Effect of pH and hydraulic retention time on volatile fatty acids production from real waste in dark fermentation process

by

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Abstract

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Waste-derived volatile fatty acids (VFAs) is an important carbon substrate for microorganisms engaged in the production of bioenergy, biodegradable plastics, and biological nutrient removal process. In this project, the generation and applications of waste-derived VFA were examined. Three solid wastes were used Primary sludge (PS), thichened waste activated sludge(TWAS) which were collected from Ashbridges Bay and source separated organics (SSO) that was collected from Disco Road facility. All the water quality analyses such as pH, TCOD, SCOD, TVFA, TSS, VSS, NH_3 and, alkalinity were monitored. The results of this study showed that with increasing the Hydraulic retention time (HRT), the percentage of acidification increased. Furthermore, the results showed that alkaline pH was better than the acid pHs.

Keywords: Total Volatile Fatty Acids, Soluble Chemical Oxygen Demand, Primary Sludge, Thickened Waste Activated Sludge, Source Separated Organics.

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Above all, I thank Almighty ALLAH, for all his blessings.

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PS	Primary Sludge
TWAS	Thickened Waste Activated Sludge
SSO	Source Separated Organics
HRT	Hydraulic Retention Time
SRT	Solids Retention Time
VFAs	Volatile Fatty Acids
F/M	Food to Microorganism
COD	Chemical Oxygen Demand
WAS	Waste Activated Sludge
WWTPs	Wastewater Treatment Plants
TCOD	Total Chemical Oxygen Demand
SCOD	Soluble Chemical Oxygen Demand
TSS	Total Suspended Solids
VSS	Volatile Suspended Solids

1 INTRODUCTION

The world has without a doubt experienced an upsurge in energy demand over the last few decades. More precisely, this upsurge has been largely accompanied with unprecedented rise in non-renewable energy consumption. Non-renewable energy, primarily from fossil fuel, has resulted into excessive greenhouse gases emission; a fete that led to increased need for renewable sources as a viable alternative. In 1776, Alessandro Volta isolated methane gas by taking an interest in "marsh gas". Methane from marshes but also rice fields, animal droppings and marine sediments is produced by the transformation of fermentable organic matter in the absence of oxygen by a microbial consortium [1]. This natural biological process that produces a mixture of gas (biogas) composed mainly of methane (60 to 70%) and carbon dioxide is called anaerobic digestion or dark fermentation process [2]. As a bioprocess, the dark fermentation process makes it possible to recycle waste organic matter from agri-food industries, sewage treatment plants, agricultural activities, but also domestic activities. During the hydrolysis of polymers such as proteins, carbohydrates and lipids are decomposed into monomers (monosaccharides, amino acids, sugars and fatty acids).

It is necessary to develop hydrogen production processes that do not emit greenhouse gases. In terms of the quantity of hydrogen produced, the synthetic pathways from fossil fuels with sequestration of CO_2 at the source will certainly be very majority [2]. The conversion of nuclear or renewable energies (geothermal, solar, hydro-electricity, wind) to hydrogen is also envisaged. Biomass, the solar energy stored as organic matter through photosynthesis, can also be converted to hydrogen by thermo-chemical or biological processes.

2 OBJECTIVE OF STUDY

As a bioprocess, not only does the dark fermentation process make it possible to recycle waste organic matter from agri-food industries, sewage treatment plants, agricultural activities, but also domestic operations. Drawing on this background, the effect of pH and hydraulic retention time (HRT) on volatile fatty acids (VFA) production in dark fermentation process were investigated in this work.

- To review the various waste types used for VFA production.
- To detail the pertinent factors affecting the performance of VFA production.
- To examine the application of waste-derived VFA.
- Experimental work is done to check how much VFA can be produced from wastes.

This flow chart shows the overview of the project how volatile fatty acids (VFA) is produce and their various application.

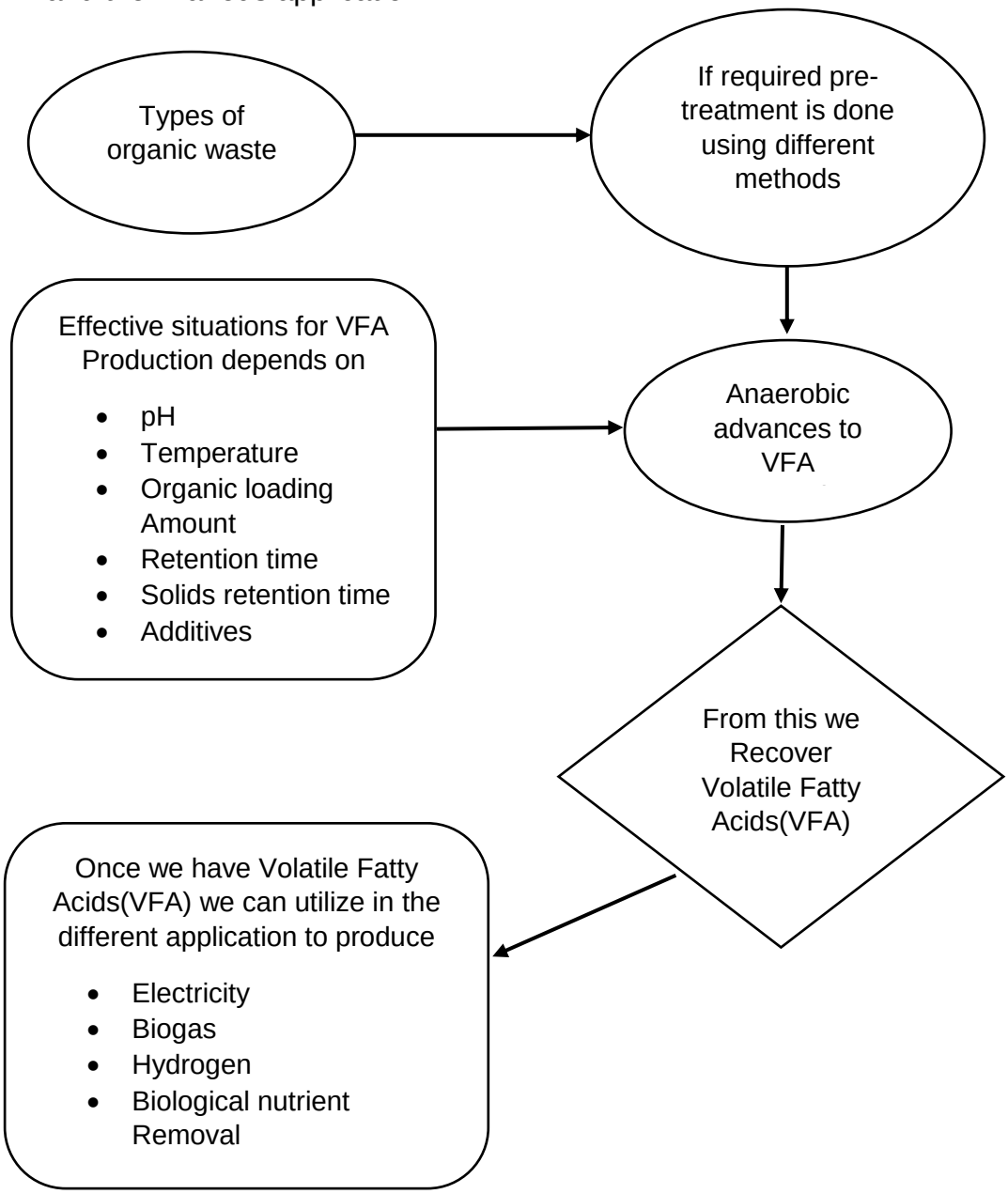


Figure 1:The Objective of the Study in Flow Chart

3.0 Dark FermentationDark fermentation is defined as the process of fermentative conversion of the organic substrate in order to produce biohydrogen [3]. The process is sophisticated, and it is enabled by multiple bacteria that facilitate a chain of biochemical reactions through a 3-stepped process of similar character to anaerobic conversion [4]. Unlike photo-fermentation, it does not require light. Dark fermentation, not only lowers organic wastes pollution but it also promotes the use of organic waste for development of renewable energy and hence sustainable development.

3.1 Main Parameters that Affect the Process (Dark Fermentation)

The bacteria present in this phase have a strict or facultative anaerobic metabolism [4, 5] and form a heterogeneous phylogenetic grouping of many bacterial groups [6]. pH and hydraulic retention time (HRT) are often considered as the limiting step in the process of anaerobic digestion in the case of hardly hydrolyzable compounds such as cellulose, starch or fats [1, 3, 7, 8]. As a matter of fact, other than the microbial community structures, HRT and pH are the operational parameters that impact hydrogen yield.

The digestion of the acid phase is influenced by a variety of factors, including wastewater characteristics, operational parameters such as HRT and solids retention time and Environmental factors such as temperature, pH, reactor configuration and oxidation-reduction potential (ORP) [9].

Operating pH, temperature, retention time, organic loading rate and the additives employed have a significant effect on the concentration, yield and composition of the VFAs produced from waste.

3.2 The temperature

The production of VFAs from waste was carried out under different temperature ranges. In the psychrophilic (4°C-20°C) and mesophilic (20°C-50°C). Product concentration, VFA production rate, and volatile fatty acid yield increased with increasing temperature [5]. Thermophilic anaerobic digestion presented higher organic matter degradation (especially fiber), higher pH and higher methane (CH_4) yield, as great as preferred rate of extreme CH_4 yield retrieved Also more level lingering CH_4 emission, at compared for mesophilic states. Over addition, easier microbial differing qualities might have been

found in the thermophilic reactors, particularly to Bacteria, the place an acceptable increased towards clostridia population parts might have been apparent. For example, the VFA production rate increased when the temperature increased from 8° C to 25° C during BP fermentation [10]. In the same way, growing the temperature from 10°C to 35°C increased the concentration of VFAs produced from the BAs by 30%. The increase is due to the presence of more carbohydrates and soluble proteins, which is the result of improved sludge hydrolysis at a higher temperature [7].

3.3 The pH

Also, a significant control parameter in the process, the pH has been a subject of extensive study. The reactor's pH value is essential for VFAs production given that acidogens are unable to withstand highly acidic conditions (pH: 3) or highly alkaline conditions (pH: 12) environments [10]. As a matter of fact, the optimum recommended pH values in volatile fatty acids' production of volatile fatty acids lies between 5.25 and 11 [5, 11].

pH has an important effect on the acidification of wastewater, but production is mainly carried out under acidic conditions with optimum pH ranges from 5.25 to 6.0 [12, 13]. The degree of acidification of wastewater from a synthetic gelatin base increased from 32.0 to 71.6% when the pH increased from 6.0 to 6.5 but dropped to 66.8% when the pH increased to 7.0. Optimum pH was found at 6.0. as a matter of fact, the type of VFA produced, whether acetic or butyric acids or any other, largely depends on the pH [5, 14].

3.4 The organic load

The organic loading rate (OLR) indicates the amount of waste, which can be expressed in terms of COD. In the literature, the influence of OLR on the production of VFAs differs from one study to another. Indeed, during an acidogenic fermentation of wastewater from the chemical-based synthesis of pharmaceutical products [11]. The concentration of VFAs increases with increasing the OLR only in the range of 7 to 13 g of COD / L / day. For example, a slight increase to 14 g COD / L resulted in a drop in the VFA concentration from 3,410 mg/L (as acetate) to 1,370 mg/L (as acetate) [13]. Conversely, the

concentration of VFAs produced from starchy wastewater increases linearly with increasing the OLR, ranging from 1 g COD/L/d to 32 g COD/L/d [15].

3.5 Hydraulic Retention time (HRT)

Several studies have shown that the application of a high HRT is more beneficial for the production of VFAs' time to react with waste. In this context, [16] showed that the production of VFAs from the dairy water almost doubled when HRT increased from 4 h to 12 h and the new increase from 16 to 24 h improved production of VFAs by 6% [7, 14]. Thus, [5] inferred that the production of VFAs from ODHF increased with HRT in a 2-6-day interval. Similarly, the yield and volumetric productivity of VFAs in the acidogenic fermentation of food waste increased when HRT increased from 96 h to 192 h [17]. However, extended HRT could lead to the stagnant production of VFAs [17, 18].

3.6 Solid retention time (SRT)

In the studies it was found that volatile fatty acids yields increased with solid retention time .[19] The retention is the time taken by organic matter to complete the degradation process. The optimum value for the SRT will be function of waste composition, operating temperature and various other process. The SRT is very important design parameter for carbon conversion as it has to be in reactor for the optimum time to perform metabolism and produce methane.[20, 21, 22]

3.7 Additives

In recent years, additives such as chemical inhibitors of methanogenesis have been used to improve the production of VFAs [23]. These inhibitors can enhance the production of VFAs by suppressing the activity of VFA-consuming methanogenic bacteria.

3.8 Optimum conditions for volatile fatty acids (vfa)

Volatile fatty acids (VFA) are a product fermentative hydrogen production. Their production takes the form of varying solvents. A majority of fatty acids were produced in the acidogenic phase of the hydrolysis process. Acetic acids, propionic acids, lactic acid, ethanol, isobutyric, and butyric acid, are the fatty acids produced [24, 25]. The distribution and fraction concentration of the acids is often used in monitoring the process. In the

treatment pH drop takes as a result of VFA accumulation and CO_2 generation in excess [23].

The VFA generation in fermentative hydrogen production process is also affected by the change in temperature with a high of 45°C for acetic and butyric acid; concentration is higher at 26-30% compared to the mesophilic temperature of 30-35°C for acetate, propionate and butyrate concentration of between 20 and 25% [18]. Also important is ethanol concentration for estimation of liquid metabolites with a high fraction of ethanol concentration fraction of between 23-40% being achieved at 30-45°C lowering hydrogen production.

