Study of the effects of temperature and pH on acidogenic fermentation process from organic fraction of municipal solid waste

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Everyday large amounts of organic waste are thrown away. A fraction of it, the Organic Fraction of Municipal Solid Waste (OFMSW), is ideal for biorefinery processes because of its high biodegradability. Hence, it is recommended to undergo acidogenic fermentation to produce valuable materials such as volatile fatty acids (VFA) that are utilized as carbon sourced for some bacteria in PHA production. The main objective is study the pH and temperature effects in VFA yield and acids distribution. For this purpose a series of pH (3 to 12) and temperature (33, 55, 70 ºC) in batch and continuous reactors were performed. The results showed that hyperthermophilic regime favour the VFA production with a maximum value of 19.56 gVFA/L compared with 13.09 gVFA/L obtained in mesophilic regime without pH correction in continuous reactor supported by the results of batch test. The VFA distribution was nearly the same but slightly better in mesophilic regime for future PHA production. Under the batch experiments of pH effect, the alkaline conditions demonstrated that it was more appropriate in VFA production avoiding methanogenic bacteria activities. On average, at pH 10 and hyperthermophilic conditions, the VFA production was 9.85 gVFA/L which was lower than expected with a bit variation in VFA distribution. The results of this study suggest that hyperthermophilic condition without pH control is suitable to maximize VFA production and mesophilic regime without pH control to achieve a better distribution for future PHA production.

Keywords: Acidogenic Fermentation, Temperature, pH, Volatile Fatty Acids

1. Introduction

The rapid growth of human population and the global economy has led to a large amount of urban organic waste generation resulting in huge important environmental problems in the world. According to the Waste Agency of Catalonia [1], these organic wastes include food waste, which is the majority proportion, paper waste, small-sized plant waste, compostable materials and other materials which are made from the mentioned resources. Besides, the European Commission (EU) currently produces 76.5 - 102.0 M t/y of food and gardening waste and 37 M t/y from the food and drinks industry [2]. Hence, the organic fraction of municipal solid wastes (OFMSW) and other easily biodegradable solid substrate have to be conveniently treated to reduce their impact and to recovery energy and material while disposal treatments (e.g. landfill or incineration) should be avoided [3]. On the one hand, landfilling or composting would occupy large quantity of valuable land [4] and releases greenhouse gases [5]; on the other hand, incineration is energy intensive and unusable due to the high moisture content (MC) [6] of OFMSW which is around 70 % [7] roughly.

The OFMSW is an ideal feedstock for biorefinery processes due to it is composed by carbohydrates (simple sugars and polysaccharides), proteins and lipids, all of which can be fermented. Therefore, this substrate can be used for the acidogenic fermentation producing valuable products as volatile fatty acids (VFA) and other low weight organic compounds such as alcohols or lactic acid. The acidogenic fermentation is an anaerobic process which is based on hydrolysis and acidogenic phases from anaerobic digestion. In the first step, the complex organic matter (such as proteins, carbohydrates, fats/oils) is broken down into simpler organic monomers (as sugars, amino acid and fatty acids) to be readily available to other bacteria. Subsequently, these organisms ferment these monomers into VFA that are short-chain fatty acids consisting of six or fewer carbon atoms which can be distilled at atmospheric pressure [8]. These acids have different applications such as

biological removal of nutrient from wastewater [9], bioenergy with H_2 [10] and biogas production [11], or bioplastics production [12].

A crucial issue that the human being is facing nowadays is the water pollution. The release of insufficient treated wastewater and the contamination of small particles residual plastic are two possible reasons which might lead to the shortages of drinking water in the near future. To solve these problems, on 16th of January 2018, European Union (EU) published "A European Strategy for Plastics in a Circular Economy" [13], with a mission of making all plastic packaging being recyclable by 2030. Polyhydroxyalkanoates (PHA), for example, is a new generation of biopolymers which can be fully biodegraded on disposal and this can definitely reduce the environmental impact. These PHA polymers are an interesting alternative to petrochemical derivative plastics because they share similar thermoplastic properties [14]. However, the PHA production seems to be hindered by some stringent requirements, production cost is one among them. Venkata Mohan and Venkateswar Reddy (2013) [15] reported that the PHA production cost is five to ten times that of conventional plastics which includes substrate cost, cultures procurement and sterilization. Due to this, the packaging trading price is not competitive between bioplastics and petrochemical-based plastics. PHA synthesis by waste-derived materials can significantly lower the primary material cost during PHA production especially when mixed microbial cultured is used. VFA are one of the main intermediates in the anaerobic fermentation and considered as the most suitable substrate for PHA storage [16].

