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**Optimization of hydraulic retention time and organic loading rate for volatile fatty acid production from low strength wastewater in an anaerobic membrane bioreactor**

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## Abstract

This study aims to investigate the production of volatile fatty acids (VFAs) from low strength wastewater at various hydraulic retention time (HRT) and organic loading rate (OLR) in a continuous anaerobic membrane bioreactor (AnMBR) using glucose as carbon source. This experiment was performed without any selective inhibition of methanogens and the reactor pH was maintained at  $7.0 \pm 0.1$ . 48,24,18,12,8 and 6 hr - HRTs were applied and the highest VFA concentration was recorded at 8 hrs with an overall VFA yield of  $48.20 \pm 1.21$  mg VFA/100 mg COD<sub>feed</sub>. Three different OLRs were applied (350, 550 and 715 mg COD<sub>feed</sub>) at the optimum 8 hr-HRT. The acetic and propanoic acid concentration maximums were ( $1.1845 \pm 0.0165$  and  $0.5160 \pm 0.0141$  mili-mole/l respectively) at 550 mg COD<sub>feed</sub>. The isobutyric acid concentration was highest ( $0.3580 \pm 0.0407$  mili-mole/l) at 715 mg COD<sub>feed</sub> indicating butyric-type fermentation at higher organic loading rate.

**Keywords** Volatile fatty acids, anaerobic membrane bioreactor, low strength wastewater, hydraulic retention time, organic loading rate

## 1. Introduction

In past decades, anaerobic bioreactors have been utilized to recover value-added chemicals and bioenergy from different waste materials (Khan et al., 2016a; Wang et al., 2018). The recent development in this technology includes coupling a membrane module with conventional an anaerobic digestion system for treating industrial and municipal wastewater (Liu et al., 2018). Although biogas has been considered as the primary resource to be recovered from wastewater, research studies have proven the technical and economic feasibility for recovering VFA and biohydrogen from anaerobic bioreactors (Liu et al., 2018; Xin et al., 2017).

So far, industrial application for anaerobic membrane bioreactors has been limited because the product revenue earned from this process is not constant. This is because of the variable production rate of VFA, biohydrogen, and methane due to the frequent change of carbon content in feed wastewater (Khan et al., 2016b; Pretel et al., 2016). In addition to this discussion, recovering VFA and biohydrogen, where biogas production can improve revenues earned from anaerobic processes and eventually improve the economic feasibility of large-scale production. Although for treating municipal wastewater, the influent COD typically varies from 150 to 350 g/L, it can be concentrated further for resource recovery purposes (Ji et al., 2014; Zheng et al., 2018).

A few studies have analyzed the economic feasibility of VFA production in contrast to biogas. The results demonstrated that production difference between VFA and the methane-containing biogas from the anaerobic process for VFA produced higher revenue compared to biogas (Khan et al., 2016b; Kleerebezem et al., 2015). VFAs have been identified as raw materials for biopolymers like Polyhydroxyalkanoate (PHA). It can also be used as a potential precursor for valuable organics, for example, alcohols, ketones, and aldehydes, biogas, biohydrogen and biodiesel (Khan et al., 2016a; Lee et al., 2014; Tao et al., 2016). VFAs are short-chain fatty acids that are produced through initial hydrolysis and the acidogenic phase in anaerobic digestion. During this process carbohydrates, proteins and fats are hydrolysed into amino acids, sugar, and fatty acids. The hydrolysis process is followed by acidogenesis where VFAs,  $\text{BioH}_2$ , and  $\text{CO}_2$  are produced. VFAs produced from initial two stages are consumed by the methanogens at the final anaerobic stage to produce biogas (Begum et al., 2018; Scoma et al., 2016).

Production of VFA can be undertaken in two major ways: Firstly, VFA can be produced as the main product of anaerobic digestion (Aydin et al., 2018). This production type involves

the inhibition of the methanogenesis process so that the methanogens cannot consume the VFA during the final stage of the anaerobic process to convert them into biogas. The selective inhibition of methanogenesis is mainly carried out by heat shock and load shock treatment. Additionally, acidic and alkaline pH treatments are also applied for selective inhibition of methanogens (Khan et al., 2016a). These methanogens have been reported to have optimum microbial growth at a pH range of 6.5 to 8.2 (Mao et al., 2015), therefore reducing the pH below 6.5 or above 8.2 can be applied to inhibit the activity of methanogens. Secondly, VFA can be simultaneously produced with biogas. This production technology involves the anaerobic process where the initial stage of acidogenesis and final stage of methanogenesis are separated through a multiple stage bioreactor design (Li & Yu, 2011; Schievano et al., 2014). In this experiment, the first type of production scheme was used to produce VFA without any selective inhibition of methanogenesis process. The reason for this approach is to improve the industrial application of the AnMBR as following the operating conditions any existing AnMBR model can be tuned to produce VFA without any design alterations.

For a specific bioreactor design, production of VFA directly depends on temperature, pH, Hydraulic Retention Time (HRT), Organic Loading Rate (OLR), pre-treatment methods of the sludge and chemical additives (Garcia-Aguirre et al., 2017; Jankowska et al., 2018; Khan et al., 2016a; Peces et al., 2016). Among these conditions, hydraulic retention time and organic loading rate can both be changed directly based on the feed composition. Kuruti et al., (2017) states that a general decrease in HRT increases the VFA production through anaerobic acidification (Kuruti et al., 2017). However, the value at which the highest VFA yield and production rate of VFA would be achieved depends on bioreactor design, microbial community, and feed characteristics. In contrast, it has been identified that an initial increase in the loading rate increases the VFA production but at the same time can affect membrane

fouling and bioreactor performance in terms of COD and nutrients removal (Khan et al., 2016b; Mao et al., 2015). An optimum value for OLR for any anaerobic process depends on the bioreactor design, and an OLR above the optimum value reduces the production of VFA significantly. Slezak et al., (2017) studied the effect of organic loading rate for VFA production in dark fermentation and identified the VFA concentration increases only up to the initial OLR of 48.2g VS/L (Slezak et al., 2017). Increasing the OLR also effects the VFA composition in the product stream. Wijekoon et al. (2011) identified that the predominant VFA component changed from acetic acid to n-butyric acid with an overall increase in VFA concentration when OLR was increased from 5 to 12 kg COD m<sup>-3</sup> d<sup>-1</sup> in a two-stage thermophilic anaerobic membrane bioreactor (Wijekoon et al., 2011).