Overall, after the first steps which induce an acidification of the medium which can reach a pH of the order of 4, the consumption of the VFAs causes a rise in the pH which also stimulates the methanogenic activity. Ultimately, the pH can reach values greater than 9, which gradually inhibit methanogenesis, in particular by the large production of ammonia (NH_4). The H_2 molecule is involved in most of these biological mechanisms. However, is not necessarily detectable if the four steps are linked together precisely, i.e. if the main limiting factor is the hydrolysis of the initial organic matter [10]. Among the microorganisms involved in the biodegradation of organic matter, the methanogenic microflora is the most vulnerable. For deep anaerobiosis conditions, optimum moisture (55 to 80% relative to the total weight) and pH between 6.8 and 8 are essential for the good progress of methanogenesis. It is also well demonstrated that a thermophilic regime (about 55°C.) is particularly favorable for methanogenesis with respect to the mesophilic regime (30-40°C.) [10]. A more acidic pH and a high concentration of salts and particularly sulfates (which are found especially in soils near the sea or in certain construction wastes such as plaster) are, on the other hand, conditions favoring sulfate-reducing bacteria. These microorganisms (Desulfovibrio, Desulfotomaculum, etc. which are also strictly anaerobic, use hydrogen, acetic acid, alcohols and VFAs to form CO_2 and hydrogen sulfide (H_2S easily detectable due to its odor rotten). This molecule is particularly toxic and volatile, so that the development of sulfate-reducing bacteria, especially from the same substrates as methanogens, tends to rapidly inhibit the entire microflora [2,12].

Since hydrogen sulfide is also harmful to the environment and to biogas treatment equipment, a limitation of this bioconversion is therefore doubly necessary.

As we have seen, aerobic/anaerobic and pH conditions influence the development of the different populations of microorganisms involved in biodegradation processes. However, it is an essential parameter, the moisture content, whose incidence is paramount to the whole biological processes. Indeed, for enzymes, but also for acid- and acetogenic or methanogenic bacteria, the activity increases as a function of the moisture content of the medium [7]. It is therefore essential to control these parameters well according to the objective pursued, whether it is to prevent or promote the biodegradation of organic matter. Production can either be in batches or continuous.

3.9 Batch vs. continuous processes

In batch processing, production is done in batches through each of the desirable processes. This implies that a certain predefined quantity or quality is produced at a time before another group is introduced. In contrast, for a continuous process, the production is run non-stop. While batch processing allows for resetting of conditions at intervals, after completion of each batch, in continuous processing, the conditions must be constantly maintained alongside production [14]. In essence, while in batch processing, bulk materials are processed from one step to another with the next batch having to await completion of the preceding batch, in a continuous flow, one unit is produced at a time with no breaks.

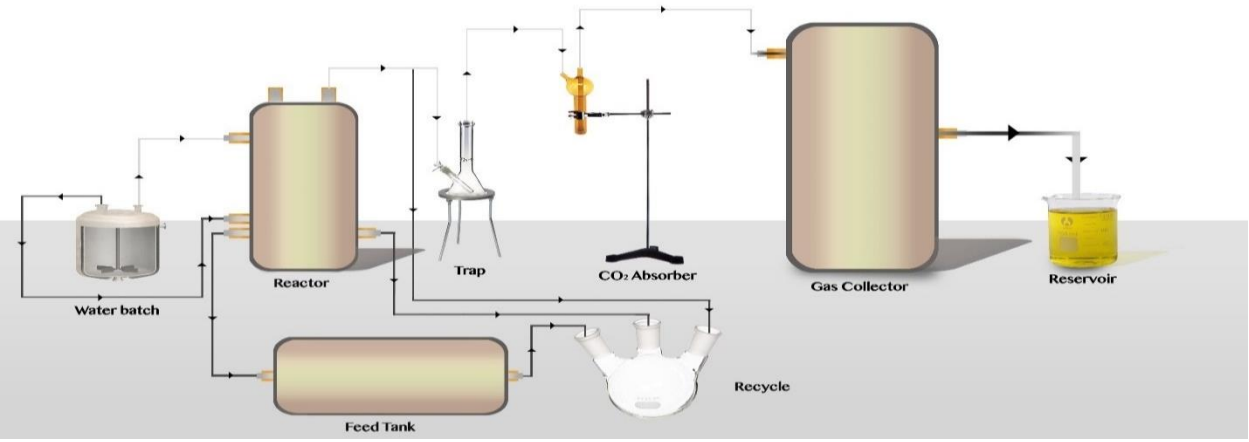


Figure 2:Continuous Process of Anaerobic Digester.

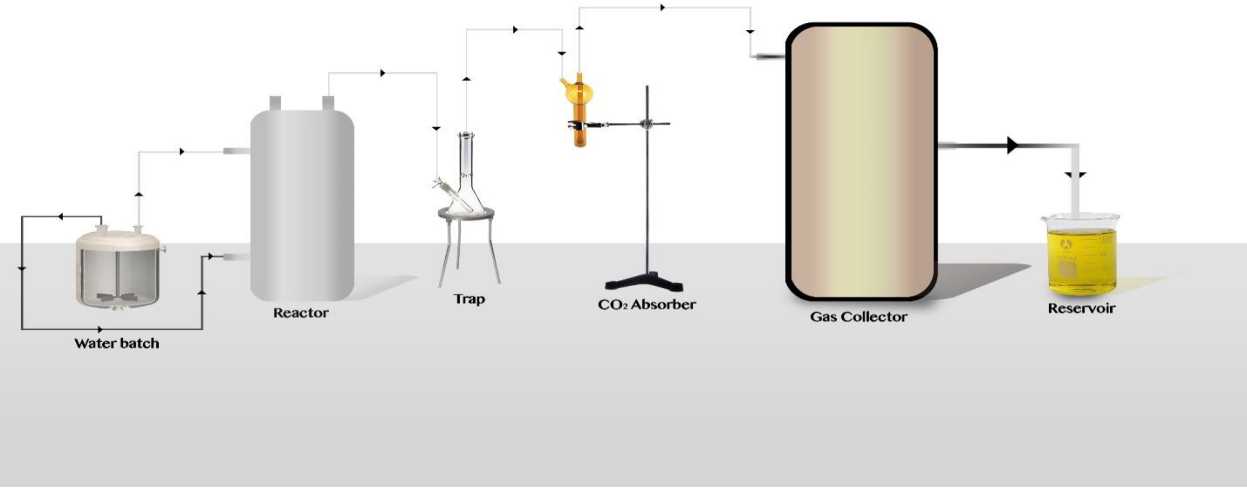


Figure 3:Batch process of Anaerobic Digester

3.10 Technological improvements

Technological improvements in dark fermentation are mainly geared towards its commercialization in which case; commercialization is largely dependent on bioprocess design and optimization advances as well as the increased understanding of the

biohydrogen-producing communities' structures as well as their improvement. These further relate to their consortium development as well as the involved organisms' microorganisms molecular understanding in the path to realizing a stable hydrogen economy [12]. As a matter of fact, experts have noted that understanding of the microorganisms is key to improving the energy yields from dark fermentation. This is however only possible if the understanding is extended to their possible interaction effects, in addition to the use of relevant substrate and strategies including hydrogen purification, solid-state fermentation, and coupling of the dark fermentation process with other processes [17]. Additionally, the technological advancements are heavily premised on improved bioreactor designs as well as integrated systems centered on process economy.

3.11 Various Biological Hydrogen Production Processes

The biological reactions of consumption or production of hydrogen correspond to various physiological roles (energy generation via aerobic or anaerobic respiration, consumption of electrons during anaerobic fermentation, nitrogen fixation, etc.) [14]. For several decades, many teams have been working on the development of biological processes that would convert solar energy or biomass into hydrogen.

Currently, 95% of the hydrogen is produced from hydrocarbons, due first of all to their integration in the petroleum industry which is one of the first consumers of hydrogen. Other reasons are, of course, their current availability as well as their chemical reactivity and the overall cost of production [18]. The predominant raw material is logically the natural gas consisting mainly of methane (chemical formula CH_4 or 4 hydrogen atoms for 1 carbon atom, this ratio of hydrogen to carbon is the highest relative to all other hydrocarbons). Other hydrocarbons may also be used.

The main production routes from hydrocarbons retained at the industrial stage pass through the production of syngas, or synthesis gas, which is a mixture of hydrogen and carbon monoxide (H_2 + CO). Hydrogen is currently used as a raw material in the chemical and petrochemical industry. Its production is therefore generally associated with other units present to minimize energy and material costs [22]. For example, in the production

of ammonia, CO_2 from the preceding hydrogen production steps is used to produce urea from ammonia.

Steam reforming, or steam reforming, consists in converting the hydrocarbons into synthesis gas by reaction with steam and in the presence of a nickel catalyst at high temperature (840 to 950 °C) and at moderate pressure (20 to 30 bar). The synthesis gas obtained is not a simple mixture of hydrogen and carbon monoxide. Because of the different reactions involved, it also contains carbon dioxide, methane, and water (H_2 + CO + CO_2 + CH_4 + H_2O) as well as residual hydrocarbons [23]. Steam reforming is generally carried out from natural gas. It can also be from methane or naphtha. Depending on the nature of the hydrocarbons used, called the steam reforming charge, and the desired hydrogen purity, different processes exist.

Partial oxidation consists in converting the hydrocarbons into synthesis gas by controlled oxidation in the presence of oxygen. This reaction takes place at high temperature (1200 to 1500°C.) and high pressure (20 to 90 bar) and does not require the presence of a catalyst [13].

Among the techniques used to produce hydrogen at the industrial stage, the two processes of steam reforming and partial oxidation give a mixture of gases containing hydrogen also carbon monoxide CO and carbon dioxide CO_2. To obtain pure hydrogen, it is necessary to add a purification step.

4.1 VARIOUS WASTES USED PREVIOUSLY FOR PRODUCTION OF VFA

Different papers are studied, and their results were shown in Table 1 below,

Table 1: Various wastes used previously for production of VFAs

Substrate	Type of system	pH range	Temperature	Optimum pH	Maximum VFAs productions	Reference	Notes
Sugary wastewater	Anaerobic microflora (continuous stirred tank reactor)	6.8	60^{o} C	6.8	Maximum Production of butyrate on 187 days is around 6100 mg/l/d	26	The pH in the reactor was kept at 6.8 by auto-matric titration with N-NaOH
Sucrose	Seed sludge (upflow anaerobic sludge blanket reactor)	5.1 to 8.2	35 ^{o} C	6.7	Acetate, propionate, butyrate, and ethanol through regular absorptions of 2678–4665, 1240–2453, 3864–4650 and 864–2715 mg COD=l	27	At an HRT of 8 h, the key effluent VFA element were 31.1% and butyrate was 54.1%.
Sucrose	Seed sludge (upflow anaerobic sludge	4.4±0.1	38 ^{o} C	4.4	At Substrate level 28.07 g-COD/L Acetate is 12.87±0.30mM. Propionate is	28	Ph was controlled by the dose of $NaHCO_3$. The concentrations

	blanket reactor)				1.57±0.20mM. At Substrate level 10.67 g-COD/L Butyrate is 0.30±0.03		of both acetate and butyrate increased with an increase in substrate concentration
Cheese whey wastewater	Indigenous microflora (continuous stirred tank reactor)	5.2	35 ^{o} C	5.2	At alkalinity controlled experiment main microbial products among metabolites measured were butyric acid which is 14.50±1.30 (g/L)	29	automatic controller/ NaHCO3 controlled ph.
Rice winery waste water	Sludge from waste treatment plant(upflow anaerobic sludge blanket reactor)	7.2 to 4.5	55 ^{o} C	6-4.5	Acetate at R2 239m g-COD/d at day 15.Propionate at R3 5.3m g-COD/d at day 4.9.Butyrate at R1 139m g-COD/d at day 22	30	Measurement of Acetate form R1 is 1.38 (mmol/d).3.73 (mmol/d) from R2.1.67 (mmol/d) from R3.Butyrate 0.87 (mmol/d)R1.0.55

							(mmol/d)R2 and 0.79 (mmol/d)R3
Starch	Sludge (anaerobic sludge blanket reactor)	5.3	35 ° C	5.3	Butyrate around 10000(mg COD/l) with in first 48 hours. Caproate around 7000 (mg COD/l) Caproate with 12 hours. Acetate production is around 5000(mg COD/l) at day 40.	31	In the reactor pH was maintained.
Sucrose	Sludge(upflow anaerobic sludge blanket reactor)	3.4-6.3	39 ° C	4.2	VFA + Eol at 4.8 pH 3880±373(mg/l). Acetate 18.8±0.6% at pH 5.3. Propionate 5.3±0.4% at pH 3.4.Butyrate 52.1±2.3% at pH 4.2.Valerate 12.2±1.2% at pH 4.8.Caproate 28.9±1.4% at pH 5.3.Ethanol 33.0±3.1% at pH 3.4.	32	summarizes the total VFA and ethanol concentrations in the effluent as well as the percentages of individual VFA and ethanol at various pH.