Many researches on this sector has been implemented in the last decades, focusing on PHA production through VFA as intermediates, such as fermentation strategies, process configuration, metabolic pathway analysis, microbial characterization and polymer characterization [12, 17-20]. Specific studies on the process operational parameters i.e. pH, temperature, hydraulic retention time (HRT) and organic loading rate (OLR), have been done by several authors [19, 21-24] because of the remarkable influences on the generation of desired products caused by the change of these process variables. Based on the previous findings, these parameters lead to a different metabolic pathway to produce various number of carbon-chain fatty acids. Zhou *et al.* (2018) [6] classified the acidogenic metabolic pathways into 6 types: (1) acetate-ethanol type, (2) propionate-type, (3) butyrate-type, (4) mixed-acid, (5) lactate-type metabolic pathway, and (6) Homoacetogenic fermentation pathway. Their results were in accordance with Jiang *et al.* (2013) [19] and Fang and Liu (2002) [25] in which acidic pH was suggested as predominant condition for VFA optimization. Nevertheless, in the general findings showed by a number of authors [16, 18, 24], basic pH was highly recommended. The pH and temperature affect the growth of bacterial communities and hydrolysis of complex organic matter into simpler monomers [19]. In acidogenic fermentation, the hydrolysis is claimed as rate limiting step [26]. To enhance the hydrolysis, this means to have more readily available substrate for the microorganisms, VFA can be produced easily.

The objective of this study was to evaluate the VFA production in acidogenic fermentation of OFMSW by manipulating pH and temperature conditions that can be performed in continuous reactors (4.5 and 30 L). In addition, the concentration of those valuable VFA (propionic and valeric) was assessed in order to observe its potential production under these process parameters, so that, a higher value on PHA production can be gained.

2. Materials and methods

2.1 Substrate and inoculum

The OFMSW used in this research was type source-sorted and was obtained from a Mechanical-Biological Treatment (MBT) plant of the Metropolitan Area of Barcelona (AMB) in which it was crushed into smaller particle size and then mixed with process water from the same plant. It was kept at 4 ºC in the refrigerator chamber. The characteristics of the substrate are presented in Table 1.

The effluent of the anaerobic digester from the Environmental Biotechnology laboratory of University of Barcelona was used to inoculate the acidogenic fermenters during this study. The inoculum was originally obtained from a full-scale anaerobic digestion plant treating residual organic matter. The inoculum was left for

one day stirring at 35 ºC before being used, the total solids (TS), volatile solids (VS) and soluble chemical oxygen demand (SCOD) were 4.65 %, 3.03 % and 71.07 g/L.

Table 1: Characteristics of OFMSW used in this study

	Units	Value
Total Solids (TS)	% w/w	6.21 ± 1.29
Volatile Solids (VS)	% w/w	4.76 ± 1.13
Soluble Chemical Oxygen Demand (SCOD)	g/L	72.53 ± 12.98
Volatile Fatty Acids (VFA)	g/L	9.57 ± 1.05
Alkalinity (Alk)	g $CaCO_3$/L	4.99 ± 0.51
Ammonium-nitrogen concentration	gNH_4^+-N/L	2.84 ± 0.66
pH	-	6.28 ± 0.36

2.2 Experimental set-up

The experiments were carried out in two modes: discontinuous (batch test: A1 and A2) and continuous (lab-scale reactor: B1, B2 and B3). Table 2 summarizes experimental conditions of each assay in this study.

Table 2: Experimental conditions of reactors

Type	Set-up	Temperature (ºC)	pH
Discontinuous	A1	35, 55, 70	Not controlled
	A2	70	3, 4, 5, 8, 9, 10, 12, not controlled
Continuous	B1	35	Not controlled
	B2	70	Not controlled
	B3	70	10

2.2.1 Batch

On the one hand, to test the influence of temperature in acidogenic fermentation of OFMSW, batch test A1 was set. Four serum bottles with working volume 200 mL (3 conditions with replicate) were filled with inoculum and substrate according to their VS contents with a ratio of 1:1 by weight. After that, each bottle was flushed with nitrogen gas for 1 minute to remove oxygen gas from the headspace and was closed with PTFE/Butyl septums. Then, these bottles were placed in two incubators (Memmert, Pass-through ovens UF750) running at 35, 55 and 70 ºC. The duration of this batch was 6 days where VFA analysis was performed every day.

On the other hand, once the effect of temperature had been confirmed, test (A2) on how the change of pH influences the production of VFA was performed. Similar to the previous batch, sixteen identical serum bottles were filled with corresponding inoculum and OFMSW. In an incubator of 70 ºC, these bottles were kept for 6 days. A total of 8 different pH ranged from 3, 4, 5, 8, 9, 10, 12 and without control (blank) were set at the beginning of experiment. Solutions of sodium hydroxide (NaOH) and hydrochloride acid (HCl) were used to adjust the pH daily after the sample was taken for VFA analysis.

