Acidogenic fermentation of vegetable and salad waste for chemicals production: Effect of pH buffer and retention time

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Abstract

This study investigated the effect of pH buffer and solids retention time (SRT) on the anaerobic fermentation of vegetable and salad waste (VSW). Experiments were carried out in batch and semi-continuous reactors at 35 °C. In the batch experiments, the effect of pH buffer on the production of volatile fatty acids (VFAs) and ethanol was investigated. Acetate and butyrate were the main fermentation products. The maximum total product concentration was 43.3 and 18.5 g COD l^{-1} in the buffered and unbuffered batch reactors resulting in a yield of 62 and 27% ($COD_{total\ product}/COD_{feed}$) respectively. Volatile suspended solids (VSS) removal was higher in the buffered semi-continuous reactor (57%) compared to the unbuffered-acidic reactor (39%), but similar yields (15%, $COD_{total\ product}/COD_{feed}$) were observed because biogas production was stimulated in the buffered reactor. The effect of SRT on the VSS removal and product distribution in unbuffered systems was investigated at 10, 20 and 30 days SRT. The VSS removal increased as the SRT increased, ranging between 18.2 – 49.1%, likewise the total product concentration, 9.1 - 19.4 g COD l^{-1}, and product yield, 7 – 24% ($COD_{total\ product}/COD_{feed}$). Acetate and butyrate were the prevalent fermentation products at all conditions followed by caproate although caproate was only detected at 20 and 30 days SRT. Total COD removal ranged between 15.2 and 35.1% with the highest removal observed at 30 days SRT.

Keywords: Volatile fatty acid (VFA), Mixed culture fermentation, Food waste, Anaerobic

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1. Introduction

Up to 10 million tonnes of food waste, including vegetable and salad waste (VSW), is generated annually in the UK, and this is associated with nearly 20 million tonnes of greenhouse gas emission. Landfill is the most common disposal method for food wastes. However, the leachate produced from landfills and the emission of greenhouse gases are

major limitations associated with landfills [1]. Food waste constitutes a nuisance in municipal landfills due to its high moisture content and biodegradability. It is characterised with high organic content, (20-45% carbon; 80-90% volatile solids), lipids (10-40%) and protein (5-10%) [2], which can be converted into value-added chemicals. Over the years, anaerobic digestion has become an interesting industrial process for the generation of biogas through the biological degradation of organic-rich feedstocks such as agricultural waste and municipal solid waste and wastewaters. Anaerobic digestion is an eco-friendly and energy efficient biological process that combines the treatment of waste with the generation of useful biogas, mainly methane and carbon dioxide, for electricity generation.

Anaerobic digestion is a complex biological process which can be divided into four stages: (1) hydrolysis of the complex substrate into smaller monomers; (2) acidogenesis of the monomers to alcohols, volatile fatty acids (VFAs), etc.; (3) acetogenesis, during which the acidogenesis products are converted into acetate, hydrogen, and CO_2 and finally, (4) methanogenesis, where the acetate and hydrogen are converted into biogas. Hydrolysis is fundamental in the fermentation process as it makes the substrate available to the microbes for metabolism [3, 4].

The diversity of biochemical pathways of microbes allows the production of a wide range of industrially relevant bio-based chemicals [5]. Bio-based chemicals have been considered as sustainable alternatives to fossil-derived chemicals in chemical reactions [6]. Chemicals that can be produced from food waste include organic acids, alcohols, hydrogen [7]. These chemicals which are produced as intermediates in the anaerobic digestion process, mainly during acidogenesis, are more valuable than the methane generated at the end of the AD process [8] and can serve as a platform from which other chemicals could be derived via biological or chemical conversion technologies [9, 10].

The AD process being a complex one requires several operational parameters to be satisfied for high productivity and process stability. The process is influenced by various physical, chemical and biological factors including pH [11-13], solids retention time, SRT [14], temperature [15, 16], nature of the substrate and organic loading rate, OLR, [15]. Substrate removal is influenced by the SRT in the reactor because there is a minimum SRT required for the substrate removal in the fermentation process and because the different classes of microorganisms in the AD process have different growth rates. For instance, methanogens are slow growers and therefore require longer residence time than the acid-

producing microbes. pH has been recognised as one of the key factors in anaerobic fermentation, and according to Zheng et al. [17], the pH determines the type of fermentation that occurs in a system. Fermentation process has been classified into four: (1) butyrate-type fermentation; (2) propionate-type fermentation [18]; (3) Ethanol-type fermentation [19]; and (4) Mixed acid-type [17].

The typical food waste in the UK may consist of drink (16%), bakery (10%), meat and fish (7%), dairy and eggs (7%), meals (8%), fresh fruit (13%), fresh vegetable and salads (23%) and other (16%) [1]. This study investigates the anaerobic fermentation of vegetable and salad waste (VSW), which is the most prominent fraction, by weight, of the food waste generated in the UK.

Although VSW has been reported as a substrate for co-digestion with sewage sludge [20], very few studies has been done on the anaerobic fermentation of only VSW [21, 22]. Moreover, these studies only focused on maximizing methane production but did not investigate in detail the influences on hydrolysis, acidification and VFAs production. Literature studies have emphasised the need for pH control in the sole digestion of VSW considering its low self-buffering capacity which consequently contributes to the excessive accumulation of VFAs [23]. On the other hand, while the accumulation of intermediate fermentation products, i.e., VFAs and alcohols are not desirable in a typical anaerobic digestion process, it can be exploited for the production of VFAs as bulk chemicals according to the carboxylate platform approach [24].

This study is therefore aimed at investigating the effect of pH buffer and SRT on the fermentation of the VSW and product formation (ethanol and VFAs) production in batch and semi-continuous processes. The degree of substrate removal and product yield were assessed by volatile suspended solids (VSS), carbohydrates (total and soluble), chemical oxygen demand (COD) and fermentation products in the liquid phase.

2. Materials and methods

2.1 *Substrate*

The VSW, which mostly comprised lettuce, spinach, onions, and carrots, was received from a food company. The sample was homogenised using a food processor/liquidiser (MGM4100GB, Bosch, UK) and it was characterised for its physico-chemical characteristics. The sample was stored in a freezer at – 22 °C and defrosted before it was fed into the reactor. Total solids (TS) and volatile solids (VS) were measured according to the standard

methods for the examination of water and wastewater [25]. The substrate contained some fermentation products, including ethanol and VFAs. The concentrations of the initial products in the feed were determined using gas chromatography. The characteristics of the VSW is shown in Table 1. No additional nutrients (nitrogen, phosphorus or other elements sources) were added to the substrate.

2.2 *Inoculum*

The inoculum, a source of active microbial population, used for the experiments was an anaerobic digester sludge obtained from Gask (mesophilic) anaerobic digester, Turriff, Aberdeenshire, UK. The sludge was refrigerated at 0 ± 2 °C until required. The large solids in the anaerobic sludge were removed by filtration through a Buchner funnel before use. The inoculum had TS content of 49.4 ± 3.7 g l^{-1} and VS content of 38.5 ± 2.7 g l^{-1}, which corresponds to 78% of the TS content.

Table 1 Characteristics of vegetable and salad waste used in the study. The values are the means (± standard deviation) of triplicates.