Sucrose	continuous-flow stirred-tank reactor	5.3±1	35 ^{o} C±1	5.3	Total VFA is 10000 mg COD/L at 12 h	33	This study shows that when we have high VFA production same time we got low alcohol and high alcohol production means low VFA production.
Food waste	Mesophilic anaerobic digested sludge from the Gaobeidian wastewater treatment plant	5.0,6.0,7.0	35 ,45 and 55 ^{o} C	6.0	At 45 ^{o} C production of VFA is 39.46 g/L	34	The maximum VFAs absorptions at pH uninhibited, 5.0, 6.0, and 7.0 were 3.94, 17.08, 39.46 and 37.09 g/L, correspondingly,
Waste activated sludge	Batch Reactor	08 to 11	60 ^{o} C	11.0	VFA production Performance is 259 mg TOC/g VSS	35	Batch was operated for 7 d, 0.02 g Sodium

							dodecylbenzene sulfonate/g VSS
Waste activated sludge	Waste Water Treatment (Winnipeg South, Manitoba) which operates high purity oxygen COD removal process with SRT of 3 d. Bench-scale batch experiments were performed in nine 1.3 L reactors which	7.02	24.6 ^{o} C, 14 ^{o} C, 4 ^{o} C	7.02	At 24.6 ^{o} C with no mixing at day 5 VFA is 20% and 60% at day 10.and at 24.6 ^{o} C with static reactors 85% at day 5 and at day 10 is 91%	36	The percentage of VFA production on day 5 and day 10 to the final VFA production considered completed after 20 d.

	conducted under three different temperature conditions						
Soft drink Wastewater(SDW)	Batch experiments (anaerobic sludge inoculum)	pH range 3.9-7.8	32±1 ^{o} C	The result is even more evident when consider that the initial pH was set at 6.0 for all vessels.	So, the final result at 21-day blank assay and R1 produces nothing NIL TVFA. And R7 produce maximum 9.4±1.5 g COD/L	37	$NaHCO_3$ was added to promote pH autoregulation. Seven anaerobic batch assays+one blank assay were run in triplicate in 320 mL glass vessel (140mL) working volume for 21 days.
Food Waste	Two types of inoculum were	4 Reactors in each group were	30±2 ^{o} C	pH 6.0	For anaerobic activated sludge VFA production performance is 0.918g/g	38	By adding 4.5 M HCl and NaOH, all reactors were

	obtained, Anaerobic activated sludge from an up-flow anaerobic sludge bed(UASB) and aerobic activated sludge from secondary settling tank of wastewater treatment plant	operated at pH 4.0, 5.0, 6.0 and pH uncontrolled			VSS $_{removal}$ .and for waste activated sludge VFA production performance is 0.482 g/g VSS $_{removal}$		mechanically stirred at 120 rpm using a magnetic stirrer throughout the experiment
Food waste	Anaerobic digester sludge	4.0, 6.0, 6.5-8.5	35 ^{o} C, 55 ^{o} C, 70 ^{o} C but optimum temperature is 35 ^{o} C	pH 7.0	At optimum pH, Maximum VFA production is 18.45g/L	39	2500 mL flask with fluid near the bottom was used as a reactor, and the working volume

							was 2L.A 1 L jar and a 1L beaker were used as the gas gathering device.
Food waste	Anaerobic digester sludge	pH 5.0, 6.0, 7.0	35 ^{o} C, 45 ^{o} C, and 55 ^{o} C(optimum temperature is 35 ^{o} C)	pH 6.0	At pH 6.0 VFA (g/L) is 39.46 and VFA (g/g $VS_{removal}$ is 0.471	40	The temperature was uncontrolled and at beginning nitrogen gas was flushed to create anaerobic conditions.
Food waste	Anaerobic digester sludge	pH 5.0, 5.5, 6.0	25 ^{o}C, 35 ^{o}C 45 ^{o} C (optimum temperature is 35 ^{o} C)	pH 6.0	Production of TVFA with temperature effect at pH 6.0 is 23.0-24.0 g/L, and with pH effect at pH 6.0 on acidogenesis food waste is 24.5-25.5 g/L	41	A 2-liter reactor mixer was salaried in this study and worked in semi-continuous approach (once a day feeding

							and drain off).
Kitchen waste	Waste activated sludge	pH 8.0	37 ° C	pH 8.0	692.4 mg-COD/g VS	42	All three reactors were operated at same pH 8,for 6 days
Food waste	Dewatered sludge	pH value is 5, 7, 9, and 11	35 ° C	pH 9.0	25 934 ± 1458 mg COD/L	43	5 identical serum bottles used with 500 ml volume of each bottle.in bottle 1-4 the pH value is controlled at 5, 7, 9, and 11 by adding 3M NaOH or 3MHCL and 5th bottle is not adjusted and

							used as a controller
Waste Activated sludge (solid Waste)	Batch Reactor	pH 4.0 to 11.0	35 ° C	pH 9	298 mg COD/g VSS	44	Organic content was 18,657 (mg COD/L)
Primary Sludge	Batch Reactor	pH 3.0 to 11.0	Room Temperature 5 days	pH10	60 mg COD/g VSS/d	45	According to this study it is clear that alkaline pH promoted the resolvable biological carbon production from PS.
Waste Activated sludge (solid Waste)	Batch Reactor	pH 4.0 to 11.0	55±2 ° C	pH 08	368 mg COD/g VSS	44	So at pH 8 and high temperature, there is more productive as compared to other.

Kitchen Waste	Batch Reactor	pH at 5, 7, 9 and 11.	35 ^{o} C	pH 7.0	36,000 mg/L	46	Five reactors were conducted. Four reactors with controlled pH and The batch were operated for 32 days.
Food Waste	Batch Reactor	Initial pH 5.5	37 ^{o} C	pH 5.5	8950 mg COD/L	47	The initial pH value of all Bottles were adjusted to 5.5 using 1 N NaOH and 1 N HCl before start the experiment. And this final VFA production is observed from untreated food waste.

4.2 FINDINGS

Several studies demonstrated in Table 1, for their possible to be used for VFAs production. A major control parameter in the process, the reactor's pH value is important VFAs' production given that acidogens are unable to withstand highly acidic conditions (pH: 3) or highly alkaline conditions (pH: 12) environments.so it is concluded that when pH is near to neutral level the condition is more suitable to produce more VFAs. Or after looking at all results when pH is controlled near to neutral level is producing more VFAs. Again, VFAs production is mainly reliable on what you have done in the pretreatment of the substrate. In addition, the research conclusions so far suggest that the optimal pH to yield a specific VFAs is extremely hooked on the kind of waste used. Besides, it was found that the application of a high HRT is more beneficial to produce VFAs' time to react with waste.

5.1 EXPERIMENTAL WORK

5.1.1 Substrate

In this study, three types of waste were obtained, and used as the substrat. Primary Sludge (PS) and, Thickened Waste Activated Sludge (TWAS) which were collected from Ashbridges Bay Wastewater Treatment Plant and Source Separated Organics(SSO) that was collected from Disco Road facility.

5.1.2 Experimental set-up

PS, TWAS, and SSO were used as substrate. The anaerobic batch fermentation tests were conducted in 250 ml each bottle. In Total, 30 bottles were used including 10 for PS 10 for TWAS and 10 for SSO. The Initially pH of PS, TWAS and SSO were 4.94, 6.43 and 4.73 respectively. For each substrate, the test was run for 5 different pH in duplicates. From reactor 1-10, 11-20, 21-30 all are adjust with same pH at 4.0, 5.5, 7.0, 8.5 and 10.0. The pH was adjusted using 3 Mole Sodium hydroxide(NaOH) and 2 Mole Hydrochloric acid(HCL) stock solution which the ammounts for each substrate are shown in Table 2, 3, 4. To prepare 200 mL of 3-mole NaOH based on acid-base molarity calculator. 31.689mL of Stock solution of NaOH was mixed with 168.311mL deionized water. For 200 mL of 2-mole HCL based on acid-base molarity calculator. 32.8 mL stock solution of

HCL was mixed with 167 mL deionized water. And the dark fermentation process was started at 150 RPM (round per minute) to sustain homogenous mixing during fermentation. Under the constant temperature at 38^{o}C±01.

Table 2:The amount of HCL and NaOH used for Primary Sludge

pH adjustment for Primary Sludge (initial pH is 4.94)			
Reactor #	Value of pH	mL NaOH /mL PS±2%	mL HCL /mL PS±2%
1 and 2	pH 4.0	-	3.2
3 and 4	pH 5.5	0.28	-
5 and 6	pH 7.0	4.28	-
7 and 8	pH 8.5	6.68	-
9 and 10	pH 10	8.36	-

Table 3:The amount of HCL and NaOH used for TWAS

pH adjustment for TWAS (initial pH is 6.43)			
Reactor #	Value of pH	mLNaOH /mL TWAS±2%	mL HCL /mL TWAS±2%
11 and 12	pH 4.0	-	20.11
13 and 14	pH 5.5	-	8.33
15 and16	pH 7.0	0.35	-
17 and 18	pH 8.5	2.35	-
19 and 20	pH 10	3.7	-

Table 4:The amount of HCL and NaOH used for SSO

pH adjustment for SSO (initial pH is 4.73)			
Reactor #	Value of pH	mL NaOH /mL SSO±2%	mL HCL /mL SSO±2%
21 and 22	pH 4.0	**-**	4.73
23 and 24	pH 5.5	3.21	-
25 and26	pH 7.0	13.21	-

27 and 28	pH 8.5	15.21	-
29 and 30	pH 10	17.58	-

All analysis were carried out in duplicates. The sealed were placed in a Thermo Scientific Benchtop Orbital shaker, Model MaxQ. Figure 4 shows the set-up that were used for Dark Fermentation test. The experiment was run for 72hours and samples were collected after every 24 hours. The pH of the effluent samples was measured immediately, and poured into 20 ml plastic bottles, and placed in the refrigerator below $4^{o}C \pm 0.5$ until wet chemistry analysis was completed. So, 90 samples were taken including three initial samples which the characteristics of these initial samples are shown in table 5 below.

Table 5:Characteristics of Initial PS, TWAS, SSO

#	Subtract	pH	TSS (mg/L)	VSS (mg/L)	NH_3 (mg/L)	Alkalinity (mg $CaCO_3$/L)	TCOD (mg/L)	SCOD (mg/L)	TVFA (mgCOD/L)
1	PS	4.94	265000.0	20933.3	322	2680	21300	12480	3914.06
2	TWAS	6.43	28800.0	22633.3	174	88.8	39900	6080	358.45
3	SSO	4.73	99400	76866.7	960	363	116500	40200	6295.88

Figure 4: Benchtop shaker used for dark fermentation test - Thermo Scientific Benchtop Orbital shaker

The analyses that were performed are pH, total solids(TS), volatile solids (VS), Total and soluble chemical oxygen demand (TCOD and SCOD), Ammonia, Alkalinity TVFAs.All tests were filtered through 0.45 µm VWR Vacuum Filtration Systems, Model 10040-462 after centrifuge in by using Thermo fisher scientific centrifuge machine model sorvall st8 at 9800RPM for 45 minutes for each sample; figure 5 shows scientific centrifuge machine to perform the soluble analysis.

Figure 5:Thermo Fisher scientific centrifuge machine

All analyses that were completed were carried out in duplicates, the procedure for each test that were completed is listed below.

pH: The pH of each sample were measured immediately it was collected using VWR Benchtop pH Meter and refillable glass probe, model B10P. This meter was calibrated twice a week with pH reference standards 4, 7 and 10 ± 0.1, See Figure 7.

Total and Volatile Solids: 5mL of each sample in aluminum plates were used to measure the solids content by following the standard guidelines provided in Methods 2540B and 2540E for TS and VS respectively [48].

Total and soluble COD: High range (20 – 1500 mg/L) COD reagent vials from HACH were used to follow Method 8000 [49]. This method is based on the reaction digestion

method developed by Jirka and Carter [50]. The COD content was then measured using HACH DR3900 spectrophotometer. which shows in figure 6.

Ammonia-Nitrogen: High range (0.4 – 50 mg/L) Amver Nitrogen Ammonia reagent set were used as per Method 10031, the Salicylate method [49]. Concentrations of ammonia-nitrogen were determined using the HACH DR3900 spectrophotometer. which shows in figure 6.

Alkalinity (Total): High range (25 - 400 mg/L $CaCO_3$) total Alkalinity TNT 870 reagent set were used as per method 10239. And concentration of Alkalinity was determined using Hach DR3900 spectrophotometer. Which shows in figure 6.

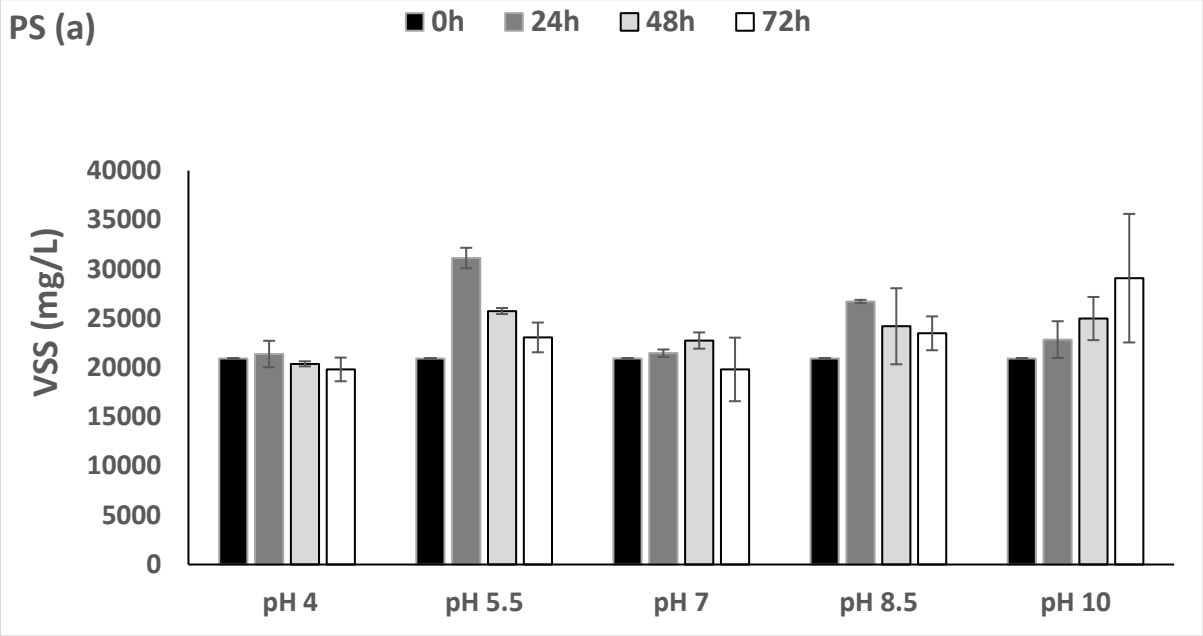
Volatile Acids: High range (50 to 2500 mg/L) CH3COOH (Acetic Acid) were used as per Method 10240, the concentration of CH3COOH were determined using the HACH DR3900 spectrophotometer, which shows in figure 6.