Although numerous studies were carried out to optimize the production of VFA, most of them utilized anaerobic digestion (AD) process. A very limited number of researches performed to produce VFA from ANMBR where potential membrane fouling is an important area of concern. Additionally, the available studies aimed to optimize VFA production mainly used different inhibition process for methanogenesis.

This is the first research which aims to find out the optimum hydraulic retention time and loading rate in the AnMBR treating low strength (synthetic) wastewater. The first stage of this experiment includes AnMBR operation in six different HRTs for 48, 24, 18,12,8 and 6 hrs. In the second stage, the same bioreactor was used with three different organic loading rates using 350, 550 and 715 mg/l COD of synthetic wastewater.

## **2. Materials and methods**

### **2.1 Characteristics of sludge and feed solution**

The AnMBR used for this reaction had mixed liquor seed sludge from two different water treatment plants in Sydney, Australia (Cronulla water treatment plant and Central Park water

treatment plant). At the beginning of each experiment, the sludge in the reactor had a mixed liquor suspended solid concentration of 10 g/l. The system was purged with nitrogen to get any unexpected air and oxygen out with diffused aeration tubes. The sludge mixture (30:70 ratios from Cronulla and Central Park respectively) was acclimatized in the reactor for 90 days until a constant COD and nutrient removal was obtained. Characteristics for mixed sludge and feed solution have been listed in Table 1.

Table 1: Characteristics for seeding sludge and feed solution

## 2.2 Experimental setup

A 3.9 L column was used in this experiment with provisions for effluent recirculation, biogas sparging and nitrogen purging from the bottom (see Figure 1). The system had a working volume of 3.5 L. The pressure sensor at the top measured the pressure in the reactor. Hollow fibre membrane (PVDF, Pore size 0.07 – 0.1  $\mu\text{m}$ ) with the inner and outer diameter of 1.0 and 2.2 mm respectively was used for this experiment. The membrane had a surface area of 0.08  $\text{m}^2$ .

Figure 1: Schematic of experimental setup for VFA production

## 2.3 The operation of anaerobic membrane bioreactor

For this experiment, the operation of anaerobic membrane bioreactor can be divided into two different stages. The first stage involves the operation of AnMBR at different hydraulic retention times (HRTs). VFA samples were analyzed at 48, 24, 12, 8 and 6 hrs of HRT. The influent COD was kept constant at 550 mg/l with an OLR of 68.75 mg COD/l.hr. The second stage involved producing VFA at different organic loading rates. The loading rates were changed by varying the influent COD in the feed wastewater. Influent COD was varied to 350, 550 and 715 mg/l with corresponding OLR of 43.75, 68.75 and 89.38 mg COD /l.hr, respectively, by keeping the HRT fixed at 8 hrs. Glucose,  $\text{NaNO}_3$ , and  $\text{KH}_2\text{PO}_4$  were used as

the main sources of carbon, nitrogen, and phosphorus respectively. The C: N: P ratio was kept constant at 100:5:1. Each trial for HRT and OLR involved 21 days of AnMBR operation in continuous mode. All relevant reactor operation conditions have been listed in Table 2.

Table 2: AnMBR Operating conditions

Throughout the period of stage 1 and 2 of bioreactor operation, the was maintained at  $7.0 \pm 0.1$  and the ambient temperature in the laboratory was kept constant at  $22 \pm 1$  °C pH fixed at 7.0. Referring the information provided in the introduction section (optimum microbial growth of methanogens at pH 6.5 – 8), no inhibition process was applied to suppress methanogenic activity.

## 2.4 Analytical methods

### 2.4.1 Solvent extraction

For preparing the sample, reactor effluent was collected and acidified to pH 2.0 to avoid any further biodegradation by the microorganisms. In order to get rid of the possible suspended matter, the acidified sample was centrifuged to 3500 rpm for 30 minutes. The next step involved taking a 4 ml sample and the addition of 1 g NaCl followed by the extraction with MTBE (2 ml). Once emulsion was formed, the sample was again centrifuged at 4000 rpm for 5 minutes.

The additional step of centrifuging was performed to break the emulsion. Once the separate organic phase was observed, it was collected with a syringe. The whole extraction process with MTBE was repeated for another time to make sure no organic content was left after extraction. Finally, the extracts were added together and taken to a separate test tube. The removed anhydrous  $\text{Na}_2\text{SO}_4$  was added to make sure no water was resent before the sample was subjected to GC-MS. After dehydration, the sample was filtered using a  $0.22 \mu\text{m}$  syringe



filter to ensure the removal of suspended particles from GC-MS. The composition of fatty acid components was measured based on the retention times and mass spectra of peaks on the chromatograms derived from the extracted sample and the standard VFA solution (Banel & Zygmunt, 2011).

#### 2.4.2 Quantification of VFA from GC-MS

Individual VFA concentrations were measured by gas chromatogram mass spectrometry method (GC-MS TQ8040, Shimadzu, Japan). For each measurement, the open tubular analytical column was used (VF-WAXms, Agilent, U.S). Helium was used as carrier gas with a flow rate of 2.05 mL/min. The temperature program started at 50°C and was held for 5 min before ramping to 250°C at 10°C/min and was then held for 10 min. Electron impact ion source was set at 230°C while the injection port and transfer line temperatures were held at 230°C. Mass spectrometer (MS) operated in a selected ion monitoring (SIM) mode and in a full scan mode ( $m/z$  15-550). Ions for detection of individual VFA in SIM mode were selected using the mass spectra of standards generated in SCAN mode.