2.2.2 Continuous

For this study, jacketed reactors with effective working volume of 4.5 L, and mechanical stirrer (IKA-Werker, RW 16 basic) functioning at approximately 170 rpm were used as fermentation reactor. Several operating conditions were applied to analyse the behaviour of acidogenic bacteria against the change of temperature and pH. The yield and distribution of VFA were assessed to better understand the best conditions for acidogenic fermentation of OFMSW.

In the experiment of effect of temperature, the effluent of anaerobic digester, treated as inoculum, was kept stirred in two jacketed reactors for 24 hours at temperature 35 ºC (mesophilic) and 70 ºC (hyperthermophilic). This was to reactivate the acidogenic bacteria in the inoculum. Based on the HRT of 3.5 days, the equivalent quantity of substrate was fed manually to reactor once per day (fed-batch culture). Nitrogen gas was used to replace possible air trapped in the headspace inside the fermenter during feeding and draw-off.

While for the experiment of effect of pH, same procedure was done to inoculate the fermentation reactor. At hyperthermophilic temperature conditions, two fermentation reactors, one at pH 10 and the other without control, were implemented to find out the optimised conditions for VFA production. The HRT was set to 3.5 days with manual draw-off and feeding. Due to the production of VFA and the acidic pH of feedstock, every day the pH was adjusted to 10 by using 10 M NaOH.

2.3 Analytical methods

TS, VS and SCOD were analysed in accordance with the standard methods [27] 2540B, 2540E and 5220D, respectively. Alkalinity was determined through titration method at pH 4.3 by using pH-Burette 24 (Crison) with 0.1 M HCl. Individual VFA (acetic, propionic, isobutyric, butyric, isovaleric, valeric, caproic and heptanoic) was measured using a gas chromatograph (Shimadzu, GC-2010 plus) equipped with capillary column (Nukol™, 15 m x 0.53 mm x 0.5 μm) with flame ionisation detector (FID). The temperature of injector and detector were 280 and 300 ºC respectively. The pH of reactor was measured with pressurized gel-electrolyte electrodes for pH (Mettler Toledo, HA405-DPA-SC-S8/225). The concentration of ammonium-nitrogen was determined using high performance ammonium ion selective electrode (Thermo Scientific, Orion 9512HPBNWP).

3. Results and discussion

3.1 Effects of temperature

Batch test was first carried out to know the performance of OFMSW when mixed with the provided inoculum. From Fig. 1, the overall production of VFA was in order of 70 ºC > 35 ºC > 55 ºC. The maximum production was observed on day between 3 and 4 in all the cases with VFA concentration of 11.51, 10.72, and 13.81 g/L, respectively. These results confirmed the HRT of 3.5 days. As previously reported, the thermophilic temperature could lead to faster biological acclimatization and more active acidogenesis as compared to those at mesophilic [16]. Unexpected results were obtained in this experiment: the production of VFA at 35 ºC was higher in comparison with those at 55 ºC. At 35 ºC, the maximum VFA produced was on the third day with a value of 11.51 gVFA/L followed by 11.43 gVFA/L on fourth day. While operating at 55 ºC, the optimal concentration reached was 10.72 gVFA/L on day 3, and on day 4 a value of 10.58 gVFA/L. Nonetheless, similar results obtained by some authors demonstrated that thermophilic temperature regime had no effect on the VFA production [24, 28]. According to Zhuo *et al.* (2012) [29], the acid-forming enzymes activities at thermophilic temperature (55 ºC) were lower than that at mesophilic temperature (37 ºC), achieving 40 % fewer in VFA production. Under hyperthermophilic conditions, there was an increment of VFA production from day 1 to 3, namely from 13.16 gVFA/L to 13.81 gVFA/L. After that, the concentration of VFA started to decrease slowly until 13.21 gVFA/L on the last day of this experiment. From the beginning, the VFA concentration at 70 ºC was far ahead from other conditions till the end of the experiment. This outperformance among mesophilic, thermophilic and hyperthermophilic temperature regimes was coincident with the results from Lu *et al.* (2005) [30], indicating they might be at least two different microbial communities exist and adequate to produce VFA at mesophilic and hyperthermophilic temperature conditions. Therefore, it is preferable to operate either at mesophilic regime (approximately 35 ºC) or hyperthermophilic regime (above 70 ºC) for optimizing the VFA accumulation.