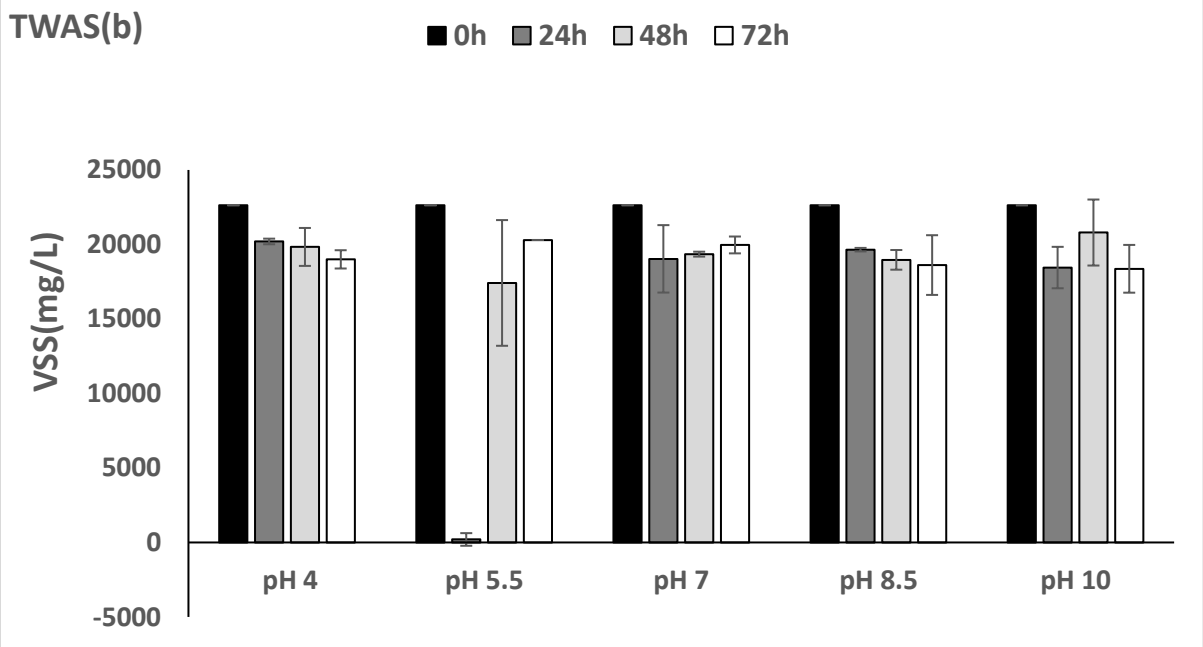
Figure 6:HACH DR3900 spectrophotometer.

Figure 7:pH meter Fisher Scientific model AB15

5.2 RESULT AND DISCUSSIONS

Figure 8 shows the VSS for the raw and fermented samples at different pHs, the initial value for VSS is presented with black bar for each substrate.





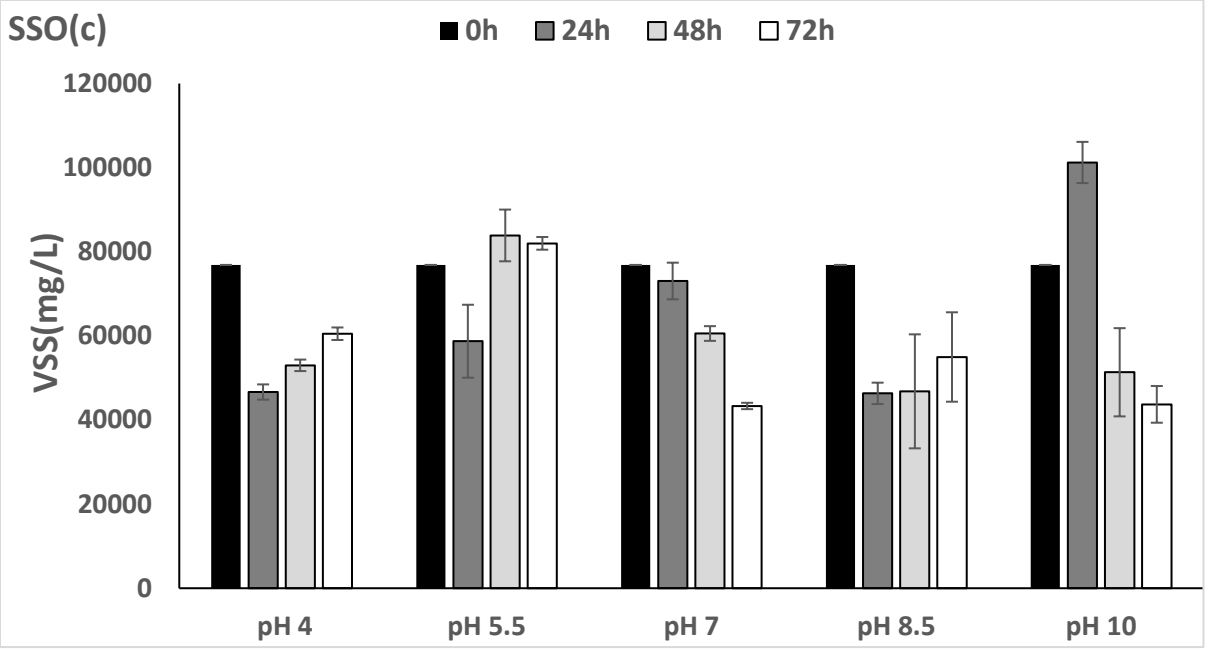


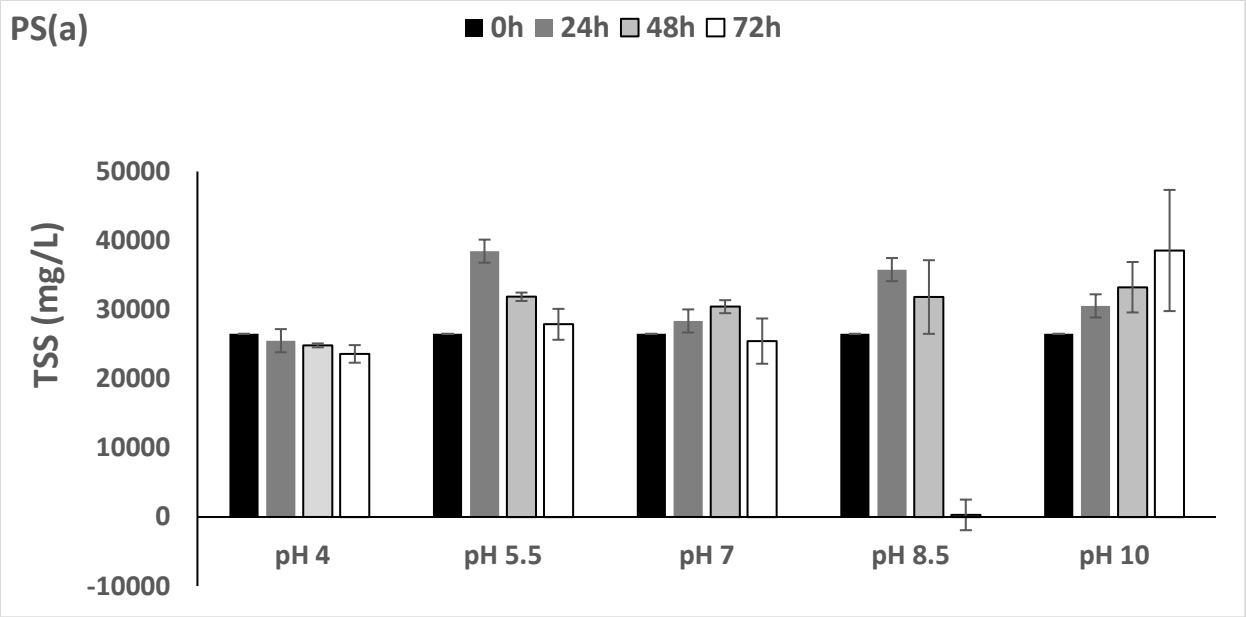
Figure 8:Volatile Suspended solids (VSS) of Primary Sludge (a), TWAS (b) and SSO (c) at different pH and sampling time

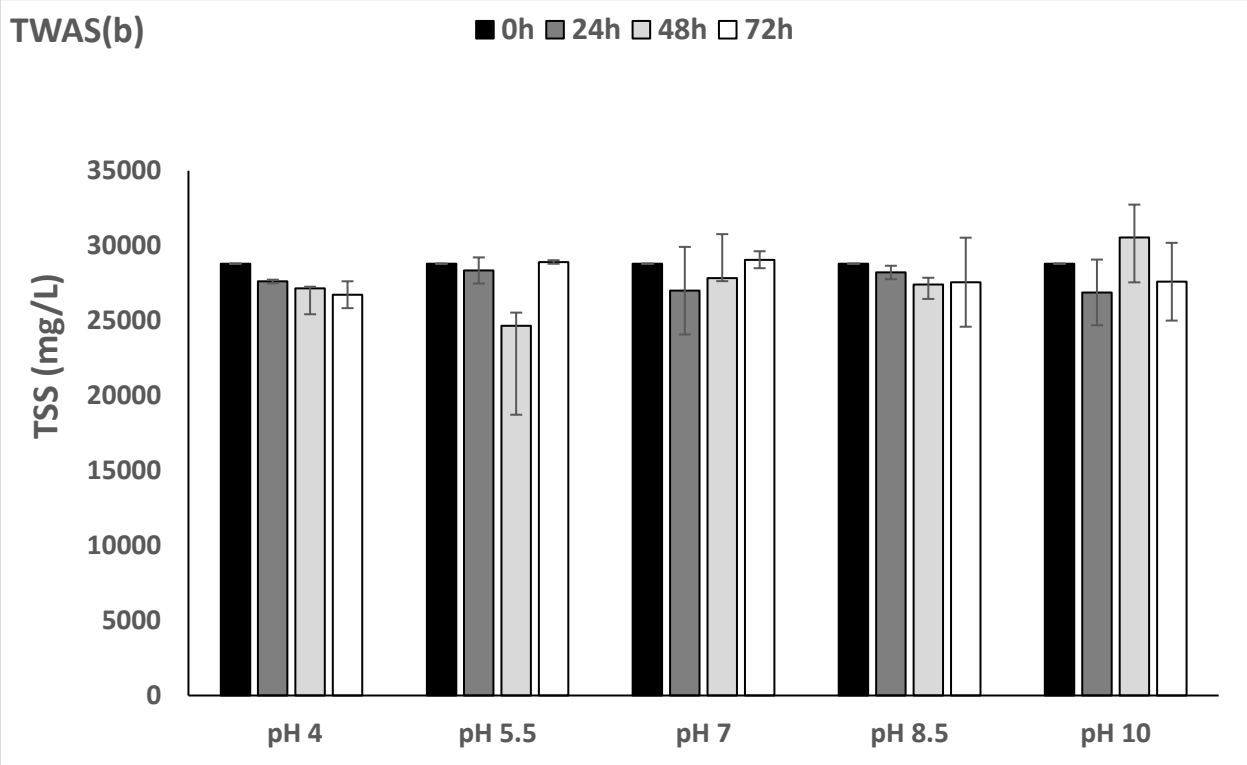
As shown in Figure 8 (a), the maximum VSS value achieved at pH5.5 for 24h. For the pH4 and pH 7 VSS remain the same as of initial value but in pH8.5 and pH10, the value increased at the different time. At pH10 VSS increased with the passage of time, whereas in pH5.5 and pH8 it decreased with time.

As shown in Figure 8 (b), the VSS shows the similar behaviour at all pH value. The VSS value during all pH range decreased from its initial concentration. The maximum VSS at pH10 for 48h, though it was less than initial concentration.

As shown in Figure 8 (c), the VSS has similar graphical pattern except for pH10. From pH 4 to pH 8.5 the VSS decreased first from its initial value at 24h and then increased at 48h to 72h. During pH10 sample, the VSS had a significant rise at 24h and then sudden fall; maximum VSS were noted at ph10 after 24h.

The results of Total Suspended solids (TSS) are shown in figure 9, the initial value for TSS is presented with black bar for each substrate.


PS(a)
0h 24h 48h 72h
TSS (mg/L)
50000
40000
30000
20000
10000
0
-10000
pH 4
pH 5.5
pH 7
pH 8.5
pH 10


TWAS(b)
0h 24h 48h 72h
TSS (mg/L)
35000
30000
25000
20000
15000
10000
5000
0
pH 4
pH 5.5
pH 7
pH 8.5
pH 10

