THE EFFECT OF pH AND HYDRAULIC RETENTION TIME ON PRODUCTION OF VOLATILE FATTY ACIDS FROM PRIMARY SLUDGE ANAEROBIC FERMENTATION

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The effect of pH and hydraulic retention time on the production of volatile fatty acids from primary sludge anaerobic fermentation

Master of Applied Science, 2018

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Abstract

Primary Sludge (PS) from wastewater treatment plants contains high biodegradable organic matter and therefore can be used to produce Volatile Fatty Acids (VFAs). The produced VFAs can be utilized in biological nitrogen and phosphorus removal processes as an external carbon source. The objective of this research was to investigate the effect of pH and hydraulic retention time (HRT) on the production of VFAs from PS through the anaerobic fermentation process. The experiments were conducted in both batch and semi-continuous flow regimes using bench scale fermenters under the mesophilic temperature. The Design of experiments included the HRT of 1 – 3 days and pH range of 4.5 - 11.0 for batch and 4.5 - 6.5 for semi-continuous modes. According to the obtained results, the VFAs production increased with an increase in HRT from 1 to 3 days. For the batch study, the pH range for maximum VFAs yield was pH 6.5 –10.0 achieved at HRT of 3 days. For the semi-continuous study, the maximum amount of VFAs production was observed at a pH of 6.5 and HRT of 3 days.

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List of Abbreviations

ABWWTP – Ashbridge's Bay wastewater treatment plant

AD – Anaerobic Digestion

BNR - Biological Nutrient Removal

BOD_5 – Biochemical Oxygen Demand

COD – Chemical Oxygen Demand

CSTR – Continuous Stirred tank reactor

HAc - Acetic Acid

HBu - Butyric Acid

HPr - Propionic Acid

MWWTP – Municipal Waste Water Treatment Plant

PS – Primary Sludge

PVC - Poly Vinyl Chloride

SCFA- Short Chain Fatty Acids

SCOD – Soluble Chemical Oxygen Demand

TCOD – Total Chemical Oxygen Demand

TS- Total Solid

TSS – Total Suspended Solids

VFAs – Volatile Fatty Acids

VSS – Volatile Suspended Solids

WAS – Waste Activated Sludge

TWAS – Thickened Waste Activated Sludge

WWTP – Wastewater Treatment Plant

1 Introduction

Waste generation and management have been a concern in the modern world. Compared to other waste management methods (i.e., landfilling, incineration, and composting), the resource recovery from organic waste provides the provision for waste minimization as well as the production of the value-added products. In this regard, the primary sludge (PS) from municipal wastewater treatment plants (MWWTP) can be considered as a rich source for the production of volatile fatty acids (VFAs) as a value-added product and therefore, it has drawn an extensive research interest through the acidogenesis process [1]. Acidogenesis is also termed as dark fermentation [2]. VFAs are short-chain fatty acids with six or fewer carbon atoms which can be used in bioplastic and bioenergy production as well as in biological nutrient removal (BNR) process as a carbon source [3].

Anaerobic Digestion (AD) has an acidification step in which VFAs such as acetic acid (HAc), propionic acid (HPr), and butyric acid (HBu) are produced as the intermediate metabolic products. VFAs production from organic wastes such as PS occurs through the dark fermentation process consists of two sequential steps, hydrolysis and acidogenesis. During the first step (hydrolysis), the high molecular weight organic compounds like complex organic polymers are broken down into simple and soluble organic monomers by the enzymes produced by the hydrolytic microorganisms. The hydrolysis step is characterized by an increase in soluble chemical oxygen demand (SCOD). The next step in the dark fermentation is the acidogenesis process during which the generated SCOD from the hydrolysis step is converted to VFAs (HAc, HPr, HBu) in the presence of the acidogenic microorganisms. Both the hydrolysis and acidogenesis processes occur in the

anaerobic condition via anaerobic microorganisms such as *Bacteriocides, Clostridia, Bifidobacteria, Streptococci, and Enterobacteriaceae* [4]. The performance of the fermentation process depends on different environmental and operational factors such as pH, temperature, solid retention time (SRT), organic loading rate (OLR), etc. Among various organic waste sludges, previous studies have mainly focused on the production of VFAs from PS, since it is one of the largely produced organic waste in MWWTPs. The total chemical oxygen demand (TCOD) of PS ranges between 15,000 mg l^{-1} and 60,000 mg l^{-1} which suggests its high potential for VFAs production [5]. One application of the produced VFAs form PS is to utilize it as a carbon source for the BNR process. On average, the removal of 1 mg of phosphorus (P) through the BNR process requires 7 to 9 mg of VFAs [6]. It was reported that the required level of the P concentration to treated wastewater would be 0.2-0.3 mg l^{-1} [7]. Considering these explanations, this research will investigate the effect of pH and SRT on the performance of the dark fermentation process to produce VFAs from PS.

2 Research Objectives

The primary objective of this study was to evaluate the effect of pH and HRT on the fermentation of the PS to maximize the SCOD and VFAs production. The goal of the project was to maximize the produced VFAs that can be used as a carbon source for the BNR process.

For this purpose, experiments were conducted under both batch and semi-continuous flow regimes. The experiments were first conducted under batch mode at different pH levels from 4.5-11.0. During the batch experiment, samples were collected at 1, 2, and 3 days intervals. After the batch test, semi-continuous fermenters were operated at three pH levels (4.5, 5.5, and 6.5) and under three HRTs of 1, 2, and 3 days. The HRT was kept low until 3 days to ensure VFAs production and inhibit the further step in AD which is methanogenesis that will consume VFAs [8]. The performance of the fermenters was evaluated by monitoring the SCOD and VFAs concentration and yield throughout the process.

3 Literature Review

3.1 Anaerobic digestion

Anaerobic digestion (AD) is a multi-step process during which organic matter is converted into methane-rich biogas in the absence of oxygen and in the presence of anaerobic microorganisms [9]. The AD process occurs through four major sequential steps including hydrolysis, acidogenesis, acetogenesis, and methanogenesis. Throughout the overall digestion process, the hydrolysis has been known as the rate limiting step [10]. The fermentation process is an AD process that progresses until the end of the acetogenesis stage. The main biochemical pathways involved in AD is shown in Figure 1. The produced biogas typically consists of 60-70% methane (CH_4) and 20-30% carbon dioxide (CO_2) and fractions of other gases (H_2 and H_2S) [9].

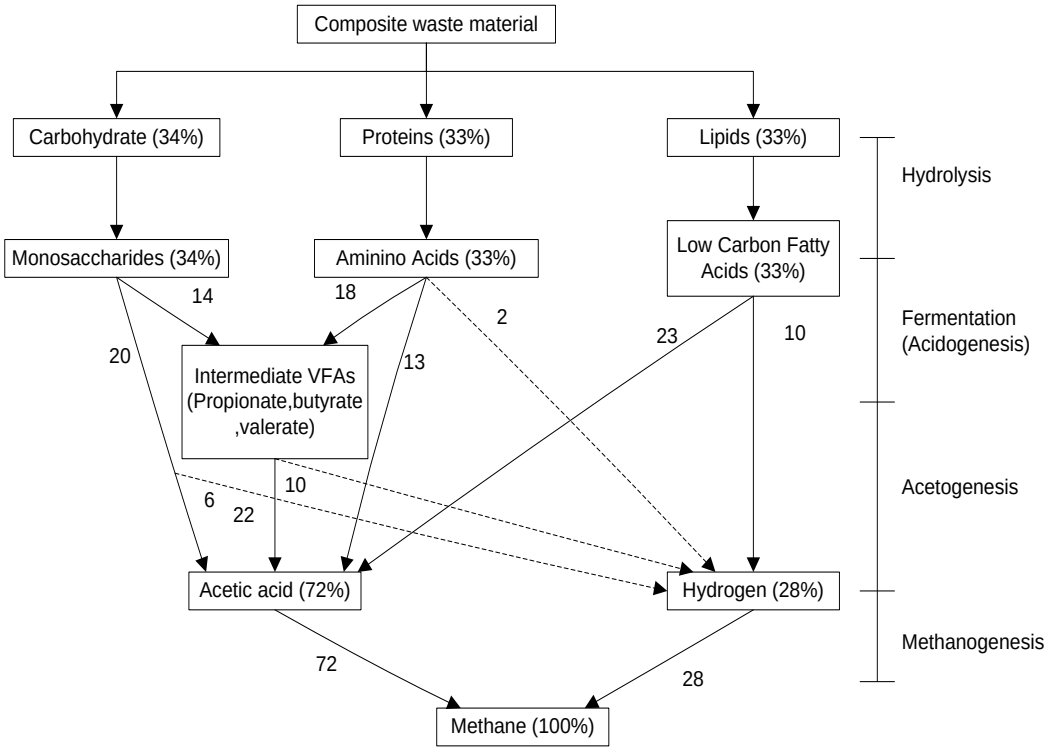

Figure 1 Pathways for anaerobic digestion (AD) process [9].

3.1.1 Hydrolysis

Hydrolysis is the first step of the digestion or fermentation process. During hydrolysis, particulate organic matter is converted to soluble compounds that can be further degraded to the simple monomers. These monomers are later used by the acidogenic bacteria to produce VFAs. For example, polymeric substrates such as cellulose, protein, and lipid (fat and oil) are converted to soluble molecules of sugar (glucose and xylem), amino acid, and long chain fatty acids (13 to 21 carbons), respectively. During the hydrolysis process, proteins are converted to amino acids by proteases secreted by proteolytic microbes. The cellulase and xylanase enzymes produced by cellulitis and xylanolytic microbes convert the complex sugar molecules into glucose and xylem, respectively. Lastly, lipases which are created by lipolytic microbes convert lipids to long-chain fatty acids and glycerol [11]. The general reactions occurring through the hydrolysis stage are shown below [12].

Hydrolysis/Liquefaction reactions

Lipid	⟶	Fatty Acids
Polysaccharides	⟶	Monnosaccharides
Protein	⟶	Amino Acids
Nucleic Acids	⟶	Purines & Pyrimidines

3.1.2 Acidogenesis

Acidogenesis is the stage through which anaerobic bacteria convert sugars, amino acids, and long-chain VFAs to acetate, hydrogen, carbon dioxide, and short chain VFAs (i.e., HPr, HBu, and HAc), ketones, alcohols, and lactic acid. Conversion of glucose to acetate, ethanol, and propionate are shown in the reactions 1, 2 and 3 below respectively [12].

$$C_6H_{12}O_6 + 2\,H_2O \longrightarrow 2CH_3COOH + 2\,CO_2 + 4\,H_2 \quad \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

$$C_6H_{12}O_6 \longrightarrow 2CH_3CH_2OH + 2\,CO_2 \quad \ldots\ldots\ldots\ldots\ldots\ldots (2)$$

$$C_6H_{12}O_6 + 2\,H_2 \longrightarrow 2CH_3CH_2COOH + 2\,H_2O \quad \ldots\ldots\ldots\ldots\ldots\ldots (3)$$

In an equilibrium condition, most of the organic matter are converted into substrates such as acetate, hydrogen, and carbon dioxide that will be used by the methanogenic microbes. As the byproduct of the amino acids fermentation, ammonia, and hydrogen sulfide are released [11]. The high concentration of these compounds can cause inhibition for AD process [12].

3.1.3 Acetogenesis

During acetogenesis, VFAs with more than two atoms of carbon, alcohols, and aromatic fatty acids are converted into acetate via obligate hydrogen-producing bacteria [12]. In this step, the products of the first phase are converted to simple organic acids, carbon dioxide and hydrogen by acetogenic bacteria, also called acid formers. The activities of different microorganisms cause the formation of different products during the acetogenesis process. These microorganisms include *syntrophobacter wolinii*, a propionate decomposer, and *sytrophomonos wolfei*, a butyrate decomposer. Also, other acid formers include cl*ostridium spp., peptococcus anerobus, lactobacillus,* and *Actinomyces*. Each group of microorganisms follows different pathways. For example, the hydrogen-producing acetogenic bacteria yield acetate, H_2 and, CO_2 from VFAs and alcohol, whereas, homoacetogenic bacteria produce acetate from CO_2 and H_2 .However, most of the acetate is formed via hydrogen producing acetogenic bacteria [12].

3.1.4 Methanogenesis

The fourth step, methanogenesis, is carried out by a group of organisms called methanogens. Two groups of methanogens are generally involved in methane production. One group is *acetoclastic methanogens* that convert acetate into methane and CO_2. The second one is hydrogen-utilizing methanogens that use hydrogen as an electron donor and CO_2 as an electron acceptor to produce methane. According to Figure 2, about 70% of the methane produced during the methanogenesis stage is from acetate. Based on the type of the substrate consumed by the methanogens, the methanogenesis process can be classified into two main categories [12]:

1) Hydrogenotrophic methanogenesis during which hydrogen and carbon dioxide are converted into methane through the following reaction:

$$CO_2 + 4H_2 \longrightarrow CH_4 + 2H_2O \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (4)$$

2) Acetoclastic methanogenesis which involves the formation of methane from the conversion of acetate by through the following reaction:

$$CH_3COOH \longrightarrow CH_4 + 2CO_2 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (5)$$

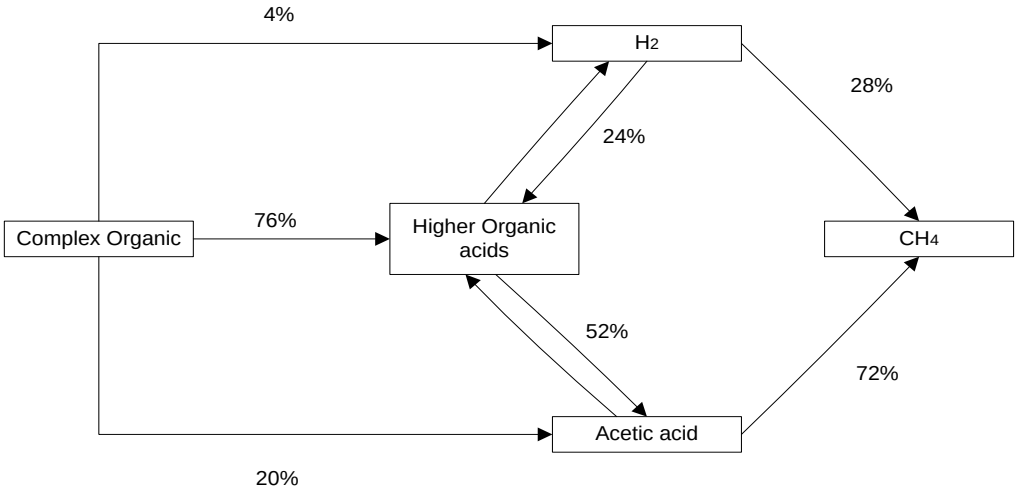

Figure 2 Carbon and hydrogen flow in the anaerobic digestion process [9]

3.2 Advantages and disadvantages of AD

AD is primarily applied for the treatment of both organic solid waste and high strength (concentrated) wastewater. In addition to the energy recovery in the form of methane, the AD has other advantages, which includes waste volume reduction, reduced odor potential, and reduced pathogen content of the of the digested compounds (digestate). Most of the previous lab and full-scale application of AD were carried on under the mesophilic temperature range (30-40°C), but several studies and field applications have been also conducted under the thermophilic condition (45-65°C) [9].

The advantages and disadvantages of the AD process is listed in Table 1

Table 1 Advantages and disadvantages of the anaerobic process compared to the aerobic process [9]

Advantages	**Disadvantages**
Less energy required	Longer startup time to develop necessary biomass recovery
Less biological sludge production	Might require alkali addition
Less nutrient required	May require further treatment with anaerobic treatment process to meet discharge requirement
Methane production is a potential energy source	Biological phosphorus and nitrogen removal is not possible
Smaller reactor volume required	More sensitive to the negative effect of lower temperatures on reaction rates
Elimination of off-gas air pollution	More susceptible to upsets due to toxic substances or broad feeding changes
Able to respond quickly to substrate addition after long periods without feeding	The potential for odor production and corrosiveness of gas
Effective pre-treatment process	
The potential for lower carbon footprint	

3.3 Dark Fermentation

During the fermentation process, the hydrolytic microorganisms hydrolyze complex organic polymers to monomers that are further converted to a mixture of low molecular weight organic acids and alcohols by acidogenic bacteria. Previously, the PS has been widely used as a potential substrate for the dark fermentation process to produce VFAs. The efficiency of the dark fermentation process depends on different factors including the pretreatment process (if applicable), operating pH, temperature, SRT, and OLR along with the characteristics of the sludge [13]. During the fermentation of PS, the carbohydrates are oxidized by electron acceptors, and eventually are converted to VFAs in the form of CH_3COOH, CH_3CH_2COOH, etc. along with some hydrogen as intermediate products [14]. This process of partial conversion of PS to produce VFAs and hydrogen is indeed called dark fermentation. The overall reactions can be expressed as follows:

$$C_6H_{12}O_6 + 2H_2O \longrightarrow 2\,CH_3COOH + 4H_2 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (6)$$

$$C_6H_{12}O_6 + 2H_2O \longrightarrow 2\,CH_3CH_2COOH + 2H_2 \ldots\ldots\ldots\ldots\ldots\ldots\ldots (7)$$

3.4 Methanogen inhibition

Compared to the other microorganisms involved in the AD process, the methanogenic bacteria are susceptible to the change in the environmental conditions. The optimal pH of methanogen is 7.0-8.5 [15]. Below pH 6.6, significant inhibition of methanogenic bacteria occurs. When pH drops below 6.2, acid conditions become inhibitory to the methanogenic bacteria as acid production continues. The fermentative bacteria continue to produce VFAs until pH drops to 4.5 or 5.0 [16]. Methanogenic bacteria become active

at pH above 6.0 [15] and therefore one way to limit their activity during the fermentation process is to keep the pH level below 6.0 throughout the process [17].

3.5 VFAs production from PS

Several studies have been conducted to evaluate the feasibility of hydrolysis and acidification process for VFAs production from PS, WAS or mixed sludge (PS + TWAS). The readily biodegradable organic content of PS is much higher than that of the WAS and therefore, the VFAs yield and SCOD yield are generally higher for the PS [9]. Since PS is abundant in most of the MWWTPs, the production of VFAs from PS gives the provision to use it as a carbon source for the on-site BNR process, reducing the cost of external carbon source as well as the transportation cost. Considering these explanations, PS was selected as the substrate for VFAs production in the current study. The main outcomes of some of the previous studies conducted for the fermentation of PS are summarized below.