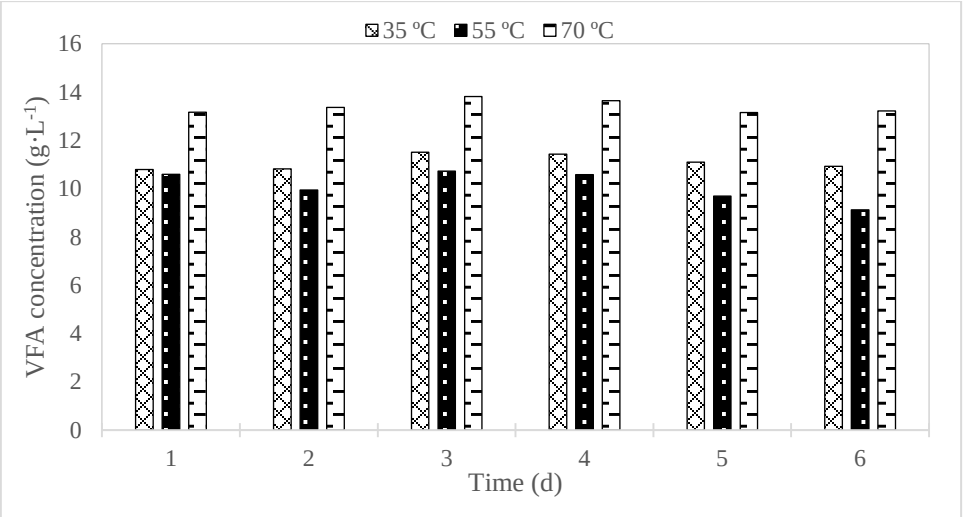

Figure 1: Effect of temperature on the evolution of VFA in batch at 35ºC, 55 ºC and 70 ºC

Once discontinuous experiments were performed to evaluate the short term effect of temperature, to receive comparable results, mesophilic and hyperthermophilic conditions were chosen to test in lab-scale reactors to assess the effect at long-term. Data of 80 consecutive days was recorded for these. As shown in Fig. 2, just like the results in batch test, the 70 ºC condition was always having higher concentration of VFA than that at 35 ºC in every single day, namely, an average of 14.59 gVFA/L and 10.91 gVFA/L (Table 3). Besides, the ratio $_{VFA}$COD/sCOD was calculated in both cases with a value of 22.59 % and 21.32 % at 35 ºC and 70 ºC respectively. This ratio at hyperthermophilic regime was slightly lower as compared to that at mesophilic regime, the small difference might be caused by the higher increment in solubility of organic compounds to VFA formation at high temperature. Apparently, the fluctuation of VFA concentration was greater at higher temperature. The inconsistent results obtained could be credited to the presence of different microbial communities in the inoculum and their activities against the variation of composition of OFMSW [8].

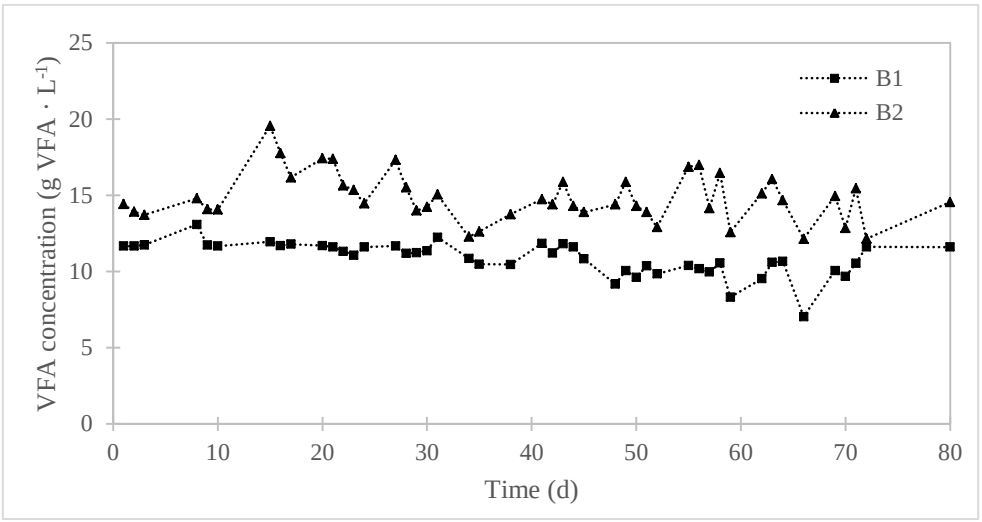

Figure 2: Effect of temperature on VFA production at 35 ºC and 70 ºC

Throughout the whole experiment, it was observed that the liquid level in the reactor B2 decreased from time to time which led to higher viscosity when compared to B1. The highest viscosity was observed on day 15 when the highest VFA concentration was reached. This provoked a need to replace the loss contents by reducing the removal volume in the draw-off in order to maintain the 4.5 L working volume in the reactor. From Table 3, the total solids of B1 and B2 were 5.82 and 9.46 % respectively, while the volatile solids were 4.31 % for B1 and 7.34 % for B2. The differences have proved that there was a clearly gap in term of moisture content in the

effluent. To identify if the loss volume was caused by the evaporation of pure water content from the aqueous solution of reactor, after the day 80, the necessary amount of tap water, after ultrafiltration, was added to the reactor so as to observe the change in VFA production. In disappointment, the concentration of VFA dropped until almost half of the previous results and it took more than one week to recover back to normal. It was assumed that part of the VFA might have been evaporated with the water. Hence, it is recommended that further study should be performed to get a detailed analysis on the lost contents.