### 3. Results and discussion

#### 3.1 AnMBR performance in COD and nutrients removal

For each stage of the AnMBR operation, bioreactor performance was analyzed in terms of COD and nutrient removal efficiency. Reactor effluents were added every 4 days during each trial for HRT and OLR. Figure 2 displays the efficiencies for COD, nitrate, and phosphorus removal at different HRTs and loading rates.

Figure 2: Nutrient and COD removal performance of continuous AnMBR

From Figure 2, it is evident that the COD removal performance was steady at approximately 70 % throughout the trials for both stages of operation.  $\text{NO}_3^-$  removal performance was maximum at the longest HRT (48 hrs) referring to the condition where the microorganisms were allowed enough time to undertake the denitrification process. As HRTs became shorter, a slight decrease in the  $\text{NO}_3^-$  removal was observed. The reason may be associated with the fact that the contact time between the feed wastewater and the denitrifying bacteria was lowered and shorter HRTs. Additionally, as the influent COD was kept constant at  $550 \pm 10$  mg/l, the organic loading rate was also increased at shorter HRTs. As the denitrification process involved processing the high organic loading, the minor decrease in the nitrate removal was expected (Wang et al., 2018). However, a minimum removal efficiency of 93.2% indicates efficient denitrification process in the bioreactor. As expected, the anaerobic process had a steady  $\text{PO}_4^{3-}$ , the removal efficiency from 0.9 to 4.6% throughout the experiment.

During the second stage, at loading rates of 350 and 550 mg COD/l, the lowest COD removal efficiency was steady at about  $70.9 \pm 1.1\%$ .  $\text{NO}_3^-$  removal was observed above  $98.2 \pm 1.7\%$  with a maximum removal efficiency of  $99.4 \pm 0.1\%$ .  $\text{PO}_4^{3-}$  removal was steady within the range of  $0.9 \pm 0.2\%$  to  $1.8 \pm 0.1\%$ . Instead, at a loading rate of  $715 \pm 10$  mg COD/l, the COD removal efficiency dropped to  $65.1 \pm 2.2\%$  and consequent  $\text{NO}_3^-$  removal efficiency dropped at  $91.9 \pm 0.5\%$ . The deterioration in the general AnMBR may be associated to multiple facts like momentary pH drops due to VFA accumulation and less contact time between the biomass and feed solution (Khan et al., 2016a; Mao et al., 2015)

### 3.2 Membrane fouling of AnMBR

Properties of the membrane, sludge characteristics, wastewater properties, and operating conditions are the key factors that control the membrane fouling in a bioreactor (Guo et al., 2012). For this experiment, the same type of membrane, sludge, and synthetic feedwater were used throughout the experiment except for changes in the HRT (stage 1) and OLR (stage 2). Instead of discussing the mechanism of membrane fouling, this section includes discussions on how different hydraulic retention times and organic loading rates change the fouling pattern in the AnMBRs. MLVSS was fixed at 10 g/L at different HRTs and membrane fouling was measured in terms of Trans Membrane Pressure (TMP). All data have been plotted in Figure 3(a) and (b).

#### **Figure 3** Variations of TMP at different operating conditions

In biological wastewater treatment, polysaccharides, EPS and organic colloids are the major contributors to membrane fouling in Membrane Bioreactors (MBRs). Additionally, the Natural Organic Matter portion in DOC, carboxylic acids, proteins, and amino acids have also been identified to have a significant effect on membrane fouling. At higher organic loading rates and shorter HRT, the trace nutrients in the synthetic wastewater ( $\text{Ca}^{+2}$ ,  $\text{Mg}^{+2}$ ,  $\text{Fe}^{+3}$ ) are perhaps responsible for creating inorganic fouling in the membrane (Guo et al., 2012).

In the beginning, the little or no significant TMP development was observed (0-7.6 Kpa) for 48 and 24 hr HRT. The results indicated that the soluble organics, trace nutrients and carboxylic acid concentration was not high enough to develop the TMP above 7.6 Kpa. However, TMP developed at a faster rate (22.5 and 33.2 and Kpa after 21 days) for 18 and 12 hrs. This is because at this loading rate the soluble organics and the nutrients present in the

feed water started to foul the membrane surface. The manufacturers' recommendation was to change/clean the membrane module once the TMP exceeds 30 Kpa, therefore during these 21 hrs of operation membrane cleaning was not performed.

At shorter HRTs (8 and 6 hrs) a steady increase in TMP development was observed until day 11 (up to 13.6 Kpa). The steady increase was followed by a rapid TMP development for both 8 and 6 hr HRT (35.2 Kpa at the end of day 20 and 35.1 Kpa at the end of day 16 respectively). The first stage of fouling (up to day 11) behaviour suggests the deposition of the foulants on the membrane surface. Later, the rapid rise in the TMP indicates blockage of the membrane pores by the formation of biofilms. Note that the results after membrane cleaning are not included in Figure 3 (a).

At low HRT, bacterial cell releases extracellular polymeric substances that eventually increases the SMP content and deflocculates the sludge. Additionally, at very low HRT, oversized and irregular flocs may have been formed in addition with the production of filamentous bacteria. The combination of these factors might have been responsible for the membrane fouling observed in 8 and 6 hr of HRT.

Membrane material also plays an important role in anaerobic wastewater treatment. Clark & Heneghan, (1991) mentioned that hydrophobic membrane materials are to suffer more membrane fouling than hydrophilic membranes (Clark & Heneghan, 1991). For this experiment, polyvinylidene fluoride (PVDF) was used as the material for membrane fabrication. As PVDF is chemically hydrophobic, a higher extent of membrane fouling was expected in this experiment.

The results for membrane fouling at different organic loading rates correlated closely with the results observed at different HRTs. These different organic loading rates (43.75, 68.75 and 89.38 mg COD/l.hr) were applied using synthetic wastewater having COD of 350, 550 and

715 mg/l. As maximum VFA concentration was found at 8 hrs of HRT at the first stage of this experiment, for all the organic loading rates, HRT was kept constant at 8 hrs.

