C/min and was then maintained at 190 °C for an additional 10 min. Both injector and detector temperature were set at 220 °C. The carrier gas was helium, and it was provided at a linear velocity of 20 cm/min. Samples were first centrifuged and the supernatants were then filtered with 0.45 μm membrane filters. One milliliter filtered samples were transferred to 1.5 mL GC vials, sealed with aluminum caps and stored frozen until the injection time. Just before the injection time, after samples were thawed, they were filtered with 0.45 μm membrane filters. One milliliter filtered samples were transferred to 1.5 mL GC vials, sealed with aluminum caps and stored frozen until the injection time. Just before the injection time, after samples were thawed, they were filtered with 0.45 μm membrane filters. One milliliter filtered samples were transferred to 1.5 mL GC vials, sealed with aluminum caps and stored frozen until the injection time. Just before the injection time, after samples reached room temperature (22 °C), acidification was conducted with 5% formic acid to lower the pH below 3. Following this, 1 μL acidified sample was injected automatically by an auto injector AOC-20i (Shimadzu, Columbia, Maryland). Solids analysis (TSS and VSS) was conducted in accordance with Standard Method sections 2,450 D and E (1995). COD analysis (total COD and soluble COD) was performed using high range (0–1,500 mg COD/L) colorimetric COD vials (Lovibond, Sarasota, Florida). High purity glycerol (assay > 99.5%) purchased from Fisher Scientific was used in this study. The glycerol concentration of the biodiesel waste was determined by two different methods: a) the modified colorimetric method (Bondioli & Della Bella 2005) in the environmental laboratory at the University of Central Florida and b) the high performance liquid chromatography (HPLC) method at Mid-West Laboratories (Omaha, Nebraska).

RESULTS AND DISCUSSION

Phase 1

In phase 1, reactors were run for eight successive weeks. However, the analysis of data from the last four weeks was taken in to final evaluation (it was assumed that the reactors achieved steady state conditions after three weeks). The VFA production was calculated by subtracting the VFA concentration in fresh primary solids going inside the reactor from the VFA concentration in the reactors at the end of each cycle (note that the same volume entered and exited the reactor at the beginning and end of each cycle). Experimental results showed that the VFA production in low-mixed reactors was higher than that of highly mixed ones. Table 4 shows the VFA production with respect to the mixing intensity. Although outliers were seen in some sampling events, overall the VFA production in Reactor # 1 with 0 rpm and Reactor # 2 with 7 rpm was much higher than that of high intensity mixed reactors. Reactor # 5 with 100 rpm showed the lowest VFA production consistently. The average VFA production in Reactor # 1 to Reactor # 5 was, in order, 9,787 ± 3,601 mg COD/L, 9,081 ± 3,151 mg COD/L, 7,057 ± 2,535 mg COD/L, 4,973 ± 3,628 mg COD/L and 3,927 ± 1,175 mg COD/L. The most probable reason for such a high standard deviation is because sampling was conducted in different weeks, so the wastewater and the primary solids characteristics, including the initial VFA, soluble COD and solids concentration, were different from one week to another. Moreover, although the medium was completely mixed at the end of each cycle and the sampling was conducted in duplicate because of the thickness of the medium, there was a high chance that the characteristics of the medium was varied from one point to another, specifically in un-mixed reactors, which showed a higher standard deviation. Regardless, the trend of lower VFA production as mixing intensified was consistent for all five reactors without a single exception. Since only 1,217 mg COD/L pure glycerol was added in Phase 1, most of the VFAs had to originate from the primary solids, and the results with respect to mixing can be generalized to primary prefermenters with no glycerol addition. Since much of the propionic acid must have originated with the primary solids, it may be that organisms producing propionic acid from glycerol used

<p>| Table 4 | Average acetic acid, propionic acid, butyric acid, valeric acid and VFA production in five reactors during phase 1 |</p>
<table>
<thead>
<tr>
<th>Reactor</th>
<th>Mixing (rpm)</th>
<th>Acetic Acid* (mg COD/L)</th>
<th>Propionic Acid (mg COD/L)</th>
<th>Butyric Acid (mg COD/L)</th>
<th>Valeric Acid (mg COD/L)</th>
<th>VFA (mg COD/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1</td>
<td>0</td>
<td>487 ± 607</td>
<td>5,397 ± 2,276</td>
<td>1,341 ± 360</td>
<td>2,562 ± 621</td>
<td>9,787 ± 3,601</td>
</tr>
<tr>
<td>R2</td>
<td>7</td>
<td>468 ± 761</td>
<td>4,564 ± 1,753</td>
<td>1,445 ± 287</td>
<td>2,605 ± 748</td>
<td>9,081 ± 3,151</td>
</tr>
<tr>
<td>R3</td>
<td>30</td>
<td>329 ± 482</td>
<td>2,530 ± 1,147</td>
<td>1,500 ± 426</td>
<td>3,356 ± 1,184</td>
<td>7,057 ± 2,535</td>
</tr>
<tr>
<td>R4</td>
<td>50</td>
<td>656 ± 1,229</td>
<td>2,250 ± 1,491</td>
<td>1,334 ± 468</td>
<td>2,045 ± 845</td>
<td>4,973 ± 3,628</td>
</tr>
<tr>
<td>R5</td>
<td>100</td>
<td>271 ± 969</td>
<td>1,773 ± 305</td>
<td>767 ± 169</td>
<td>1,428 ± 184</td>
<td>3,927 ± 1,175</td>
</tr>
</tbody>
</table>