Despite the fact that at hyperthermophilic temperature could produce more total VFA, these results denoted that the reactor at 70 ºC did not made much contribution to the production of PHA with higher quality (polyhydroxyvalerate, PHV) based on the ratio (C2+C4)/(C3+C5) of 1.85. This is because, at the meantime, similar ratio, 1.76 was obtained at 35ºC in which favours the PHV production with a small extent. But still, even with this negligible difference, the hyperthermophilic temperature regime could be of great interest in some extendable studies.

Table 3 presents the characterisation of effluent of continuous reactors. When compare the production of short-chain fatty acid with less or equal to 4 carbons, the increment in VFA concentration was obvious. Meanwhile, almost no variation was observed in the concentration of caproic and heptanoic acids as they were minorities. In terms of NH_4^+-N and sCOD, B1 was found to have a light increment to 3.14 gNH_4^+-N /L and 76.66 gCOD/L, while these occurred more intensively in B2 with 4.83 gNH_4^+-N /L and 95.1 gCOD/L. This is in accordance with the results from Jiang *et al.* (2013) [19], showing that more soluble organic compounds were found as a result of hydrolysis. The alkalinity of B1 decreased while increased in B2, given that 3.01 and 7.34 $gCaCO_3$/L respectively. This explained why the pH drop was more in B1 than B2, reached 5.96 and 6.17 respectively.

Table 3: Characterisation of effluent of continuous reactors

	Units	B1	B2	B3*
TS	% w/w	5.82 ± 0.78	9.46 ± 3.49	9.21 ± 1.32
VS	% w/w	4.31 ± 0.69	7.34 ± 2.98	6.00 ± 1.21
Alk	$gCaCO_3$/L	3.01 ± 2.82	7.34 ± 1.42	9.36 ± 0.30
NH_4^+-N	gNH_4^+-N/L	3.14 ± 0.10	4.83 ± 0.81	1.42 ± 0.67
pH	-	5.96 ± 0.32	6.17 ± 0.33	9.95 ± 0.18
sCOD	g/L	76.66 ± 12.48	95.1 ± 16.0	84.72 ± 15.41
$_{VFA}$COD/sCOD	%	22.59 ± 3.78	21.32 ± 4.22	19.19 ± 5.07
VFA	g/L	10.91 ± 1.08	14.59 ± 2.54	9.85 ± 1.04
Acetic	%	38.16 ± 4.21	39.81 ± 2.67	38.77 ± 1.61
Propionic	%	20.93 ± 1.83	21.65 ± 0.91	22.10 ± 1.26
Butyric	%	18.67 ± 2.06	19.19 ± 2.52	16.29 ± 1.89
Valeric	%	10.83 ± 1.27	9.93 ± 0.82	9.92 ± 0.96
$(C_2+C_4)/(C_3+C_5)$	-	1.76 ± 0.23	1.85 ± 0.09	1.79 ± 0.09

*B3 experiment started in parallel with the experiment B1 and B2 after day 55.

In the bar chart of individual VFA concentration at different temperature (Fig. 3), an amplification scale can be seen as moving from 35 ºC to 70 ºC. The amplify factor was between 1.46 and 1.22 (data not shown), for acetic, propionic, butyric and valeric acids. With respect to the caproic and heptanoic acids, since their concentrations, after sample was diluted, were too small to be detected by the gas chromatograph, the difference was insignificant as compared to other short chain fatty acids. In both conditions, the concentration of acetic acid (C2) was about two times that of butyric acid (C4). Same thing happen between propionic acid (C3) and valeric acid (C5).

