

ACETOGENESIS OF FRUIT AND VEGETABLE WASTES (FVW): EFFECT OF TEMPERATURE, HYDRAULIC RETENTION TIME, ORGANIC LOADING RATE AND Fe_3O_4 NANOMATERIAL WASTE

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ABSTRACT

Acetogenesis of Fruit and vegetable wastes (FVW) was performed in a 250 ml batch reactor with semi continuous mode operation to study the effect of Temperature, Hydraulic retention time (HRT), organic loading rate (OLR) and nanomaterial waste on volatile fatty acids (VFA) production from FVW. Optimum conditions for enhancement of the acetogenesis process in order to obtain maximum VFA production were determined by varying the temperature, HRT, OLR and nanomaterial wastes in successive four cycles and studying their impact on concentration of VFA production and pH. In cycle 1, increase in temperature resulted in increase of VFA concentration with maximum VFA of 2316.88 ± 185.19 mg/L obtained at 55p C. In cycle 2, increase in VFA concentration was observed with increase in HRT with maximum VFA of 2413.13 ± 145.83 mg/L obtained at 7-day HRT, but the degradation rate was considerably reduced after 5 days which indicates process inhibition after 5-day HRT. In Cycle 3, VFA increased with the increase in OLR with maximum VFA concentration of 2951.67 ± 137.12 mg/l obtained at an OLR of 1.4 gVS/l.d. However at OLR of 1.2gVS/l. d and 1.4 gVS/l.d the pH was reduced below 4.0 which could result in process inhibition. Use of 100 nm size Grade II iron oxide (Fe_3O_4) nanomaterial waste as a catalyst with FVW resulted in decrease of VFA concentration and was ineffective in enhancing the acetogenesis process.

KEY WORDS : Fruit and vegetable wastes (FVW), Acetogenesis, Volatile fatty acids (VFA), Nanomaterial wastes, Organic loading rate (OLR), Hydraulic retention time (HRT), Temperature.

INTRODUCTION

India produces 150 million tons of fruits and vegetable and almost 33% of it is wasted annually (Singh and Gu, 2010). Fruits and vegetable are wasted because of inadequate storage, transportation, handling, preservation and putrefaction due to microorganisms (Singh *et al.*, 2007) and these wastes are disposed off along with municipal solid wastes in open dumpsites (Joshi and Ahmed, 2016; Parihar *et al.*, 2017) which is responsible for foul odor, pests and greenhouse gas emissions. In a city of Bhopal, it was revealed that residential areas produce 50% and fruits and

vegetable markets produce 45% of the total food and fruits wastes which is 40-45% of the total Municipal solid waste (MSW) (Parihar *et al.*, 2019). In terms of quantity fruits and vegetable markets produce about 9.61 hundred thousand tons of fruit and vegetable market waste annually (Zia *et al.*, 2020) which could be utilized for energy recovery through anaerobic digestion.

Due to characteristics like high moisture content, high organic solids and high biodegradability fruits and vegetable wastes (FVW) are identified as the most suited substrate for anaerobic digestion (Arvanitoyannis and Varzakas, 2008; Appels *et al.*, 2011). Their organic characteristics include 75%

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sugars and hemicelluloses, 9% cellulose and 5% lignin (Verrier *et al.*, 1987; Raynal *et al.*, 1998).

Due to the high amounts of carbohydrates in FVW it produces volatile fatty acids (VFA) at a very rapid rate (Mata-Alvarez, *et al.*, 2000) and may result in accumulation of VFA which reduces pH, increases acidity and inhibits the process. The intermediate compounds during acetogenesis representing VFA are acetic acid, propionic acid, butyric acid, valeric acid, caproic acid, enanthic acid (Labatut and Gooch, 2012), but mainly acetic acid or acetate is produced through aceto-clastic and hydrogenotrophic pathways. VFAs, if produced in excess, can be inhibitory to the anaerobic digestion process leading to less methane production and thus decreased anaerobic digestion efficiency. The factors affecting VFA production and its conversion to methane are selection of proper reactor type, OLR, temperature, pH, hydrogen partial pressure and chemical additives (Khan *et al.*, 2016).