Parameter	VSW
TS (%)	4.82 (0.36)
VS (% TS)	77.95 (0.36)
TSS (g l^{-1})	22.97 (3.44)
VSS (g l^{-1})	22.79 (3.50)
TCOD (g l^{-1})	56.76 (3.59)
SCOD (g l^{-1})	28.29 (4.70)
Total carbohydrate (g l^{-1})	14.13 (2.00)
pH	4.63 (0.07)
Ethanol (g COD l^{-1})	3.75 (1.56)
Acetate (g COD l^{-1})	1.64 (0.23)
Propionate (g COD l^{-1})	0.06 (0.03)
Butyrate (g COD l^{-1})	0.21 (0.09)
Caproate (g COD l^{-1})	0.004 (0.00)
$\sum COD_{\text{initial products in feed}}$ (g COD l^{-1})*	5.91 (1.68)

*Sum of ethanol, butyrate, propionate, butyrate, and caproate

2.3 *Experimental set-up*

2.3.1 Batch reactor: Effect of pH buffer

Experiments were carried out in two batch reactors under similar conditions to determine the effect of pH buffer on the fermentation of VSW. The headspace of the reactors was sparged with N_2 before hermetic closure to ensure anaerobic conditions. In each experiment, 300 ml of VSW was inoculated with 5% (v/v) inoculum. The temperature of the reactor was controlled at 35 °C with a water jacket connected to a thermostatic water bath (Julabo, Germany). The reactors were mechanically mixed at an initial speed of 350 rpm by a magnetic stirrer due to the initial high viscosity of the slurry. The stirring speed was subsequently reduced to 200 rpm after a week owing to the solubilisation of the substrate during the hydrolysis period, consequently leading to a reduction in the viscosity of the culture.

The effect of pH buffer was investigated by comparing the reactor performance with and without the addition of $NaHCO_3$. In the buffered reactor, 158.7 mM (13.3 g l^{-1}) $NaHCO_3$ were added at the beginning of the test and additional 39.7 mM (3.3 g l^{-1}) $NaHCO_3$ was added to the batch reactor when a decrease in pH was observed.

2.3.2 Semi-continuous reactor: Effect of pH buffer and SRT

The effect of pH buffer on the fermentation of VSW was investigated under semi-continuous conditions by operating the reactors at 20 days SRT with buffered and unbuffered feed. In the buffered reactor, the pH of the feed was adjusted to neutrality with 600 mM $NaHCO_3$.

The effect of the SRT was investigated by operating the unbuffered reactors at 10, 20 and 30 days SRT. Semi-continuous reactors with working volume of 200 ml were inoculated with 5% (v/v) anaerobic digester sludge as in the batch experiments. The headspace was flushed with N_2 at the start of the experiment. The reactor was connected to a volumetric gas counter (MilliGas counter, Ritter, Bochum, Germany) working at atmospheric pressure. The feed tank was maintained at 4 °C to prevent fermentation before feeding to the reactor and continuously stirred to ensure consistency. The VSW was fed to the reactor semi-continuously, controlled by the SRT, using a peristaltic pump (VELP SP 311, Italy). The feeding pump was controlled by a programmable power management system (Energenie, ENER019, UK). The reactors were fed once-daily, and the effluent overflowed from the

reactor by gravity through a U-tube trap which prevented gas escape. The reactors were maintained at 35 °C and continuously stirred at 200 rpm. There was a 7-day start up period during which the inoculum was acclimated to the VSW, and no fresh feed was added to the reactor during this phase. The start-up period was followed by daily feeding of the VSW substrate to the reactor, and the fermentation process performance was monitored through periodic sampling. The 30 days SRT reactor was connected to BlueSens gas sensors (Herten, Germany) for online gas measurement (H_2, CO_2, and CH_4).

2.4 *Analysis*

Fermentation products. Fermentation products (acetate, propionate, butyrate, caproate, and ethanol) were analysed by collecting 3 ml samples from the reactor. The samples were centrifuged at 8000 r min^{-1} for 10 minutes followed by filtration through 0.45 µm syringe filters (Merck Millipore, MCE membrane). 1 ml of the supernatant was acidified (to pH < 2) with, 200 µl, 2-Ethylbutyric acidification solution (0.6 w/v i.e. 6 g of 2-Ethylbutyric acid in 1 l of 30% v/v concentrated phosphoric acid) according to Raposo et al. [26]. Acidified samples were analysed using gas chromatography (Thermoscientific, Trace 1300) equipped with a flame ionization detector (FID) in split mode and a TG-Wax MS A capillary column (30 m x 0.25 mm x 0.5 µm). The initial temperature of the column was 80 °C held for 2 min followed by an increase to 200 °C at a rate of 20 °C min^{-1} and further held for a minute; the injector and detector temperatures were set at 250 °C. Hydrogen was used as the carrier gas at a flowrate of 35 ml min^{-1}. The sample injection volume was 1 µl.

Other parameters. The TSS and VSS were determined according to standard methods [25]. Total COD (TCOD) and soluble COD (SCOD) were analysed using the Spectroquant cell test method (Merck Millipore, method number 114555) and the Spectroquant Nova 60 photometer. Total and soluble carbohydrate were estimated using Anthrone method with glucose as the standard. The solution was digested at 100 °C for 10 min in a thermoreactor and thereafter cooled to room temperature for 30 min. The absorbance of the solution was measured with a spectrophotometer (Jenway 6314, Staffordshire, UK) at 620 nm. SCOD and soluble carbohydrate were analysed using the centrifuged and filtered sample described above (fermentation products analysis). pH was measured with a pH metre (Mettler Toledo, Switzerland) equipped with a P12/BNC probe.

The VSS removal is defined as

$$VSS\ removal\ (\%) = \frac{(VSS_{feed} - VSS_{sample})}{VSS_{feed}} * 100 \quad (1)$$

Where VSS_{feed} is the average amount of VSS in the feed over the period of the experiment and VSS_{sample} is the average amount of VSS in the reactor at steady state.

2.5 Process yield calculation

The product concentration was converted to COD basis using the theoretical COD conversion factors of the different fermentation products. The conversion factors used for the calculations are acetate, 1.07; ethanol, 2.09, propionate, 1.51; butyrate, 1.82 and caproate, 2.21. The product yield (g $COD_{fermentation\ products\ produced}$ g^{-1} COD_{feed}, %) was calculated according to Eq. (2) below

$$Product\ yield\ (\%) = \frac{\sum COD_{measured\ fermentation\ products} - \sum COD_{initial\ products\ in\ feed}}{TCOD_{feed} - \sum COD_{initial\ products\ in\ feed}} \times 100 \quad (2)$$

In Eq. (2) $\sum COD_{measured\ fermentation\ products}$ represent the sum of the COD of the measured fermentation products in the reactors. $TCOD_{feed}$ and $\sum COD_{initial\ products\ in\ the\ feed}$ are the total COD of the feed and the sum of the COD of the measured products in the feed (given in Table 1).

The presented data and error bars are the average and standard deviation of mean calculated from the steady state values in the semi-continuous experiments. The reactors were assumed to be at steady state when the reactor parameters were found to be stable over a period.

3. Results and discussion

3.1 *Effect of pH buffer on the fermentation products in batch experiments*

The effect of pH buffer on the product concentration and distribution was evaluated in two batch reactors. The pH in the unbuffered reactor was between 5.0 and 6.0 for the duration of the experiment. On the other hand, the pH in the buffered reactor was 7.8 at the start of the test which subsequently decreased to 6.9 on day 2 due to acetate production (5.5 g l^{-1}) and to 6.44 on day 14. The pH was thereafter adjusted to 7.3 by adding 39.7 mM (3.3 g l^{-1}) of $NaHCO_3$ on day 14 to maintain the pH of the culture between 6.8 and 8.0.

Fig. 1 shows the metabolites production/consumption profiles for the buffered and unbuffered reactors.