The mesophilic fermentation of PS, TWAS, and mixed sludge under the mesophilic temperature of 37ºC and a SRT of 5 days was studied and resulted in the specific VFAs production rate of 270 mg COD g^{-1} VSS, 62 mg COD g^{-1} VSS, and 114 mg COD g^{-1} VSS, respectively [18]. And also the effect of PS samples from different origins was studied in another experiement with PS VSS content ranging from 15,290 to 29,100 mg l^{-1} [18]. The results showed consistent VFAs production potential and composition regardless of the initial VSS concentration [18]. It is known from the literature that the VFAs production from PS can be maximized by adjusting the pH and HRT conditions [19]. In terms of SRT, under long SRTs, the production of methane will begin which will reduce the VFAs yield. It was reported at a temperature of 25ºC, the digestion of sludge at a SRT of 8 days

resulted in methanogenic activity [20]. On the other hand, higher SRT leads to higher reactor volume associated with higher capital cost. Therefore, the SRT is kept as low as possible to avoid methanogenesis process and to minimize the cost. According to the literature, the optimum SRT of PS is suggested in a range of 3-5 days [21]. In another study and under semi-continuous flow regime, the SRT of 5 days was investigated to prevent the growth of methanogen and thereby to avoid the consumption of readily degradable CODs including VFAs [18]. PS generally contains a higher percentage of organic matter. The higher organic matter content of PS results in more bacterial activity and subsequently higher concentration of enzyme produced by hydrolytic bacteria [22]. In PS, organic matters are more biodegradable while for WAS organic matters are in polymer form which is difficult to degrade. The rigid cell structure of WAS made up of glycan and peptide make it intractable for microbial degradation [22].WAS cells are held together with extracellular polymeric substances (EPS) which also makes WAS challenging to biodegrade. An external mechanism such as pre-treatment is required to disintegrate WAS which is not necessary in the case of PS.

Volatile suspended solids (VSS) in PS were more biodegradable (87%) than that of WAS (43%) [23]. The ratio of SCOD to TCOD in a batch reactor operated at 20ºC for five days for TWAS was significantly lower than that of PS [18]. The concentration of VSS in PS is a good indication of the biodegradable substrate. It was stated that the rate of the reaction was directly proportional to the initial biodegradable substrate concentration measured as VSS [24]. The effect of SRT on hydrolysis, acidification, and methanogenesis of PS in UASB and CSTR reactors at 25°C was studied by [20].This study revealed that SRT $\leq$ 8 days resulted in acidogenic conditions and SRT $\geq$ 8 days shows methanogenic conditions

[20]. The authors reported that the most substantial increase in hydrolysis and acidification of TCOD of PS occurs between SRT 1-3 days [20].

3.6 Factors affecting dark fermentation

Environmental conditions such as temperature and pH have an essential role in the selection, growth, and survival of microorganisms. Generally, the optimal growth of microorganism happens in a narrow range of temperature and pH, but they can survive in broader limits. The previous studies confirmed that the effects of temperature in anaerobic fermentation are significant [9]. In general, the growth rate doubles for every 10°C increase in temperature until it reaches to the optimum temperature [9].The typical and optimum temperature range for microorganisms are shown in Table 2 for psychrophilic, mesophilic, and thermophilic conditions [9].

Table 2 Temperature classification for the biological process with its optimum range [9]

Type	Temperature range (°C)	Optimum range (°C)
Psychrophilic	10-30	12-18
Mesophilic	20-50	25-40
Thermophilic	35-75	55-65

The pH of the environment is also a key factor for the metabolism and growth of microorganisms. Most bacteria cannot tolerate a pH level below 4.0 and above 9.5. The optimum pH for anaerobic bacterial growth lies between 6.5 and 7.5 [9]. Along with the progression of acid fermentation, VFAs are produced and the accumulation causes a drop in pH and thereby inhibiting the methanogenic activity. If the pH can be controlled below

7.0, suitable pH environment can be kept for VFA production. Biochemical reactions of the AD process are catalyzed by enzymes. These organisms' dominant for each step of the AD process has an optimum pH when the rate of reaction is maximum. For methanogens, pH 7.0-8.0 is optimal; for fermentation pH 6.5 - 8.5 is operational, pH 5.0-7.0 is optimal; and for hydrolysis pH 5.0-7.0 is optimal [15]. Deviation from optimum pH value during a fermentation process can be caused by the influent pH, and the accumulation of acidic products such as VFAs or basic products such as ammonia [15].

Several studies have been conducted to evaluate the effect of the environmental and operational parameters including pH, temperature, and SRT. The current section will summarize some observations from those studies. The yield and composition of VFAs are dependent on operational temperature, pH, HRT and OLR [20].

3.6.1 Effect of pH

Comparatively, few studies have been conducted to investigate the effect of pH on VFAs production from municipal waste sludge including PS and WAS especially under pH value below 5.0 [25]. Previous studies that have been conducted to identify the effect of pH on VFAs and biohydrogen production revealed that the optimum pH range to achieve maximum VFAs and hydrogen yield is in the range of 5 to 6 using either pure or mixed culture bacteria [26]. Previous research also showed that the level of pH affects the type, composition, and concentration of VFAs produced during the fermentation process [1]. The influence of pH on the acidification of PS in the complete mix fermenter was investigated and determined to be optimum at 6.8 at a temperature of 50°C [17]. No significant increase in VFAs production from PS fermentation at a controlled pH of 7.0 was observed compared to the uncontrolled pH at a range of 5.9-6.4 [17]. The acidic pH

range in bioreactor inhibited methanogenesis with no methane gas production in reactor [27].

The influence of pH on acid phase anaerobic digestion of PS was examined in another study [25]. In this study, controlled and uncontrolled pH experiments were conducted using bench scale completely mixed reactor (CMR) with clarifier and solids recycle unit as well as a UASB reactor [25]. Specific VFA production rate, COD solubilization, and VSS reduction percentage for both CMR and UASB reactor were not affected by pH variation in a range of 4.3-5.2. However, at higher pH values (5.9-6.2), 25 - 30% reduction was observed for the parameters [25]. Analysis of degradation behavior of carbohydrates, proteins, and lipids revealed that each organic class followed an individual trend concerning pH changes. Regardless of the level of pH, HAc was the dominant VFAs product with an average of 45% in both reactors [25]. However, the composition of the produced VFAs was different in terms of HPr and HBr depending on the pH level [25].

The effect of pH on the production of VFAs from glucose was investigated in a CSTR for the pH range of 4.0-7.0. The authors reported the optimum pH for maximum VFA production to be 5.5 [28]. The study showed that butyrate and acetate were the two abundant VFAs in the effluent. Within the pH level between 4.0 and 6.0, the effluent contained 41.4 -32.4% butyrate and 15.3 -29.5 % acetate. However, the pH levels of 6.5 to 7.0 resulted in an increase in acetate (33.1-34.1%) and a decrease in butyrate (31.5-31.2 %) concentration [28].

Another study was conducted to evaluate the effect of pH and temperature to produce soluble organics through the fermentation of PS [29]. In this study, parallel experiments were conducted under different pH and temperature conditions. It was reported that at a

temperature of 20°C, the uncontrolled pH resulted in SCOD and VFAs concentration of 14 mg l^{-1} and 9.2 mg-COD l^{-1} [29]. According to this study, not only the concentration but the composition of the VFAs also changed at higher pH levels. According to this research, at the low range of pH, about 45% of the total produced VFAs was HAc. However, the HAc content of total VFAs reduced at higher pH levels [29]. Similar results were reported [30]. It is noteworthy that out of different VFAs, HAc is the preferred carbon source to be used in the BNR process [31]. PS from primary sediment was also investigated for producing carbon source through the fermentation process [32]. The system condition was as follows: temperature: 35ºC, SRT: 3 days, HRT: 28 hours. This system resulted in the SCOD and VFAs concentration of 975.5 mg l^{-1} and 516.4 mg l^{-1} [32].

3.6.2 Effect of SRT

In acidogenic fermentation of PS to produce VFAs, retention time is a critical parameter. Among the operational parameters of anaerobic digestion or dark fermentation, HRT (or SRT) is one of the most influential parameters on the growth of acidogens. HRT affects the net VFA production as it directly links to the contact time between substrate and microorganisms [31].

HRT is the amount of the theoretical time that water or liquid takes to travel through the entire system. HRT is calculated by the following equation (8).

$$HRT = \frac{Volume\ of\ the\ reactor, V}{Influent\ flow\ rate, Q} = \frac{L}{\frac{L}{d}} = d \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (8)$$

SRT is the average time the cell mass stays in the reactor (9). For a CSTR, SRT can be calculated as follows:

$$SRT = \frac{Mass\ of\ the\ reactor}{Mass\ rate\ of\ the\ solids\ leaving} = \frac{V * TSS}{Q_{Out} * TSS} = \frac{V}{Q_{out}} = HRT\ (d) \ldots\ldots\ldots (9)$$

For a CSTR semi-continuous system, HRT is equal to SRT. However, for a reactor with different HRT and SRT (with an sludge recycle line), under longer SRTs, microorganisms get more time to react with waste, but at longer HRTs, VFAs production becomes stagnant [33]. This can result in high organic loading rate [34].

Effect of HRT on PS acid-phase anaerobic fermentation was investigated using bench scale continuous flow reactors [25]. Results indicated that both VFAs and SCOD increased with increase in HRT up to 12 hrs, but drop down moderately at longer HRTs [25]. HAc (46%) and HPr (32%) were the main components of the VFAs formed [25]. Variation of HRT significantly impacts the organic substrate degradation [25]. Two completely mixed reactors (CMR) with 3L volume and solid recycling ability were used to investigate the effect of HRT and temperature on VFAs production [7]. One reactor was filled with PS only, while the other one was fed with PS and industrial wastewater full of starch. VFAs and SCOD concentration reached maximum values at HRT 30 hrs at 25°C [7].

Two bench-scale CMRs were used to investigate the acidogenesis of PS at different HRT and temperature [27]. Increasing the HRT from 18 to 30 hours improved the substrate solubilization in both the reactors [27]. At the HRT of 18 hrs., temperature of 22ºC, and pH of 5.63-5.77, the net VFAs production was 273 ± 61 mg l^{-1} [27]. VFAs production was increased by 14% (329 ± 52 mg l^{-1}) with an increase in HRT to 30 hours and temperature to 30ºC [27]. But with further increase in temperature at 35ºC, VFAs production

decreased, however, the amount of VFAs generated was enough for using in BNR process [27].

Effect of HRT on VFAs production was studied in a CMR using diluted PS [6]. VFAs and SCOD concentration as well as specific VFAs production rate were found maximum at the temperature of 25°C, HRT of 30 hours, and a SRT of 10 days [6]. Specific VFAs production rate at the HRT = 30 hours and a temperature of 25°C was 0.0306 mg VFAs g^{-1}VSS d^{-1} [6]. HAc was the dominant VFAs produced with an average percentage content of 61-67%, followed by HPr (24-35 %) [6].

Two bench scale fermenters were used to analyze the effect of PS fermentation on VFAs production [8]. Experiments were conducted at a SRT of 4-10 days, total volatile solids concentration of 0.6-2.8%, and under two different temperatures of 20°C and 30°C [8]. The results from this study indicated the importance of feed sludge characteristics on VFAs yield. High VFAs yield was observed at high total volatile solids concentrations above 23,000 mg l^{-1} [8]. When SRT increased from 4 days to 6 days, a significant decrease in VFAs yields was observed [8]. Increasing the temperature increased the VFAs yields dominantly as a result of improved hydrolysis of particulate organic matter [8].

The effect of SRT in a range of 3-15 days was investigated on PS hydrolysis, acidification and methanogenesis [20]. The research demonstrated that SRT ≤ 8 days resulted in acidogenic conditions with negligible biogas production, whereas SRT ≥ 8 days resulted in methanogenic conditions [20]. The hydrolysis of carbohydrate and lipids increased as SRT increased [20].

3.6.3 Effect of Temperature

PS fermenters have been operated at ambient and controlled temperatures in various studies. It was observed that when temperature increases, hydrolysis, and acidification of PS improves [25]. It was reported that temperature had a significant effect on the hydrolysis of protein, carbohydrate, and lipids content of PS. Hydrolysis rate was higher at 35°C than 25°C [10]. Additionally, a higher rate of hydrolysis was observed at 55°C, compared to PS fermentation at 20°C and 35°C [35]

Production of VFAs had been studied in various temperature ranges and found that if the temperature is increased within psychrophilic (12-18°C) and mesophilic temperature ranges (25-40°C), the concentration of VFAs production increases [36]. In addition, the rate of the VFAs production will increase [6]. The study shows that VFAs yield also increases with temperature [8]. By increasing the temperature from 10 to 35°C, the VFAs concentration from WAS increased by 30% [37]. In the case of the fermentation of PS, VFAs concentration rate improved six-fold as the temperature was increased from 8-25 °C [6]. It has been reported that thermophilic temperatures lead to faster biodegradation and more active acidogenesis compared to that of mesophilic temperature [38]. A batch experiment conducted on activated sludge with 1% glucose showed that at pH 5.0 and 6.0, the higher the temperature, the higher the fermentation rate is as microbial metabolism increased with the increasing temperatures [39].

It has been observed that VFAs production from PS consistently improved with the temperature increase from 10°C to 30°C [16]. However, at 30°C and under uncontrolled pH and HRT of 9 days, the net VFAs production dropped from 115 mg l^{-1} as HAc at 20°C to 103 mg/ as HAc at 30°C [40]. Temperature effect on VFA production was also studied

for PS fermentation in a temperature range of 10-24°C [29]. According to this study, the VFA production increases significantly because of temperature increase. At 10°C, 610 mg l^{-1} VFAs was produced, while it increased to 2950 mg l^{-1} at 24 °C [29]. The effect of SRT and process temperature on the hydrolysis and acidification of PS was investigated in CSTR reactors. The SRT and temperature have a substantial effect on the hydrolysis of protein, carbohydrates, and lipids [10]. The hydrolysis rate constant of all solid substance was significantly affected by temperature [10]. Biodegradability of PS shows no temperature dependency for a range of 15-35 °C [10].

The above studies recapitulate the fact that increasing the fermentation temperature rises the production of VFAs as the kinetics and rate of reaction is increased. Higher temperature such a thermophilic temperature range is most likely require less HRT for higher VFAs production.

3.7 Alkaline fermentation of PS sludge

Alkaline fermentation of sewage sludge can also be applied for enhanced VFAs production from PS. As opposed to using caustic soda or other chemicals to achieve alkaline pH that causes higher cost, the anaerobic supernatant can be used to adjust pH of the fermenter. The effects of mesophilic (35°C) and thermophilic (55°C) temperatures, retention time (1- 8 days), pH (8.0 -11.0) and initial TS concentrations (4.5 -6.5%) have been studied in various studies to optimize the VFAs production under alkaline fermentation. Similar results were obtained in terms of VFA production when anaerobic fermenter supernatant of sludge was used for pH adjustment compared to the caustic soda. The highest VFA concentration was achieved at pH 10.0 and 11.0, TS 6.5%, and a SRT of 6 days under a temperature of 55°C [41]. Design conditions for the batch test

was as follows: temperature: 35°C & 55°C, pH: 8.0-11.0, retention time: 1- 8 days, initial TS: 4.5% & 6.5%. The VFAs concentration of 122 mg COD g^{-1} VS fed was obtained at pH = 8 & 9, retention time 6-7 days, TS=6.5%, and temperature of 37°C [41]. The VFA concentration was increased to 298 mg COD/g VS fed (2.5 times higher) at pH = 10 & 11, retention time 6 days, TS=6.5% ,Temperature 55°C [41].

The effect of pH ranging from 5.0 to 12.0 on PS fermentation for VFA production was examined [42]. The experiment result indicated that hydrolysis was accelerated and sludge solubilization was greatly enhanced as high concentrations of SCOD were produced at alkaline pH conditions [43]. The study also demonstrated a decrease in VFAs production due to inhibition of acidification in extremely alkaline conditions of pH 11.0-12.0 [43]. However, between pH 8.0 and 9.0, more VFAs accumulation was observed, even though SCOD production was less compared to extreme alkaline conditions [43]. HAc and HPr were dominant constituents for VFA produced in this study.

The mechanism for VFAs accumulation through PS fermentation has been examined [44]. The result indicated that highest VFAs yield (312.9 mg COD g^{-1}VSS) was achieved at pH 10.0 to 11.0 over five days retention time [44]. Composition and distribution of the VFAs generated from PS was HAc 49.4%, HPr 34.4%, iso-HBu 14.6%, and n-HBu 12.2% [44]. VFAs production at pH 10 was 1.8 times higher than that of neutral and acid pH. Maximum yield of SCOD (5755 mg l^{-1} or 343 mg SCOD g^{-1} VSS) was achieved at pH 10.0 [45]. The SCOD yield (4003 mg l^{-1} or 192.8 mg SCOD/g VSS) at pH of 7.0 was 1.8 times higher than the yield of SCOD (3755 mg l^{-1} 173.2 mg SCOD) at pH of 4.0 [44].

3.8 Application of VFAs

The primary application of VFAs are as follows:

a) Bioplastics production – polyhydroxyalkanoates (PHA) are biodegradable polymers which are created by microorganisms using VFAs [3]. PHA is environment-friendly and has widespread applications in the industry that substitutes petrochemical-based plastics with high production cost [3].

b) Generation of Bioenergy – In the current world of the energy crisis, the waste-derived VFAs can be considered an alternative source for producing bioenergy [3]. For example, it is possible to generate electricity from PS derived VFAs using a microbial fuel cell [3]. VFAs can also be used to yield fuels such as biogas, hydrogen, and biodiesel [3].

c) Biological Nutrient Removal (BNR) process – Additional carbon substrates like VFAs are required for stable BNR process because the carbon substrate in treated municipal wastewater is insufficient to remove P and N [3]. Carbon to nitrogen requirement should be 5-10 mg COD/mg N for combined nitrification /denitrification process [19]. A range of 7.5-10.7 mg COD required to remove 1 mg of P. PS derived VFAs is more economical than using synthetic VFAs [46]. For the BNR process, the success criterion is to provide enough biodegradable organic substance. The substrates can be supplied to biomass by either dosing chemicals or by exploiting internal carbon sources of the system [3]. Hydrolysis and acid fermentation converts the particulate and slowly soluble biodegradable substances of PS into readily biodegradable substrates such as VFAs. Feasibility of using the VFAs produced through the mesophilic fermentation of PS on the BNR process was investigated by

[47]. The results of this study showed that the denitrification efficiency increased by 4-10% after the addition of VFAs as a carbon source [47].

4 Materials and Methods

4.1 PS and inoculum source

The PS that was used in this work was collected from Ashbridge's wastewater treatment plant (ABWWTP). ABWWTP is the largest of four wastewater treatment plants operated by the City of Toronto. Located in Toronto's east end, the plant has a nominal treatment capacity of 818,000 m^3day^{-1} and serves an equivalent population of 1,524,000. The average daily influent flow rate in 2016 was 549.8 ml day^{-1} [48]. Raw wastewater flows into two preliminary treatment facilities where grit and screenings are removed. There are twelve Primary Clarifiers with a total installed peak flow capacity of 2,730,999 m^3/day. The inoculum was also collected from the digester in the same plant. The PS and TWAS are mixed at the ratio of 1:4 (by volume) and the mixed sludge stream is then fed to twenty digesters operated at the mesophilic condition (34-38°C). The average SRT and OLR of the digesters are 18.1 d and 1.1 kg VS m^{-3}.

Characteristics of PS used in the batch test for bench scale experiment has been shown in Table 3. The mean value of TSS and VSS concentration of PS were 36,005 mg l^{-1} and 28,723 mg l^{-1}, respectively. TCOD and SCOD were 53,386 mg l^{-1} and 3016 mg l^{-1}, respectively. The ratio of mean SCOD/TCOD ratio was 5.6%, and VFAs/SCOD ratio was 42%.