OLR is an important parameter and it directly affects the quantity and conversion of VFA. Due to the difference in the growth rates of methanogens and VFA producing bacteria, a high OLR leads to accumulation of VFA and may affect the overall biomethanation efficiency. Also, a high OLR can encourage methane inhibition due to the changes in composition of VFA compounds from acetic acid to n-butyric acid (Wijekoon, *et al.*, 2011). OLR adjustments by reduced or intermittent feeding had been employed by many researchers effectively for improving the VFA utilization (Wijekoon, *et al.*, 2011; Liu *et al.*, 2012; Sarker and Møller, 2014). In a batch digestion of mixed vegetable waste at 5% TS and OLR of 1.06 kgVS/m³.d biogas of 0.16 m³/kgVS was produced after 47 days of digestion (Rajeshwari *et al.*, 1998). In a single stage mesophilic CSTR treating Barcelona central food market waste 0.47 m³/kgVS of methane was produced at an OLR of 1.6 kgVS/m³.d (Mata-Alvarez *et al.*, 1992). In a similar study a single stage CSTR treating FVW had a methane potential of 0.45 m³/kgVS at an OLR of 2 kgVS/m³.d (Ganesh *et al.*, 2014). In a single stage continuous tubular digester treating FVW 0.452 m³/kgVS of methane was produced at an OLR of 2.8 kgVS/m³.d (Bouallagui, Haouari, *et al.*, 2004), although higher OLR in the range of 5.7 to 7.7 could also be achieved for FVW in two stage digestion system (Verrier *et al.*, 1987; Viturtia *et al.*, 1989; Rajeshwari *et al.*, 2001; Bouallagui *et al.*, 2004; Dinsdale *et al.*, 2000). For batch digestion optimum OLR is reported close to 1 kgVS/m³.d and for single

stage continuous system OLR should be less than 2.0 kg VS /m³.d (Shen *et al.*, 2013) and for two stage system it should not be greater than 6 kgVS/m³.d (Bouallagui *et al.*, 2005).

In addition to OLR temperature also has a considerable impact on VFA production and its utilization. For production and conversion of VFA, presence of microorganisms, their growth and inter specie communication are necessary which are dependent on temperature. As the temperature increases, hydrolysis rate increases increasing the solubility of carbohydrates and proteins resulting in an accelerated VFA production. However, impact of temperature on VFA composition is limited. In a study a temperature rise of 4 °C to 14 °C resulted in 55-43% reductions in acetate production (Yuan, *et al.*, 2011). In another study sudden temperature change from 63-55 pC have resulted in lesser propionate conversion and affected methanogenic populations (Öztürk, 1994). In a study thermophilic temperature resulted in a 32% increase in methane yields over mesophilic temperature while treating FVW in a semi continuously mixed tubular digester at an HRT and OLR of 20 days and 2.8 gVS/l.d respectively (Bouallagui, *et al.*, 2004) and the VS removal percentage observed at mesophilic and thermophilic temperatures were 76% and 87% respectively. In another study thermophilic and mesophilic conditions resulted in degradation of organic compounds in 11 days and 27 days respectively. Although temperature directly affects the degradation rate, HRT and VFA during acetogenesis, however for the subsequent methanogenesis mesophilic temperatures yield better results (Parawira *et al.*, 2007).

Hydraulic retention time (HRT) of substrate and inoculum sludge in the acetogenesis reactor treating FVW is an important parameter in VFAs production. Two stage systems are not preferred in real applications because they are costly and have complexity in design (Lissens *et al.*, 2001) while the single stage systems are still dominant because of their lesser cost and simple design (Lissens *et al.*, 2001). In a batch digestion system both the substrates and microorganisms are present in the reactor for the same duration thus their HRT and solids retention time (SRT) are equal, however in completely mix reactors there may be a wash out of the microorganisms which may lead to instability and a reduced degradation rate (Wong *et al.*, 2019). Effect of HRT on methane production and VS reduction was investigated for a mixture of Waste

activated sludge and 25% FVW in two stage systems. VS reduction increased by 4% when the HRT in the acidogenic reactor increased from 3 to 4 days (Dinsdale *et al.*, 2000). It was revealed that the HRT in the acidogenic state could fluctuate from 4 to 26 days and for methanogenic stage it can fluctuate between 10 to 65 days. Thus, HRT needs to be investigated to achieve maximum VS removal percentage, and optimum VFA for maximum biogas production.

Also, addition of few elements or their compounds in trace amounts have shown to enhance and stimulate the anaerobic digestion of FVW. In one study addition of Fe, Zn, Mn, Cu and Mo to a laboratory scale anaerobic digester treating FVW resulted in an increase of 88 to 96% organic solids conversion (Lane, 1984). Few other studies have also reported improvement in stability and performance of anaerobic digestion process after the addition of trace elements like Fe, Cu, Zn, Mn, Mo, Co, Al and Se to the FVW (Kumar *et al.*, 2006). In one study hydrothermal pretreated Fe_3O_4 nanoparticles in the size range of 15 to 22 nm were found to be effective in improving the methane production from municipal solid waste when used in concentration of 50 mg/l and 75 mg/l (Ali *et al.*, 2017).