*The negative values show minor acetic acid consumption in the Reactor #3 and #4.
the associated enzymes to ferment primary solids also. Solubilization occurred in the reactors. The SCOD/TCOD of the reactors’ influent varied between 0.07 and 0.09, and it was increased up to 0.23 at the end of some treatment cycles. More VFAs at lower mixing for primary solids alone is also supported by the literature (Denesh & Oleszkiewicz 1997). In the current study, lowering the mixing energy from 100 rpm to 0 rpm increased the propionic acid production almost three times on average. On the other hand, acetic acid production was inconsistent, and in some cases, acetic acid consumption was observed. However, the amount was small, implying that acetoclastic methanogenesis was not significant. TCOD data showed little decrease, which was also consistent with low methanogenesis. Propionic acid was consistently produced, and production was much higher at 0 compared with 7 rpm. Butyric acid production was significant but did not vary much with mixing except at 100 rpm. Valeric acid production was very significant and peaked at 30 rpm, where its concentration exceeded that of propionic acid. Total VFA correlated inversely with mixing, largely due to propionic acid.

Table 5 shows the specific VFA production rate of the five reactors. Reactor # 1 (0 rpm) had the highest VFA specific production rate (30.3 mg COD/g VSS/h). Specific and volumetric VFA production rates inversely correlated with mixing for the most part. Comparing the results from the current study (Table 5) with the literature shows that the production rates in this study were much higher than those of the literature except for the 50 and 100 rpm reactors (i.e. R4 and R5 in Table 5). For example, specific rates in the literature varied from 2.95 to 3 mg COD/g VSS/h, and 11.98 when 750 mg COD/L of molasses was added (Danesh & Oleszkiewicz 1997; Zeng et al. 2006). The mixed liquor volatile suspended solids (MLVSS) in these studies varied from 2.7 to 8.45 g/L. Bengtsson et al. (2008) did manage to achieve 55 ± 6.3 mg COD/L/h volumetric rates, but that was with dairy whey wastewater, not domestic primary solids. The higher rates in Table 5 might be related to the higher MLVSS solids concentration in the study and the higher soluble COD content (e.g. external substrate addition), or it may be due to high mixing in the references from the literature (unknown).

A hypothesis to explain phase 1 data

A plausible theory to explain our observations is that in non-methanogenic pre fermenters acetic acid is a hydrogen producing reaction and propionic, butyric, and valeric acid (i.e. C3 to C5 VFAs) are hydrogen consuming reactions, much as they are in methanogenic processes (Vavilin et al. 1995; see Figure 1). The balancing of the reactions depends on hydrogen transfer from obligate hydrogen producing acetogens (OHPA) to hydrogen consuming acidogens that produce C3 to C5 VFAs such as those observed in this study (i.e. propionic, butyric, and valeric acids). Just as in methanogenic reactors, hydrogen transfer requires proximity of the OHPA to the hydrogen consumers (Speece 2008). It has been shown that completely mixed reactors (e.g. continuously stirred tank reactors or CSTRs) result in poor hydrogen transfer between OHPA and hydrogen consuming methanogens (in methanogenic reactors) relative to stratified or biofilm reactors (Speece 2008). This same phenomenon is consistent with the lower C3 to C5 production, and lower total VFA production, observed in the highly mixed reactors of this study. To prove this, further work would need to be conducted, but it does provide a plausible explanation for understanding why lower mixing is better for prefermenter performance. It also provides a hypothesis that could serve as a starting point for future research.