The results found in this step of this experiment were interesting. When the influent COD was dropped to 350 mg/l (corresponding loading rate of 43.75 mg/l.h) TMP only went up to 24.9 Kpa at the end of day 21 compared to than that of 35.2 Kpa at the end of 20 days of operation using 550 mg/l COD in the feed. The lower organic loading at 350 mg COD/l involved less amount of organic acid, EPS and organic colloids deposit in the membrane surfaces. Therefore, membrane fouling was not severe at this organic loading.

In contrast, for 715 mg COD in the feed wastewater (loading rate of 89.38 mg/l.h), a rapid 35.4 KPa TMP was developed at the end of 15 days of operation. At this operating condition, high amounts of SMP and bound EPS were generated that resulted in a decrease in sludge filterability and filtration index. As the concentration of different foulants were high due to the high loading rate, a combination of these factors may be responsible for membrane fouling during this condition (Chen et al., 2018; Guo et al., 2012).

### **3.3 VFA concentration at different HRT**

The major components of VFA include acetic acid, propanoic acid, butyric acid, and valeric acid. The components are mainly produced in the acidogenic phase of anaerobic digestion.

Among these VFA components, acetic, propanoic and butyric acids are predominant during VFA production from the anaerobic process. According to literature, 65 to 95% of methane present in biogas is directly produced from butyric and acetic acid (Khan et al., 2016a; Mamimin et al., 2017; Morgan-Sagastume et al., 2011).

Table 3: Concentration of VFA components at different HRT

From the experiment, it has been observed that the acetic acid concentration was nearly doubled (from  $0.4922 \pm 0.0134$  to  $0.8321 \pm 0.0160$  mili-mole/L) when the HRT was reduced from 48 hrs to 24 hrs. Production of acetic acid was increasing gradually when HRT was shortened and the maximum concentration was achieved at 8 hrs ( $1.1845 \pm 0.0165$  mili-mole/l). The change from 48hrs to 24 hrs indicates a shift in microbial activity from methanogenesis to acidogenesis. A gradual increase in acetic acid concentration was observed when the HRT was reduced further to 18,12 and 8 hrs. These trials with shorter HRT involved higher organic loading rates as the COD of influent wastewater was kept constant at COD of  $550 \pm 10$  mg/l. Although the increase in acetic acid concentration had the highest degree of increase during the first change from 48hrs to 24 hrs, the following increase in the trend for acetic acid was associated with the high amount of organics and nutrients loading the bioreactor at a fixed MLVSS of  $10.1 \pm 0.1$ .

A further decrease in the HRT (from 8 to 6 hrs) reveals a drop in acetic acid concentration to  $1.0095 \pm 0.008$  mili-mole/l. Although the initial decrease in HRT supported acetic acid production, an HRT below 8 hrs indicates an imbalance in the initial hydrolysis and acidogenesis process. More explicitly, the high amount of organics fed into the reactor at this HRT had a faster rate of initial hydrolysis whereas the acidogenic bacteria could not perform their action by consuming amino acids, sugar and other fatty acids that are produced through the initial hydrolysis process in anaerobic digestion.

For isobutyric acid, the concentration initially increased from  $0.1128 \pm 0.008$  to  $0.2836 \pm 0.0005$  mili-mole/l when the HRT was shortened from 48 to 24 hr period. Similarly, for acetic acid, the results suggest that the methanogens could not convert the produced VFA into methane and  $\text{CO}_2$  during this change (Braguglia et al., 2018). However, no significant rise in the butyric concentration was observed when in shorter HRTs (18,12,8 and 6 hrs). The

reasons may be associated with operating pH factors in the reactor. According to the literature, pH values of 6.0-7.0, 4.0-5.0 and 11.0 have been referred as optimum for acetic acid, propanoic acid, and butyric acid production respectively (Begum et al., 2018; Lin & Li, 2018; Yu & Fang, 2003). As the experiment involved maintaining the reactor pH level to  $7.0 \pm 0.1$ , the butyrate type fermentation was not predominant during this experiment.

A gradual increase in the concentration of propanoic acid has been observed when the HRT was shortened from 48 hrs to 6 hrs. The highest concentration was observed at 6 hrs ( $0.5293 \pm 0.03$  mili-mole/l). Propanoic acid, unlike acetic and butyric acid, remains unconverted during the final stage of anaerobic digestion as the conversion is thermodynamically less favourable compared to the other two major VFA components (Yu et al., 2016). As a result, it accumulates in the bioreactor at high organic loading rates or shorter HRT. The literature explains that this single VFA component is responsible for rapid acidification in anaerobic bioreactors that eventually leads to the conditions of microbial stress, sharp pH drop and reactor instability (Wang et al., 1999). For this study, the reactor pH was maintained to  $7.0 \pm 0.1$  by adjusting the pH of the feed solution. Therefore, the possibility of propanoic acid accumulation was very small during this experiment.

The remaining components present in our analysis included isovaleric, n-valeric, iso-caproic, n- caproic and heptatonic acid. Although no particular trend was observed in their concentration, an overall decrease in their concentration was observed when the HRT was shortened. The results may be associated with the fact that shorter HRT encouraged the production of major VFA components like acetic, butyric and propanoic acid. Consequently, VFA production shifted towards acetic, butyric and propanoic acid at shorter HRTs.

Table 3 also shows the overall percentage of VFA yield in mg VFA/mg COD in the feed solution. A change from 48hrs to 24 hrs records a rapid increase in VFA yield from  $13.39 \pm 1.21\%$  to  $32.88 \pm 2.56\%$ . In unit time, the carbon content in the feed solution increased in shorter HRTs, the VFA yield increased up to  $48.20 \pm 1.21\%$  at 8 hrs HRT. A further decrease in the HRT (6 hrs) caused a drop in the overall VFA yield  $42.32 \pm 2.32\%$  (mg VFA/mg  $\text{COD}_{\text{feed}}$ ) indicating insufficient contact time between the microbes and the feed solution. As discussed previously, the predominant VFA components (acetic, propanoic and butyric acid) only had a rise in concentration up to the HRT of 8 hrs. The drop in their individual concentration triggered an overall decrease of the VFA yield at 6 hrs operation.