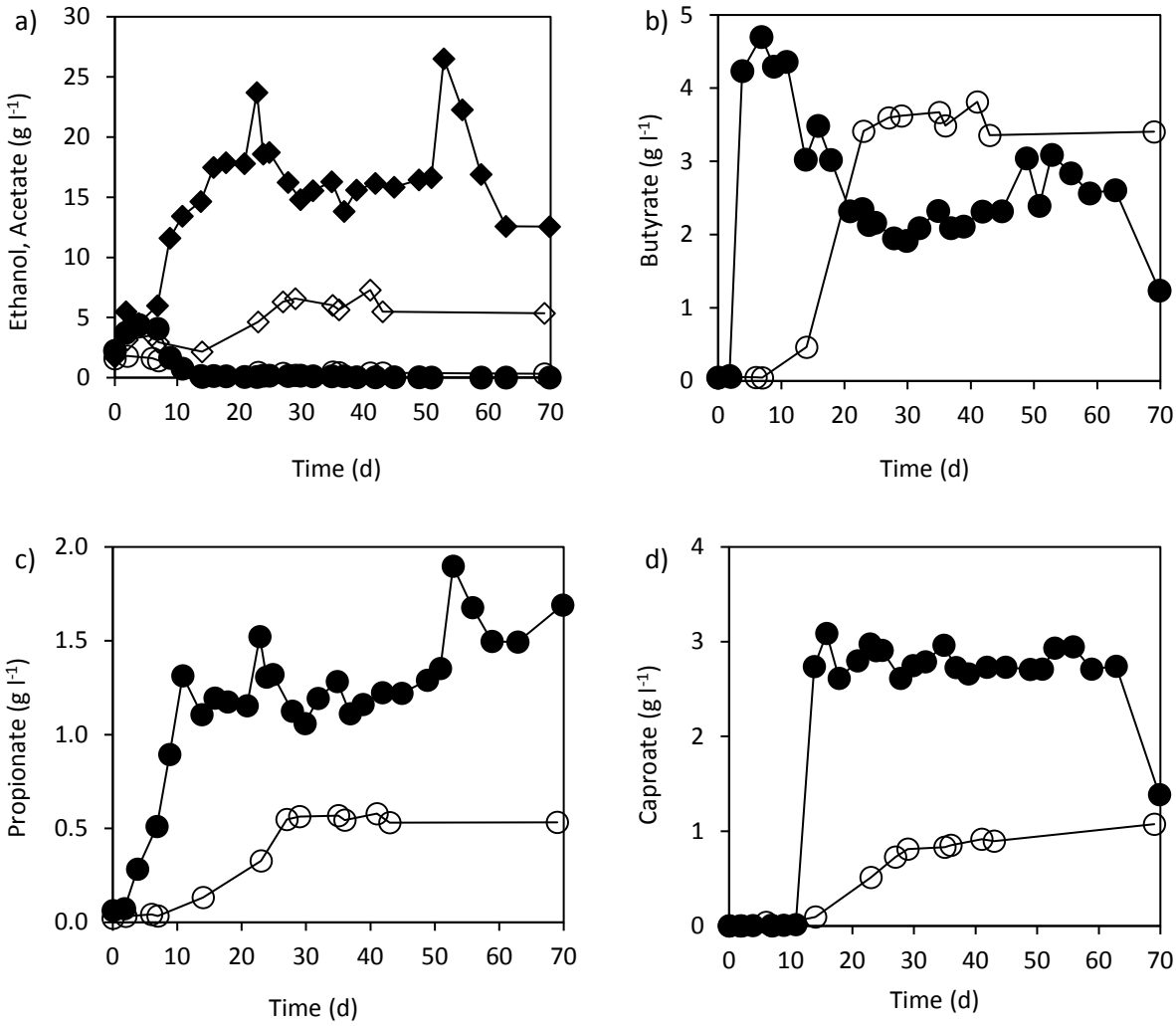
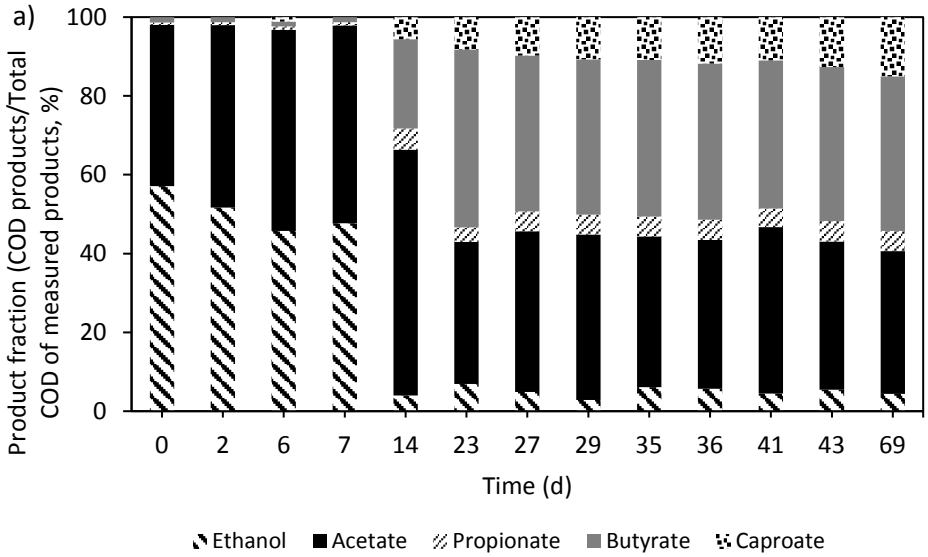


Figure 1 Monitoring profiles of the measured fermentation products in the unbuffered and buffered batch reactors a) ethanol (symbol: circle) and acetate (symbol: diamond); b) butyrate; c) propionate and d) caproate. Empty symbols represent the unbuffered reactor and filled symbols, the buffered reactor.