As is shown in Table 4, VFAs were produced mostly in the form of propionic acid, whereas acetic acid production in the reactors was the lowest of the C2 to C5 VFAs. A slight COD depression at the end of some sampling cycles (24 h fermentation) in addition to the acetic acid consumption in some observations suggested that there may

Table 5 | VFA specific and volumetric production rates in five reactors during phase I. All reactors were dosed with 1,217 mg COD/L pure glycerol

<table>
<thead>
<tr>
<th>Reactor #</th>
<th>Mixing (rpm)</th>
<th>MLVSS (g/L)*</th>
<th>Total VFA (mg COD/L)</th>
<th>Specific rate (mg COD/g VSS/hr)</th>
<th>Volumetric rate (mg COD/L/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1</td>
<td>0</td>
<td>15.7</td>
<td>9,787 ± 3,601</td>
<td>30.3 ± 29.1</td>
<td>101.9 ± 37.6</td>
</tr>
<tr>
<td>R2</td>
<td>7</td>
<td>17.4</td>
<td>9,081 ± 3,151</td>
<td>20.3 ± 7.4</td>
<td>94.9 ± 32.8</td>
</tr>
<tr>
<td>R3</td>
<td>30</td>
<td>16.1</td>
<td>7,057 ± 2,535</td>
<td>16.2 ± 12</td>
<td>73.5 ± 26</td>
</tr>
<tr>
<td>R4</td>
<td>50</td>
<td>15.3</td>
<td>4,973 ± 3,628</td>
<td>7.7 ± 10</td>
<td>51.8 ± 37.8</td>
</tr>
<tr>
<td>R5</td>
<td>100</td>
<td>15.8</td>
<td>3,927 ± 1,175</td>
<td>9.4 ± 2.4</td>
<td>40.9 ± 11.5</td>
</tr>
</tbody>
</table>

*The presented value for R1, R2, R3, R4 and R5 was the average of four, seven, five, three, and four observations for which solids data were available.
have been at most a minor amount of acetoclastic methanogenesis active in the reactors. The average VFA production and composition in the reactors are stated in Table 4. Experimental results from Phase 1 showed that the COD value of VFAs that were produced during the fermentation process of all reactors was higher than the COD added into the reactors in the form of glycerol. This meant that most of the VFAs were generated from primary solids fermentation. Therefore, in order to increase the contribution of the glycerol to the fermentation process, the primary solids were diluted with the wastewater in the same week at a ratio of 1 to 1 (V: V), and the initial glycerol concentration was increased from 1,000 mg/L to 4,333 mg/L.

### Phase 2

Experimental results of Phase 2 revealed that, compared with the Control Reactor (#1), adding either pure glycerol or biodiesel waste increased the VFA production in the reactors, especially in Reactor # 2, Reactor # 3 and Reactor # 5 (Table 6). However, the biodiesel waste-fed reactors had the best performance. Although the glycerol concentration in the biodiesel waste was about 20%, the high VFA production might be related to the other organics/carbon sources present in the biodiesel waste (COD analysis showed that the COD of biodiesel waste was 1.95 mg COD/mg-biodiesel waste, which is 60% higher than that of pure glycerol at 1.217 mg COD/mg-glycerol). Reactor # 4 (7 rpm, pure glycerol) had the lowest efficiency among the fed reactors, with no VFA production in half of sampling events and a slight production in the other half. It produced more VFA than the Control Reactor (#1) but far less than the other reactors. The reason for its failure is uncertain, since the 0 rpm reactor dosed with glycerol (#2) produced over 3,400 mg/L VFA (Table 6). The Control Reactor (#1) had diluted primary solids but produced very little VFAs, but Reactors # 3 and # 5 produced VFAs as COD equal to the COD of their biodiesel waste dosages (Table 6). Table 7 shows that VFA volumetric production rates were high compared with most of the reactors in Phase 1 (Table 5) and specific rates were higher.

Phase 2 data show that biodiesel waste (or at least this specific biodiesel waste) can be used to enhance VFA production in prefermenters. The results with glycerol were inconsistent in Phase 2, but it is likely that the 7 rpm reactor (#4) was the outlier of the two.

Comparing the results from Reactor # 1 of Phase 1 (0 rpm, 1,217 mg COD/L pure glycerol, 15,700 mg/L MLVSS) with Reactor # 2 of Phase 2 (0 rpm, 5,273 mg COD/L pure glycerol, 5,434 mg/L MLVSS) revealed that although Reactor # 2 of Phase 2 was dosed with a significantly higher amount of glycerol, it produced less VFA than Reactor 1 of phase 1 (the average VFA in Reactor # 1 of phase 1 and Reactor # 2 of Phase 2 were 9,821 ± 3,597 mg COD/L and 3,415 ± 1,856 mg COD/L, respectively). This difference was most likely related to the lower influent VSS concentration of Reactor 2 during Phase 2 (8,043 mg VSS-COD/L) compared with that of R1 (23,236 mg VSS-COD/L), since VSS solubilization and fermentation was a major part of VFA production along with glycerol in Phase 1. Average COD mass balances showed no significant change in TCOD across the reactors, and thus no methanogenesis took place in the reactors.