Table 3 Characteristics of feed PS used in batch fermentation studies

Parameter	units	Mean	Std Dev
pH		5.6	0.11
TSS	mg l^{-1}	36,000	5,179
VSS	mg l^{-1}	28,300	4,340
NH_3-N	mg l^{-1}	35	7
Alkalinity	mg $CaCO_3$ l^{-1}	675	322
TCOD	mg l^{-1}	53,400	6,208
SCOD	mg l^{-1}	3,000	90
$TBOD_5$	mg l^{-1}	21,200	5,296
VFAs	mg COD l^{-1}	1,266	99

4.2 Experimental Setup

Both batch and semi-continuous flow experiments were conducted in a cylindrical shape anaerobic fermenter with 4L working volume capacity, see Figure 10. Each fermenter has temperature display with a temperature probe submerged in the liquid. The temperature was controlled using a water heating bath. Plastic tubing from the water tank was wrapped around the digesters, and the reactor then covered with reflective material to retain heat. The water bath was set up to heat the containing water to 50°C to ensure that the internal reactor temperature was 35 ± 2°C. The reactor has a propeller connected with the shaft and rotated by a NEMA 17 motor to provide the required mixing. The pH in the fermenter was controlled using a pH probe at the bottom of the reactor and connected to a DLX pH-RX/MBB meter pump with a display showing the pH. A schematic diagram of the experimental set up is displayed in the below Figure 3 and Figure 4.

Figure 3 Photograph for bench scale PS anaerobic acid fermentation experimental set up

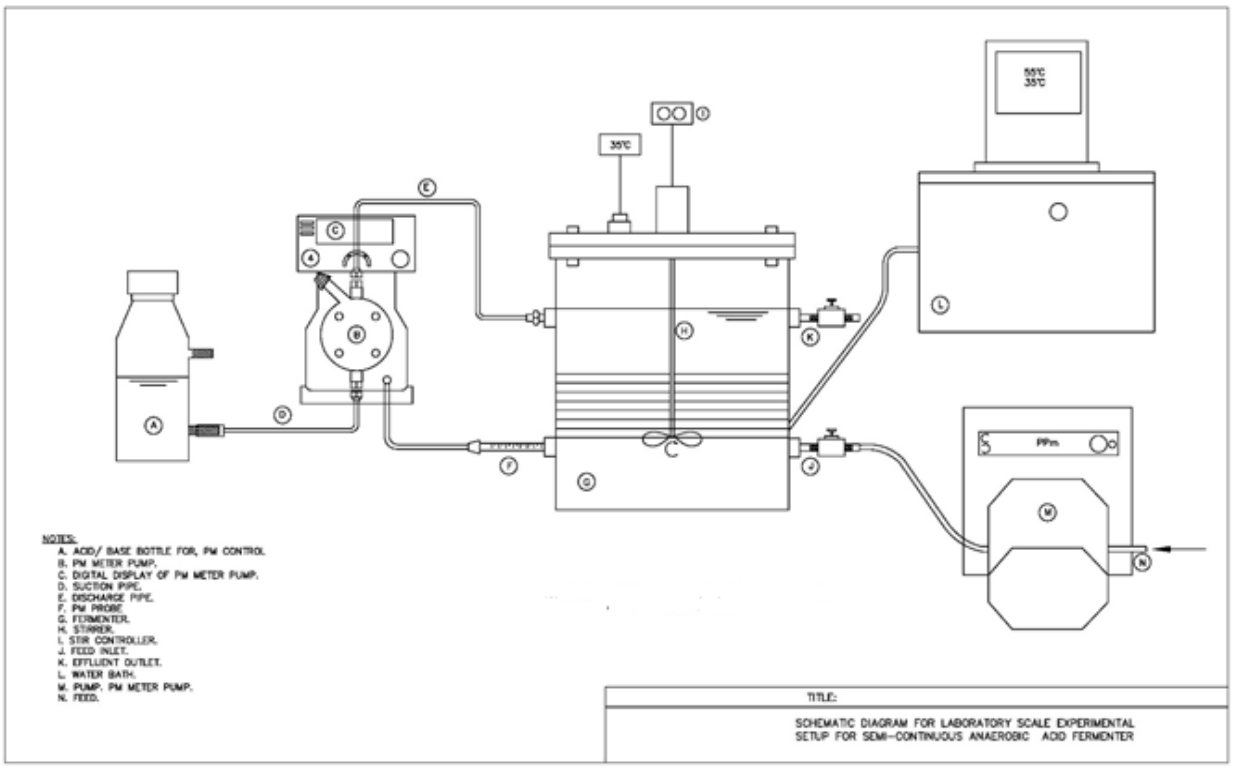

Figure 4 Schematic diagram for a bench scale experimental setup for PS anaerobic acid fermentation

4.3 Batch experiments

The batch tests were conducted in a 4L reactor with 3L working volume at mesophilic temperature (35°C). One Liter of PS was added to two liters of seed. Batch tests run at 7 different pH conditions 4.5, 5.5, 6.5, 8.0, 9.0, 10.0, and 11.0. Each experiment runs for three days, and the samples were collected and analyzed every 24 hours (24, 48, and 72 hrs). Each sample was analyzed in triplicate.

4.4 Semi-continuous experiments

The semi-continuous flow experiments were conducted using three fermenters (working volume of 4 L). Three different SRT of 1 day, 2 days, and 3 days were tested at three different pH values of 4.5, 5.5, and 6.5. The feed and effluent volume was calculated based on the SRT, Master flex L/S digital pump system and Master Flex C-flex tubing were used for feeding the PS and collecting the effluent. The semi-continuous process was run for three times the designed HRT for start-up period, after that steady state starts to reach. As soon the steady state reaches the reactor run for more for three times the designed HRT duration. During the steady state period, the liquid samples (6 samples for each run) were collected. The pH and temperature of the effluent samples were measured immediately, poured into plastic 250 mL sample bottles, and placed in the refrigerator below 4°C until analysis was completed.

4.5 Feeding substrate and collecting effluent semi-continuous study

While running a semi-continuous system, the effluent is always collected before feeding the system. The volume collected should equal quantity fed. For this semi-continuous study working volume was 4L. Every day while the experiment continues 2L of effluent

was collected, and then 2L of PS fed to the system through the feed pump. The system was kept well mixed throughout the total reaction time. Routine was kept consistent each day.

The amount collected and fed to the system each day will depend on the system working volume and required HRT as per equation (8).
For HRT of 1 day or less, the system will need to be fed twice or more each day as per equation (8).

The terminology HRT can be used instead of SRT for a continuous flow system anaerobic acid fermentation process. For Suspended Growth, Continuous Stirred Tank Reactor (CSTR) HRT can be considered same as SRT, as flow rate (Q), Q_{in} is equal to Q_{out} and Total Suspended Solids (TSS), $TSS_{in}=TSS_{out}$.

The effluent was collected from the bottom sprout; the substrate was fed through the top sprout. While obtaining the effluent sample, the outlet sprout valve was opened, and the effluent sample was collected. Whereas while supplying, the tube was connected through the pump with one side with feed inlet jar and another side with the reactor inlet sprout. It was ensured that the valve was open before running the pump and the valve was shut before disconnecting the tubing. Out of total reaction time first six days was considered as a startup on the 7^{th} days until the entire reaction time total six effluent samples were collected from the fermenter every day for HRT of 1 day and 2 days and every consecutive day for HRT of 3 days.

4.6 Sample analysis

The analyses that were performed are TSS, VSS, TCOD and SCOD, ammonia-nitrogen (NH_3-N), alkalinity, $CBOD_5$, VFAs. The samples were filtered through Acrodisc® 32 mm Syringe filter with 0.45 µm Supor® membrane, to perform the soluble analysis. All analyses were carried out in triplicates. The pH for each sample was measured immediately using VWR Benchtop pH Meter and refillable glass probe, model B10P. Total and Volatile Suspended Solids was measured using methods 2540B and 2540E for TS and VS respectively [49]. Total and soluble COD was measured using COD reagent vials from HACH, method 8000. Ammonia-Nitrogen was measured using the Amver Nitrogen Ammonia reagent set, method 10031. Alkalinity was measured with colorimetric method 10239 using TNT plus 870 Total Alkalinity test kit. Total VFAs were measured by the Esterification Method as per method 10240 at a range of 50 - 2500 mg l^{-1} as CH_3COOH (Acetic Acid) using TNT plus™ 872 Vials.

4.7 Calculations

Several calculations formulae used in the experiment has been described below

- The degree of solubilization batch experiment

$$Degree\ of\ solubilization\ (\%) = \frac{Mass\ of\ SCOD_{Produced}}{pCOD_{in}} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (10)$$

$$Mass\ of\ SCOD_{Produced} = (SCOD_{Effluent} - SCOD_{in}) \times Volume_{Total} \ldots\ldots\ldots\ldots\ldots (11)$$

$$SCOD_{in} = \frac{SCOD_{PS} \times V_{PS} + SCOD_{Seed} \times V_{seed}}{V_{PS} + V_{Seed}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (12)$$

$$pCOD_{in} = (TCOD_{PS} - SCOD_{PS}) \times V_{PS} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (13)$$

- Degree of solubilization semi-continuous experiment

$$Degree\ of\ solubilization\ (\%) = \frac{Mass\ increase\ in\ SCOD}{pCOD_{in}} \times 100 \ldots\ldots\ldots\ldots (14)$$

$$Mass\ increase\ in\ SCOD = \left(SCOD_{Effluent} - SCOD_{in}\right) \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (15)$$

$$Particulate\ pCOD_{in} = \left(TCOD_{Effluent} - SCOD_{in}\right) \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (16)$$

- VFAs yield

$$VFAs\ Yield = \frac{VFAs\ Produced}{PS\ VSS\ feed} = \frac{\frac{mg\ COD}{l}}{\frac{mg\ VSS_{feed}}{1000 \times l}} = \frac{mg\ COD}{gm\ VSS_{feed}}$$

$$= mg\ COD\ gm^{-1} VSS_{feed} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (17)$$

- SCOD yield

$$SCOD\ Yield = \frac{SCOD\ Produced}{PS\ VSS\ feed} = \frac{\frac{mg\ SCOD}{l}}{\frac{mg\ VSS_{feed}}{1000 \times l}} = \frac{mg\ SCOD}{gm\ VSS_{feed}}$$

$$= mg\ SCOD\ gm^{-1} VSS_{feed} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (18)$$

- VFAs/SCOD ratio

$$\frac{VFAs}{SCOD} = \frac{VFAs\ Produced}{SCOD\ Produced} = \frac{\frac{mg\ COD}{l}}{\frac{mg\ SCOD}{l}} \times 100 = \% \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (19)$$

5 Results and Discussion

5.1 Batch Experiments

Batch tests were used to evaluate the effect of pH on both VFAs production and solubilization. Samples were collected on day 1, 2, 3 (i.e. after 24, 48, and 72 hr) to assess the impact of different HRTs of 1, 2, 3 days on the VFAs production and solubilization.

5.1.1 VFAs production and Soluble COD

Figure 5 shows the VFAs production during the fermentation of PS in a batch reactor. It reveals that with increasing HRT, VFAs production increased for all the pHs. However, VFAs production increased with increasing pH until pH 8.0 and then it starts to decrease. Comparing acidic and alkaline pH conditions, the results showed that the alkaline pH (pH 8.0-11.0) produced higher VFAs compared to acidic pH range (pH4.5-6.5), 1954 – 2587 mg COD l^{-1} for alkaline pH versus 1316-1852 mg COD l^{-1} for acidic pH. Maximum VFAs production was observed at pH 8.0 for all the different HRTs. The maximum VFAs concentrations of 2587, 1820, and 1,455 mg COD l^{-1} were achieved for HRTs of 1 day, 2 days, and 3 days, respectively. The VFAs production, normalized by mass of VSS added, ranged from 43 to 234 mg COD g $^{-1}VSS_{feed}$ for pH 4.5-11.

As shown in Figure 5, the SCOD increased with increasing the HRT for all pH values. The highest SCOD concentration of 10640 mg l^{-1} was achieved at pH 10.0 and HRT 3 days. On the other hand, for all HRTs, the SCOD increased with increasing the pH values until pH 10.0, after which the SCOD decreased. The batch study data indicates that as the pH goes from acidic to alkaline solubilization of the substrate increases.

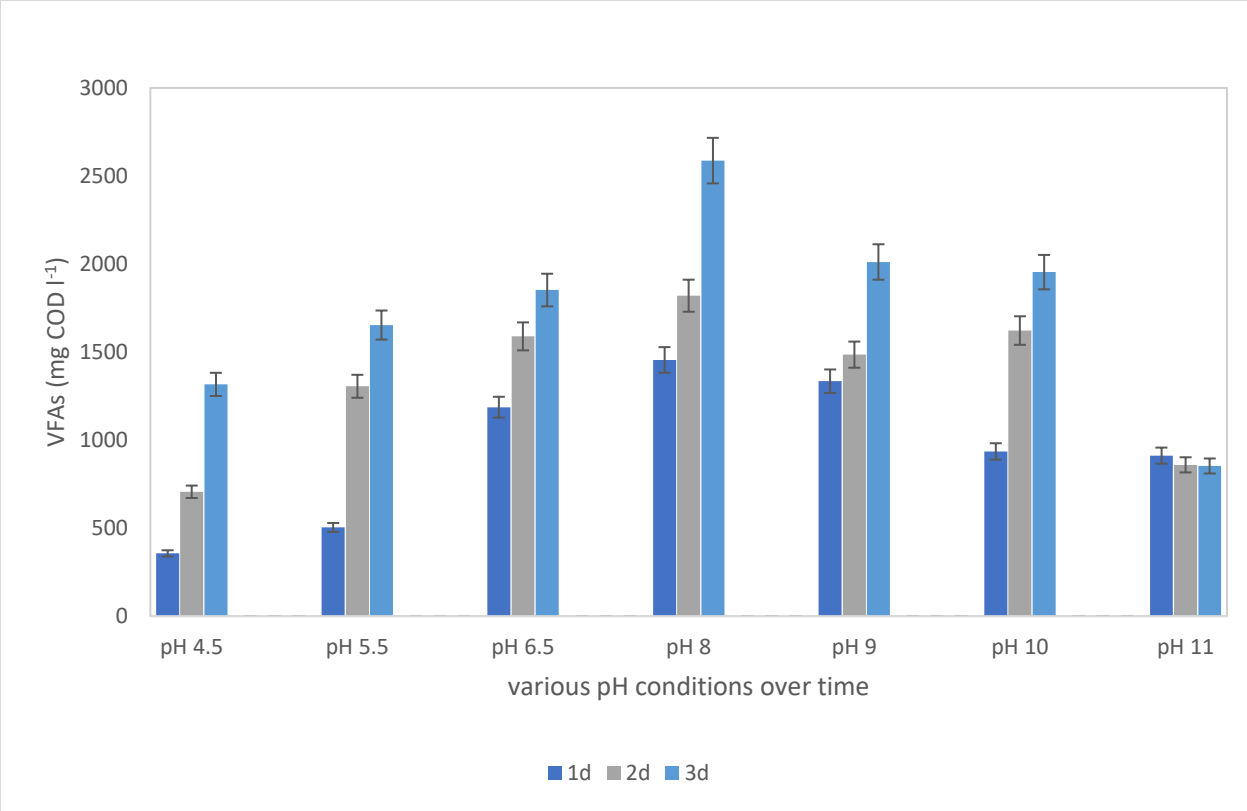

Figure 5 VFAs production during batch studies through PS anaerobic acid fermentation

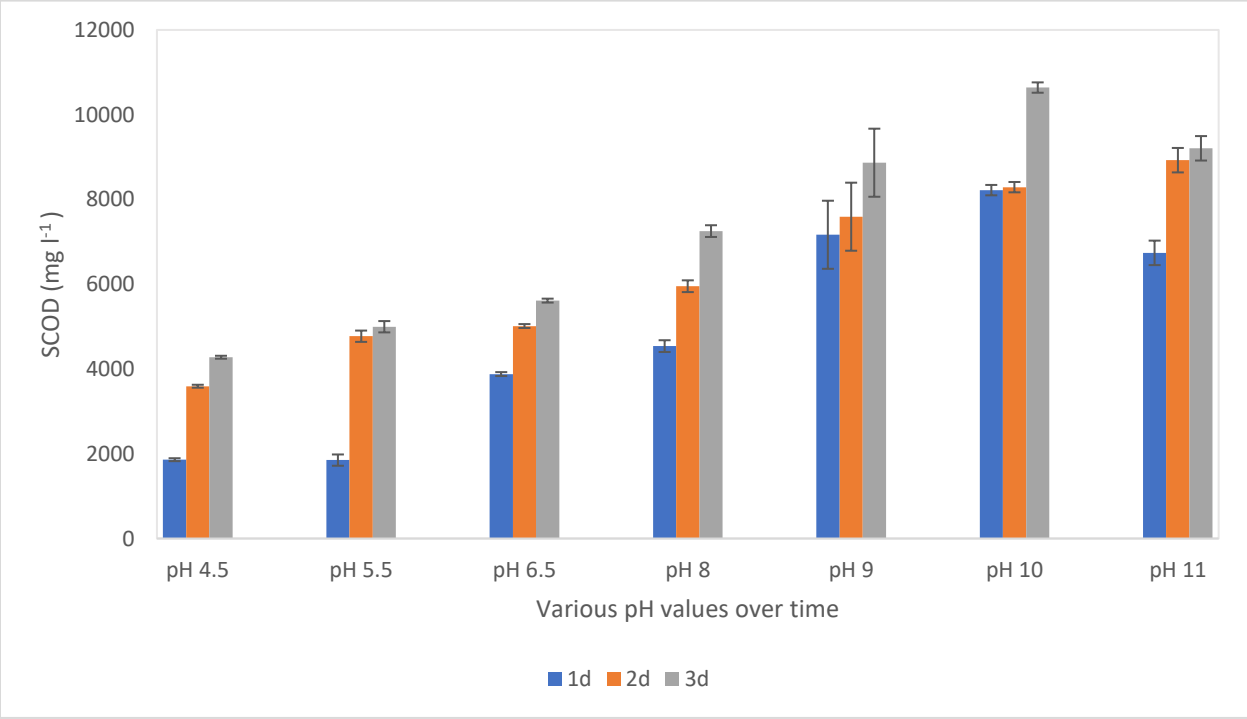

Figure 6 SCOD data for the batch experiment

5.1.2 The degree of solubilization

One of the main advantages of anaerobic fermentation is the solubilization of particulate organic fractions and converting them into soluble substances. One of the primary indicators for the fermentation efficiency is the degree of solubilization. The degree of solubilization is the fraction of the particulate COD (pCOD) that converted into SCOD during the fermentation process. The degree of solubilization for the batch experiment was calculated using the equation (10).

Figure 7 shows the degree of solubilization for the different pH values at different HRTs. It demonstrates that the degree of solubilization ranged from 2.0% to 48.0. The degree of solubilization increased with increasing the pH and reached a maximum of 48% at pH 10.0 and HRT 3 days. The maximum degree of solubilization for acidic pH of 30% was observed at pH 6.5. For pH 5.5, there were no significant differences between the degree of solubilization at HRTs 2 and three days. The results showed that the maximum degree of solubilization that achieved with acidic pH could be achieved with much shorter HRT at alkaline pH. For example, to obtain a degree of solubilization of about 30% with acidic pH, the HRT should be three days, however, to achieve the same degree of solubilization with alkaline pH, HRT of only one day is required.