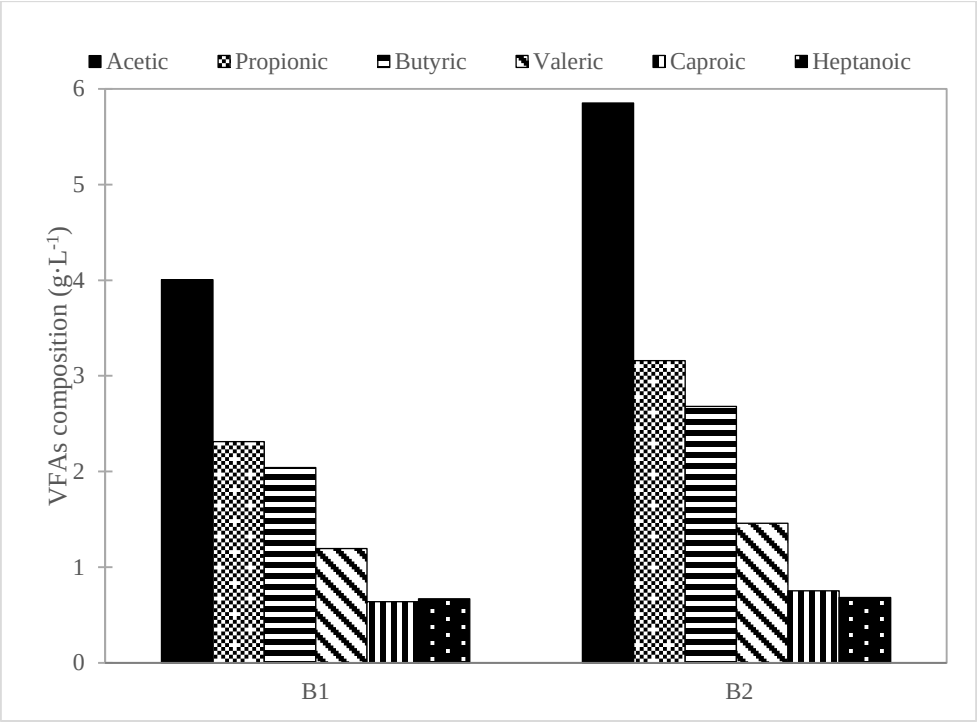

Figure 3: Average concentration of individual VFA at different temperature (35 ºC and 70 ºC)

Fig. 4 and Fig. 5 explain the evolution of individual VFA concentration for fermenter B1 and B2. In both conditions, the highest particular VFA produced was acetic acid, followed by propionic, butyric and valeric, while the least produced were obviously to be caproic and heptanoic. It is not surprising that acetic acid was the dominant acidogenic fermentation product because there are actually many metabolic pathways which end up with the generation of acetic acid or acetate [6]. The results obtained were inversely proportional to the number of carbon that the particular acid holds (C2 > C3 > C4 > C5 > C6 > C7). Under mesophilic conditions, the fermenter took around two week to be stabilised. In contrast, the reactor pretended to be more stable since the beginning of the experiment under hyperthermophilic conditions. There was one point during the experiment at which after that time, a higher degree of fluctuation was detected, it was the moment when the stock of OFMSW was finished and replaced by a new one. This observation can be clearly located on day 48, the VFA composition of both effluents were affected, especially acetic, propionic and butyric acids.

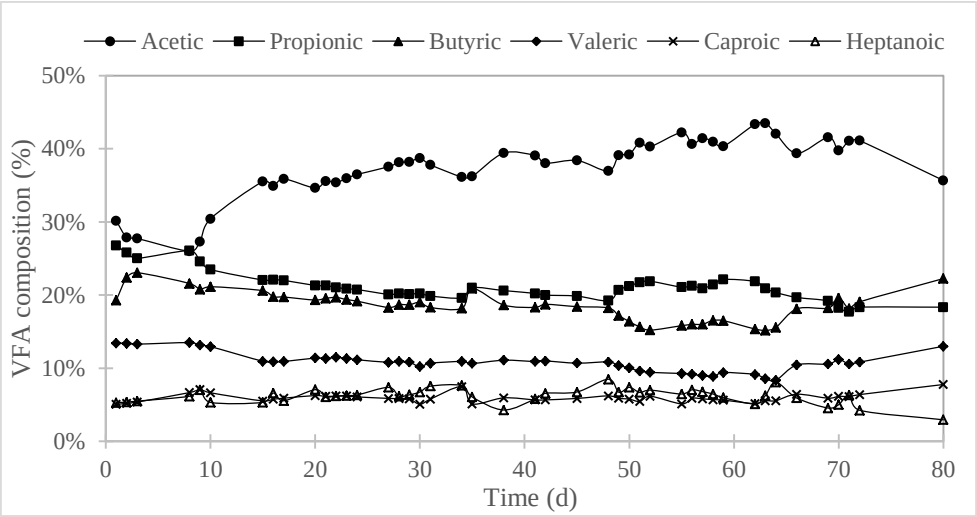

Figure 4: Selected particular VFA production under mesophilic conditions (35 ºC)