### Table 6 | Average VFA production and composition in five reactors at room temperature and SRT of four days during phase 2 (the presented data are the average of eight data points)

<table>
<thead>
<tr>
<th>Mixing (rpm)</th>
<th>Substrate</th>
<th>Dosage (mg COD/L)</th>
<th>Acetic acid (mg COD/L)</th>
<th>Propionic acid (mg COD/L)</th>
<th>Butyric acid (mg COD/L)</th>
<th>Valeric acid (mg COD/L)</th>
<th>VFA (mg COD/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1</td>
<td>0</td>
<td>–</td>
<td>–48 ± 157</td>
<td>43 ± 88</td>
<td>27 ± 45.3</td>
<td>0</td>
<td>22 ± 227</td>
</tr>
<tr>
<td>R2</td>
<td>0</td>
<td>G</td>
<td>5,274</td>
<td>506 ± 789</td>
<td>1,075 ± 419</td>
<td>393 ± 813</td>
<td>1,441 ± 1,690</td>
</tr>
<tr>
<td>R3</td>
<td>0</td>
<td>BDW</td>
<td>8,450</td>
<td>–368 ± 134</td>
<td>5,386 ± 1,412</td>
<td>23 ± 54</td>
<td>3,371 ± 1,316</td>
</tr>
<tr>
<td>R4</td>
<td>7</td>
<td>G</td>
<td>5,274</td>
<td>–299 ± 222</td>
<td>147 ± 372</td>
<td>100 ± 236</td>
<td>332 ± 437.2</td>
</tr>
<tr>
<td>R5</td>
<td>7</td>
<td>BDW</td>
<td>8,450</td>
<td>–269 ± 188</td>
<td>5,049 ± 1,891</td>
<td>214 ± 220</td>
<td>3,909 ± 2,300</td>
</tr>
</tbody>
</table>

*G = glycerol.

*BDW = biodiesel waste.

*The negative values show minor acetic acid consumption in corresponding reactors.
Phase 1 mass balances for all reactors showed an average decrease in TCOD of 5.2% (STD DEV = 21.1%). In Phase 2 there was an average increase of 8.4% (STD DEV = 21.6%). Considering the high background level of TCOD and the relatively small ΔTCODs, these averages are insignificant and probably just represent zero change in ΔTCOD, with analytical variability causing the observed values. pH is another factor that needs to be discussed, since pHs less than the pKas of the acids (acetic pKa = 4.76; propionic pKa = 4.7, etc.) result in the undissociated acids being the dominant form. These are cell membrane permeable and often inhibitory to fermentation. In this study the results from Phase 1 showed final pH to be lower than the pKas for all the acids quantified, yet VFA production correlated with mixing. In Phase 2 there was a much broader range of final pHs (3.9 – 5.8), but the VFA production did not correlate with pH.

**CONCLUSIONS**

- Low or no mixing favored production of C₃ and/or C₅ volatile fatty acids or VFAs (propionic and/or valeric acid) and also increased the total amount of VFAs produced. Propionic acid was the main VFA to increase as mixing intensity decreased. This has important operational implications for prefermenters and potentially any downstream EBPR process.
- The increased production of C₃ to C₅ VFAs under low mixing conditions may have been due to improvements in hydrogen transfer from OHPAs to acidogenic fermenters.
- The biodiesel waste-fed reactors had a better performance than the pure glycerol-fed reactors in terms of VFA production and VFA production rate. The main VFA was still propionic acid, with significant valeric acid production. Although the glycerol concentration in biodiesel waste-fed reactors was less than that of pure glycerol-fed reactors (glycerol constituted 20% of the biodiesel waste), other carbon sources present in the biodiesel waste were probably involved in VFA production.
- Primary solids were an important part of VFA production along with glycerol. It may be that some of the bacteria capable of fermenting the glycerol to propionic acid also used the associated enzymes to ferment primary solids, and this would explain why propionic acid was the dominant end-product even when primary solids were the dominant substrate.

**ACKNOWLEDGEMENTS**

The authors wish to thank the City of Orlando’s Wastewater Division and the Staff at the Iron Bridge Regional Water Reclamation Facility and the city of Lakeland, Florida, Glendale Wastewater Treatment Plant. This work was funded by SAKAL LLC, a United States subsidiary of Grupo Lakas SA.

**REFERENCES**


First received 5 September 2017; accepted in revised form 27 December 2017. Available online 10 January 2018