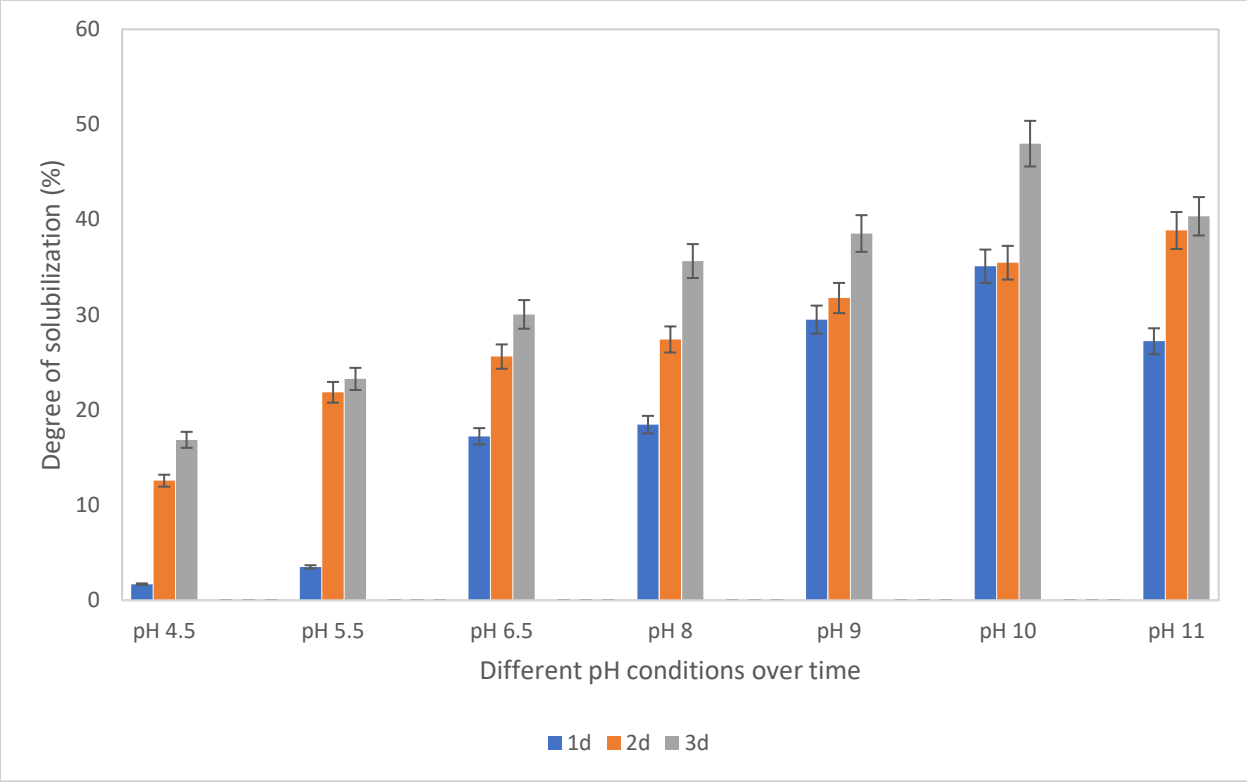

Figure 7 Degree of solubilization for the batch experiment

5.1.3 VFAs yield, SCOD yield

Due to the variable composition of the PS collected several times of the batch experiment, overall yield has been calculated to evaluate the acid fermentation potential. Both VFAs and SCOD yield have been calculated as the mass of VFAs and SCOD fermenter effluent produced per unit mass of VSS feed. The batch studies performed with PS indicates that the soluble COD produced during the fermentation is converted to VFAs, i.e., short chain fatty acids.

As shown in Table 4, the VFAs yield increased with increasing the HRT for all pH values. For acidic pH, the VFAs yield increased with increasing the pH and reached a maximum of 222 mg VFAs $g^{-1}VSS_{feed}$ at pH 6.5 and HRT of 3 days. For alkaline pH, there were no significant differences for pH 8, 9, and 10. However, the yield dropped significantly at pH

Table 4 Data for batch study, VFAs, SCOD, VFAs and SCOD yields for PS fermentation

pH	Time	PS TSS	PS VSS	Effluent VFAs	Effluent SCOD	VFAs/ SCOD	VFAs yield	SCOD yield	degree of solubilization
	(d)	(mg l^{-1})	(mg l^{-1})	(mg COD l^{-1})	(mg l^{-1})	mg COD mg $^{-1}$ SCOD %	(mg COD g $^{-1}$VSSfeed)	(mg SCOD g^{-1} VSS feed)	%
4.5	1	31889	24978	356	1880	19	43	226	2
4.5	2	31889	24978	706	3593	20	85	432	13
4.5	3	31889	24978	1316	4277	31	158	514	17
5.5	1	31889	24978	504	1850	27	61	222	4
5.5	2	31889	24978	1305	4773	27	157	573	22
5.5	3	31889	24978	1653	4997	33	199	600	23
6.5	1	32589	25011	1187	3800	31	142	456	17
6.5	2	32589	25011	1589	5013	32	191	601	26
6.5	3	32589	25011	1852	5613	33	222	673	30
8.0	1	43189	34100	1455	4610	32	128	406	19
8.0	2	43189	34100	1820	5953	31	160	524	27
8.0	3	43189	34100	2587	7253	36	228	638	36
9.0	1	32890	26130	1334	7000	19	153	804	29
9.0	2	32890	26130	1485	7593	20	171	872	32
9.0	3	32890	26130	2012	8867	23	231	1018	39
10.0	1	32589	25011	935	8340	11	112	1000	36
10.0	2	32589	25011	1622	8980	18	195	1077	39
10.0	3	32589	25011	1954	10640	18	234	1276	48
11.0	1	36700	29733	912	6740	14	92	680	27
11.0	2	36700	29733	859	8927	10	87	901	39
11.0	3	36700	29733	853	9207	9	86	929	40

11.0. The maximum VFAs yield observed was 234 mg VFAs $g^{-1}VSS_{feed}$ followed by 231 mg VFAs $g^{-1}VSS_{feed}$ both were corresponding to HRT of 3 days and pH 10.0 and pH 9.0,

respectively. The lowest VFAs yield of 43 mg VFAs $g^{-1}VSS_{feed}$ was observed at HRT of 1 day and pH 4.5.

Figure 8 shows the SCOD yield normalized per mass of VSS added. It shows that the SCOD yields increased with increasing the pH and reached a maximum of 1276 mg SCOD g^{-1} VSS at pH 10 and HRT of 3 days, after which it dropped to about 900 mg SCOD g^{-1} VSS for pH 11. The lowest SCOD yield of about 200 mg SCOD g^{-1} VSS was observed at pH 4.5 and HRT of 1 day. The SCOD yields for all alkaline pH values (except pH 8.0) were higher than those for acidic pH.

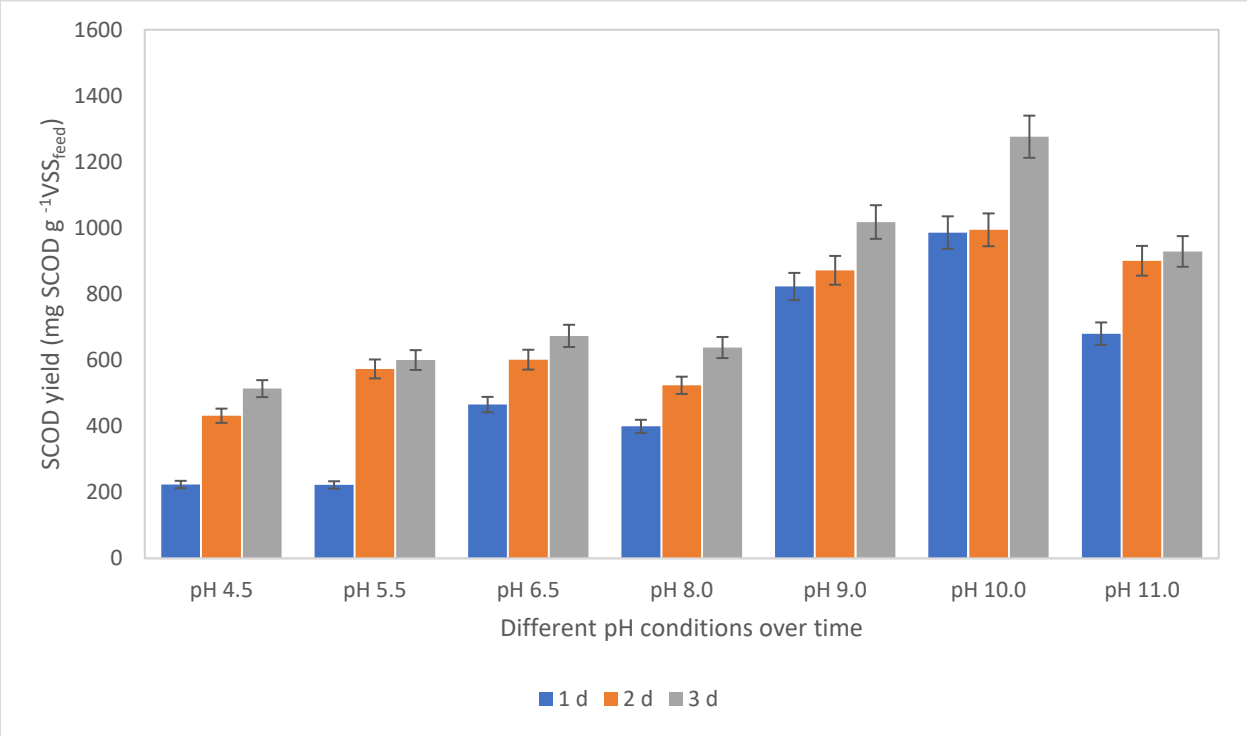

Figure 8 SCOD yield subject to VSS_{feed} in batch studies of acid fermentation of PS

Figure 9 illustrates the ratios of VFAs to SCOD for the different operating conditions. As depicted in the figure, VFAs/SCOD ratios for acidic pH (4.5-6.5) were higher than those for alkaline pH (9.0-11.0). The VFAs/SCOD ratio ranged from 9% to 36%. The maximum

VFAs/SCOD ratio of 36% was achieved at pH 8.0 and HRT of 3 days. For most pH values, except pH 10.0 and 11.0, there was no significant difference between HRT 1 and two days. However, this ratio was higher for HRT of 3 days at all pH values except pH 11.0.

In contradicting with most of the results observed in this study, for pH 11.0, the VFAs/SCOD ratio at HRT of 1 day was higher than those for 2 and 3 days. The highest VFAs/SCOD ratio achieved for acidic pH was about 32%, this ratio was observed at HRT of 3 days regardless of the pH (4.5 or 5.5 or 6.5). The highest VFAs/SCOD ratio achieved for alkaline pH was about 23%, this ratio was observed at HRT of 3 days and pH 9.0. The results also demonstrated an increasing pattern on VFAs/SCOD ratio with increasing the HRT for all the pH values except 11.0.

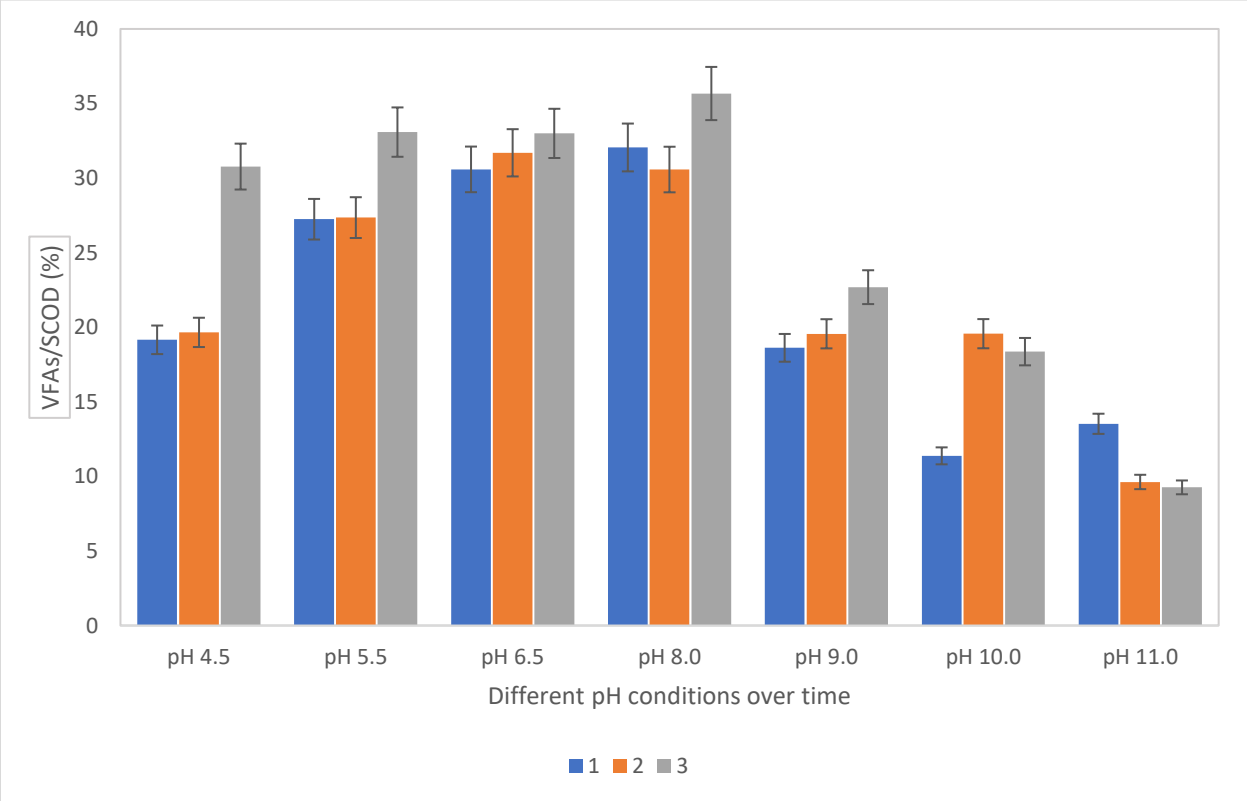

Figure 9 VFAs/SCOD as a percentage for acid fermentation of PS in batch studies

Previous experiment results support the fact that pH significantly impacts fermentation efficiency of the fermenter and plays a vital role on anaerobic solubilization of PS. pH performs a significant function in increasing the production rate of VFAs as well VFAs yield regarding VFAs produced per unit mass VSS added. This batch study reveals that at a pH level of 8.0, VFAs accumulation was maximum. Whereas the other experimental study supports that maximum VFAs accumulation was obtained at pH 10.0, where alkaline fermentation of PS for SCFAs was studied [44]. This experiment result reveals that pH range 8.0-10.0 caused higher VFAs production than pH range 4.5 – 6.5. Between pH range 4.5 -10.0, VFAs yield increased rapidly with fermentation time, i.e., HRT and reached maximum on HRT 3 days. Further increase in pH did not result in an increase of VFAs production at HRT 3 days, somewhat decreased for pH 11.0.

The above result reveals the fact that though VFAs production was maximum at pH 8.0 2587 mg COD l^{-1} (SCOD 7253 mg l^{-1}) in comparison to that of pH 10.0 ,VFAs 1954 mg COD l^{-1} (SCOD 10640 mg l^{-1}) but VFAs yield (mg COD $g^{-1}VSS_{feed}$) achieved maximum when fermentation pH was 10.0 with a value of 234 mg COD $g^{-1}VSS_{feed}$ .The reason for lesser VFAs production at pH 11.0 and 4.5 during HRT 1-3 days was due to inhibition of acidogenic bacteria to extreme alkaline and acidic condition [44].

5.1.4 Discussion of batch experiment results

According to literature studies, production of VFAs depends more on HRT than temperature [50]. With an increment of HRT, the acidogens get more contact time to convert the waste, i.e., particulate organic carbon matters into soluble matters which favors the VFAs yield [1]. HRT of the system depends on the type, composition, and solid content of the substrate [51]. HRT favors the production of VFAs up to a specific value

while prolonged HRT up to five days causes accumulation of VFAs in the fermenter. VFAs yield does not have any significant difference for HRT of 3 days and pH 6.5 – 10.0 with p value ranging 0.303 – 0.993 i.e., ≥ 0.05 (appendix C). The batch study reveals the fact that VFAs yield increased as time increases from 1 day to 3 days. VFAs yield for batch experiment has significant difference over time (1 day -3 days) with p value ranging $p \leq 0.05$ (appendix C). Methanogens grow at a slower rate than acidogens when the substrate is particulate organics. It is essential to operate the process at low SRT, preferably less than five days for better performance of the fermenter. If the SRT is low, it does not allow methanogens to grow and consumes the VFAs and convert them to methane and carbon dioxide [3]. The results of this batch study indicate the VFAs accumulation increases with time and no gas production was observed.

Previous researchers reported that fermentation of PS at higher pH 6.5 -10.0 caused higher VFAs accumulation than at pH 4.5-5.5 [44]. This study showed a similar pattern of VFAs yield supporting the literature claim. Henceforth, it became essential to analyze the mechanism of VFAs accumulation under alkaline condition.

Hydrolysis is the rate-limiting step for anaerobic fermentation which can be expressed by the change in SCOD [52]. Figure 8 displays changes in SCOD yield for PS hydrolysis for 3 days of fermentation time.

pH has significant impact on SCOD yield as p value ≤ 0.05 (appendix C). A per statistical analysis there is no significant difference on SCOD yield on 3 days reaction time over pH 5.5, 6.5 and 8.0 with p value ranging 0.295 - 0.956 i.e., $p > 0.05$ (appendix C). However, there is significant difference on SCOD yield on 3 days reaction time for pH 4.5, 9.0, 10.0, 11.0 as p value ≤ 0.05 (appendix C). The SCOD yield shows a gradually increasing trend

for PS fermentation at 3 days reaction time at different pH conditions which is also evident in statistical analysis with p value ≤ 0.05 (appendix C). The data reveals that the alkaline state prompts more solubilization of organic matter over acidic condition.

VFAs/SCOD ratio is an indicator of acidogenic activity [27]. VFAs/SCOD ratio at different pH conditions for HRT of 3 days is shown in Figure 9. The previous research reported that the VFAs/SCOD ratio was higher in alkaline pH than that of an acidic pH condition, i.e., the degree of acidification was higher in alkaline pH conditions [44].

On the other hand, this batch experiments showed that the VFAs/SCOD ratio of 19-36% was higher for pH range 4.5 – 8.0, compared to 9-23 % for pH range 9.0-11.0. The reason for this result is that the higher SCOD was produced at alkaline pH while higher VFAs was produced at acidic pH. Hence while calculating the VFAs/SCOD percentage the denominator being a more substantial value than the numerator ratio becomes lower during alkaline pH condition.

5.2 Semi-continuous Experiment

Based on the result of the batch experiment of PS fermentation for VFAs production, it was observed that the VFAs yield was more for pH 6.5-10.0. VFAs yield (222 – 234 mg COD g^{-1} VSS_{feed}) on pH range 6.5 -10.0 for 3 days reaction time doesn't have any significant difference. On the other hand, as the pH moves upward to alkaline condition 8.0 -10.0, solubilization of pCOD to SCOD increases which is evident from SCOD concentration and degree of solubilization data. The primary objective of this study is to maximize the VFAs production. The pH of PS is around 5.5-6.8, to raise the pH and to conduct the experiment to satisfy the objective pH 6.5 has been chosen. Raising the pH

to 6.5 consumes less chemical and incurs less cost than it requires for pH 10.0. The semi-continuous experiments were designed to investigate the effect of three different HRTs of 1 day, 2 days, and 3 days and three acidic pH values of 4.5, 5.5 and 6.5. Table 5 shows the operating and environmental conditions of the semi-continuous experiment.