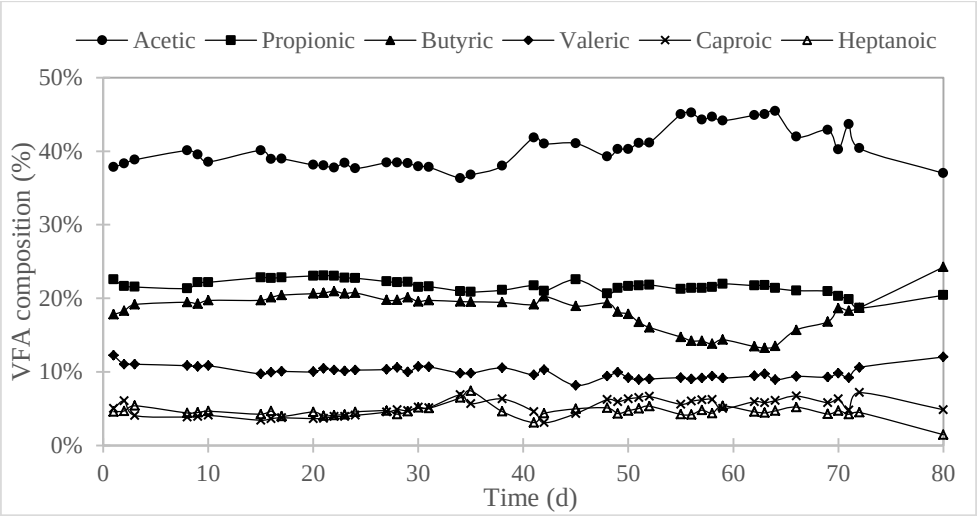

Figure 5: Selected particular VFA production under hyperthermophilic conditions (70 ºC)

3.2 Effects of pH

As what have been explained before, pH can influence the VFA yield and distribution [31]. During the acidogenic fermentation process, pH can affect not only the hydrolysis, but also the acidogenesis [32]. In this case, the optimal pH for acidogenic fermentation will able to optimise these two steps. A batch test at 70 ºC was carried out at a series of pH ranged from 3 to 12 and uncontrolled as described earlier in Table 2 to examine the VFA production under a combination of these two important process parameters. From Fig. 6, the VFA production was decreasing at acidic pH (3, 4 and 5) from the first day until the end of the batch test. In contrast, generally, higher VFA produced at alkaline pH was achieved. On fourth day, a maximum value of 15.10 gVFA/L was reached at pH 8 followed by pH 9 and pH 10 with 13.17 and 13.01 gVFA/L, respectively. This batch test demonstrated that at alkaline conditions in hyperthermophilic regime favoured the VFA yield after operated for 3 or 4 days.

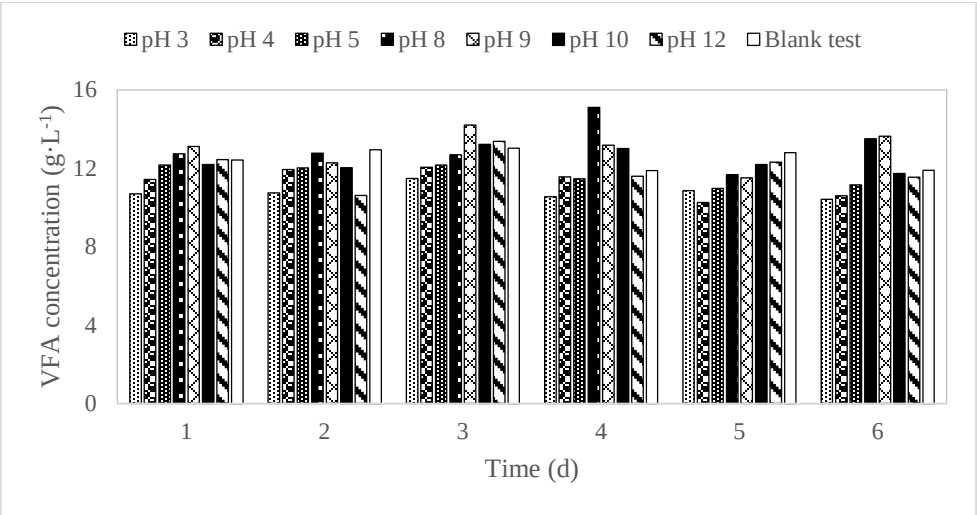

Figure 6: Effect of pH on VFA production in batch

With the aim of avoiding methanogenic bacteria activities, it was suggested to operate the reactor out of the range of pH 7.8-8.2 [33]. Also, several researches have proved that the methanogenesis can be inhibited by increasing or decreasing to an extreme pH [34, 35]. For this reason, pH 10 was chosen to be applied in continuous reactor (B3). Moreover, this batch test showed that was interesting to work with HRT of 3.5 days.