Experiments have shown that, for VFA production, at pH 5.5 acetic acid is the major VFA component whereas at pH 11 butyric acid is the predominant VFA component (Begum et al., 2018; Jankowska et al., 2017). In this case, an alteration of reactor pH to acidic (pH 5.5) or alkaline (pH 11.0) can be beneficial for acetic and butyric acid production. Therefore, maximizing the concentration of individual VFA component can improve the overall VFA yield from this process.

### 3.4 VFA concentration at different OLR

Three different organic loading rates were applied (43.75, 68.75 and 89.38 mg COD /l.h) using an influent COD of 350, 550 and 715 mg/l at 8 hr HRT period. For 350 mg/l COD in feed solution, acetic, propanoic and isobutyric acid concentrations  $0.7602 \pm 0.014$ ,  $0.2707 \pm 0.011$  and  $0.2393 \pm 0.007$  mili-mole/l respectively with an overall VFA yield of  $35.39\% (\pm 3.52\%)$  mg VFA/ 100 mg  $\text{COD}_{\text{feed}}$ . At this loading rate, the soluble organics and nutrients were not enough for the acidogenic bacteria present in the reactor. Therefore, both individual VFA concentrations and overall VFA yield were relatively low during this condition.

Figure 4: VFA concentration at different OLR



At a loading rate of 550 mg/l COD in feed, highest acetic acid concentration was observed ( $1.1845 \pm 0.0165$  mili-mole/l) along with an increase for propanoic acid (from  $0.27070 \pm 0.03$  to  $0.5160 \pm 0.0104$  mili-mole/l). This initial increase in the influent COD increased the supply of organics and nutrients to the microbes that were performing acidogenesis.

However, at this loading rate the butyric acid concentration dropped  $0.2393 \pm 0.0406$  to  $0.2284 \pm 0.0023$  mili-mole/l. The subsequent drop in butyric acid concentration may be associated with the fact that it was degraded to acetic acid by acetic acid producing bacteria (Shen et al., 2018). Another reason for this drop in the butyric acid concentration is linked to the fact that, the system was not supported with the optimum pH (5.5 to 6.5) for butyrate type fermentation (Kuruti et al., 2017). An increase in the propanoic acid concentration was observed at this loading rate due to the reason that it was not consumed by any other acidogenic bacteria or methanogenic archaea (Khan et al., 2016a).

Finally, for 715 mg/l COD at the influent, there was a decrease in acetic acid and propionic acid concentration (from  $1.1881 \pm 0.0081$  mili-mole/l to  $1.1385 \pm 0.0081$  and  $0.5160 \pm 0.03$  to  $0.4167 \pm 0.03$  mili-mole/l respectively). In addition to this decrease, an overall drop in AnMBR performance was also observed (COD removal rate dropped to  $65.1 \pm 2.2\%$  and the  $\text{NO}_3^-$  removal rate dropped to  $91.9 \pm 0.5\%$ ). In contrast, an increase in the trend of isobutyric acid concentration was observed ( $0.2284 \pm 0.0117$  to  $0.3580 \pm 0.0407$  mili-mole/l) in this condition. A possible reason may be at this loading rate VFA accumulation in the reactor triggered a momentary drop of the reactor pH below 6.5 that encouraged butyrate type fermentation. In summary, the high organic loading rate can be referred to as a trade-off between AnMBR performance and maximizing butyric acid production.

A possible future improvement opportunity can be operating the bioreactor by altering the pH condition into the acidic zone (5.5 to 6.5). Where the acetic acid and propanoic acid production can be maximized at this pH range (Begum et al., 2018), it would be interesting to

see the possibility of overall VFA concentration exceeding the values that are obtained in the loading rate of 550 mg COD /l.

### 3.5 Advantages of VFA production from continuous AnMBR

The current study utilizes a continuous AnMBR to produce VFA from low-strength synthetic wastewater. Over the past few years, there have been a lot of experiments to extract VFA using anaerobic digestion, but most of these researches involve anaerobic digestion process in batch operation. For example, Begum et al. (2018) used anaerobic batch reactors to produce VFA using landfill leachate. The reactor was operated at different pH conditions (pH: 5.5 / 11.0) and at a temperature of  $37 \pm 2$  °C. The highest VFA yield from this research was 48% VFA/ COD feed. Garcia-Aguirre et al. (2017) investigated the production of VFA using different carbon sources (Slaughterhouse wastewater, Papermill wastewater, and glycerol) using batch fermentation process. The range of overall VFA yield from paper mill wastewater and glycerol were 32 - 47 %. Both studies used adequate inhibition of the methanogenic activity of microorganisms through pH and temperature control in the batch mode of operation. The research by Li & Li (2017) carried out an experiment to produce VFA using iron-flocculation batch reactor using wastewater and food waste and achieved a conversion of 66% of food waste into VFA. Yarimtepe, Oz & Ince (2017) achieved a 68% overall VFA yield in an anaerobic sequencing batch reactor using olive mill wastewater. Therefore, the currently available research studies for VFA production have achieved a high percentage of VFA yield through utilizing inhibition of methanogenic activity in the batch mode of operation. There has been a limited no of research that involves VFA production from low strength municipal wastewater in a continuous AnMBR.