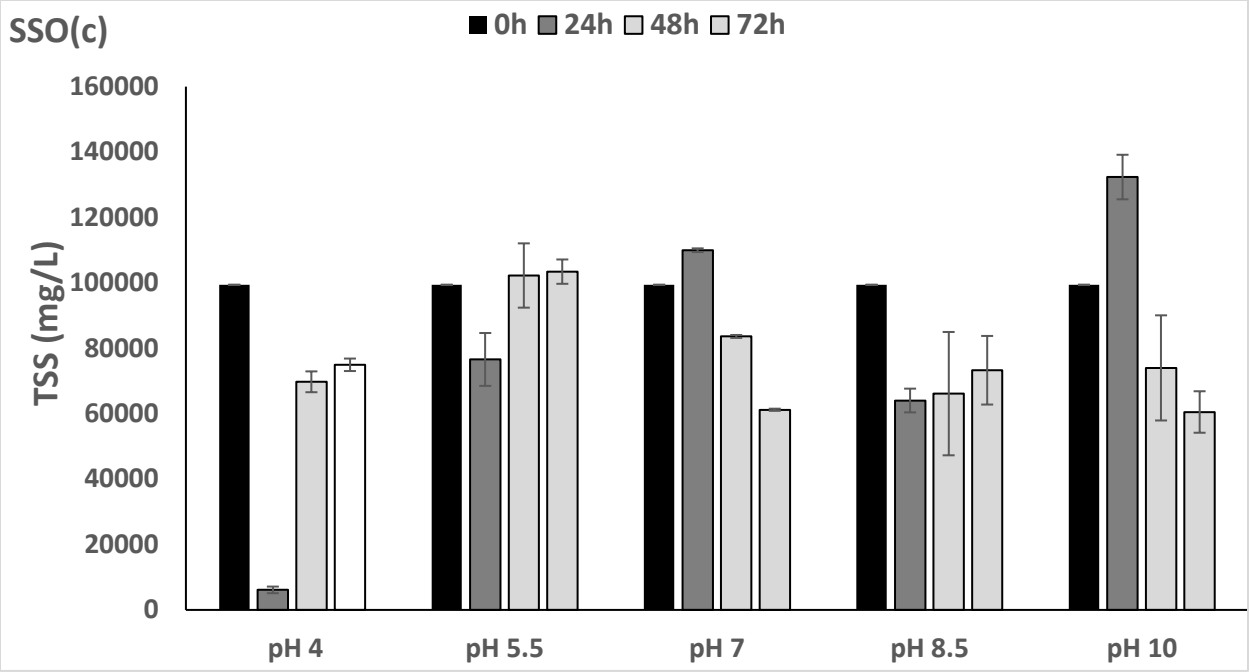


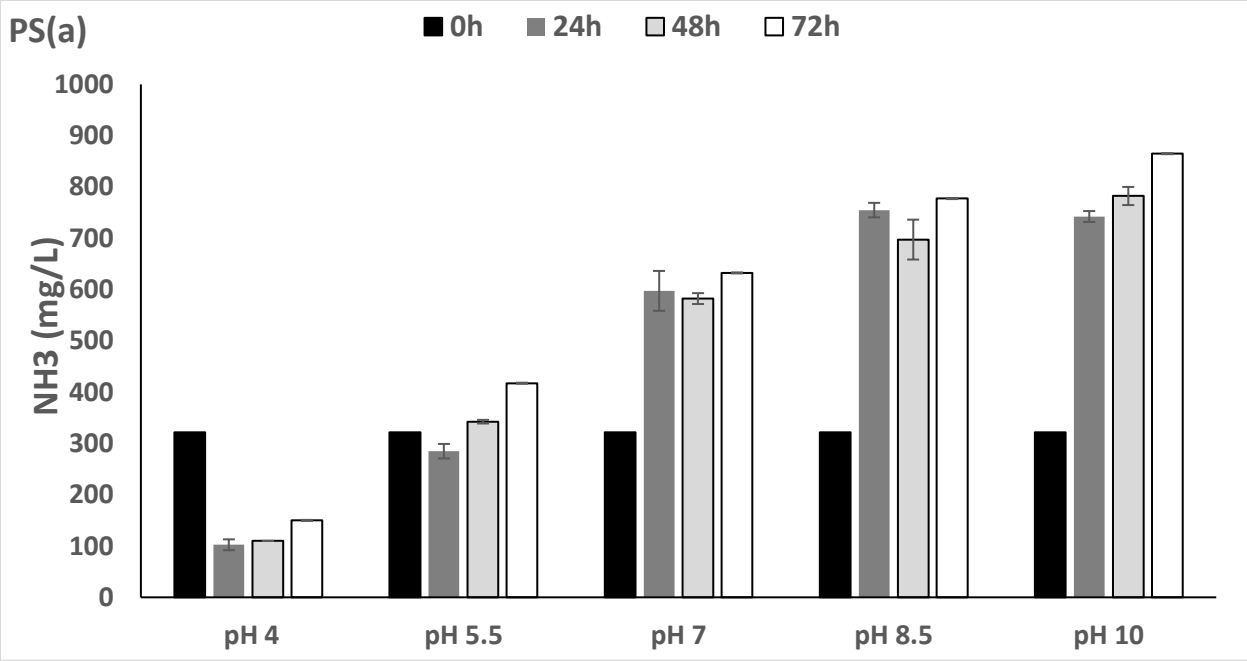
Figure 9:Total Suspended solids (TSS) of Primary Sludge (a), TWAS (b) and SSO (c) at different pH and sampling time

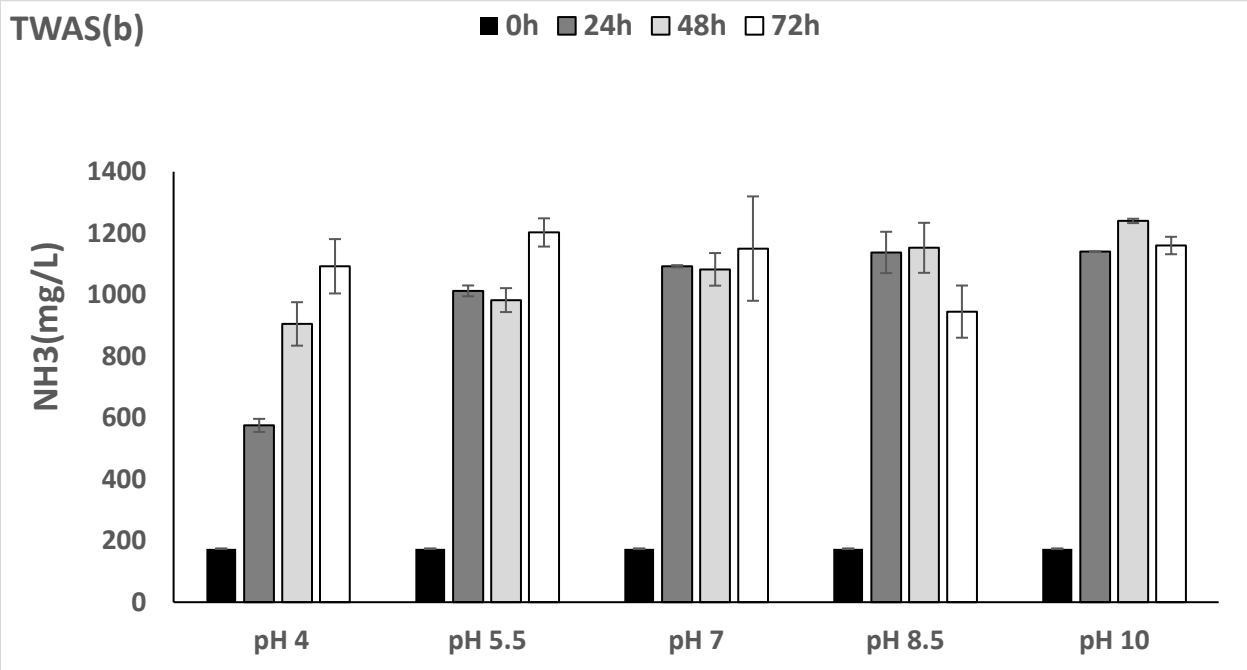
For pH4 and pH7, the TSS value are almost stable to the initial value. At pH5.5 and pH8, TSS value increased at 24h, but later it decreased. At ph10, the value kept on increasing with the passage of time.

comparing pH4 and pH8.5, the TSS value had the almost same value, but it was noted that the value for TSS in all pH during different time has less value than initial value except for pH10 at 48h, where it showed maximum value.

The pH value at the different time showed much variation from 9 (a) and 9 (b). The pH4 and pH8.5 had similar graphical representation. The initial value had gradual decreased and slight increase after 24h, and vice versa behaviour in pH5, pH10 and again pH10 at 24h has a maximum value.

The results of Ammonia (NH_3) are presented in Figure 10, the initial value for NH_3 is presented with black bar for each substrate.


PS(a)
0h
24h
48h
72h
NH3 (mg/L)
1000
900
800
700
600
500
400
300
200
100
0
pH 4
pH 5.5
pH 7
pH 8.5
pH 10


TWAS(b)
0h
24h
48h
72h
NH3(mg/L)
1400
1200
1000
800
600
400
200
0
pH 4
pH 5.5
pH 7
pH 8.5
pH 10

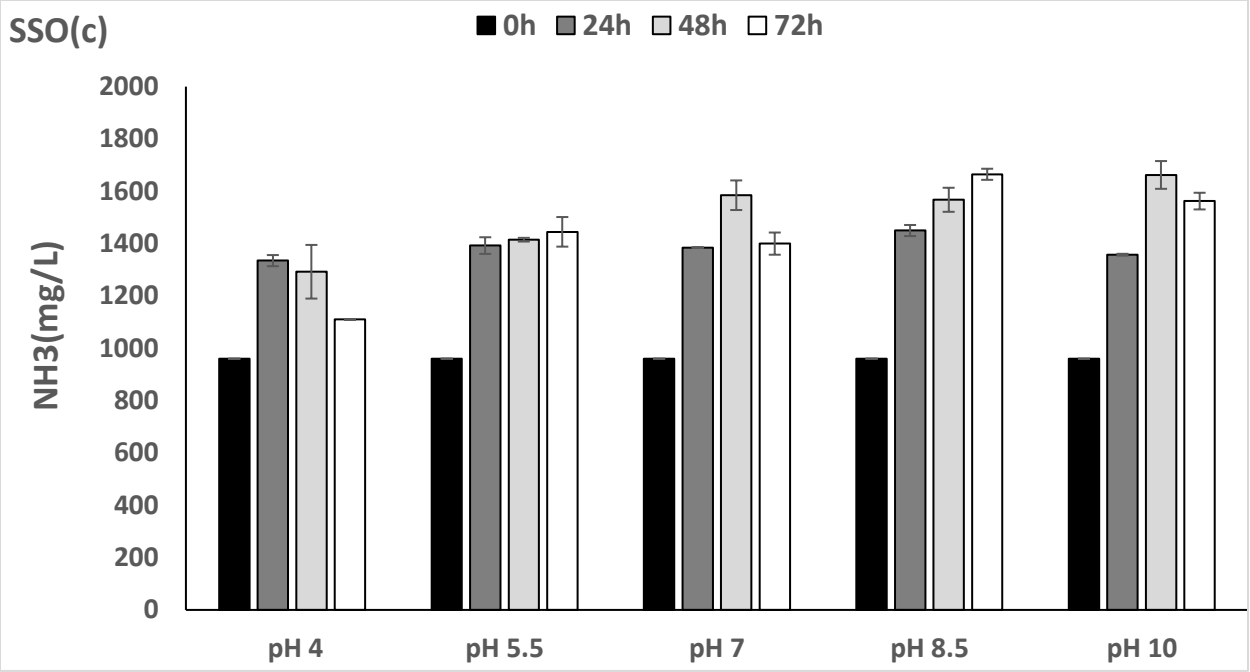
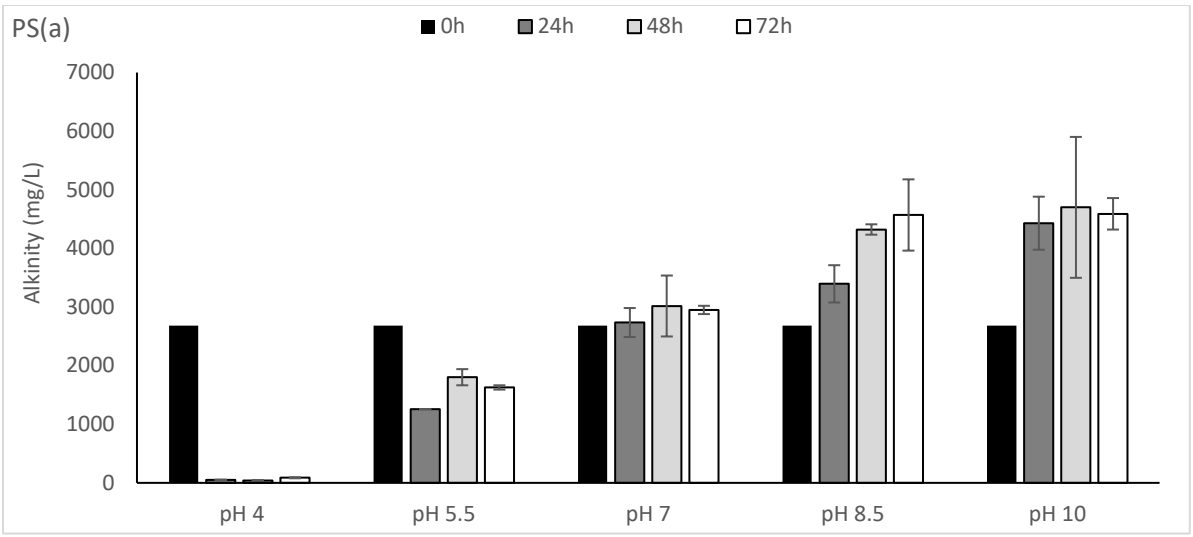
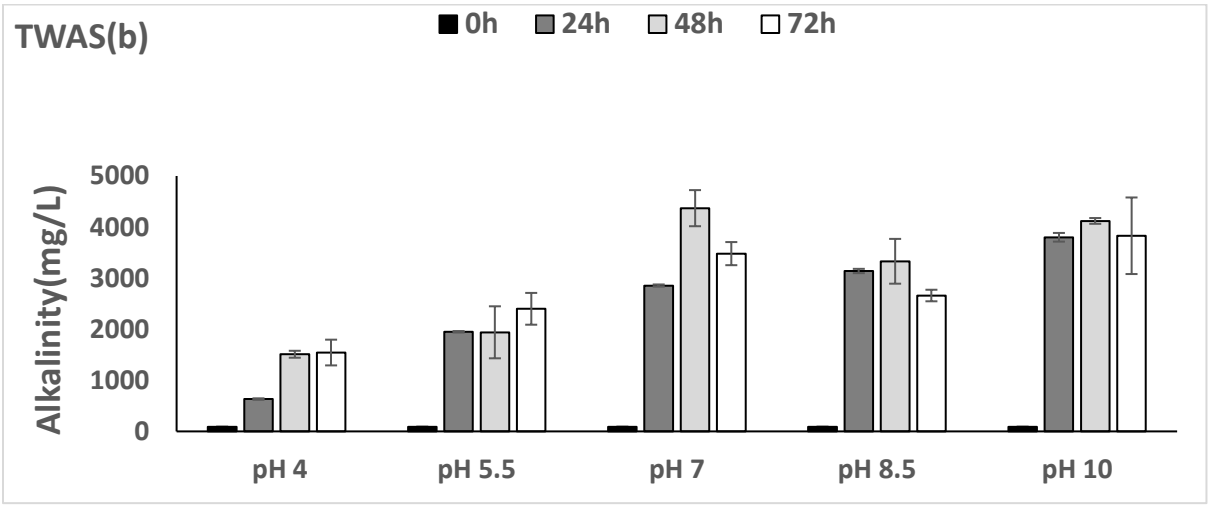


Figure 10: Ammonia (NH3) of Primary Sludge (a), TWAS (b) and SSO (c) at different pH and sampling time

Ammonia was checked initially and the findings were 322 mg/L, 174 mg/L and 960 mg/L in PS, TWAS and SSO respectively. Figure 10 shows the values of Ammonia in all waste. In PS, pH4 is dramatically decrease to 102.5 mg/L at 24 h, and increase to 150 mg/L at 72 h. It was demonstrated that increase in time and pH would result in increased of Ammonia. In PS, the highest value of Ammonia which were recorded 865 mg/L at 72 h pH10. In TWAS the maximum value for Ammonia was 1240 mg/L at 48 h pH10. The result of SSO were similar to TWAS, and the maximum recorded ammonia was 1662.5mg/L at 48 h pH10. So, it was demonstrated that increasing of pH and time will break down more organic nitrogen form of urea and fecal material and produce more NH_3.