At the meantime reactor B2 was in function, on day 55, reactor B3 was set up for data collection. In addition to the stability reaction produced by hyperthermophilic temperature, pH 10 also contributed to establish stable acidogenic fermentation in this experiment. As well-known, acetic acid could be degraded directly by methanogens. However, the proportion of acetic acid in this experiment was considerably constant, which might be caused by the inhibition of activities of methanogenic bacteria at pH 10. Even though the inhibition of methanogenesis was achieved, the results showed that the VFA production was not as high as what was expected, reaching only 9.85 gVFA/L on average. This number was even lower than that at mesophilic temperature without pH control. In Table 3, the characterisation of B2 and B3 is shown. The TS did not show much change between them, giving that 9.46 and 9.21 % for B2 and B3 respectively. Fewer VS content was found in B3 with a value of 6.00 % as compared to B2 (7.34 %). During the experiment, the pH of reactor B3 was adjusted to 10 once per day. This would definitely increase the alkalinity of the reactor. The alkalinity of reactor at pH 10 was 9.36 $gCaCO_3$/L, approximately two times of that of the reactor at pH without control, 4.83 $CaCO_3$/L. But, the production of VFA will cause it to decrease. An average of pH 9.95 was registered. The ammonium-nitrogen concentration of B3 recorded 1.42 gNH_4^+-N /L, which was half of the OFMSW at the end of the experiment. At basic conditions in which there are more hydroxide ions (OH^-) in the aqueous solution, the ammonium ion (NH_4^+) will form ammonia gas (NH_3) to achieve the equilibrium. This equilibrium shift happened in reactor B3 and provoked the drop in NH_4^+-N due to ammonia stripping. This low concentration of NH_4^+-N will be beneficial for the posterior PHA selection process. The sCOD of B3 was 84.72 g/L and it was less than that of B2. This can be explained by the loss of volume in the reactor B2. But in general, it has risen from the beginning of 72.53 g/L.

Comparing the VFA composition, it was found that the distribution was similar between the fermenters at pH without control and at pH 10. However, at pH 10, lower concentration of acetic and butyric acids was produced while more propionic acid was formed. According to Jie *et al.* (2014) [18], who worked with excess sludge at pH 10, the distribution was similar to this experiment with acetic (38.77%) as most prevalent VFA followed by propionic, accounting for 22.10 %. In experiment B3, the ratio of (C2+C4)/(C3+C5) was found to be 1.79 which was lower than B2. This implies that these conditions will promote the production of copolymer poly-(3-hydroxybutyrate-co-3-hydroxyvalerate), PHBV in the next process.

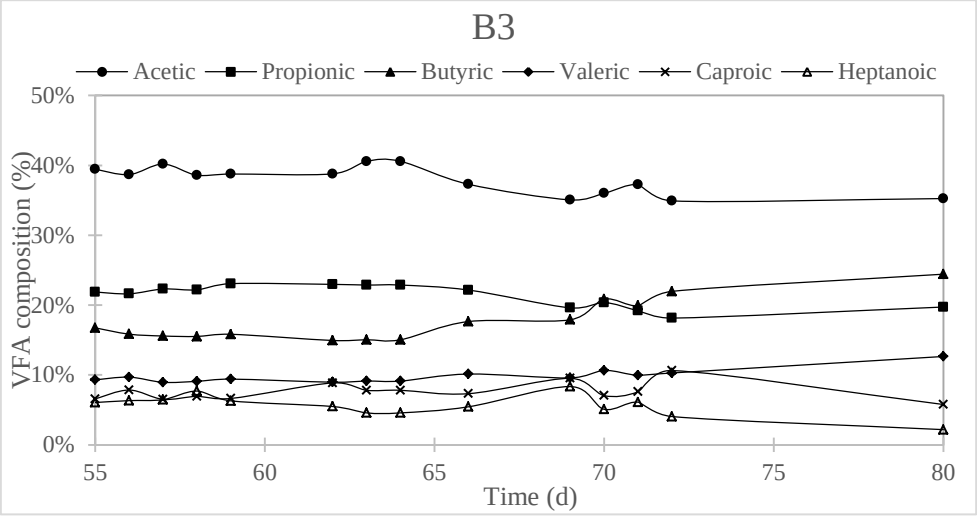

Figure 7: VFA composition at pH 10 and hyperthermophilic temperature

Jiang *et al.* (2013) [19] has done a study of OLR effect on VFA production and showed that operation of a reactor by using food waste at high OLR (16 gTS/L·d) was unstable because the fermentation broth was very viscous and. This led to some undesirable fluctuations during the experiment and caused difficulties on the way to find out the optimal process conditions, especially in the batch tests. The experiment was carried out at roughly 18 gTS/L·d which was higher than 16 gTS/L·d. Thus, a future experiment could be performed with

lower OLR to observe whether under these conditions, the VFA composition can be improved to reach to a higher level.

4. Conclusions

The conclusions were: (i) the hyperthermophilic range at 70 ºC is more favourable for maximum VFA production; (ii) continuous experiments shown that at 70 ºC without pH control, the production of VFA was optimum, the ratio (CODVFA)/(CODS) and (C2+C4s)/(C3+C5s) will favour the PHB production; (iii) it seems to be in basic conditions (pH 8-10), in the two ranges of temperature, the concentration of VFA was ever to reach to a higher level than neutral and acidic conditions; (iv) operating at higher pH (pH 10) in continuous reactor did not promote VFA production, but it lowered the concentration of NH_4^+-N and altered slightly the composition of VFA promoting PHBV production; (v) the high OLR led to failure of the reactor and it is recommended to operate at lower OLR.

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