Hence, a continuous AnMBR can offer many advantages over the currently available VFA production processes. Firstly, VFA production in the continuous mode of operation makes it more applicable for wastewater treatment whereas the batch mode of operation is more practical for anaerobic digestion of organic waste. Secondly, this study has achieved a maximum VFA yield of  $48.20 \pm 1.21\%$  (mg VFA/mg COD in feed solution) without the inhibition of methanogenic activity. Therefore, the result can be used in the simultaneous production of VFA and methane can increase the amount of revenue earned from the AnMBR. Thirdly, acidification is a major operational problem in AnMBR that are primarily caused by VFA accumulation (Khan et al., 2016b). Throughout this experiment, the reactor pH was maintained at  $7.0 \pm 0.1$ . Recovering VFA from this process offered an operational benefit by reducing the chance of rapid acidification. Finally, the membrane fouling profile during VFA production under different operating conditions has not yet completely discovered. Therefore, the findings from this research study could be beneficial to reduce/eliminate membrane fouling in the future.

#### 4. Conclusion

The experimental results concluded that the highest individual VFA concentration was observed at HRT 8hrs with a corresponding yield of  $48.20 \pm 1.21\%$  without any selective inhibition of methanogenesis. From different organic loading rates, highest acetic and propanoic acid concentration were  $1.1845 \pm 0.0424$  and  $0.5160 \pm 0.0322$  mili-mole/l respectively at  $550 \text{ mg/l COD}_{\text{feed}}$ . An increase in high organic loading at  $715 \text{ mg COD/feed}$  suggested a future research option by operating the AnMBR at different pH levels. Additional operating conditions like reactor pH and temperature can be altered to maximize the production rate and yield of VFA.

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**Figure captions**

Figure 1: Schematic of experimental setup for VFA production

Figure 2: Nutrient and COD removal performance of Continuous AnMBR

Figure 3: Variations of TMP at different operating conditions

3(a): TMP at different HRTs (hrs)

3(b): TMP at different OLRs (mg/l)

Figure 4: VFA concentration at different OLRs

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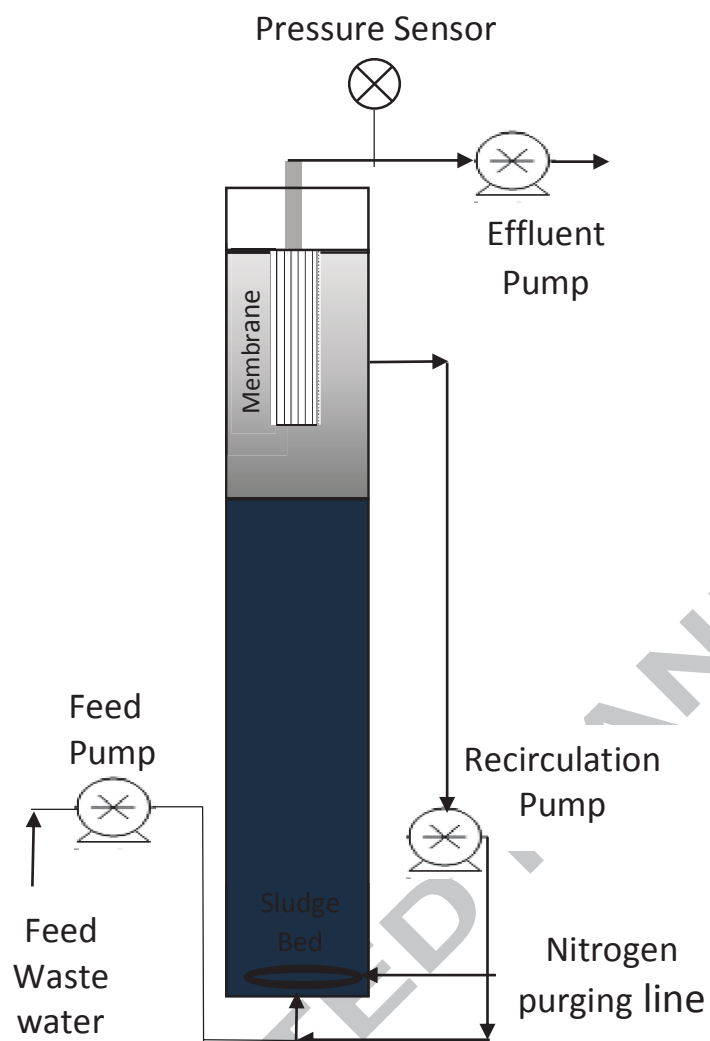