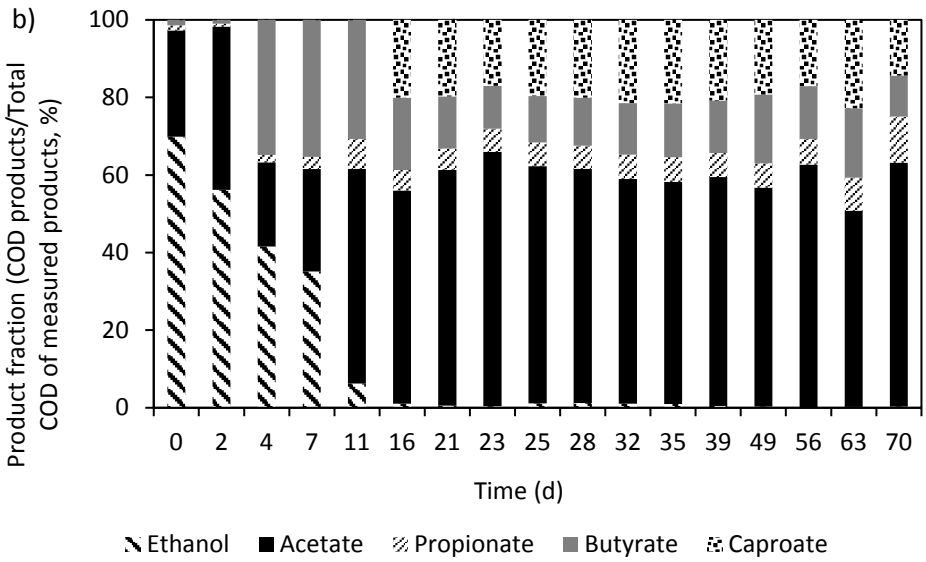


Figure 2. Product distribution in a) unbuffered and b) buffered batch reactors

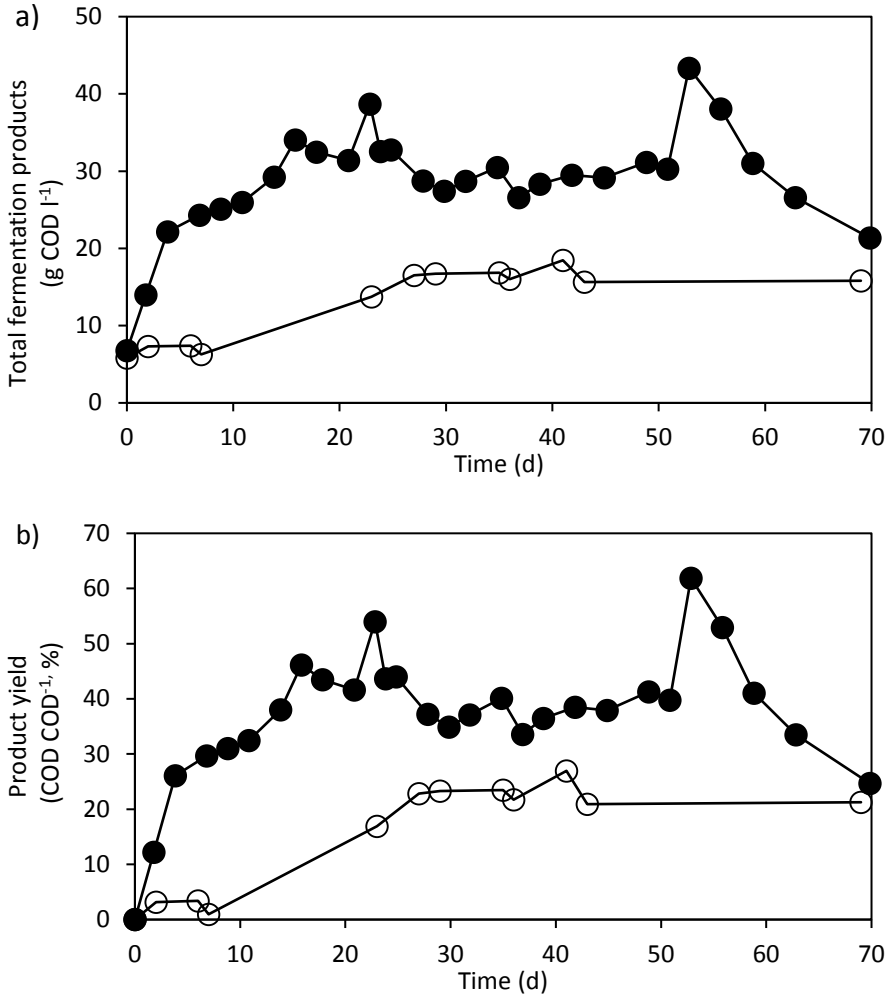


Figure 3. a) The total fermentation products and b) product yield profile in the unbuffered (○) and buffered (●) batch reactors

The reactors were run until there was no further increase in the VFAs production. A stable total product concentration of about 16.0 g COD l^{-1} was sustained between days 43 and 69

of the fermentation in the unbuffered reactor (Fig. 3a). Acetate, butyrate, and caproate were the main fermentation products under both conditions. The presence of these VFAs in the mixed liquor was predictable since they are usually the main products of acidogenic fermentation [27]. The fractions of the individual fermentation products are shown in Fig. 2. From this Figure, it can be observed that the compositions of the products varied between the two batch conditions. Caproate production was higher in the buffered reactor, amounting up to 3 g l^{-1} and 23% of the total products in the liquid phase, while its concentration was less than 1.0 g l^{-1} in the unbuffered reactor. The individual product concentrations were in the order acetate > butyrate > caproate > propionate, with maximum concentrations of 7.8, 6.9, 2.4, 0.9 g COD l^{-1} respectively in the unbuffered reactor and 28.4, 8.6, 6.8, 3.0 g COD l^{-1} in the buffered reactor (Fig. 3). The higher acetate concentration in the buffered reactor agrees with the study by Mohan et al. [28] where it was observed that alkaline pH improved the production of acetate and its fraction in the total VFAs.

Ethanol was not produced in either reactor, but the fraction of ethanol present in the feed was used up by other reactions. For instance, in the buffered reactor, the ethanol percentage composition in the total products was reduced from was 70% to 1% by day 14 (Fig. 2). Most likely due to chain elongation of short chain carboxylates such as acetate into longer chain carboxylates like butyrate and caproate *via* the reverse β-oxidation (Fig. 1). The reverse β-oxidation pathway is a cyclic process. An acetyl-CoA molecule derived from ethanol is added to a carboxylate thus elongating its carbon chain length with two carbons (C2) at a time (i.e. acetate (C2) to butyrate (C4), butyrate (C4) to caproate (C6), caproate (C6) to caprylate (C8), propionate (C3) to valerate (C5), valerate (C5) to heptanoate (C7), etc.) [29].

Butyrate production started sooner in the buffered reactor than in the unbuffered one. However, butyrate concentration subsequently decreased in the buffered reactor while it remained stable in the unbuffered one. The decrease in butyrate corresponded to increased caproate production in the buffered reactor (Fig. 1). This phenomenon may be explained by the occurrence of reverse β-oxidation; however, it is also possible that butyrate was converted to acetate (acidogenesis) and that that caproate production was due to the conversion of a fraction of the COD which had remained unconverted in the initial stage of the fermentation. Propionate was present in lower fractions than acetate

and butyrate (less than 2 g l^{-1} under both conditions) even though the concentration was higher in the buffered reactor. The total products concentration was much lower in the unbuffered reactor, and this may be because lower pH values suppress microbial growth and activity due to the higher fractions of undissociated VFAs [30].

No decrease was observed in the acetate concentration throughout the length of the experiment in the unbuffered reactor; this indicates that methane was not produced under this condition. Most methanogenic bacteria function in the pH range 6.6 – 7.6 with an optimum near pH 7.0 even though methanogenic activity has been reported at pH values up to 9.0 [14, 31]. The pH in the unbuffered reactor was less than 6.0 throughout the experiment, which is outside the active pH range for methanogenic bacteria, and this explains the absence of methane production in this reactor.

On the other hand, the total COD decreased by 29% during the length of the experiment in the buffered reactor [See supplementary Fig. S1]. The gas phase at the end of the experiment mainly comprised of methane (75% v/v) with much lower concentrations of carbon dioxide and hydrogen (6% and 5% v/v). The remainder of the gas composition comprised of the nitrogen used to strip out the gas phase at the beginning of the experiment [Supplementary Fig. S2]. The onset of methane production agrees with the decline in acetate production (Fig. 1a).

The maximum product yield (COD COD^{-1}) in the buffered and unbuffered reactor was 27% (day 41) and 62% (day 53) respectively. The final yield (day 70) was 21% and 25% respectively (Fig. 3). Higher product yield and the rapid production of fermentation products in the buffered reactor was stimulated by the alkaline conditions.

3.2 *Semi-continuous reactors*

3.2.1 Effect of pH buffer

The effect of pH buffer on the fermentation process was investigated at 20 days SRT. Fig. 4 and 5 shows the effect of pH buffer on the product concentration and distribution and the substrate consumption based on total carbohydrate consumption, COD removal and VSS removal in the buffered and unbuffered reactors.