The results of Alkalinity are shown in Figure 11, the initial value for Alkalinity is presented with black bar for each substrate.





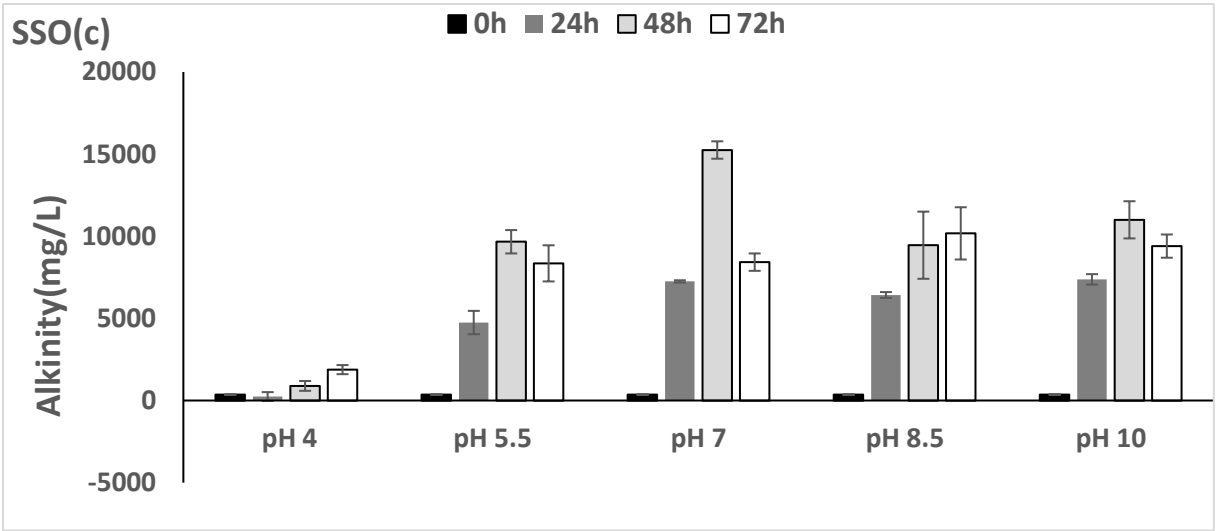
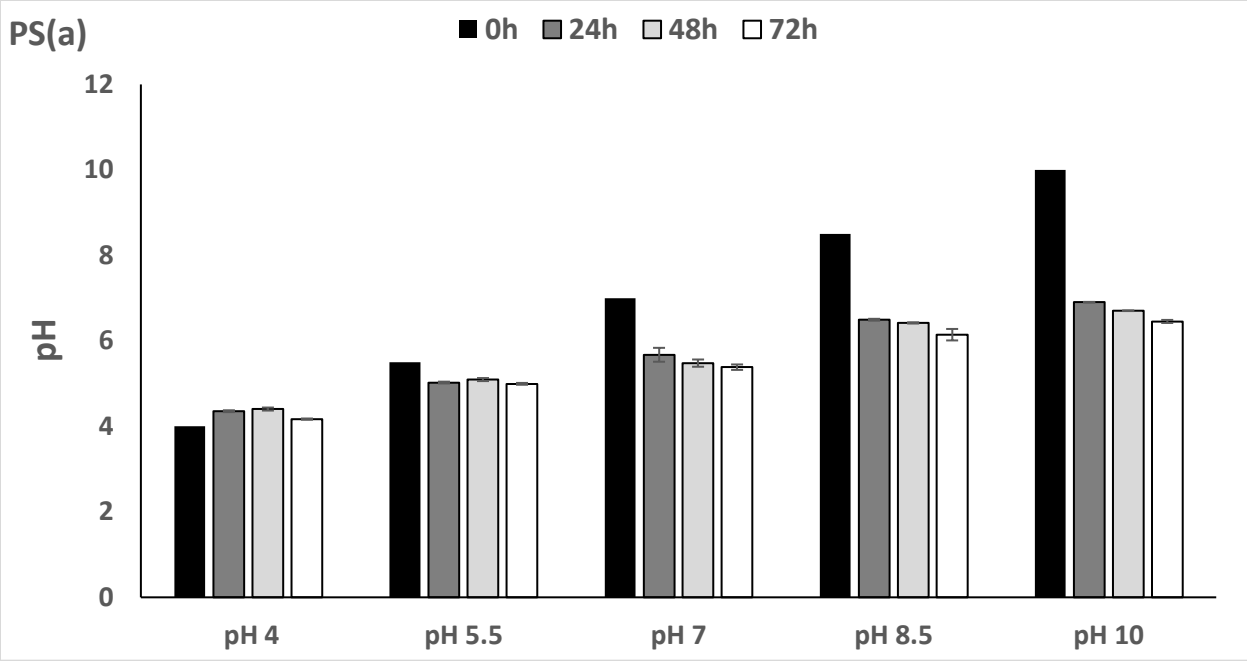
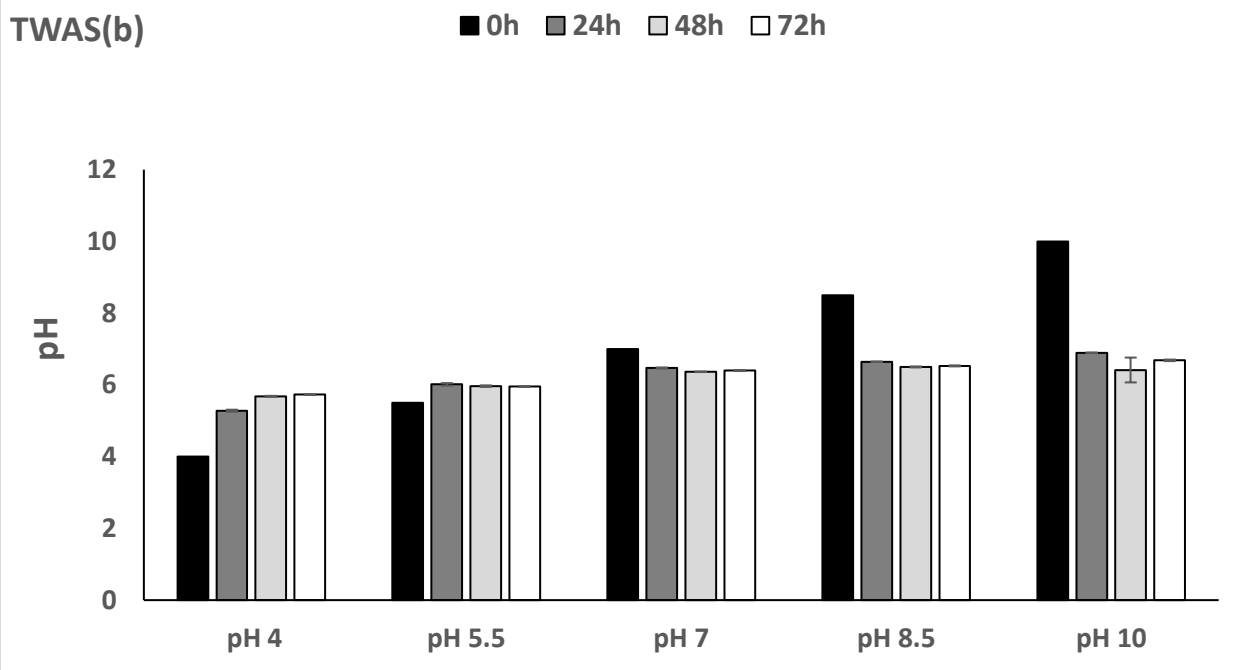


Figure 11:Alkalinity of Primary Sludge (a), TWAS (b) and SSO (c) at different pH and sampling time

Alkalinity was every so often used as a pointer of biological activity. Increase in Alkalinity means more biological processes. Figure 11 shows trend of alkalinity for PS, TWAS, SSO is increasing in pH. Initially, Alkalinity were checked for samples and found 2680 mg/L for PS, 88.8 mg/L for TWAS and 363 mg/L for SSO. Moreover, the initial value for PS shows very high range which is 2680 mg/L. It means that the initial sample of PS has some problem while taking a reading, or characteristics of the sample were changed due to some reason. The maximum alkalinity which were recorded for PS was 4700 mg/L at pH10 in 48 h. Moreover, it was found that the minimum alkalinity was recorded on pH4 at 24 h was 52mg/L, and at 72 h it was 87 mg/L. TWAS graph(b) for alkalinity showed the increase in pH which was highly increased in alkalinity. The maximum value of alkalinity which was measured for TWAS at pH7 48 h was 4370 mg/L and then it dropped to 3480 mg/L at 72h, which means they produce methane. pH8.5 at 48h shows same trend value of alkalinity which was 3330 mg/L and then drop to 2660 mg/L at pH8.5 at 72h. It was demonstrated that the SSO and TWAS shows the same trend of alkalinity which did not change much at pH4 at time 24 h, 48 h, and 72 h. However, It highly increased at pH7 48h to 15250 mg/L and then decreased to 8425 mg/L at pH7 72 h, this was due to methane production.

The results of pH are presented in Figure 12, the initial value for pH is presented with black bar for each substrate.