Figure1 Schematic of experimental setup for VFA production

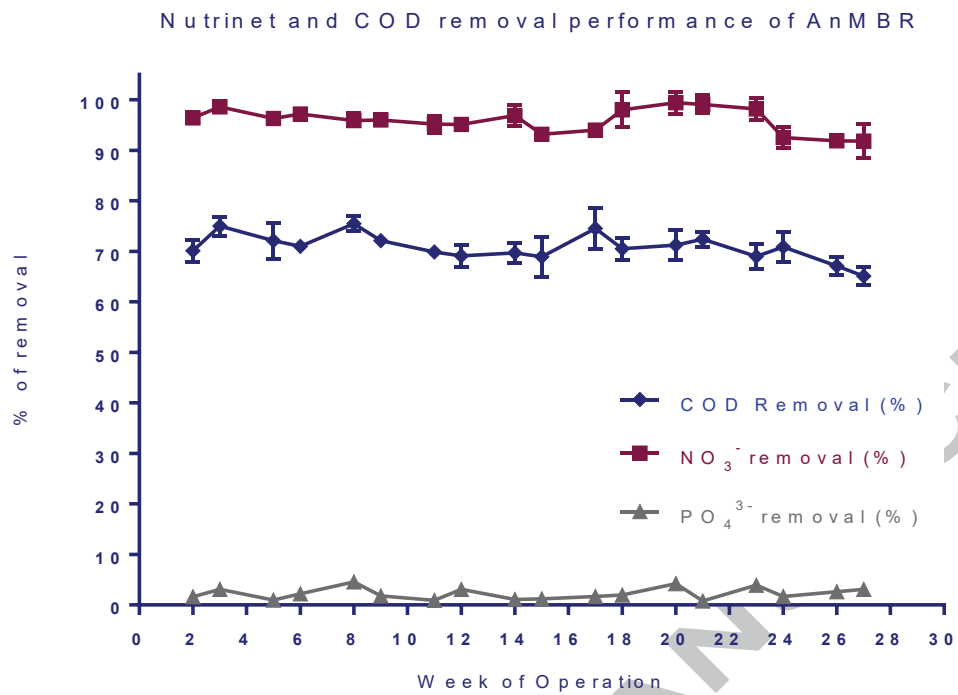
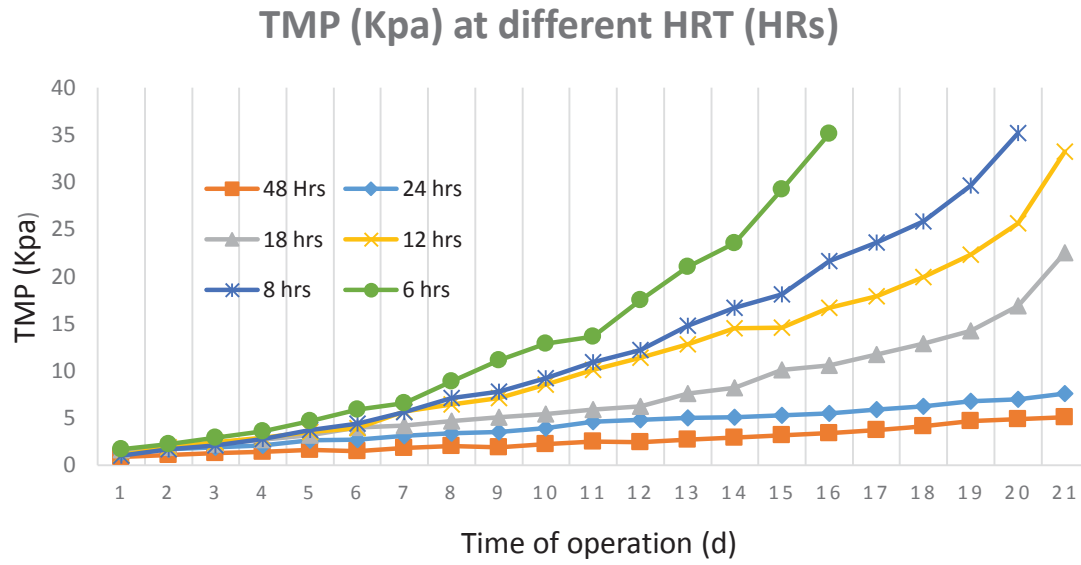
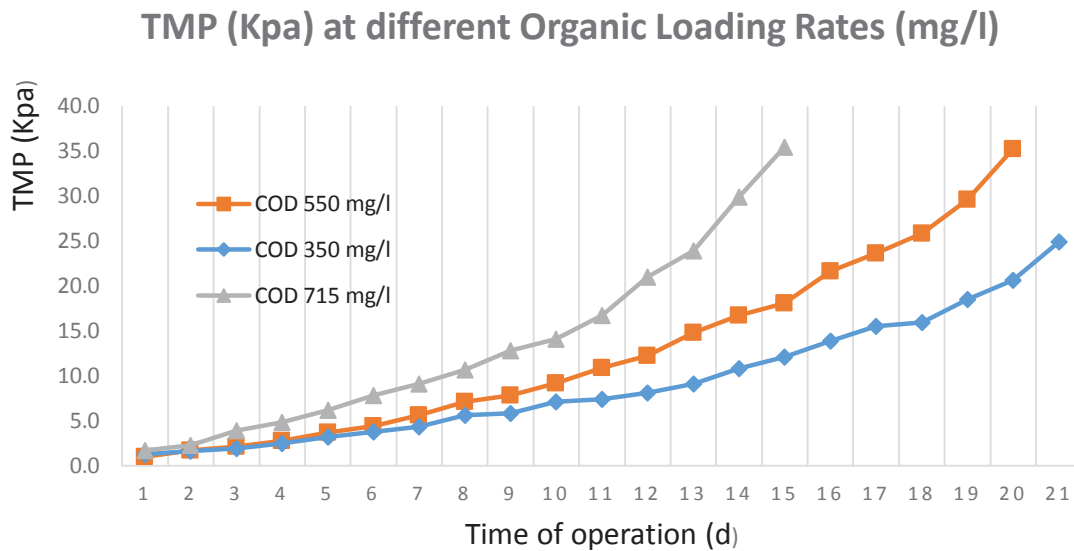


Figure 2: Nutrient and COD removal performance of continuous AnMBR

3(a) TMP at different HRTs (hrs)



3(b) TMP at different OLRs (mg/l)