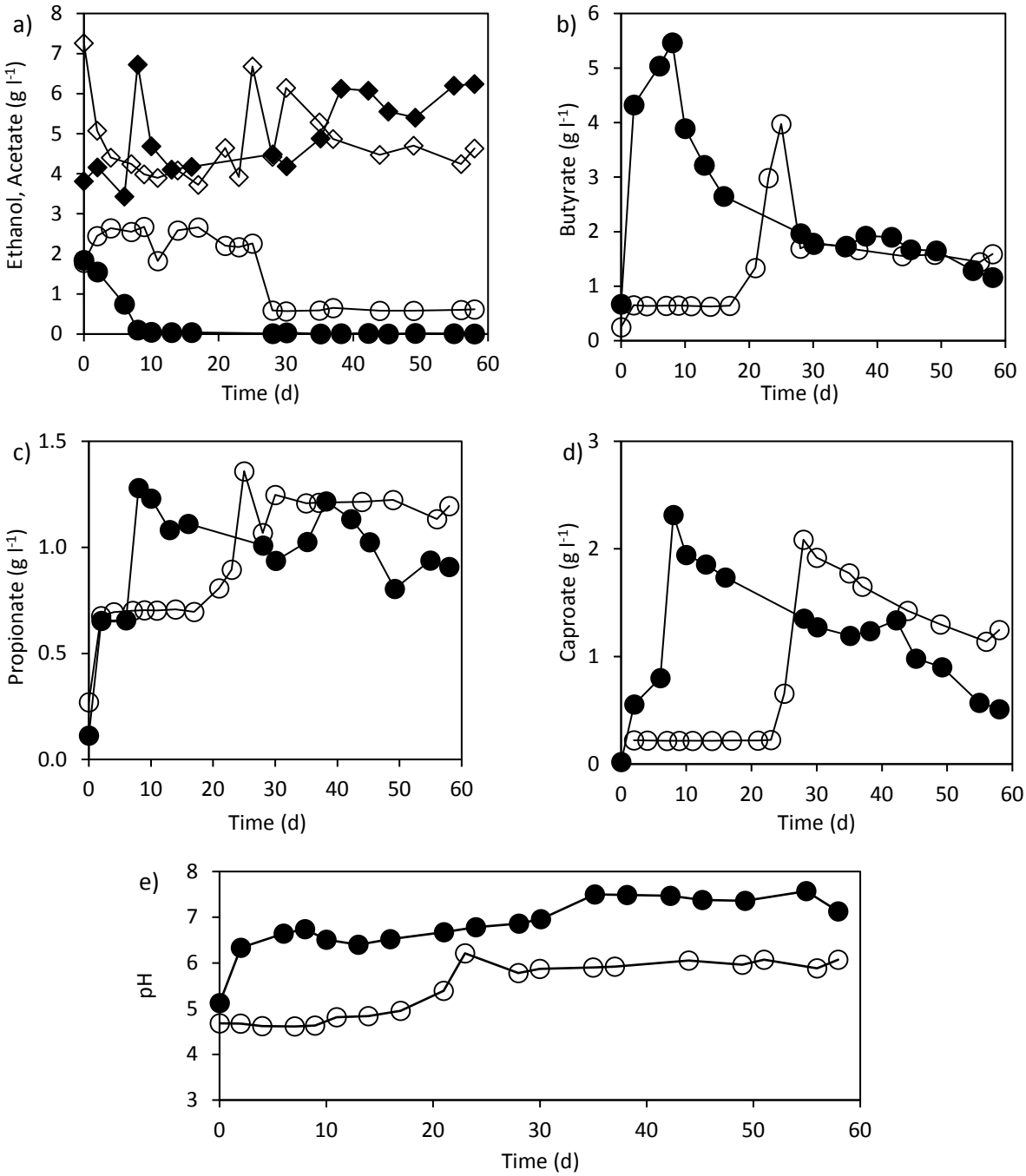


Figure 4 Monitoring profiles of the measured fermentation products in the unbuffered and buffered semi-continuous reactors at 20 days SRT a) ethanol (symbol: circle) and acetate (symbol: diamond); b) butyrate; c) propionate d) caproate e) pH profile. Empty symbols represent the unbuffered reactor and filled symbols, the buffered reactor.

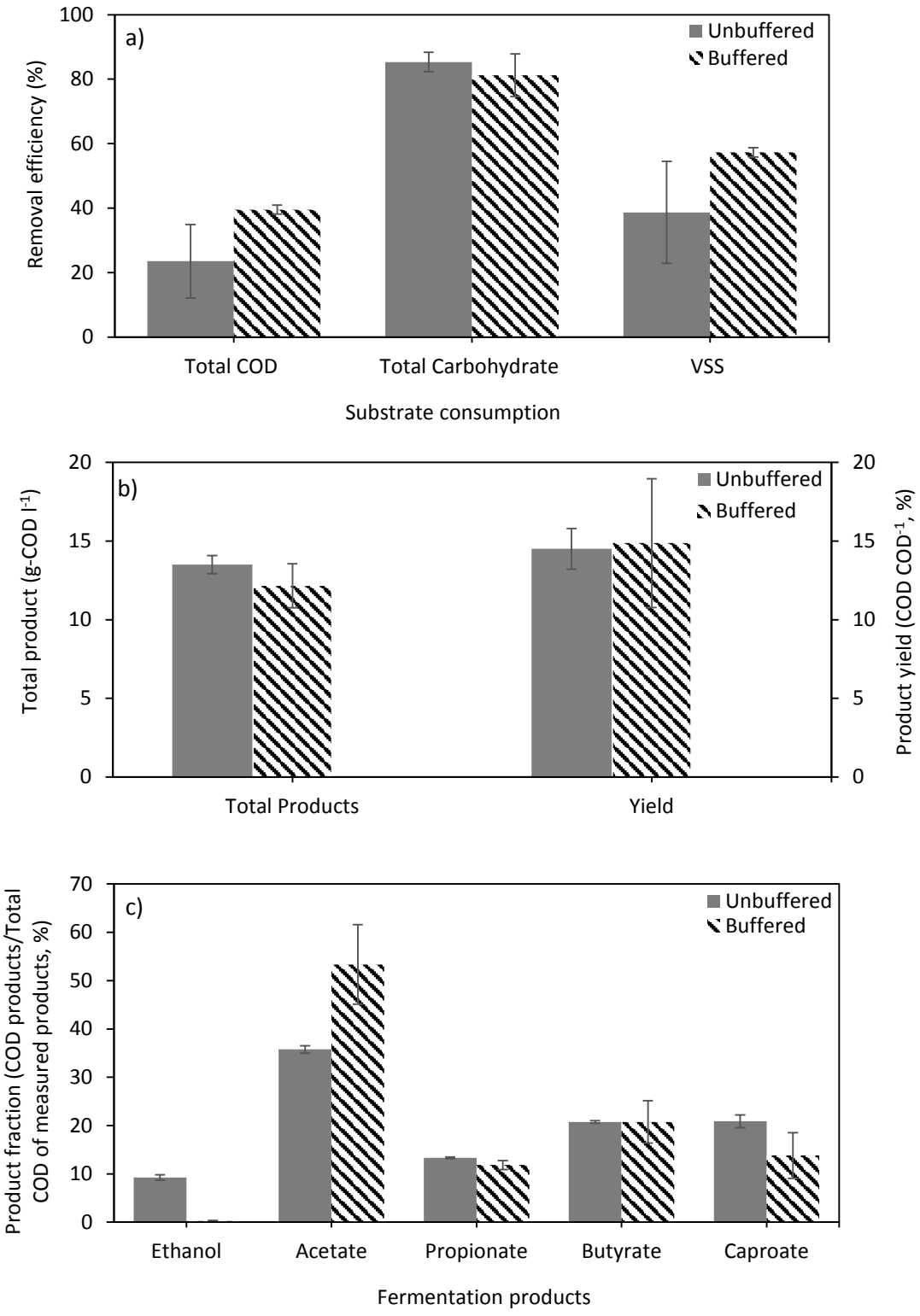


Figure 5 a) Average substrate consumption based on the COD, total carbohydrate and VSS removal b) average total products and yield and c) product distribution at steady state

VFAs were the main fermentation products and similar VFAs were produced in both reactors. Acetate and butyrate were the most prevalent VFAs. Ethanol was not generated in either experiment, and most of the ethanol in the feed was converted to products. Butyrate reached a maximum concentration on day 25 and day 8 in the unbuffered and

buffered reactor respectively. In the case of caproate, a maximum was reached on day 28 and day 8 in the unbuffered and buffered reactor respectively. Propionate did not exceed 1.4 g l^{-1} in both experiments (Fig. 4). There was no significant difference in the percent distribution of propionate and butyrate accounting for 11.8% and 21.1% in the buffered and 13.3% and 20.7% in the unbuffered reactor respectively. Acetate and caproate accounted for 52.7% and 14.2% in the buffered and 35.7% and 20.9% in the unbuffered reactor respectively (Fig.5c).