Table 5 Semi-continuous experiments conditions.

Run	Temp	HRT (d)	pH	Minimum duration of the run (d)
Run 1	(35±3) °C	1	4.5	9
Run 2	(35±3) °C	2	4.5	12
Run 3	(35±3) °C	3	4.5	18
Run 4	(35±3) °C	1	5.5	9
Run 5	(35±3) °C	2	5.5	12
Run 6	(35±3) °C	3	5.5	18
Run 7	(35±3) °C	1	6.5	9
Run 8	(35±3) °C	2	6.5	12
Run 9	(35±3) °C	3	6.5	18

5.2.1 Characteristics of feed stock i.e., PS

For the semi-continuous study, PS was collected from Ashbridge's Bay WWTP on a weekly basis and stored in a refrigerator at 4°C. Characteristics of feed PS used in semi-continuous bench scale experiment is shown in Table 6. The mean value of TSS and VSS concentration of PS were in the typical range 34,000 mg l^{-1} and 27,100 mg l^{-1}, respectively. TCOD and SCOD were 50,600 mg l^{-1} and 3,700 mg l^{-1}, respectively. The ratio of SCOD/TCOD was about 7%.

Table 6 Characteristics of feed PS for the semi-continuous reactor.

Parameter	Mean	Stdev
pH	6	0.13
TSS (mg l^{-1})	34,000	7,690
VSS (mg l^{-1})	27,100	6,230
TCOD (mg l^{-1})	50,700	3,670
SCOD (mg l^{-1})	3,700	327
NH_3-N (mg l^{-1})	51	14
Alkalinity (mg l^{-1})	1,000	190
VFAs (mg COD l^{-1})	1,400	157
VFAs/VSS (mg COD g^{-1} VSS_{feed})	54	10
$TBOD_5$ (mg l^{-1})	19,700	4,740

The data in this table is the average and standard deviation of six samples

5.2.2 VFAs and SCOD production for pH 4.5

For the semi-continuous experiments, six liquid samples were collected for each run after reaching a steady state, i.e., after 3 times HRT, for example, for HRT of 3 days, the liquid samples were collected after 9 days of starting the experiment. Figure 10 and Figure 11 show the VFAs production and SCOD production trends, respectively, for pH 4.5 and different HRTs of 1 day, 2 days, and 3 days for the six samples.

As shown in Figure 10, the VFAs concentrations for HRT of 1 day were higher than those for 2 and three days. Furthermore, there was no significant difference between the VFAs at HRT of 2 days and HRT of 3 days. As depicted in Figure 10 and Figure 11, for HRT of 1 day, the average VFAs production was (3587± 276) COD l^{-1} which was corresponding to average SCOD of (7173 ± 236) mg l^{-1}. For HRT of 2 days, the average VFAs production was (2769 ± 290) mg COD l^{-1} which was corresponding to average SCOD of (9255 ± 638)

mg l^{-1}. For HRT of 3 days, the average VFAs production was (2534 ± 241) mg COD l^{-1} which was corresponding average SCOD of (9103 ± 811) mg l^{-1}.

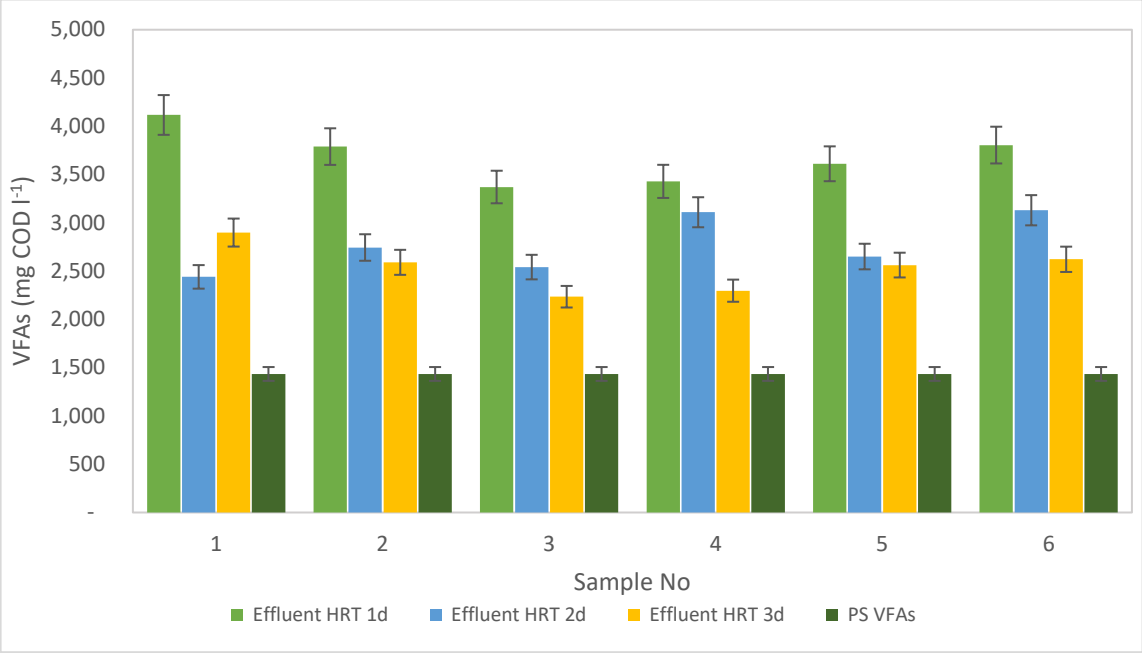

Figure 10 VFAs production data for pH 4.5 and different HRTs

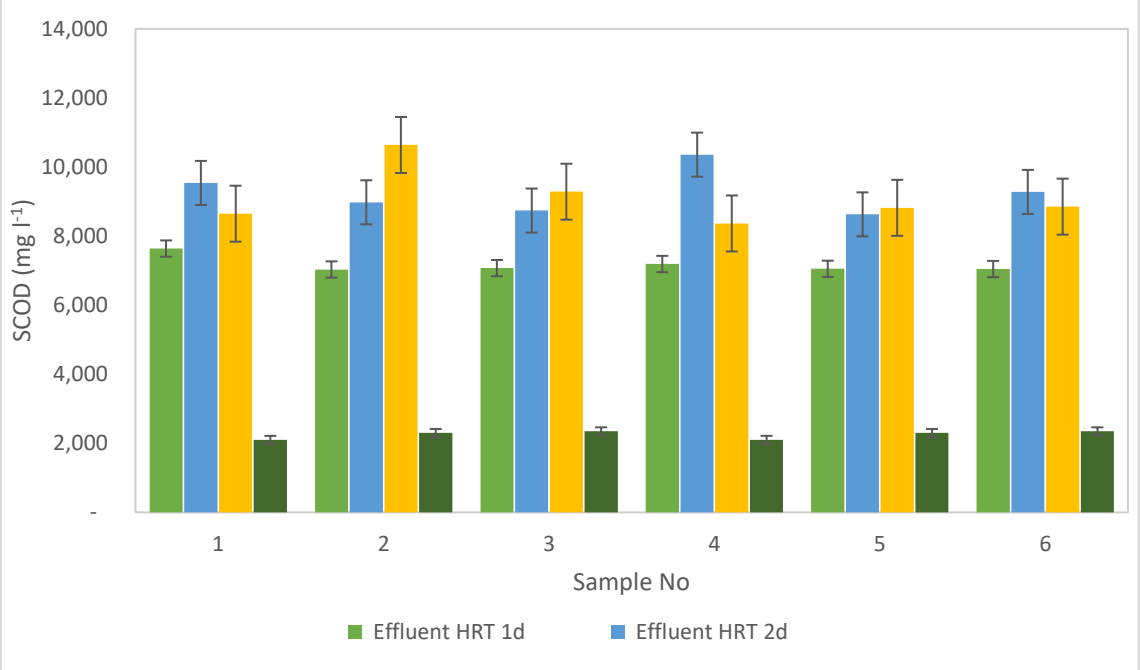

Figure 11 SCOD data for pH 4.5 and different HRTs

5.2.3 VFAs and SCOD production for pH 5.5

Figure 12 and Figure 13 describes the VFAs production and SCOD production trend respectively for pH 5.5 and HRT 1day, 2 days and 3 days for six samples for the semi-continuous experiment of primary sludge fermentation. As demonstrated in Figure 12 and Figure 13, for HRT of 1 day, the average VFAs production for pH 5.5 was (3400 ± 297) COD l^{-1} which was corresponding to average SCOD of (6238 ± 551) mg l^{-1}. For HRT of 2 days, the average VFAs production was (3,933 ± 561) mg COD l^{-1} which was corresponding to average SCOD of (9,188 ± 876) mg l^{-1}. For HRT of 3 days, the average VFAs production was (3,197 ± 373) mg COD l^{-1} which was corresponding average SCOD of (10,157 ± 448) mg l^{-1}.

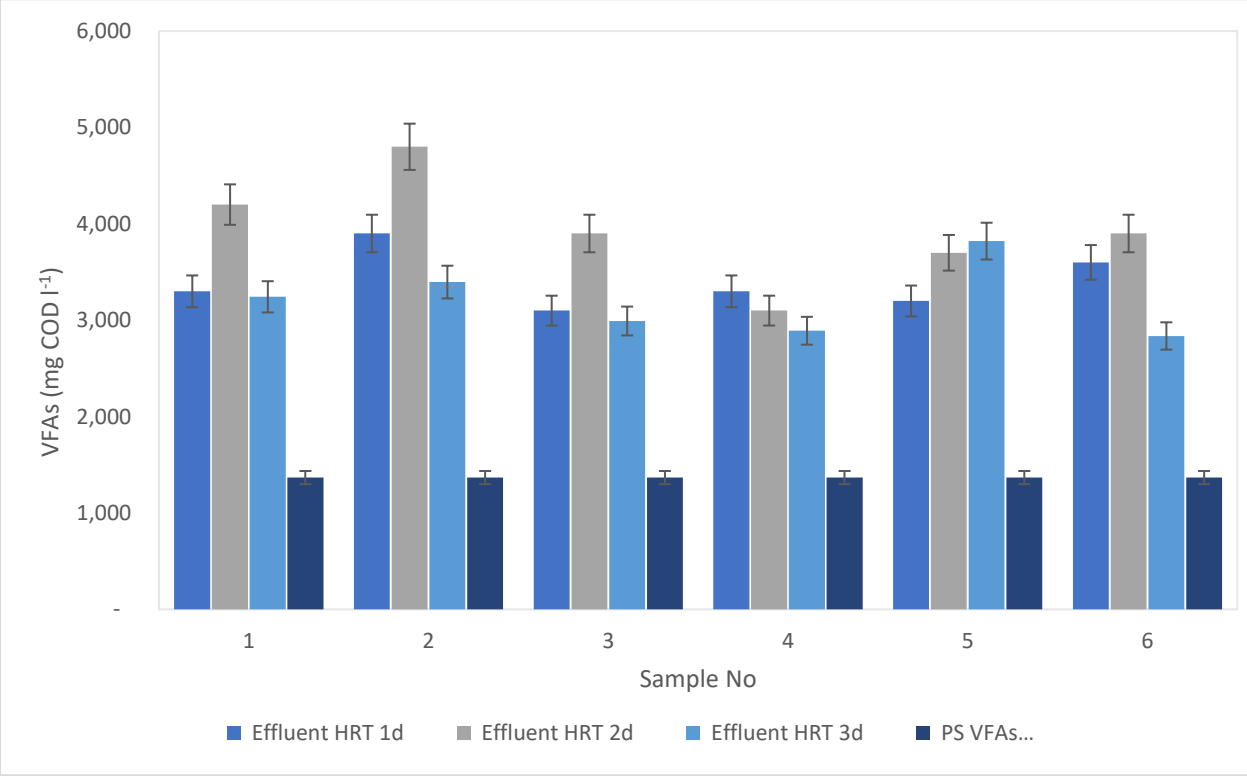

Figure 12 VFAs data for pH 5.5 and different HRTs

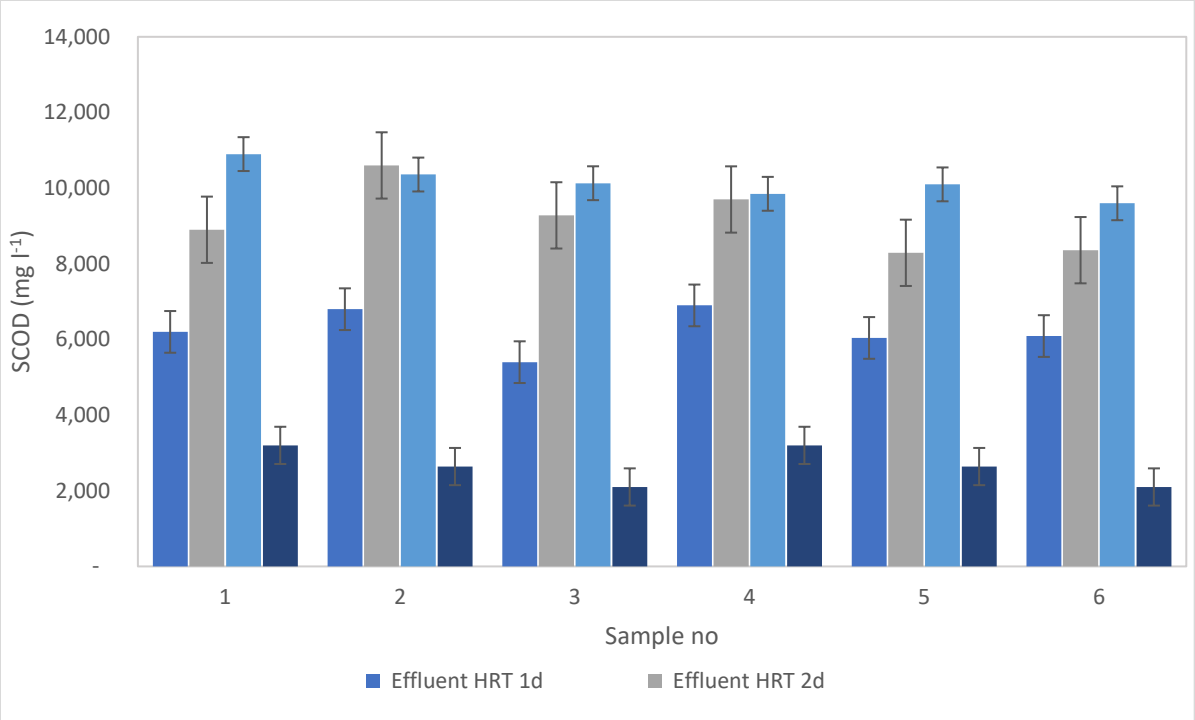

Figure 13 SCOD data for pH 5.5 and different HRTs

5.2.4 VFAs and SCOD production for pH 6.5

Figure 14 and Figure 15 describes the VFAs production and SCOD production trend respectively for pH 6.5 and HRT 1 day, 2 days and 3 days for six samples. At the mesophilic temperature and pH 6.5, as described in Figure 14 and Figure 15, for HRT of 1 day, the average VFAs production was (2,469 ± 451) COD l^{-1} which was corresponding to average SCOD of (7,255 ± 101) mg l^{-1}.

For HRT of 2 days, the average VFAs production was (4,530 ± 860) mg COD l^{-1} which was corresponding to average SCOD of (14,377 ± 205) mg l^{-1}. For HRT of 3 days, the average VFAs production was (6,549 ± 528) mg COD l^{-1} which was corresponding average SCOD of (14,554 ± 414) mg l^{-1}.