**Figure 3** Variations of TMP at different operating conditions

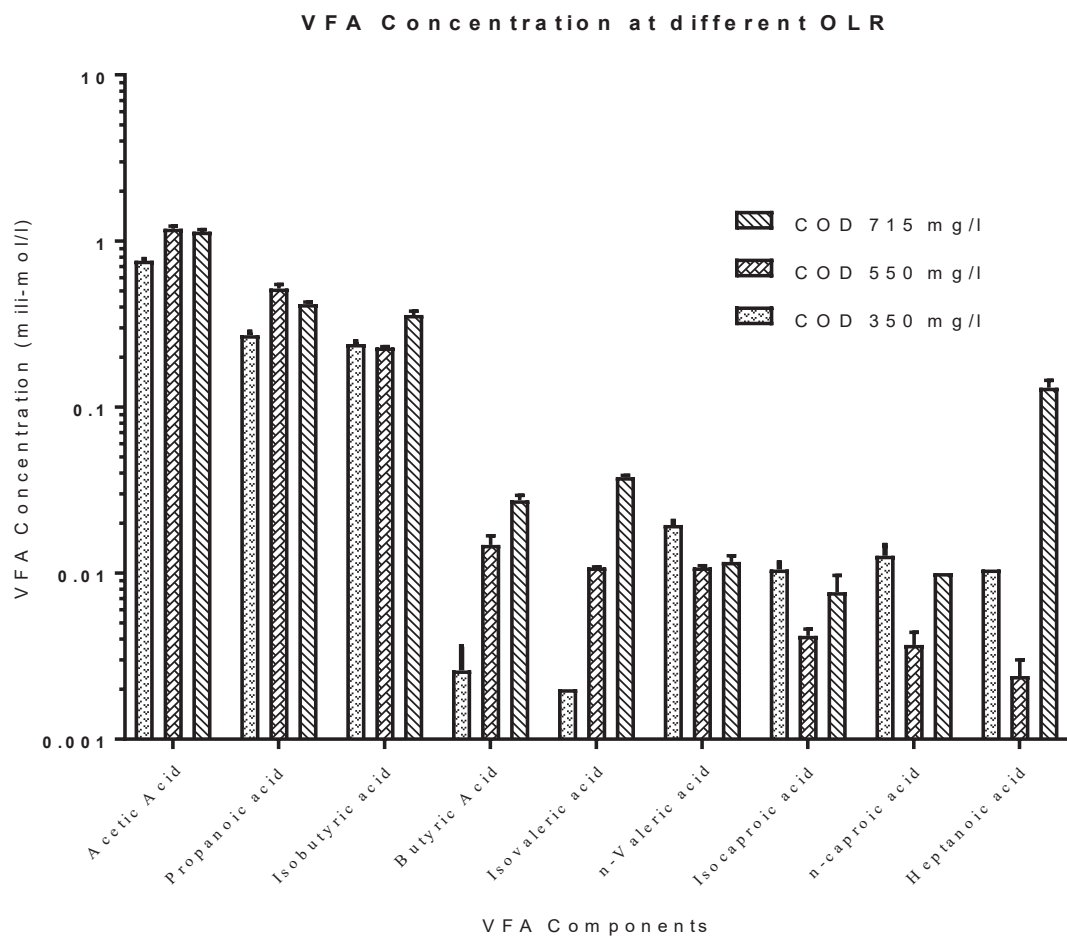


Figure 4: VFA concentration at different OLRs

Table 1: Characteristics for seeding sludge and feed solution

<b>Parameters</b>	<b>Units</b>	<b>Cronulla WW treatment plant</b>	<b>Central Park WW Treatment plant</b>
pH	–	$7.1 \pm 0.2$	$7.3 \pm 0.2$
TSS	% w/w	$14.5 \pm 0.5$	$11.3 \pm 0.5$
VSS/TSS	% w/w	$74.12 \pm 1.9$	$68.30 \pm 0.5$
COD	mg/L	$1102 \pm 10$	$890 \pm 2$
TN	mg/l	$117.2 \pm 2.5$	$142.91 \pm 3.1$
TP	mg/l	$27.24 \pm 1.2$	$21.36 \pm 1.5$

Table 2: AnMBR Operating conditions

<b>Operating Parameter</b>	<b>Stage 1 (At different HRT)</b>	<b>Stage 2 (At Different OLR)</b>
MLVSS (g/L)	10.1 ± .1	10.1 ± .1
COD in feed (mg/l)	550 ± 10	350 ± 10, 550 ± 10, 715 ± 10
HRT (h)	48, 24, 18, 12, 8, 6	8
Loading rate (mg COD /l. h)	68.75	43.75, 68.75, 89.38
SRT (d)	∞	∞
DO (ppm)	0.01	0.01
Temperature (°C)	22 ± 1	22 ± 1
pH	7.0 ± 0.1	7.0 ± 0.1

Table 3: Concentration of VFA components at different HRT

<b>VFA Component</b>	<b>Concentration (mili-mole/L)</b>
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HRT (hrs)	48	24	18	12	8	6
Acetic Acid	0.4922 ± 0.0134	0.8321 ± 0.0160	0.8753 ± 0.0062	1.1451 ± 0.0175	1.1844 ± 0.0165	1.0090 ± 0.0081
Propanoic acid	0.2172 ± 0.0126	0.4376 ± 0.0198	0.4632 ± 0.0035	0.3185 ± 0.0431	0.5160 ± 0.0141	0.5293 ± 0.0300
Isobutyric acid	0.1128 ± 0.0008	0.2836 ± 0.0005	0.2880 ± 0.0212	0.2801 ± 0.0141	0.2283 ± 0.0117	0.2398 ± 0.0406
Butyric Acid	0.0084 ± 0.0011	0.0001 ± 0.0000	0.0035 ± 0.0013	0.0051 ± 0.0007	0.0148 ± 0.0009	0.0155 ± 0.0034
Isovaleric acid	0.0003 ± 0.0001	0.0044 ± 0.0008	0.0164 ± 0.0002	0.0103 ± 0.0002	0.0108 ± 0.0008	0.0093 ± 0.0008
n-Valeric acid	0.0193 ± 0.0025	0.0019 ± 0.0004	0.0113 ± 0.0005	0.0119 ± 0.0004	0.0108 ± 0.0013	0.0143 ± 0.0023
Isocaproic acid	0.0110 ± 0.0008	0.0030 ± 0.0001	0.0033 ± 0.0002	0.0023 ± 0.0003	0.0041 ± 0.0008	0.0049 ± 0.0002
n-caproic acid	0.0971 ± 0.0075	0.0253 ± 0.0011	0.0040 ± 0.0008	0.0005 ± 0.0004	0.0037 ± 0.0006	0.0085 ± 0.0023
Heptanoic acid	0.0286 ± 0.0015	0.0129 ± 0.0009	0.0029 ± 0.0012	0.0017 ± 0.0001	0.0023 ± 0.0001	0.0038 ± 0.0018
Overall VFA Yield	13.39 ± 1.21%	32.88 ± 2.56 %	35.35 ± 1.89 %	38.83 ± 3.25 %	48.20 ± 1.21%	42.32 ± 2.32 %