The removal of total carbohydrates was similar and high under both conditions, accounting for more than 80% removal. The concentration of the total carbohydrate decreased rapidly at the start of the fermentation period and remained stable around 1.3 – 2.0 g l^{-1} untill the end of the fermentation. The stability can be attributed to the balance between the dissolution and consumption of the carbohydrate [32]. The VSS removal was 57% and 39% in the buffered and unbuffered reactors respectively, indicating that the solubilisation of the VSW was more efficient under alkaline conditions (Fig. 5a). The decrease in the VSS demonstrates the solubilisation of the particulate organic fractions of the substrate. Chen et al. [33] reported a significant increase in the hydrolysis and VFAs production under alkaline conditions.

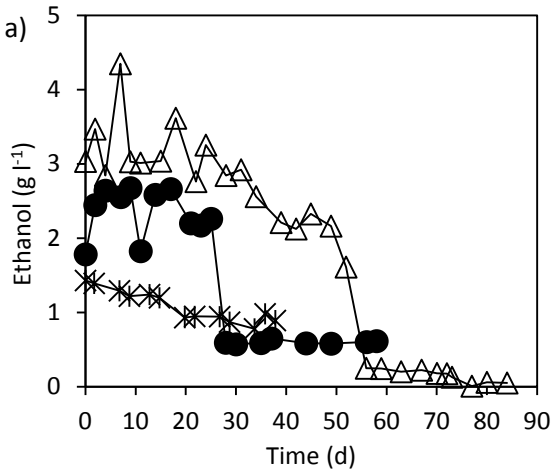
The total COD removal in the buffered and unbuffered reactor was 40% and 24% respectively (Fig. 5a). The lower COD removal in the unbuffered reactor is an indication of less biogas production than in the buffered reactor. The accumulation of acetate suggests that the biogas was probably hydrogen and not methane. However, this is contrary to what was observed in the buffered batch experiment where methane was the primary gas produced. Methane production started after 60 days in the buffered batch reactor suggesting that the growth or activity of methanogenic bacteria in the buffered semi-continuous reactor may have been limited by the SRT of 20 days. At the beginning of the continuous runs, the pH in both reactors was acidic, because the pH was not buffered during the start-up phase. The pH in the buffered reactor increased steadily during the semi-continuous fermentation, reaching an average steady state value of 7.4. The average pH in the unbuffered reactor was 6.0 (Fig. 4e).

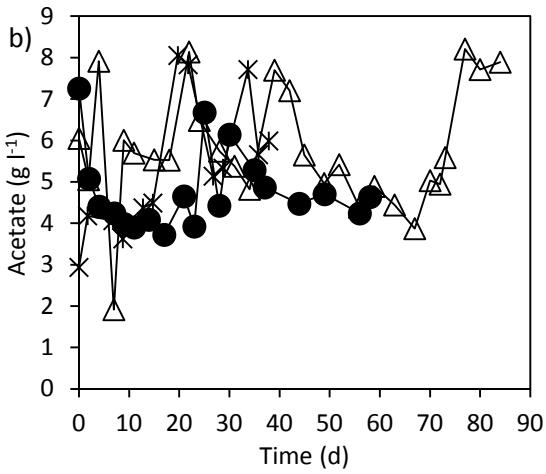
The increased VSS hydrolysis rate in the buffered reactor (Fig. 5a) did not correspond to a higher product yield (Fig. 5b), because of the higher gas production under this condition, which was demonstrated by the higher total COD removal (Fig. 5a). The product yield was

15% ($COD_{total\ product}/COD_{feed}$) under both conditions. Butyrate generation was steady in the uncontrolled reactor whereas a rapid decrease was observed in the buffered reactor after the peak concentration of 5.5 g l^{-1} was attained. A similar behaviour was observed for caproate generation, but its production in the unbuffered reactor was delayed, as in the batch test. The decreased concentration of butyrate, propionate, and caproate in the buffered reactor was probably due to their conversion to acetate, which was subsequently converted to gases at the same rate at which it was produced (the acetate concentration in the unbuffered reactor remained constant (Fig. 4a).

Effect of SRT

Fig. 6 and 7 shows the time-course of products formation, pH and substrate conversion at different SRT. VFAs were the main fermentation products. At steady state, the total carbohydrate removal was in the order SRT20 > SRT30 > SRT10 ranging between 52% and 85% (Fig. 7a). The high rate of carbohydrate consumption indicates that the carbohydrate fraction was readily available to the microbes. The COD and VSS removal increased with SRT. TCOD and VSS removal increased with SRT, with the highest removal of 35% and 49% respectively at 30 days SRT.