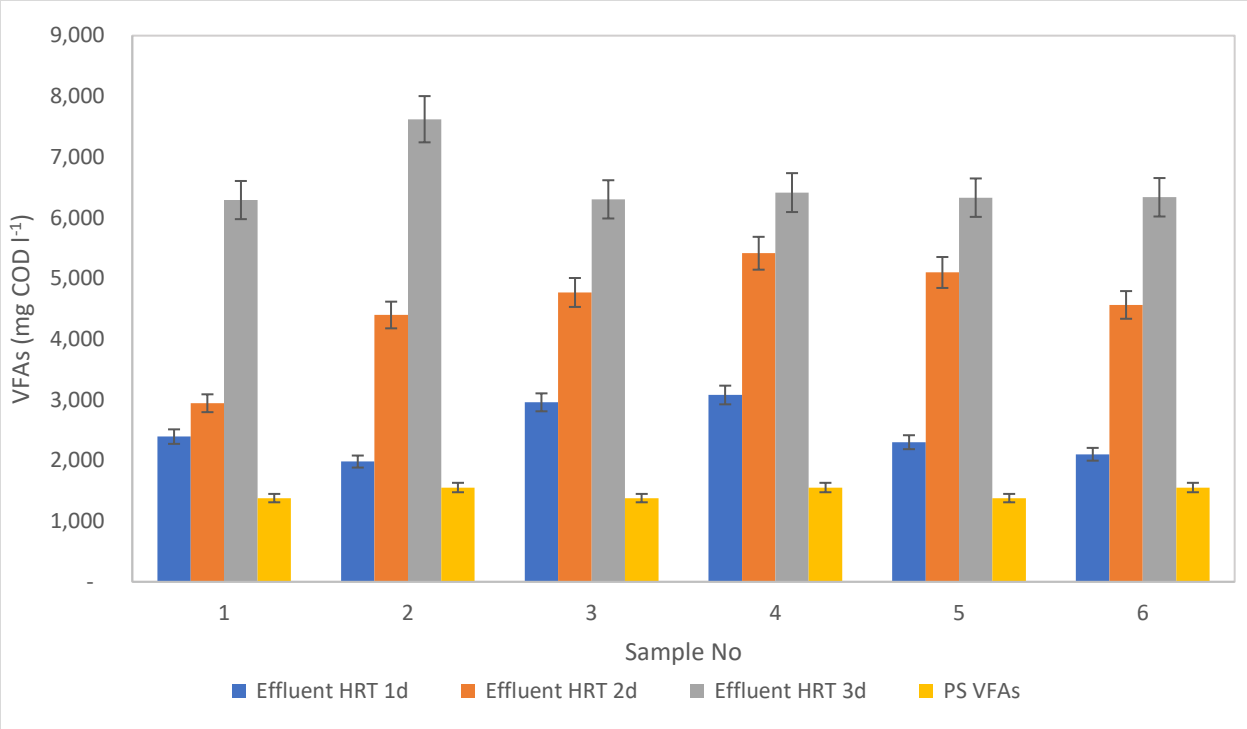

Figure 14 VFAs data for pH 6.5 and different HRTs

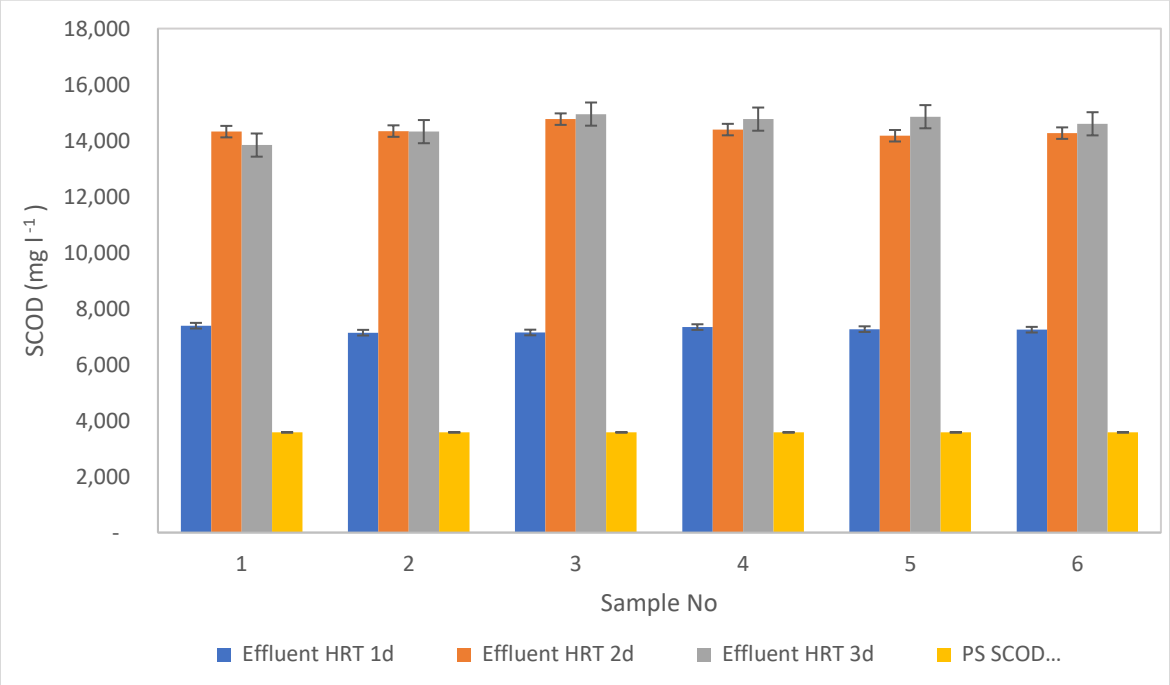

Figure 15 SCOD data for pH 6.5 and different HRTs

5.2.5 VFAs and SCOD production for pH 4.5, 5.5, 6.5 and different HRTs

PS was fermented under the designed condition to produce VFAs and optimize the design conditions to maximize the VFAs production. Figure 16 describes the comparison of VFAs production on pH 4.5, 5.5, 6.5 based on average VFAs data for the semi-continuous experiment of PS anaerobic fermentation. Each pH was fixed over three HRTs 1 day, 2 days, and 3 days. pH 5.5 shows a gradual increase of VFAs production from HRT 1 day until 2 days but it dropped in HRT of 3 days. Whereas pH 6.5 depicts a significant increase in the production of VFAs from HRT 1 day to 3 days. pH 4.5 can be considered as an extremely acidic condition which acts as the inhibitory situation for acidogens for VFAs production as the graph does not shows any increment in VFAs accumulation rather shows a decreasing trend.

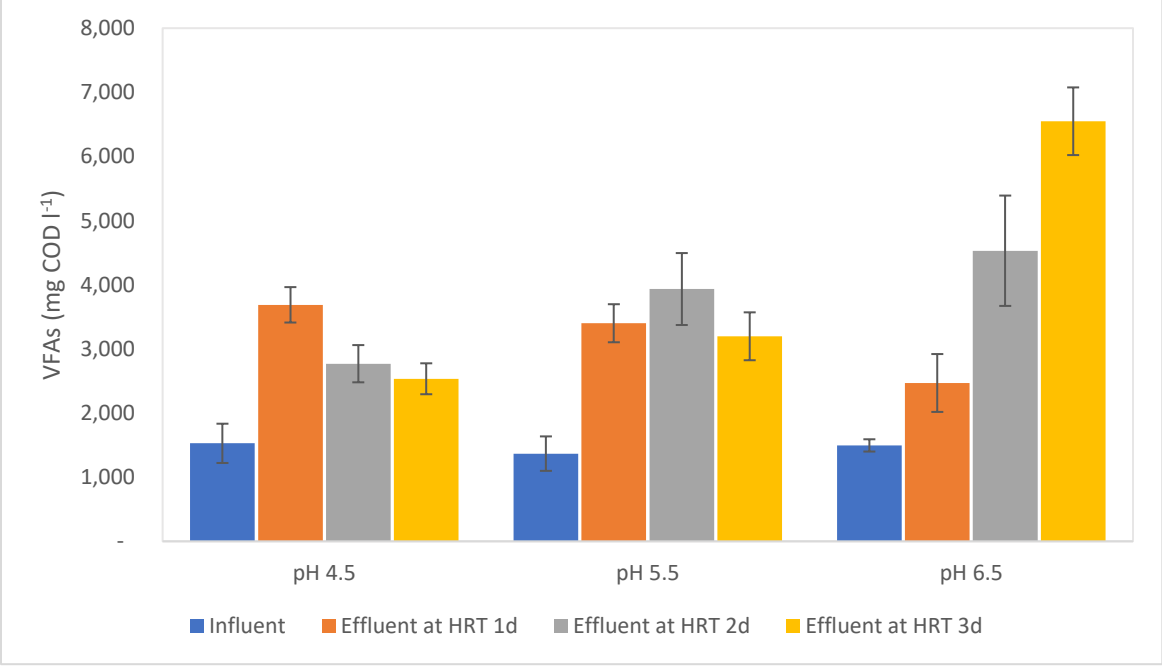

Figure 16 VFAs data for different HRTs and pHs for semi-continuous experiment

Figure 17 shows the solubilization trend of pCOD to SCOD for semi-continuous studies. The graph shows a gradual increase in SCOD with increasing HRT from 1 day to 3 days for pH 5.5 and 6.5. However, data for pH 4.5 which is more acidic condition does not comply this trend. For pH 4.5, SCOD increases until HRT 2 day but decreases with the increase of HRT to 3 days. pH 6.5 and HRT 3 days shows maximum SCOD (14,554 ± 414 mg COD l^{-1}) production which in agreement with the VFAs data. SCOD data also shows an increasing trend over an increment of HRTs for all the pH values.

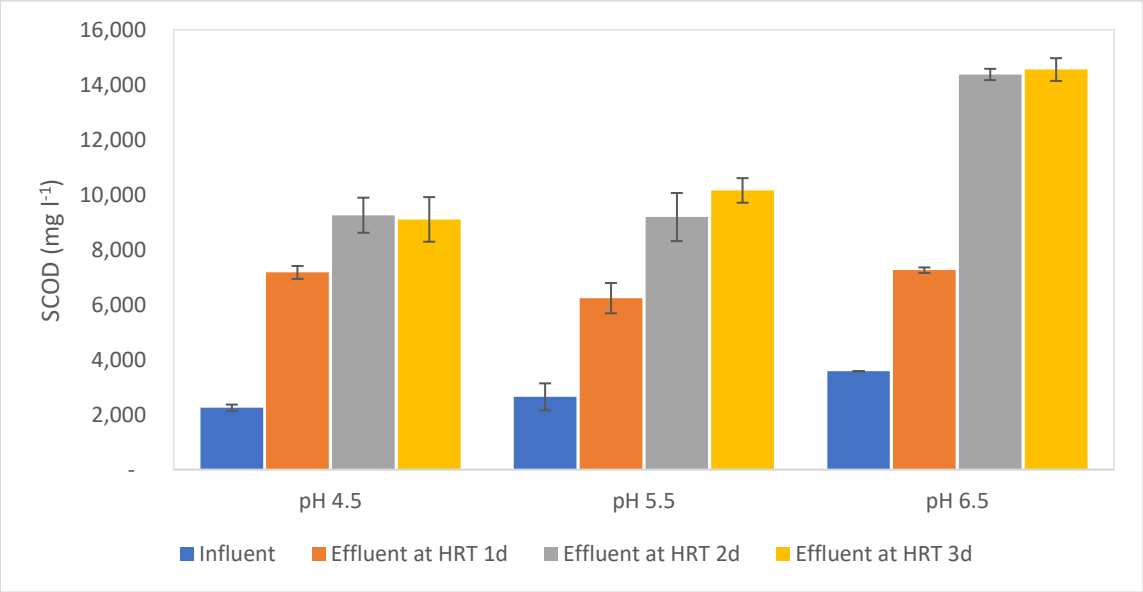

Figure 17 SCOD data for different HRTs and pHs for semi-continuous experiment

5.2.6 The degree of solubilization for pH 4.5,5.5,6.5 and different HRTs

The degree of solubilization is a measure to what extent the particulate or solid fraction of PS is solubilized, i.e., converted to SCOD during the fermentation process. The degree of solubilization for the fermenter during the semi-continuous experiment was measured using equation (14).

As shown in Figure 18, the maximum degree of solubilization of 25% along with corresponding SCOD of 14,554 mg l^{-1} and VFAs of 5,549 mg COD l^{-1} were achieved at pH 6.5 and HRT of 3 days. Data for pH 6.5 and HRT of 2 days also shows a degree of solubilization 25% with a lesser value of SCOD 14,377 mg l^{-1} and VFAs 4,782 mg COD l^{-1} which almost equal to those of HRT 3 days. The degree of solubilization for HRT of 1 day was only 8%. For the of 5.5, the degree of solubilization increased with increasing the HRT, it was 7%, 15%, and 20% for HRT of 1, 2, and 3 days, respectively.

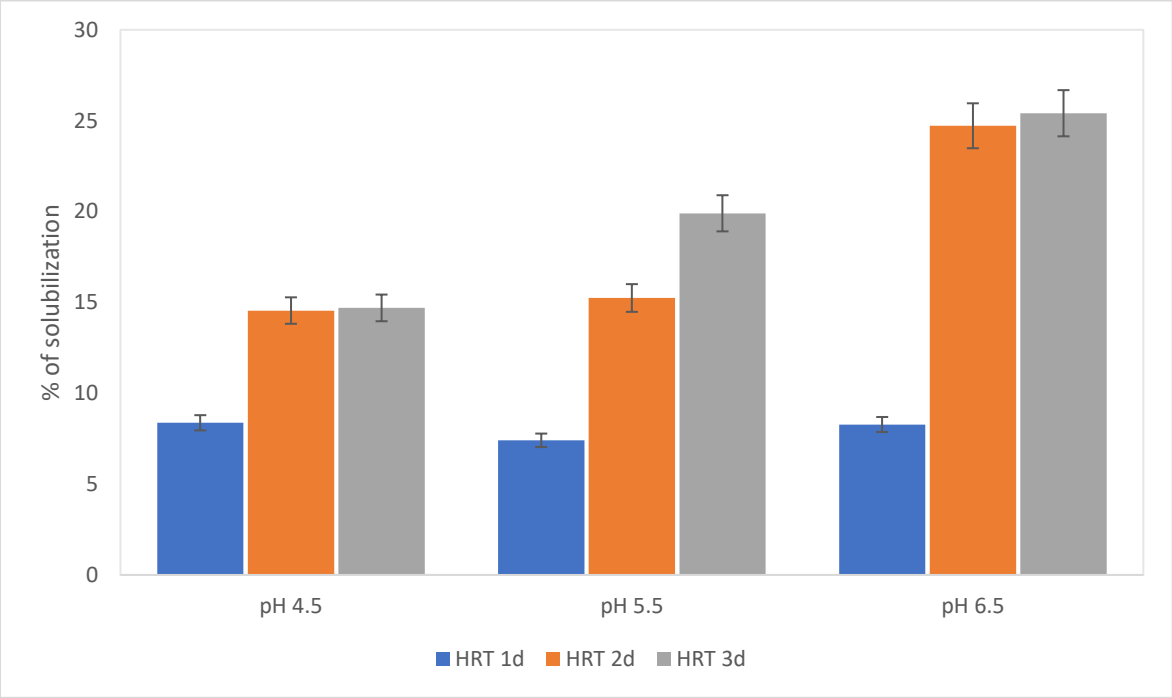

Figure 18 Degree of solubilization different HRTs and pHs for semi-continuous experiment

For pH 4.5, the degree of solubilization was 8%, 15%, and 15% for HRT of 1, 2, and 3 days, respectively. The above data emphasis that the degree of solubilization for HRT 2 days and 3 days does not have significant difference.

5.2.7 VFAs yield, SCOD yield, VFAs/SCOD ratio

VFAs yield and VFAs/SCOD ratio is the measure of accomplishment of acid fermentation or acidification, representing the amount of VFAs converted from solubilized matter, i.e., SCOD. VFAs yield as mg COD g^{-1} VSS_{feed} and VFAs/SCOD ratio (%) are shown in Figure 19 and Figure 20, respectively. The semi-continuous study of PS fermentation indicates that for pH 4.5, VFAs yield decreases gradually as the HRT increases from 1day to 3 days: VFAs yields of 150, 94, and 86 mg COD g^{-1} VSS_{feed} were observed at HRT of 1, 2, and 3 days, respectively. However, for pH 5.5, VFAs yield increases from 150 mg COD g^{-1} VSS_{feed} at HRT 1 day to192 mg COD g^{-1} VSS_{feed} at HRT of 2 days and decreased to 130 mg COD g^{-1} VSS_{feed} as HRT increases to 3 days.

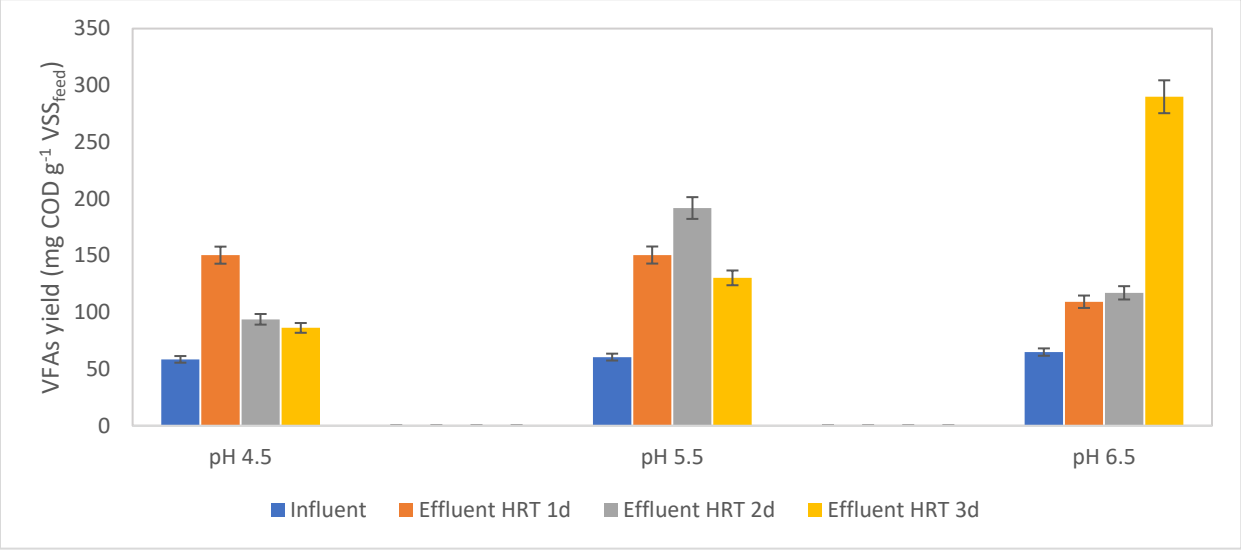

Figure 19 VFAs yield data for different HRTs and pHs for semi-continuous experiment

For pH 6.5, VFAs yield shows opposite trend to that of pH 4.5. For pH 6.5, VFAs yield clearly shows a steady increasing pattern from 1 day to 3 days: 109 mg COD g^{-1} VSS_{feed} (HRT 1 day) < 117 mg COD g^{-1} VSS_{feed} (HRT 2 days) < 290 mg COD g^{-1} VSS_{feed} (HRT 3 days). The highest value of VFAs yield of 290 mg COD g^{-1} VSS_{feed} was achieved at pH

6.5 and HRT 3 days. The probable reason for decreasing VFAs yield from 1 day to 3 days for pH values of 4.5 and 5.5 could be due to the consumption of the VFAs to produce alcohol i.e., ethanol. The optimum pH for ethanol production is pH 4.5-5.0.

Figure 20 shows the VFAs/SCOD ratios for different pHs and HRTs. As shown in the figure, the VFAs/SCOD ratio showed gradual decreasing pattern for pH 5.5 and 6.5 with an increase of HRT from 1 day until 3 days. VFAs/SCOD ratios for pH 5.5 of 51%, 30%, and 28%, were observed for HRTs of 1, 2, and 3 days, respectively. For pH 6.5, VFAs/SCOD ratios of 55%, 43%, and 31% were observed for HRTs of 1, 2, and 3 days, respectively. However, VFAs/SCOD ratio at pH 6.5 shows a slight decrease from HRT 1 day (32%) to 2 days (34%) then an increase with the increase of HRT from 2 days until 3 days (45%).

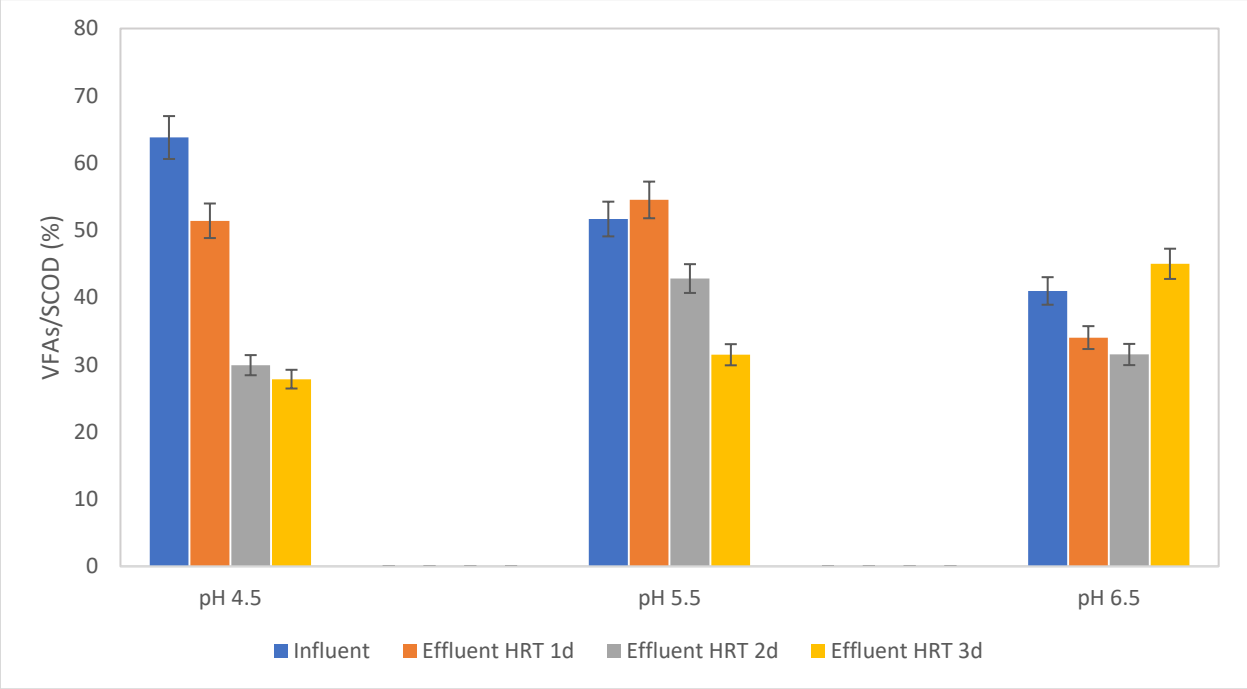

Figure 20 VFAs/SCOD ratio for different HRTs and pHs for semi-continuous experiment

Figure 21 displays the average SCOD yield data as mg SCOD $g^{-1}VSS_{feed}$ for semi-continuous fermentation. SCOD yield data demonstrates an increasing trend for all the pHs values when HRT increased from 1 day to 3 days. SCOD yield data for pH 4.5 ranged from 292 mg SCOD $g^{-1}VSS_{feed}$ (at HRT 1day) to 310 mg SCOD $g^{-1}VSS_{feed}$ (at HRT 3 days). The maximum SCOD yield of 644 mg SCOD $g^{-1}VSS_{feed}$ was achieved at Ph of 6.5 and HRT of 3 days. For pH 6.5 the SCOD yield data for HRT 2 days and 3 days does not have significant difference.