PS(a)
0h
24h
48h
72h
12
10
8
6
4
2
0
pH
pH 4
pH 5.5
pH 7
pH 8.5
pH 10


TWAS(b)
0h
24h
48h
72h
12
10
8
6
4
2
0
pH
pH 4
pH 5.5
pH 7
pH 8.5
pH 10

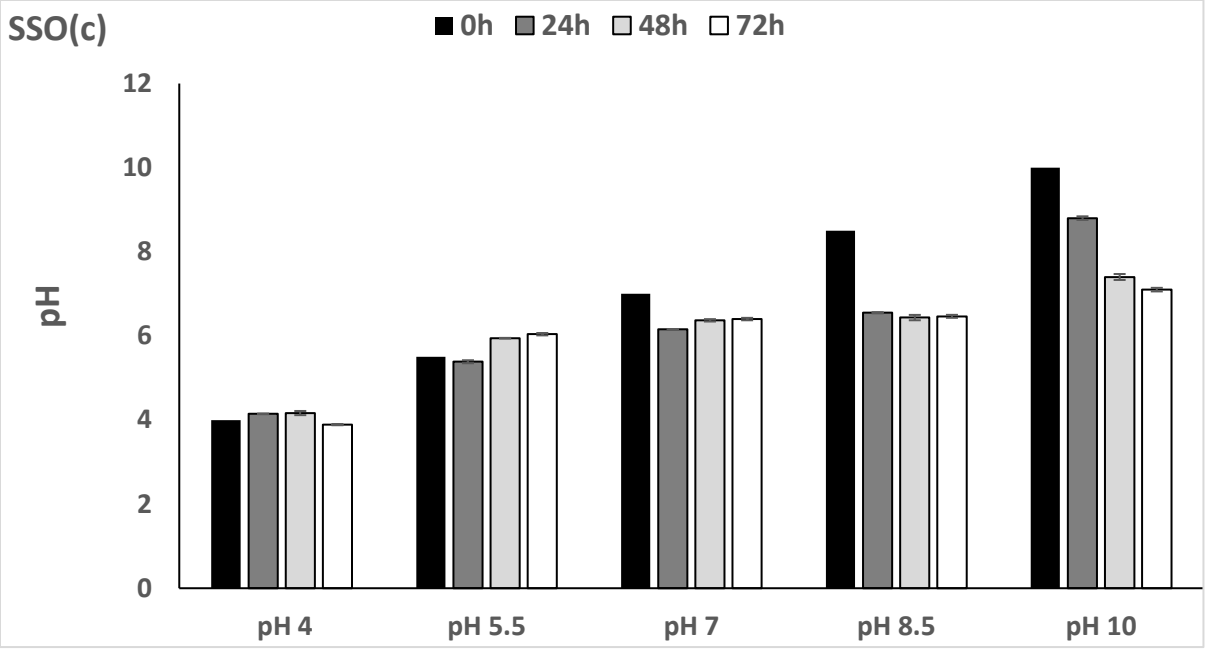
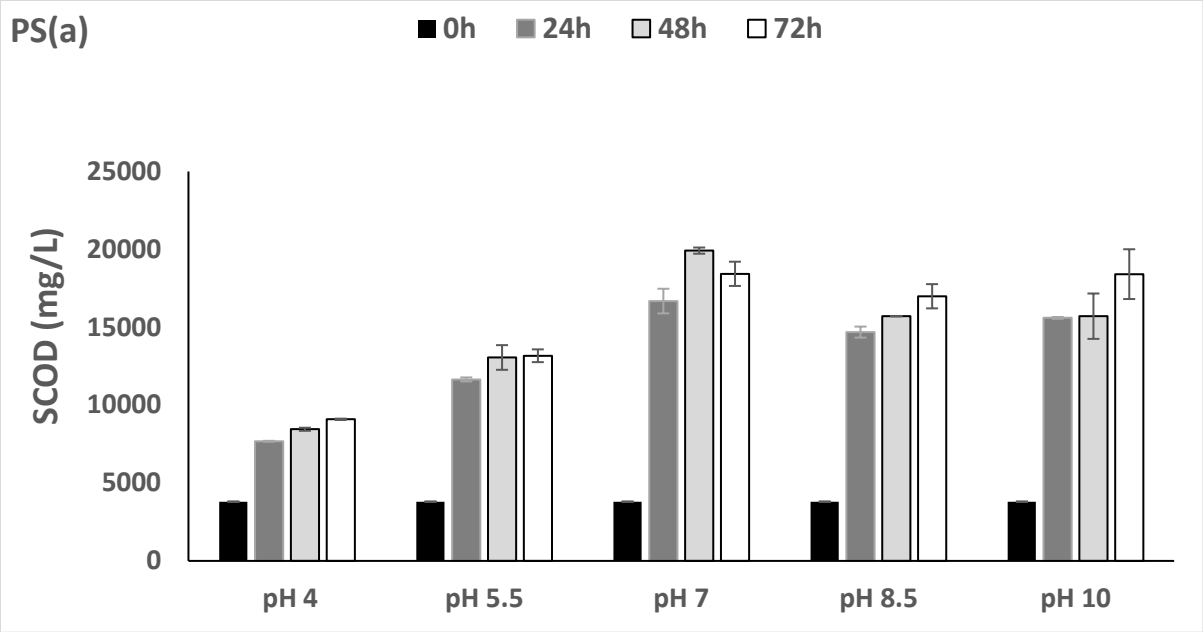
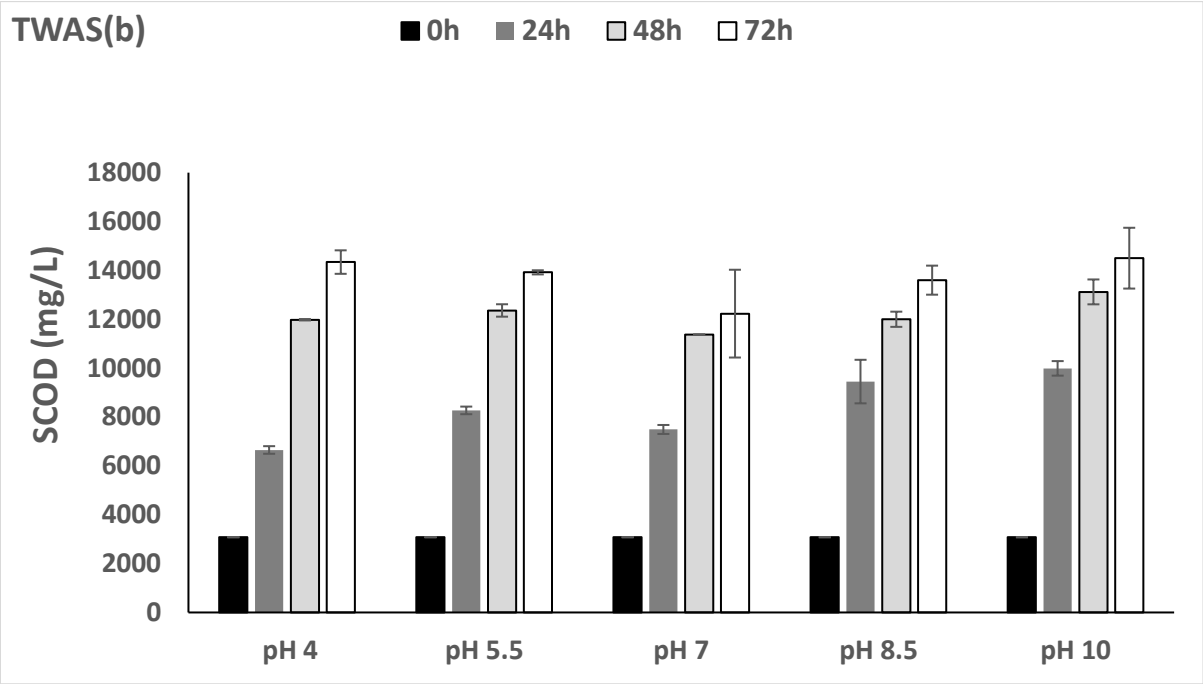


Figure 12:pH of Primary Sludge (a), TWAS (b) and SSO (c) at different pH and sampling time

As shown in Figure 12, the acid concentration in the aqueous system is expressed by pH value which means the concentration of hydrogen ions. In all the waste, the pH level falls with the passage of time after pH7 but remains the almost same at pH5.5. After pH 7, from all three types of wastes, it can be seen that pH falls after 24h and keeps falling until 72h or remain same at the value of 24h to 72h. So the drop down in pH level indicates the accumulation of acetogenesis acids which is another reason for low pH value in the reactor. In PS, the pH10 has a drastic fall to pH 6.9 after 24h and remains almost the same until 72h. Whereas SSO has the same behaviour till pH8.5 but in pH10 fall in the level is not as drastic as PS. PS and TWAS have the same behaviour concerning time over pH as shown in figure 12(a) and 12(b).

The results of Soluble Chemical Oxygen Demand (SCOD) are presented in figure 13 below, the initial value for (SCOD) is presented with black bar for each substrate.


PS(a)
0h
24h
48h
72h
SCOD (mg/L)
25000
20000
15000
10000
5000
0
pH 4
pH 5.5
pH 7
pH 8.5
pH 10


TWAS(b)
0h
24h
48h
72h
SCOD (mg/L)
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
pH 4
pH 5.5
pH 7
pH 8.5
pH 10

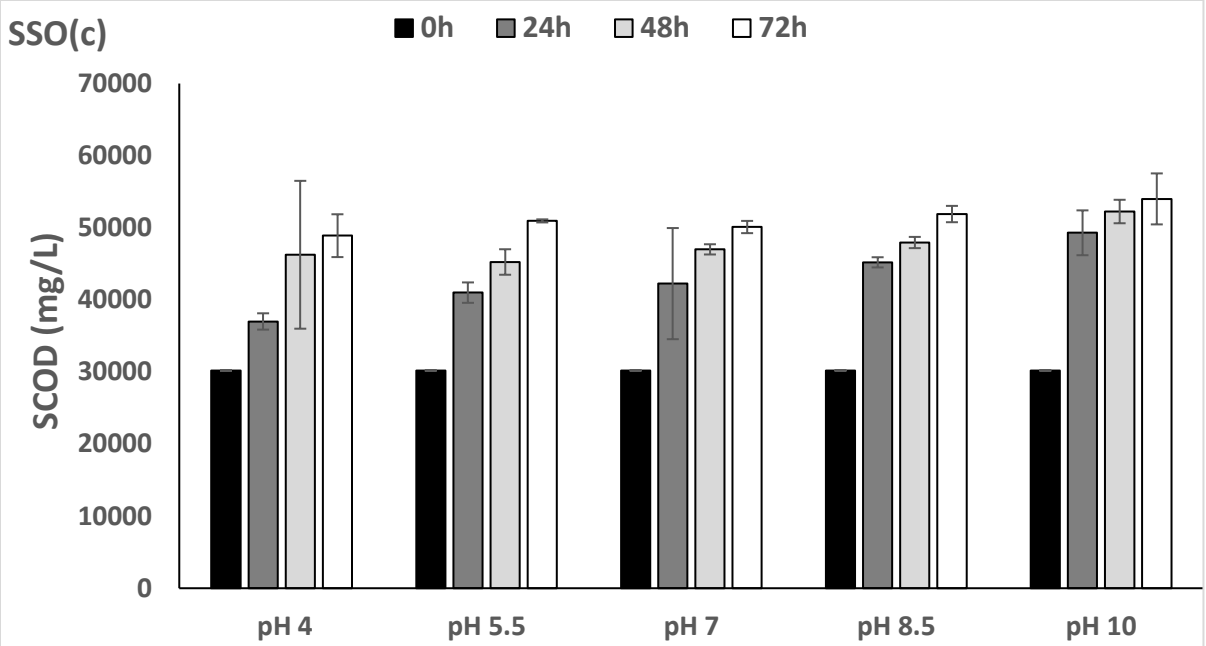


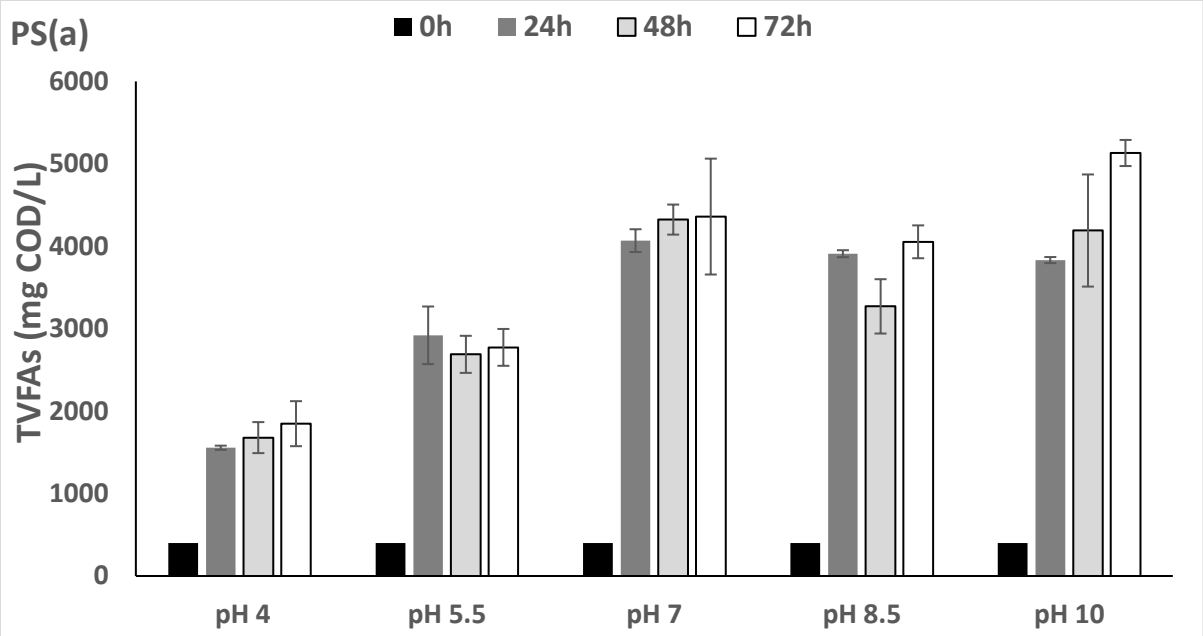
Figure 13:Soluble Chemical Oxygen Demand (SCOD) of Primary Sludge (a), TWAS (b) and SSO (c) at different pH and sampling time

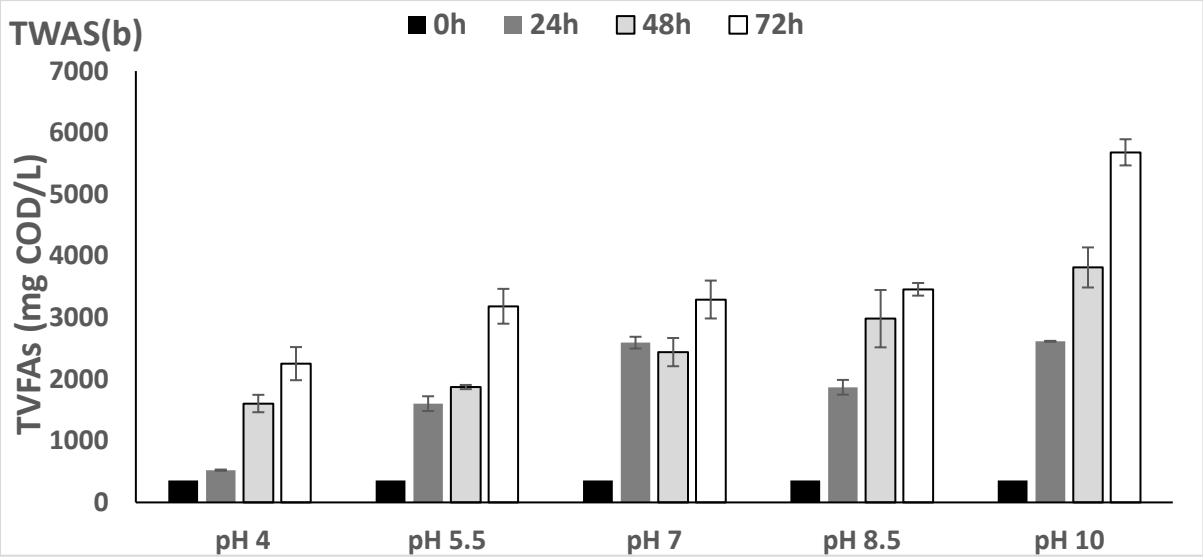
As shown in Figure 13(a), the highest concentration at pH7 at 48h was noted to be 19220 mg\L from 3800 mg\L from the low initial concentration of the sample. The more concentration were observed at higher pH level after 24h.

As shown in Figure 13(b), the SCOD concentration of TWAS increased rapidly at pH4 and pH5.5. The concentration of SCOD at pH4 and pH10 is almost the same approximately 14500 mg\L. After pH7 SCOD level raised rapidly only at 24h, after that there were not much variations in the concentration.

As shown in Figure 13(c), the Initial concentration level of the SSO is too high even more than the highest concentration observed in PS and TWAS. The high concentration level observed at pH10 at 72 h on an average, it can be seen that there was an increase in 20000 mg\L on each pH level from 0h to 72h. Overall from the figure 15, it can be concluded that high pH level with more retention time produced a high concentration of SCOD.

The results of Total Volatile Fatty Acids (TVFAs) are shown in figure 14 below, the initial value for (TVFAs) is presented with black bar for each substrate.