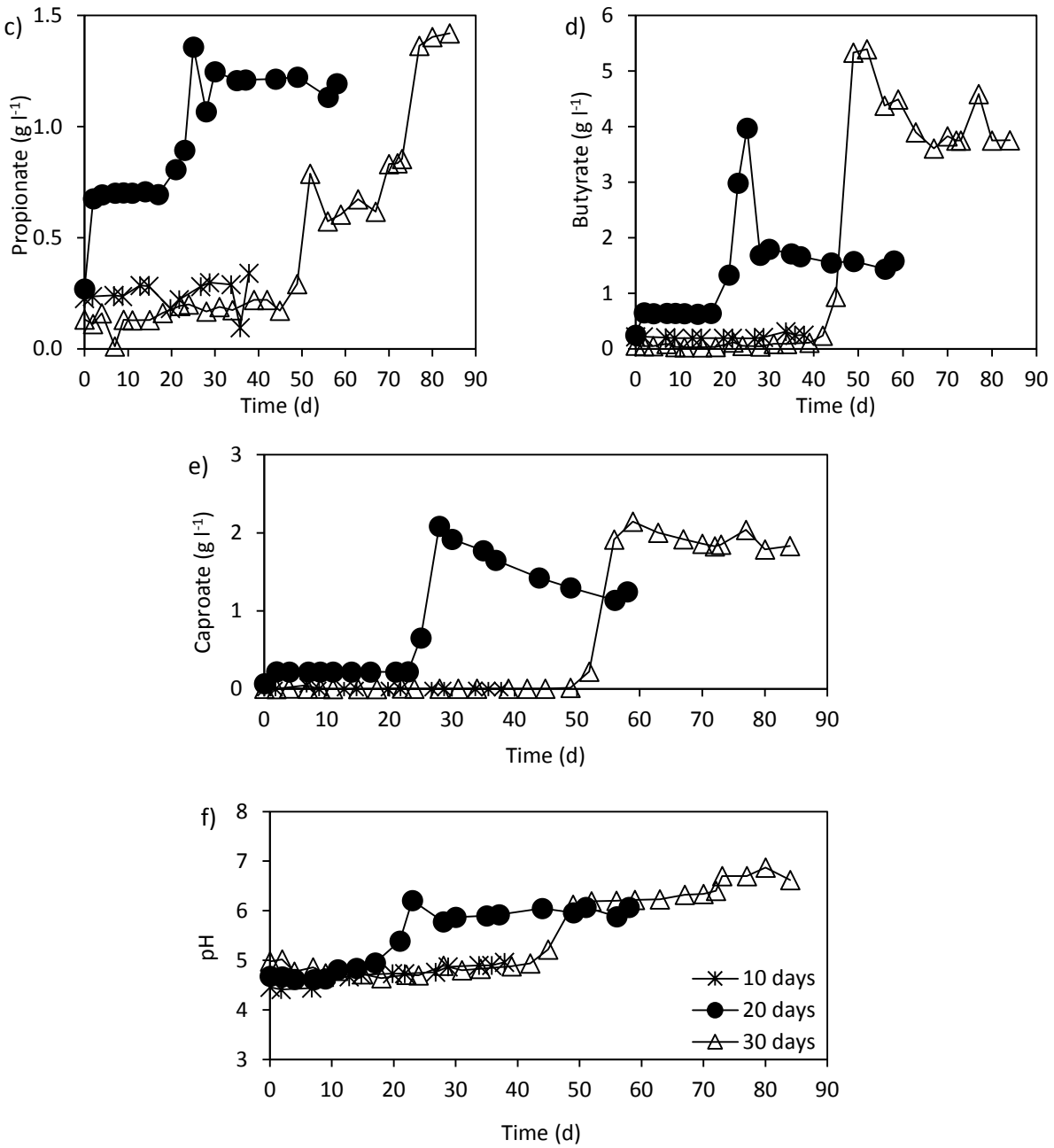


Figure 6 Monitoring profiles of the measured fermentation products at different SRTs a) Ethanol; b) Acetate; c) Propionate; d) Butyrate e) Caproate day and f) pH profile over the experimental period (✳ 10 days, 20 days ●, 30 days△)

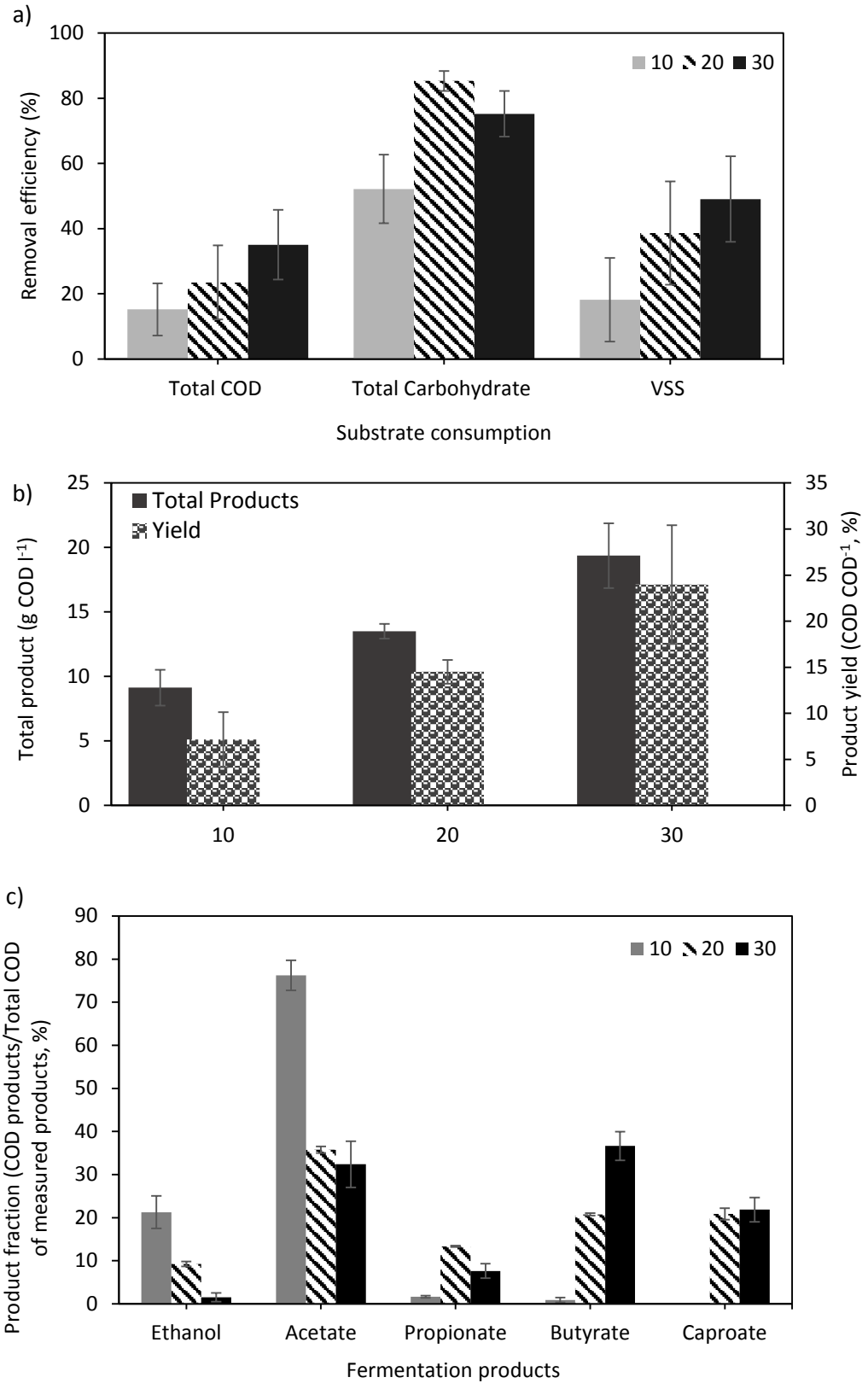


Figure 7 Average substrate consumption at different SRTs, based on the COD, total carbohydrate, and VSS removal b) average total products and yield and c) product distribution at steady state

In principle, prolonged digestion time should enhance the substrate solubilisation and consequently the product yield. It also stimulates biogas production due to methanogenic activity which leads to a decrease in the liquid-phase products. The increased COD removal

at 30 days SRT indicates higher conversion of the liquid fermentation products to biogas by the hydrogen-producing acetogens. The data obtained from the gas sensors shows that hydrogen was generated (up to 45%) and methane was negligible under this condition (less than 1%) [Supplementary Fig. S3]. There is an indication that unbuffered conditions (pH < 7) favoured hydrogen production whereas methane production was favoured under buffered conditions (pH ≥ 7). This observation shows that hydrogen can be produced at pH values unsuitable for methane production.

The average pH values were 4.84, 6.02 and 6.54 at 10, 20 and 30 days SRT respectively, the higher pH at the 30 days SRT experiment may have contributed to the increased COD removal. Fig. 6f shows the pH trends for the respective conditions. The extent of pH increase was dependent on the SRT. The pH at 30 days SRT increased by 1.6 units compared to 0.5 units at 10 days SRT. The total products concentration and product yield increased with increasing SRT. Acetate was the most predominant metabolite accounting for 76%, 36% and 32% at of the total fermentation products 10, 20 and 30 days SRT respectively (Fig. 7c). Propionate, butyrate, and caproate accounted for 13.3%, 20.8%, and 20.9% respectively at 20 days SRT and 7.6%, 36.6%, and 21.9% respectively at 30 days SRT while they were present in lower fractions at 10 days SRT. The fraction of ethanol was 21.3%, 9.3% and 1.5% at 10, 20 and 30 days SRT. The concentration of total VFAs were 9.1, 13.5 and 19.4 g COD l^{-1} at 10, 20 and 30 days SRT respectively (Fig. 7b). The highest VFA production was observed at 30 days SRT. Acetate accumulated at the shorter SRT because acetate-producing bacteria have shorter doubling time than butyrate-producing bacteria, therefore as the retention time increases, the product shifts from more oxidised compounds like acetate towards more reduced compounds such as butyrate or caproate [29].