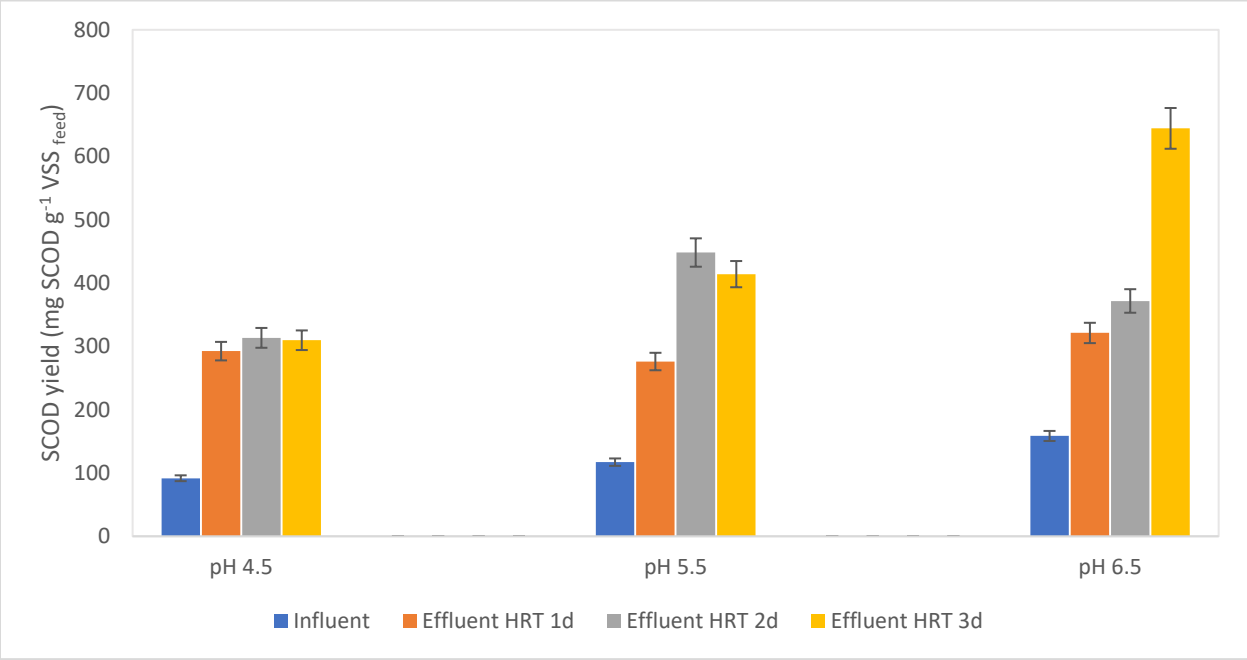

Figure 21 SCOD yield data for different HRTs and pHs for semi-continuous experiment

5.2.8 Discussion on Semi-continuous experiment results

pH of a fermenter has a direct influence of VFAs production as the growth rate microbes and microbial activities are significantly impacted by the changes of pH conditions [53]. The semi-continuous study cases higher VFAs production as the pH increases and was higher for pH 5.5 and 6.5 for HRT 2 days and 3 days respectively. The hydrolytic enzymes, as well as the hydrolysis and disintegration, can be affected by various pH

conditions [35]. Microbial activities and hydrolysis process are also facilitated by multiple non-biological processes, i.e., physiochemical methods such as acidic, alkaline, steam explosion, ultrasonic or microwave, etc. [46]. By comparing both the batch and semi-continuous experiment results of this experiments, it can be decided that alkaline pH conditions could be a considerable alternative as compared to acidic pH conditions for higher hydrolysis and disintegration.

Both SRT and HRT controls the efficiency of VFAs production from anaerobic fermentation process [54]. Lower HRT can lead to a risk of biomass washout for semi-continuous experiment resulting in a low VFA yield. Higher SRT can cause the production of methane. However, shorter SRT compared to the optimum value causes more VFAs accumulation, increased alkalinity and methanogens washouts [26]. HRT should be determined to bear in mind the operational temperature and the content of the organic substrate of the specific fermenter.

As per statistical one-way ANOVA analysis VFAs yield shows significant difference as demonstrates p value ≤ 0.05 for pH 4.5 to 5.5 with p value 0.01 (appendix D), pH 4.5 -6.5 with p value 0.003 (appendix D). But pH 5.5 -6.5 does not show any significant difference with p value 0.66 (appendix D). SCOD yield follows the same pattern as VFAs yield. SCOD yield shows significant difference as displays p value ≤ 0.05 for pH 4.5 to 5.5 with p value 0.000 (appendix D), pH 4.5 -6.5 with p value 0.000 (appendix D). But pH 5.5 -6.5 does not show any significant difference with p value 0.119 (appendix D).

For this experiment acidogenic fermentation of semi-continuous study under mesophilic conditions shows highest VFAs production (6549 ± 528 mg COD l^{-1}), VFAs yield (290 mg COD $g^{-1}VSS_{feed}$), SCOD yield (644 mg SCOD $g^{-1}VSS_{feed}$) and degree of solubilization 25

% for pH 6.5 with HRT 3 days. So, for semi-continuous acidogenic fermentation pH, 6.5 and HRT 3 days was recommended pH under mesophilic condition.

6 Conclusions

6.1 Anaerobic fermentation of PS batch study

Based on the batch fermentation of PS at different pHs and different HRTs, the following points can be concluded:

- The maximum VFAs yield was achieved at HRT of 3 days, However, there were no significant differences when the pH changed from 6.5 to 10.0, i.e., there was no significant effect of the pH in the range of 6.5 to 10 on the VFAs yields.
- The HRT has a significant effect on the VFAs yield for all pH values except pH 11.0.
- The optimum conditions for SCOD yield and the degree of solubilization were HRT of 3 days and pH 10.0.
- The VFAs/SCOD ratio was higher for acidic pH compared to alkaline pH.
- The maximum VFAs yield 230 mg COD g^{-1} VSS_{feed} was observed at pH 10.0 and HRT of 3 days; corresponding to the maximum SCOD concentration of 10,700 mg l^{-1}, SCOD yield of 1,300 mg SCOD $g^{-1}VSS_{feed}$ and the highest degree of solubilization of 48%.

  For the batch study, the pH range for maximum VFAs yield is pH 6.5 –10.0 and HRT of 3 days.

6.2 Anaerobic fermentation of PS semi-continuous study

Based on the semi-continuous flow fermentation of PS at different pHs and different HRTs, the following points can be concluded:

- VFAs production increases with an increase in HRT.

- Maximum VFAs production & yield, SCOD yield observed at pH 6.5, HRT of 3 days (35°C).
- The highest VFAs concentration 6,549 mg COD l^{-1}, SCOD concentration 14,600 mg SCOD l^{-1} and degree of solubilization 25% were achieved at pH 6.5 and HRT of 3 days.
- The maximum VFAs yield 290 mg COD gm $^{-1}$ VSS_{feed}, SCOD yield 644 mg SCOD g $^{-1}$ VSS_{feed} were achieved as well for pH 6.5 and HRT of 3 days.
- The high SCOD concentration of 9,000 to 14,500 mg l^{-1} was achieved at SRT of 3 days for the three pHs.

For the semi-continuous study, the maximum amount of VFAs production observed at pH 6.5 and HRT of 3 days.

7 Recommendations and Future Research

The results of this study reveal that the PS has excellent potential to produce VFAs by dark fermentation. However, the highest VFAs production was achieved at high HRT of 3 days. From a practical point of view, this HRT is high, and thus it is required to try to push this HRT to one day by combining the dark fermentation with another technology that has low footprint such thermal hydrolysis pre-treatment of combining the dark fermentation with microbial electrochemical cells. Another technique to reduce the HRT is doubling the SRT from HRT using solid-liquid separation method and recirculate the solids back to the fermenter. It is also recommended to investigate VFAs production through the semi-continuous experiment at alkaline pH (8 - 11) conditions as well as the fermentation process at different pH at the thermophilic temperature condition.

Appendices

A. Batch experiments

Table 7 Data for batch test of PS anaerobic fermentation pH 4.5 at 35 °C

	TSS	VSS	NH_3-N	Alkalinity	TCOD	SCOD	$TBOD_5$	VFAs
	mg l^{-1}	mg l^{-1}	mg l^{-1}	mg $CaCO_3$ l^{-1}	mg l^{-1}	mg l^{-1}	mg l^{-1}	mg COD l^{-1}
Influent	31,889	24,978	32	223	50,767	2,973	26,280	1,193
Effluent 1d	21,189	15,156	487	352	31,650	1,860	10,500	356
Effluent 2d	20,244	14,711	632	553	31,800	3,593	12,060	706
Effluent 3d	19,244	13,633	650	474	31,533	4,277	13,560	1,316

Table 8 Data or batch test of PS anaerobic fermentation pH 5.5 at 35 °C.

	TSS	VSS	NH_3-N	Alkalinity	TCOD	SCOD	$TBOD_5$	VFAs
	mg l^{-1}	mg l^{-1}	mg l^{-1}	mg $CaCO_3$ l^{-1}	mg l^{-1}	mg l^{-1}	mg l^{-1}	mg COD l^{-1}
Influent	31,889	24,978	32	223	50,767	2,973	26280	1,193
Effluent 1d	20,322	13,322	236	180	36,850	1,850	10320	504
Effluent 2d	19,533	13,311	567	892	34,133	4,773	10440	1,305
Effluent 3d	18,356	11,700	604	1,110	34,667	4,997	10620	1,653

Table 9 Data for batch test of PS anaerobic fermentation pH 6.5 at 35 °C.

	TSS	VSS	NH_3-N	Alkalinity	TCOD	SCOD	$TBOD_5$	VFAs
	mg l^{-1}	mg l^{-1}	mg l^{-1}	mg $CaCO_3$ l^{-1}	mg l^{-1}	mg l^{-1}	mg l^{-1}	mg COD l^{-1}
Influent	32,589	25,011	27	943	43,433	2,847	23,040	1,193
Effluent 1d	21,156	13,989	574	1,520	27,767	3,880	13,200	1,187
Effluent 2d	19,411	13,156	634	1,560	27,067	5,013	12,480	1,589
Effluent 3d	19,100	2,322	569	1,670	27,233	5,613	13,560	1,852

Table 10 Data for batch test of PS anaerobic fermentation pH 8.0 at 35 °C.

	TSS	VSS	NH_3-N	Alkalinity	TCOD	SCOD	$TBOD_5$	VFAs
	mg l^{-1}	mg l^{-1}	mg l^{-1}	mg $CaCO_3$ l^{-1}	mg l^{-1}	mg l^{-1}	mg l^{-1}	mg COD l^{-1}
Influent	43,189	34,100	43	858	50,433	3,080	13,800	1,402
Effluent 1d	21,456	15,556	689	3,630	27,500	4,540	11,640	1,455
Effluent 2d	20,856	14,122	692	3,990	24,767	5,953	12,000	1,820
Effluent 3d	19,756	13,578	772	3,860	24,567	7,253	13,140	2,587

Table 11 Data for batch test of PS anaerobic fermentation pH 9.0 at 35 °C.

	TSS	VSS	NH_3-N	Alkalinity	TCOD	SCOD	$TBOD_5$	VFAs
	mg l^{-1}	mg l^{-1}	mg l^{-1}	mg $CaCO_3$ l^{-1}	mg l^{-1}	mg l^{-1}	mg l^{-1}	mg COD l^{-1}
Influent	32,890	26,130	43	858	59,433	3,080	13,800	1,402
Effluent 1d	20,833	13,244	444	4,700	22,300	7,167	9,660	1,334
Effluent 2d	20,578	12,944	588	6,000	25,833	7,593	9,780	1,485
Effluent 3d	20,489	12,300	750	5,775	25,000	8,867	9,240	2,012

Table 12 Data for batch test of PS anaerobic fermentation pH 10.0 at 35 °C

	TSS	VSS	NH_3-N	Alkalinity	TCOD	SCOD	$TBOD_5$	VFAs
	mg l^{-1}	mg l^{-1}	mg l^{-1}	mg $CaCO_3$ l^{-1}	mg l^{-1}	mg l^{-1}	mg l^{-1}	mg COD l^{-1}
Influent	32,589	25,011	27	943	59,433	3,080	23,040	1,193
Effluent 1d	21,011	12,967	489	5,380	28,433	8,220	11,700	935
Effluent 2d	20,967	12,844	573	6,250	29,767	8,290	11,580	1,622
Effluent 3d	20,778	16,856	666	6,185	27,567	10,640	11,520	1,954

Table 13 Data for batch test of PS anaerobic fermentation pH 11.0 at 35 °C

	TSS	VSS	NH_3-N	Alkalinity	TCOD	SCOD	BOD5	VFAs
	mg l^{-1}	mg l^{-1}	mg l^{-1}	mg $CaCO_3$ l^{-1}	mg l^{-1}	mg l^{-1}	mg l^{-1}	mg COD l^{-1}
Influent	36,700	29,733	43	680	59,433	3,080	21,840	1,284
Effluent 1d	21,550	13,633	375	6,550	24,833	6,740	11,580	912
Effluent 2d	21,100	12,467	345	8,180	25,100	8,927	11,880	859
Effluent 3d	21,150	13,083	367	9,770	25,133	9,207	12,900	853

B. Semi-continuous experiments

Table 14 Data for semi-continuous PS anaerobic fermentation pH 4.5 HRT = 1 days ,35°C.

		TSS	VSS	NH_3-N	Alkalinity	TCOD	SCOD	$TBOD_5$	VFAs	VFAs/ VSS $_{feed}$
		mg l^{-1}	mg l^{-1}	mg l^{-1}	mg $CaCO_3$ l^{-1}	mg l^{-1}	mg l^{-1}	mg l^{-1}	mg COD l^{-1}	mg l^{-1}
	Influent	30,983	24,528	40	1,133	62,680	2,100	24,300	1,714	70
Effluent	Sample 1	36,933	29,789	194	1,600	56,750	7,640	29,400	4,117	168
	Sample 2	34,300	27,878	161	1,100	58,833	7,033	28,020	3,790	154
	Sample 3	31,167	25,100	397	1,265	58,100	7,073	29,400	3,371	137
	Sample 4	34,233	27,289	352	1,310	61,667	7,193	27,900	3,429	140
	Sample 5	33,311	26,644	362	1,305	60,800	7,053	29,040	3,611	147
	Sample 6	31,911	25,400	315	1,285	55,167	7,047	27,960	3,805	155
	Average	33,643	27,017	297	1,311	58,553	7,173	28,620	3,687	150
	Std Dev	1864	1577	88	148	2226	215	672	252	10

Table 15 Data for semi-continuous PS anaerobic fermentation pH 4.5 HRT = 2 days ,35°C

		TSS	VSS	NH_3-N	Alkalinity	TCOD	SCOD	$TBOD_5$	VFAs	VFAs/ VSS $_{feed}$
		mg l^{-1}	mg l^{-1}	mg l^{-1}	mg $CaCO_3$ l^{-1}	mg l^{-1}	mg l^{-1}	mg l^{-1}	mg COD l^{-1}	mg l^{-1}
	Influent	37,667	29,528	51	1,072	50,100	2,300	22,530	1,714	58
Effluent	Sample 1	31,633	24,256	324	832	48,100	9,540	30,660	2,440	83
	Sample 2	32,222	21,978	270	644	46,400	8,980	28,260	2,744	93
	Sample 3	33,150	24,644	242	625	41,133	8,740	28,800	2,542	86
	Sample 4	34,500	27,078	228	660	53,267	10,360	29,280	3,110	105
	Sample 5	28,211	21,733	243	1,081	46,670	8,630	29,940	2,651	90
	Sample 6	32,756	25,467	219	1,035	44,667	9,280	27,300	3,130	106
	Average	32,079	24,193	254	813	46,706	9,255	29,040	2,769	94
	Std Dev	1943	1876	35	186	3658	582	1094	265	9

Table 16 Data for semi-continuous anaerobic fermentation pH 4.5 HRT = 3 days ,35°C

		TSS	**VSS**	**NH_3-N**	**Alkalinity**	**TCOD**	**SCOD**	**$TBOD_5$**	**VFAs**	**VFAs/ VSS $_{feed}$**
		mg l^{-1}	**mg l^{-1}**	**mg l^{-1}**	**mg $CaCO_3$ l^{-1}**	**mg l^{-1}**	**mg l^{-1}**	**mg l^{-1}**	**mg COD l^{-1}**	**mg l^{-1}**
	Influent	37,022	29,394	44	597	48,317	2,347	21120	1,155	39
Effluent	Sample 1	32,678	25,000	413	770	52,150	8,650	22140	2,899	99
	Sample 2	30,656	24,478	361	747	55,300	10,640	22320	2,591	88
	Sample 3	31,156	25,922	318	597	49,467	9,287	22080	2,235	76
	Sample 4	30,078	25,600	262	495	46,933	8,367	22560	2,297	78
	Sample 5	30,300	25,533	247	621	45,833	8,820	22860	2,562	87
	Sample 6	30,600	23,111	279	825	46,233	8,853	22680	2,622	89
	Average	30,911	24,941	313	676	49,319	9,103	22,440	2,534	86
	Std Dev	857	941	58	114	3444	740	284	220	7

Table 17 Data for semi-continuous PS anaerobic fermentation pH 5.5 HRT = 1 days ,35°C

		TSS	**VSS**	**NH_3-N**	**Alkalinity**	**TCOD**	**SCOD**	**$TBOD_5$**	**VFAs**	**VFAs/ VSS $_{feed}$**
		mg l^{-1}	**mg l^{-1}**	**mg l^{-1}**	**mg $CaCO_3$ l^{-1}**	**mg l^{-1}**	**mg l^{-1}**	**mg l^{-1}**	**mg COD l^{-1}**	**mg l^{-1}**
	Influent	28,500	22,600	79	894	44,200	3,200	22100	1,194	53
Effluent	Sample 1	21500	18300	353	1590	42800	6200	21400	3,300	146
	Sample 2	22200	19200	245	1280	41900	6800	20950	3,900	173
	Sample 3	20900	17800	216	1217	43700	5400	21850	3,100	137
	Sample 4	23500	18000	210	1102	38600	6900	19300	3,300	146
	Sample 5	21600	16500	203	1247	40200	6040	20100	3,200	142
	Sample 6	21800	16400	183	1163	39400	6090	19700	3,600	159
	Average	21,917	17,700	235	1,266	41,100	6,238	20,550	3,400	150
	Std Dev	807	987	56	156	1837	503	918	271	12

Table 18 Data for semi-continuous PS anaerobic fermentation pH 5.5 HRT = 2 days ,35°C.