PS(a)
0h
24h
48h
72h
TVFAs (mg COD/L)
6000
5000
4000
3000
2000
1000
0
pH 4
pH 5.5
pH 7
pH 8.5
pH 10


TWAS(b)
0h
24h
48h
72h
TVFAs (mg COD/L)
7000
6000
5000
4000
3000
2000
1000
0
pH 4
pH 5.5
pH 7
pH 8.5
pH 10

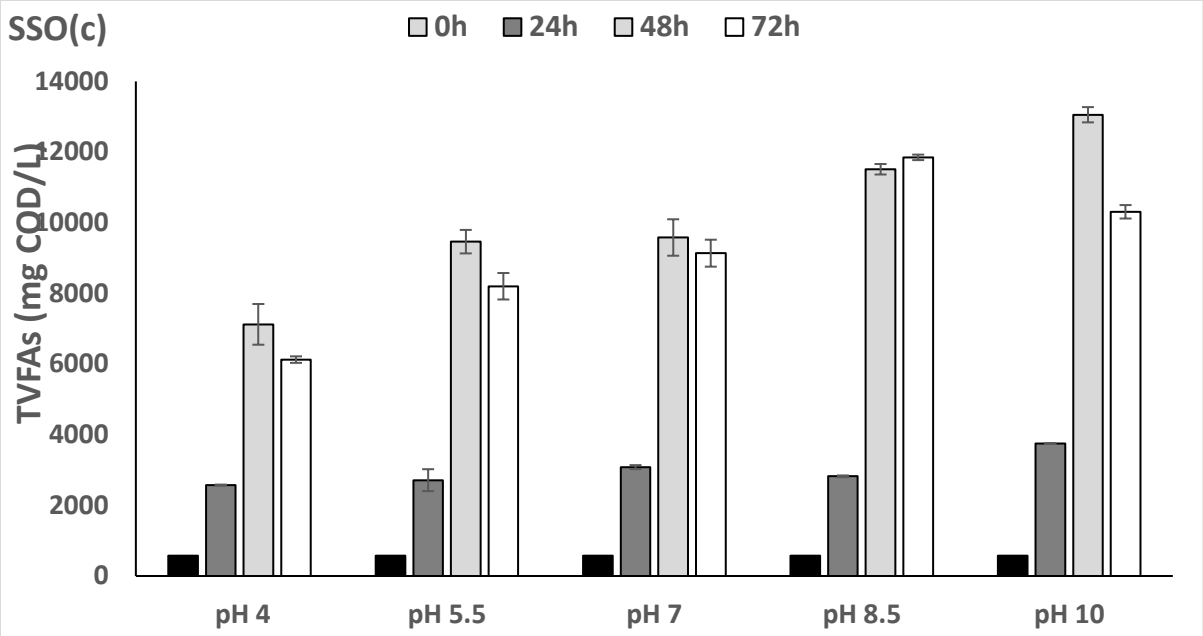
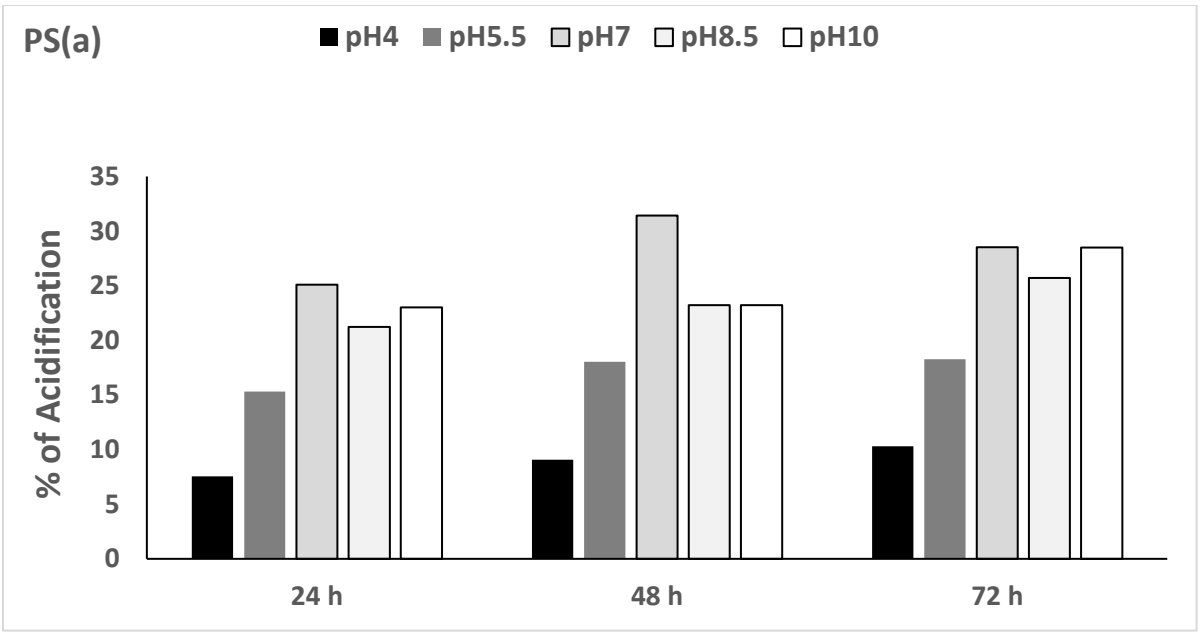
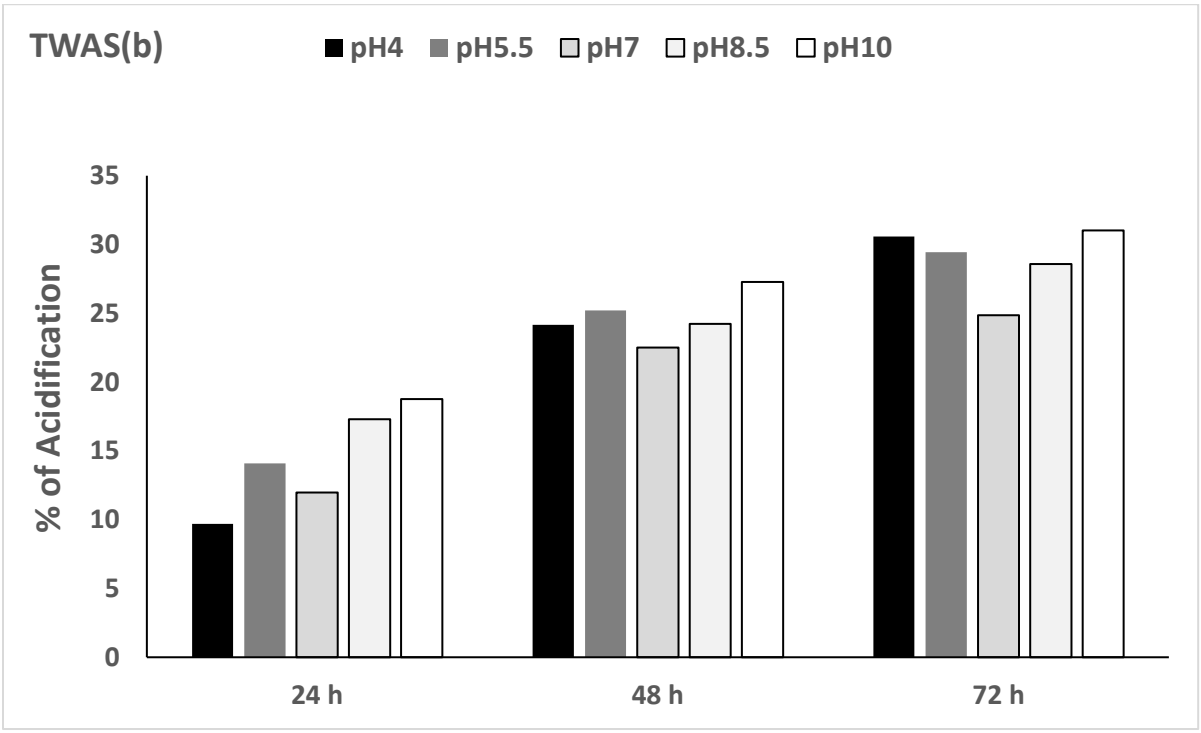


Figure 14:Total Volatile Fatty Acids (TVFAs) of Primary Sludge (a), TWAS (b) and SSO (c) at different pH and sampling time

As shown in Figure 14(c), the TVFAs production was at most in SSO pH10 at 48h, whereas in TWAS it was noted least at pH4 at 24h, which are 13056 mg COD/L and 523 mg COD/L respectively. On comparison of these three graphs, it can be analyzed that the higher concentration of TVFAs in SSO and poor concentration in TWAS. The production of TVFAs rises significantly in SSO after 24h. In TWAS the production of TVFAs increased gradually with time at almost in each pH value, but in PS the pH level decreased or remain the same. Overall it can be concluded that the production of TVFAs has more higher pH value or after pH7, where in PS, TWAS and SSO, the waste produced the higher concentration of TVFAs at 72h.

The results of Percent (%) of Acidification are shown in figure 15 below, the initial value for Percent (%) of Acidification is shown with black bar for each substrate.


PS(a)
pH4
pH5.5
pH7
pH8.5
pH10
% of Acidification
35
30
25
20
15
10
5
0
24 h
48 h
72 h


TWAS(b)
pH4
pH5.5
pH7
pH8.5
pH10
% of Acidification
35
30
25
20
15
10
5
0
24 h
48 h
72 h

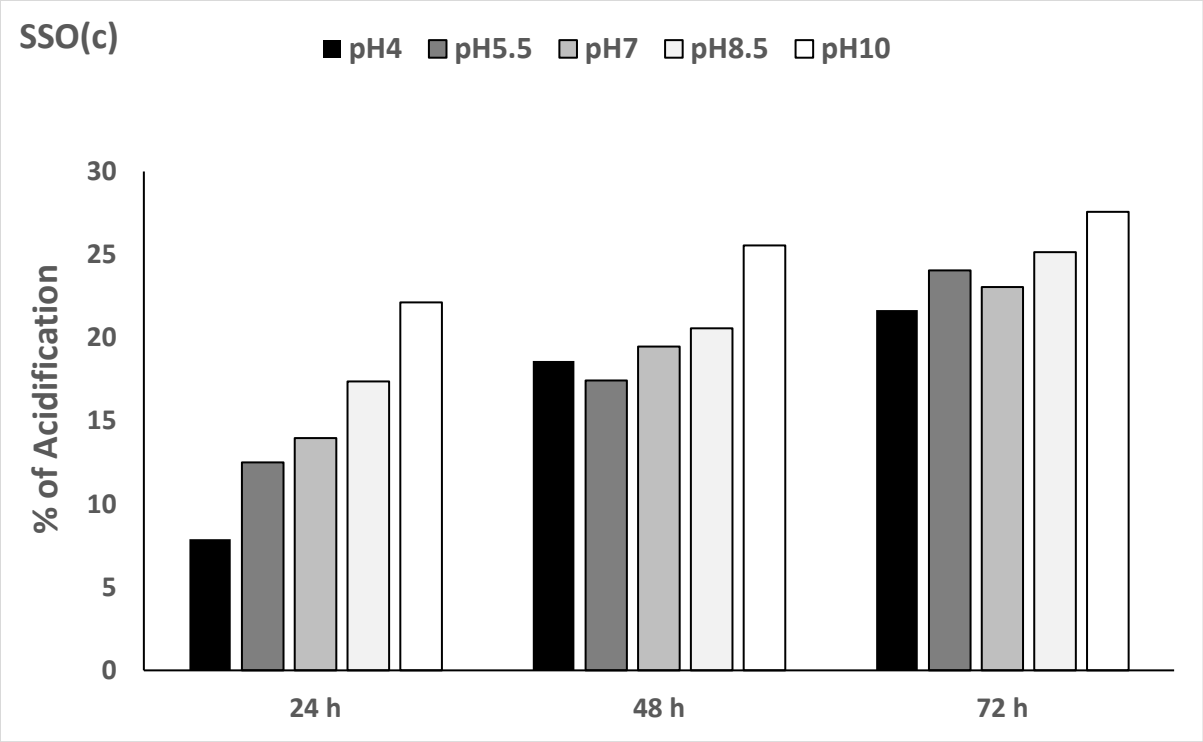


Figure 15: Percent (%) of Acidification of Primary Sludge (a), TWAS (b) and SSO (c) at different pH and sampling time

As shown in Figure 15(a), the high percentage of acidification was noted at pH7, value for every time interval, whereas 31% at pH7 at 48h was the highest reading and 8% at pH4 24h was the lowest reading.

In figure 15(b), acidification does not change much in respect to the pH value for all time interval, but at pH4 and pH10 72h have the same acidification percentage. During 48h there was not much variation in the percentage as it lies between 23% - 27%.

In the graph 15(c), SSO sample shows a significant rise in acidification during first 24h in pH level, but acidification percentage for all pH level during 48h and 72h does not have much variations. At 48h the pH level rise from 19% to 26% in the graph. It can be concluded that the high percentage of acidification was noted in PS and TWAS at 48h on pH7 and 72h on pH4 and pH10 respectively.

5.3 CONCLUSIONS AND RECOMMENDATIONS

The interrelationship amongst energy and water and the natural substance of sewage can support energy recovery operations from numerous conceivable sources, including city wastewater treatment offices. As the world population is growing and humans are producing too much waste, which is affecting our environment. Nowadays, the trend is not to consider the wastes as a martials that need need to be treated but it is a graet source of many valuable-added products such as methane, hydrogen, nutrients, and volatile fatty acids. On the other hand, sewage treatment is an energy-intensive, and energy recovery from these wastes have the potential to balance increases in electricity consumption. Energy recovery from sewage treatment plants gives an opportunity for successful and sustainable management of energy resources.

In this study, the effect of pH and HRT on production of VFAs from three wastes (primary sludge, thickened waste activated sludge, and source separated organics) were investigated.

The results show that with increasing the HRT, the VFAs production increased. On the other hand, the alkaline pH produced higher VFAs compared to the acid pH. For primary sludge, the VFAs increased by 1183% after 72h at pH10 compared to the initial value. For, thickened waste activated sludge, VFAs increased by 1485% after 72h at pH10 and Source separated organics increased to 2070% after 48h at pH10.

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