Ethanol was not produced in any of the reactors, and as discussed for the batch experiment, the ethanol present in the feed was used up in other metabolic reactions. Acetate production followed a similar pattern in all the reactors and propionate concentration did not exceed 1.5 g l^{-1} under all conditions (Fig. 6). The propionate production was lower at 10 days SRT, and this can be attributed to the lower pH. Similarly, Dareioti et al. [34] observed lower propionate concentrations (< 2 g l^{-1}) at lower pH values (≤ 6) and up to 4.0 g l^{-1} at pH 7.0 whereas Zheng et al. [17] observed up to 2.7 g l^{-1} at pH 5.0 – 5.5. Butyrate production increased suddenly after day 17 and declined after day 25 at 20 days SRT. The reason for

the decline is not apparent, but it coincided with the decrease in ethanol concentration. Microorganisms can use VFAs as electron acceptors and hydrogen or ethanol as an electron donor to produce medium chain fatty acids such as valerate and caproate. The consumption of ethanol and the increase in the butyrate and caproate production has been described by Eq. (3) – (5) [9, 24, 29, 35].

$$C_2H_3O_2^- + C_2H_5OH \rightarrow C_4H_7O_2^- + H_2O \qquad (\Delta G_r^0 = -38.6 \text{ KJ mol}^{-1}) \qquad (3)$$

$$C_2H_3O_2^- + 2C_2H_5OH \rightarrow C_6H_{11}O_2^- + 2H_2O \qquad (\Delta G_r^0 = -77.4 \text{ KJ mol}^{-1}) \qquad (4)$$

$$C_2H_3O_2^- + C_4H_7O_2^- + 2H^+ + 2H_2 \rightarrow C_6H_{11}O_2^- + 2H_2O + H^+ \quad (\Delta G_r^0 = -48.1 \text{ KJ mol}^{-1}) \qquad (5)$$

Alternatively, butyric acid may be formed by the condensation of two moles acetic acid with two moles of hydrogen:

$$2C_2H_3O_2^- + H^+ + 2H_2 \rightarrow C_4H_7O_2^- + 2H_2O \qquad (\Delta G_r^0 = -48.1 \text{ KJ mol}^{-1}) \qquad (6)$$

However, there was no significant decrease in the production of acetate even though Eqs. (3) – (6) shows that acetate consumption is associated with the production of butyrate and caproate. The reason might be that there was a balance between the production and consumption of the acetate.

The gas production was only monitored at 30 days SRT. The H_2 composition at the end of the acclimation phase was around 10% until day 43. The gas content increased rapidly between days 43 and 46, up to 46%, after which a decline was observed. Correspondingly, there was a rapid increase in the butyrate concentration from 0.2 to 5.0 g l^{-1} between days 42 and 49; this was due to the increased hydrogen partial pressure in the reactor (Eq. 6). According to Agler et al. [24], propionate production is favoured by increased hydrogen partial pressure since hydrogen is consumed during the reaction but the concentration of propionate in the experiments were not significant relative to the other products. This is similar to what Arslan et al. [36] observed in their study where they concluded that propionate production is not always higher at elevated hydrogen partial pressure. Fig. 6a and 6d show that 20 and 30 days SRT exhibited a similar behaviour relating to ethanol consumption which coincided with caproate production (Eq. 5).

Even though the composition of fermentation products was similar at the different SRTs, the results show that the metabolic pathways for the VFAs production were different. The

difference in the pH values may have contributed to the variation in the metabolic pathways. Microorganisms often alter their environment because of their growth activities and sometimes as a means of improving their competitive advantage against other organisms. Their response to their environment is reflected in both physical and chemical mechanisms which provides them with a selective advantage [37].

The fermentation shifted from acetate-type fermentation to butyrate-type fermentation at 20 and 30 days SRT; this shift may have resulted from a change in the pH and hydrogen partial pressure of the system. A similar change in the product distribution was reported by Horiuchi et al. [38]. They concluded that the change was due to an adjustment in the dominant microbial population in the system in response to the pH shift, rather than a change in the metabolic pathway of the same microbial population. The change in the dominant microbial population often occurs because of the difference in the optimal pH for the various microbes.

The yields obtained at the investigated SRTs ranged from 7 - 24% ($COD_{total\ product}/COD_{feed}$), this suggests that the studied SRTs was sufficient for an appropriate level of substrate degradation (Fig. 7). Similarly, Lim et al. [15] reported an increase in the yield (g VFA/VS $_{added}$) with an increase in the SRT, from 26 – 32% at 4 days SRT to 36 – 39% at 12 days SRT. The production of butyrate and caproate at 20 and 30 days SRT contributed to the higher yield. The product yield is associated with the extent of substrate conversion to liquid products, and the lower product yield at 10 days SRT shows that a substantial fraction of the VSW remained in the reactor unutilised.

The fermentation experiments in this study were evaluated under batch and semi-continuous modes. The experimental results showed the product yield to be higher under batch conditions than the continuous counterpart, and the variation can be attributed to the SRT. The extended retention time in the batch reactors allowed maximum conversion of the substrate whereas the lower SRT limited the semi-continuous fermentation. Also, all the microbes in the seed inoculum may take part in the fermentation process under batch conditions whereas microbes with lower growth rate are washed out of the reactor in semi-continuous systems. Semi-continuous fermentation was more stable than batch fermentation because microorganisms are more tolerant to changes in environmental conditions in continuous processes than in batch processes. The dilution effect of the fresh feed minimises toxicity to microorganisms in continuous processes. Nevertheless, some

shortcomings of these systems include reduced conversion efficiency due to the loss of unconverted VSW in the effluent and the requirement of large reactor volume at prolonged SRTs [39]. A batch reactor that is not optimised for chemicals production will lead to methane formation, consequently decreasing the process yield.

The industrial applications, market price and production rates of the considered fermentation products have been discussed in a recent paper by this research group [7]. Caproate was the most valuable fermentation product in this study based on its current market value of 1.6 $ kg^{-1} [40]. Caproate production from VSW is of practical relevance due to its market value and the ease of separation compared to the short chain carboxylates. Long SRT favoured caproate production; thus, it would be advantageous to operate at long SRTs to steer the process towards its production.

Conclusions

This study investigates the anaerobic fermentation of VSW under a range of SRTs and different pH conditions. In the batch tests, the total product yield was higher in the buffered reactor (pH ≥ 7). In the semi-continuous reactors, VSS removal was higher under buffered-alkaline condition (57%) compared to the unbuffered-acidic conditions (39%). Indeed, the pH has an impact on the hydrolysis of the complex substrates to simpler molecules. Prolonging the SRT enhanced the total carbohydrate and VSS removal. The yield based on the measured liquid products at 10, 20 and 30 days SRT was 7%, 15%, and 24% $COD_{total\ product}/COD_{feed}$ respectively. The highest yield obtained in the batch experiment was 62%. Increasing the SRT increased the VFAs concentrations, but equally stimulated biogas formation, with the maximum total product obtained at 30 days SRT (19.4 g COD l^{-1}). Less COD removal was observed at 10 and 20 days SRT. The product distribution was significantly affected by the SRT with a metabolic shift from acetate production to butyrate and caproate production at prolonged SRT. Indeed, further studies are required to verify the results of this study at a larger scale considering the small scale at which the laboratory studies were carried out.

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