		TSS	**VSS**	**NH_3-N**	**Alkalinity**	**TCOD**	**SCOD**	**$TBOD_5$**	**VFAs**	**VFAs/ VSS_{feed}**
		mg l^{-1}	**mg l^{-1}**	**mg l^{-1}**	**mg $CaCO_3$ l^{-1}**	**mg l^{-1}**	**mg l^{-1}**	**mg l^{-1}**	**mg COD l^{-1}**	**mg l^{-1}**
	Influent	28,000	20,500	204	1,270	45,600	2,640	22,800	1,194	58
Effluent	Sample 1	22,300	18,000	471	2,470	40,300	8,900	20,150	4,200	205
	Sample 2	20,700	17,600	439	2,640	42,130	10,600	21,065	4,800	234
	Sample 3	21,900	18,100	324	1,561	39,900	9,280	19,950	3,900	190
	Sample 4	21,100	17,700	299	2,025	38,900	9,700	19,450	3,100	151
	Sample 5	18,600	16,400	230	2,727	41,500	8,290	20,750	3,700	180
	Sample 6	22,100	17,100	253	1,745	40,200	8,360	20,100	3,900	190
	Average	21,117	17,483	336	2,194	40,488	9,188	20,244	3,933	192
	Std Dev	1258	581	90	445	1057	800	529	512	25

Table 19 Data for PS anaerobic fermentation semi-continuous study pH 5.5 HRT = 3 days ,35°C

		TSS	**VSS**	**NH_3-N**	**Alkalinity**	**TCOD**	**SCOD**	**$TBOD_5$**	**VFAs**	**VFAs/ VSS_{feed}**
		mg l^{-1}	**mg l^{-1}**	**mg l^{-1}**	**mg $CaCO_3$ l^{-1}**	**mg l^{-1}**	**mg l^{-1}**	**mg l^{-1}**	**mg COD l^{-1}**	**mg l^{-1}**
	Influent	30,983	24,528	40	1,133	42,580	2,100	21,300	1,714	70
Effluent	Sample 1	24,056	16,267	304	2,090	43,650	10,900	23,220	3,243	132
	Sample 2	25,133	18,189	137	1,460	40,460	10,360	22,320	3,396	138
	Sample 3	23,600	14,611	293	2,210	38,300	10,130	24,240	2,992	122
	Sample 4	24,178	16,144	282	2,135	45,050	9,850	22,800	2,891	118
	Sample 5	19,422	17,467	262	1,590	40,670	10,100	24,060	3,821	156
	Sample 6	22,756	15,678	245	1,520	39,270	9,600	24,960	2,837	116
	Average	23,191	16,393	254	1,834	41,233	10,157	23,600	3,197	130
	Std Dev	1828	1165	56	315	2373	409	903	341	14

Table 20 Data for semi-continuous study of PS anaerobic fermentation pH 6.5 HRT = 1 days ,35°C

		TSS	**VSS**	**NH_3-N**	**Alkalinity**	**TCOD**	**SCOD**	**$TBOD_5$**	**VFAs**	**VFAs/ VSS $_{feed}$**
		mg l^{-1}	**mg l^{-1}**	**mg l^{-1}**	**mg $CaCO_3$ l^{-1}**	**mg l^{-1}**	**mg l^{-1}**	**mg l^{-1}**	**mg COD l^{-1}**	**mg l^{-1}**
	Influent	27,922	22,589	47	657	47,967	3,580	17,100	1,379	27,922
Effluent	Sample 1	21,989	16,778	359	2,545	49,650	7,390	15,300	2,393	21,989
	Sample 2	22,211	16,944	323	2,270	43,100	7,140	15,540	1,982	22,211
	Sample 3	21,444	16,122	365	2,840	48,100	7,147	15,840	2,957	21,444
	Sample 4	21,722	16,578	341	2,545	49,367	7,340	15,060	3,078	21,722
	Sample 5	21,333	16,044	377	2,810	47,500	7,267	15,660	2,301	21,333
	Sample 6	21,844	16,200	373	2,460	52,933	7,247	15,360	2,101	21,844
	Average	21,757	16,444	356	2,578	48,442	7,255	15,460	2,469	21,757
	Std Dev	302	342	19	197	2945	92	254	412	302

Table 21 Data for semi-continuous study of PS anaerobic fermentation pH 6.5 HRT = 2 days ,35°C

		TSS	**VSS**	**NH_3-N**	**Alkalinity**	**TCOD**	**SCOD**	**$TBOD_5$**	**VFAs**	**VFAs/ VSS $_{feed}$**
		mg l^{-1}	**mg l^{-1}**	**mg l^{-1}**	**mg $CaCO_3$ l^{-1}**	**mg l^{-1}**	**mg l^{-1}**	**mg l^{-1}**	**mg COD l^{-1}**	**mg l^{-1}**
	Influent	48,011	38,678	47	1,145	46,067	3,580	13,680	1,554	
Effluent	Sample 1	28,022	20,100	468	4,655	52,750	14,320	33,773	4,453	76
	Sample 2	34,956	26,144	458	3,400	52,967	14,340	34,359	4,397	114
	Sample 3	30,467	22,556	464	3,800	46,500	14,767	35,281	4,768	123
	Sample 4	29,822	20,456	361	3,520	51,333	14,393	32,348	5,414	140
	Sample 5	28,911	19,722	377	3,105	52,633	14,173	32,097	5,097	132
	Sample 6	30,411	20,711	312	3,315	54,567	14,267	31,510	4,562	118
	Average	30,431	21,615	407	3,633	51,792	14,377	33,228	4,782	117
	Std Dev	2197	2215	60	503	2547	187	1342	366	20

Table 22 Data for semi-continuous study of PS anaerobic fermentation pH 6.5 HRT = 3 days ,35°C

		TSS	VSS	NH_3-N	Alkalinity	TCOD	SCOD	$TBOD_5$	VFAs	VFAs/ VSS_{feed}
		mg l^{-1}	mg l^{-1}	mg l^{-1}	mg $CaCO_3$ l^{-1}	mg l^{-1}	mg l^{-1}	mg l^{-1}	mg COD l^{-1}	mg l^{-1}
	Influent	27,922	22,589	47	657	47,967	3,580	13,680	1,554	27,922
Effluent	Sample 1	25,889	18,044	393	3,870	45,100	13,840	20,520	6,291	25,889
	Sample 2	27,833	19,811	465	4,270	50,167	14,320	19,920	7,623	27,833
	Sample 3	25,544	17,622	546	3,900	48,100	14,947	18,120	6,302	25,544
	Sample 4	27,489	18,833	508	4,335	50,867	14,767	15,180	6,414	27,489
	Sample 5	25,544	16,589	510	3,875	45,200	14,853	12,780	6,330	25,544
	Sample 6	29,100	20,133	454	4,255	52,900	14,600	14,100	6,337	29,100
	Average	26,900	18,506	479	4,084	48,722	14,554	16,770	6,549	26,900
	Std Dev	1339	1233	49	204	2888	378	2926	482	1339

Table 23 VFAs yield, SCOD yield , VFAs/SCOD , degree of solubilization data for semi-continuous study of PS anaerobic fermentation 35°C

pH	HRT	VFAs/SCOD	VFAs yield	SCOD yield	degree of solubilization
	d	mg COD mg $^{-1}$ SCOD (%)	mg COD g $^{-1}$$VSS_{feed}$	mg SCOD g^{-1} VSS_{feed}	%
4.5	0	64	58	92	
4.5	1	51	150	292	8
4.5	2	30	94	313	15
4.5	3	28	86	310	15
5.5	0	52	61	117	
5.5	1	55	150	276	7
5.5	2	43	192	448	15
5.5	3	31	130	414	20
6.5	0	41	65	158	
6.5	1	34	109	321	8
6.5	2	32	117	372	25
6.5	3	45	290	644	25

C. One-way ANOVA, Fisher pairwise comparison for batch experiment

One-way ANOVA: VFAs yield versus pH

Method

Null hypothesis	All means are equal
Alternative hypothesis	At least one mean is different
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
pH	7	4.5, 5.5, 6.5, 8.0, 9.0, 10.0, 11.0

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
pH	6	10468	1744.7	7.82	0.000
Error	56	12501	223.2		
Total	62	22970			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
14.9412	45.57%	39.74%	31.12%

Means

pH	N	Mean	StDev	95% CI
4.5	9	31.70	16.89	(21.72, 41.68)
5.5	9	46.18	20.43	(36.20, 56.15)
6.5	9	61.69	11.64	(51.71, 71.67)
8.0	9	54.37	16.15	(44.40, 64.35)
9.0	9	61.63	11.80	(51.65, 71.60)
10.0	9	60.12	17.99	(50.14, 70.10)
11.0	9	29.415	0.972	(19.438, 39.392)

Pooled StDev = 14.9412

Fisher Pairwise Comparisons

Fisher Individual Tests for Differences of Means

Difference of Levels	Difference of Means	SE of Difference	95% CI	T-Value	Adjusted P-Value
5.5 - 4.5	14.48	7.04	(0.37, 28.59)	2.06	0.045
6.5 - 4.5	29.99	7.04	(15.88, 44.10)	4.26	0.000
8.0 - 4.5	22.67	7.04	(8.56, 36.78)	3.22	0.002
9.0 - 4.5	29.93	7.04	(15.82, 44.04)	4.25	0.000
10.0 - 4.5	28.42	7.04	(14.31, 42.53)	4.04	0.000
11.0 - 4.5	-2.28	7.04	(-16.39, 11.83)	-0.32	0.747
6.5 - 5.5	15.51	7.04	(1.40, 29.62)	2.20	0.032

8.0 - 5.5	8.20	7.04	(-5.91, 22.31)	1.16	0.250
9.0 - 5.5	15.45	7.04	(1.34, 29.56)	2.19	0.032
10.0 - 5.5	13.94	7.04	(-0.17, 28.05)	1.98	0.053
11.0 - 5.5	-16.76	7.04	(-30.87, -2.65)	-2.38	0.021
8.0 - 6.5	-7.32	7.04	(-21.43, 6.79)	-1.04	0.303
9.0 - 6.5	-0.06	7.04	(-14.17, 14.05)	-0.01	0.993
10.0 - 6.5	-1.57	7.04	(-15.68, 12.54)	-0.22	0.825
11.0 - 6.5	-32.27	7.04	(-46.38, -18.16)	-4.58	0.000
9.0 - 8.0	7.25	7.04	(-6.86, 21.36)	1.03	0.308
10.0 - 8.0	5.75	7.04	(-8.36, 19.86)	0.82	0.418
11.0 - 8.0	-24.96	7.04	(-39.07, -10.85)	-3.54	0.001
10.0 - 9.0	-1.51	7.04	(-15.61, 12.60)	-0.21	0.832
11.0 - 9.0	-32.21	7.04	(-46.32, -18.10)	-4.57	0.000
11.0 - 10.0	-30.71	7.04	(-44.81, -16.60)	-4.36	0.000

Simultaneous confidence level = 57.58%

One-way ANOVA: VFAs yield versus Time

Method

Null hypothesis	All means are equal
Alternative hypothesis	At least one mean is different
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Time	3	1, 2, 3

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Time	2	9389	4694.7	20.74	0.000
Error	60	13580	226.3		
Total	62	22970			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
15.0446	40.88%	38.91%	34.82%

Means

Time	N	Mean	StDev	95% CI
1	21	34.79	13.15	(28.23, 41.36)
2	21	48.45	14.42	(41.88, 55.01)
3	21	64.66	17.27	(58.09, 71.23)

Pooled StDev = 15.0446

Fisher Pairwise Comparisons

Fisher Individual Tests for Differences of Means

Difference of Levels	Difference of Means	SE of Difference	95% CI	T-Value	Adjusted P-Value
2 - 1	13.65	4.64	(4.37, 22.94)	2.94	0.005
3 - 1	29.87	4.64	(20.58, 39.15)	6.43	0.000
3 - 2	16.21	4.64	(6.93, 25.50)	3.49	0.001

Simultaneous confidence level = 87.91%

One-way ANOVA: SCOD yield versus pH

Method

Null hypothesis	All means are equal
Alternative hypothesis	At least one mean is different
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
pH	7	4.5, 5.5, 6.5, 8.0, 9.0, 10.0, 11.0

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
pH	6	405009	67501	42.50	0.000
Error	56	88946	1588		
Total	62	493955			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
39.8537	81.99%	80.06%	77.21%

Means

pH	N	Mean	StDev	95% CI
4.5	9	129.9	43.3	(103.3, 156.5)
5.5	9	174.5	43.2	(147.9, 201.1)
6.5	9	193.3	35.1	(166.7, 219.9)
8.0	9	173.5	34.6	(146.9, 200.1)
9.0	9	301.40	29.84	(274.79, 328.01)
10.0	9	371.0	49.1	(344.4, 397.6)
11.0	9	278.8	40.5	(252.2, 305.5)

Pooled StDev = 39.853

Fisher Pairwise Comparisons

Fisher Individual Tests for Differences of Means

Difference of Levels	Difference of Means	SE of Difference	95% CI	T-Value	Adjusted P-Value
5.5 - 4.5	44.6	18.8	(7.0, 82.3)	2.38	0.021
6.5 - 4.5	63.5	18.8	(25.8, 101.1)	3.38	0.001
8.0 - 4.5	43.6	18.8	(6.0, 81.2)	2.32	0.024
9.0 - 4.5	171.5	18.8	(133.9, 209.2)	9.13	0.000
10.0 - 4.5	241.2	18.8	(203.5, 278.8)	12.84	0.000
11.0 - 4.5	149.0	18.8	(111.3, 186.6)	7.93	0.000
6.5 - 5.5	18.8	18.8	(-18.8, 56.5)	1.00	0.320
8.0 - 5.5	-1.0	18.8	(-38.7, 36.6)	-0.05	0.956
9.0 - 5.5	126.9	18.8	(89.3, 164.5)	6.75	0.000
10.0 - 5.5	196.5	18.8	(158.9, 234.2)	10.46	0.000
11.0 - 5.5	104.4	18.8	(66.7, 142.0)	5.55	0.000
8.0 - 6.5	-19.9	18.8	(-57.5, 17.8)	-1.06	0.295
9.0 - 6.5	108.1	18.8	(70.4, 145.7)	5.75	0.000
10.0 - 6.5	177.7	18.8	(140.1, 215.3)	9.46	0.000
11.0 - 6.5	85.5	18.8	(47.9, 123.1)	4.55	0.000
9.0 - 8.0	127.9	18.8	(90.3, 165.6)	6.81	0.000
10.0 - 8.0	197.6	18.8	(159.9, 235.2)	10.52	0.000
11.0 - 8.0	105.4	18.8	(67.8, 143.0)	5.61	0.000
10.0 - 9.0	69.6	18.8	(32.0, 107.3)	3.71	0.000
11.0 - 9.0	-22.5	18.8	(-60.2, 15.1)	-1.20	0.235
11.0 - 10.0	-92.2	18.8	(-129.8, -54.6)	-4.91	0.000

Simultaneous confidence level = 57.58%

One-way ANOVA: SCOD yield versus Time

Method

Null hypothesis	All means are equal
Alternative hypothesis	At least one mean is different
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Time	3	1, 2, 3

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Time	2	71636	35818	5.09	0.009
Error	60	422318	7039		
Total	62	493955			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
83.8966	14.50%	11.65%	5.74%

Means

Time	N	Mean	StDev	95% CI
1	21	187.3	87.2	(150.7, 224.0)
2	21	239.0	76.3	(202.4, 275.6)
3	21	269.0	87.7	(232.4, 305.6)

Pooled StDev = 83.8966

Fisher Pairwise Comparisons

Fisher Individual Tests for Differences of Means

Difference of Levels	Difference of Means	SE of Difference	95% CI	T-Value	Adjusted P-Value
2 - 1	51.7	25.9	(-0.1, 103.5)	2.00	0.050
3 - 1	81.6	25.9	(29.9, 133.4)	3.15	0.003
3 - 2	30.0	25.9	(-21.8, 81.8)	1.16	0.252

Simultaneous confidence level = 87.91%

D. One-way ANOVA, Fisher pairwise comparison for semi-continuous experiment

One-way ANOVA: VFAs yield versus pH

```
Method

Null hypothesis         All means are equal
Alternative hypothesis  At least one mean is different
Significance level      α = 0.05

Equal variances were assumed for the analysis.


Factor Information

Factor  Levels  Values
pH           3  4.5, 5.5, 6.5


Analysis of Variance

Source  DF  Adj SS  Adj MS  F-Value  P-Value
pH       2   32228   16114     5.64    0.006
Error   51  145783    2858
Total   53  178012


Model Summary

      S    R-sq  R-sq(adj)  R-sq(pred)
53.4649  18.10%     14.89%       8.19%


Means

pH    N    Mean  StDev       95% CI
4.5  18  110.11  30.85  ( 84.81, 135.41)
5.5  18  157.55  32.16  (132.25, 182.85)
6.5  18   165.4   81.2  ( 140.1,  190.7)

Pooled StDev = 53.4649
```

Fisher Pairwise Comparisons

```
Fisher Individual Tests for Differences of Means

Difference  Difference       SE of                          Adjusted
of Levels     of Means  Difference      95% CI     T-Value   P-Value
5.5 - 4.5         47.4        17.8  ( 11.7, 83.2)     2.66     0.010
6.5 - 4.5         55.3        17.8  ( 19.5, 91.1)     3.10     0.003
6.5 - 5.5          7.9        17.8  (-27.9, 43.7)     0.44     0.660

Simultaneous confidence level = 87.93%
```

One-way ANOVA: SCOD yield versus pH

```
Method

Null hypothesis         All means are equal
Alternative hypothesis  At least one mean is different
Significance level      α = 0.05

Equal variances were assumed for the analysis.


Factor Information

Factor  Levels  Values
pH           3  4.5, 5.5, 6.5


Analysis of Variance

Source  DF   Adj SS  Adj MS  F-Value  P-Value
pH       2   496490  248245    15.77    0.000
Error   51   802990   15745
Total   53  1299480


Model Summary

      S    R-sq  R-sq(adj)  R-sq(pred)
125.479  38.21%     35.78%      30.72%


Means

pH    N   Mean  StDev      95% CI
4.5  18  217.5  138.3  (158.1, 276.8)
5.5  18  379.4   81.7  (320.1, 438.8)
6.5  18  445.7  146.4  (386.4, 505.1)

Pooled StDev = 125.479
```

Fisher Pairwise Comparisons

```
Fisher Individual Tests for Differences of Means

Difference  Difference       SE of                            Adjusted
of Levels     of Means  Difference      95% CI      T-Value   P-Value
5.5 - 4.5        162.0        41.8  ( 78.0, 246.0)     3.87     0.000
6.5 - 4.5        228.3        41.8  (144.3, 312.3)     5.46     0.000
6.5 - 5.5         66.3        41.8  (-17.7, 150.3)     1.58     0.119

Simultaneous confidence level = 87.93%
```

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