Various studies have also reported stimulation of the anaerobic digestion by the addition of trace elements (Speece *et al.*, 1983; Williams *et al.*, 1986; Florencio, 1994; Gonzalez-gil and Kleerebezem, 1999; Gonzalez-Gil *et al.*, 2003; Osuna, *et al.*, 2003; Worm *et al.*, 2009). Biochemical reactions require essential enzymes and these enzymes have also coenzymes and cofactors which are made of metals like nickel, iron, zinc, cobalt, tungsten and molybdenum. The roles of these enzymes in the biochemical reactions are demonstrated in several studies (Beveridge and Doyle, 1989; Feroso *et al.*, 2008). Thus, the biochemical reactions can be stimulated by the addition of essential trace elements.

The purpose of the present research was to investigate the effect of Temperature, hydraulic retention time (HRT), organic loading rate (OLR), and nanomaterial waste, i.e. Grade II iron oxide (Fe_3O_4) on VFA production from FVW. Single stage batch reactor was used for the present study and the effect of variation of design parameters on VFA production was analyzed in a series of experiments.

MATERIALS AND METHODS

Reactor Configuration

Erlenmeyer flasks of 250 ml capacity with a working volume of 240 ml were used as a batch reactor for the acetogenesis of FVW as shown in Figure 1. The reactor had a sampling port and a feeding tube from which the FVW could be withdrawn and added respectively and semi-continuous operation was achieved. The reactor was covered with aluminum foil to prevent the penetration of light. The flask and the feeding tube are provided with a rubber cap to prevent the occurrence of any aerobic condition in the reactor. The air present in the headspace is insufficient to cause any change to the redox reactions in the reactor. Total 25 such reactors were prepared for the study. Feeding was done daily to maintain a constant level in the digester with regular checks and the temperature was controlled through incubation.

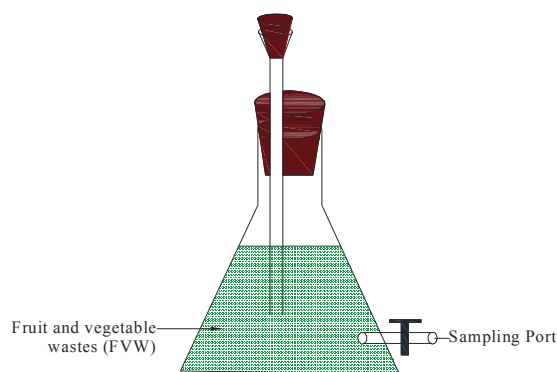


Fig. 1. Acetogenesis reactor

Reactor Feed and inoculum

The fruits and vegetable wastes (FVW) were prepared and selected based on the arrival data of major fruits and vegetable at the Azadpur fruits and vegetable market (FVM) collected from Agricultural Produce Marketing Committee (APMC), Azadpur New Delhi. Since the fruits and vegetable sold are changing throughout the year, the wastes were selected based on average amount of fruits/vegetable sold annually. Also due to large quantities of waste requirement for a laboratory experiment direct collection and sorting was not possible. Therefore, discarded fruits and vegetable namely apple, banana, mango, potato, onion and tomato were collected from the nearby fruits and vegetable

market. FVW was prepared according to the percentage arrival of each commodity at the FVM as in (Mata-Alvarez *et al.*, 1992). The arrival data of major fruits and vegetable from 2013 to 2018 at the Azadpur FVM was obtained from the APMC, Azadpur and their percent contribution in the prepared feed of FVW are shown in Table 1.

The fruits and vegetable are shredded to an approximate size of less than 5 mm in a household fruit and vegetable cutter as shown in Figure 2. The fruits and vegetable are weighed gravimetrically in the proportion as shown in Table 1. The fruit and vegetable are mixed and stored at 4 °C. The wastes were diluted with distilled water for characterization and with tap water for feeding as substrate to the reactor according to the required organic loading rate (OLR).



Fig. 2. Household fruit and vegetable cutter.

The inoculum/seed sludge was obtained from an anaerobic biodigester treating food wastes in a 50 kg Biogas plant located at Holy family hospital, Jamia Nagar, New Delhi. The inoculum had the same microbes as required for fruits and vegetable wastes. The inoculum was mixed in a proportion of 20:100 to suit the fast degrading FVW. The wastewater was collected in a 5 kg container and

stored at 4 °C. The food waste has a very high organic matter and solids concentration and thus inoculum was diluted with distilled water before determining the BOD, COD, VFAs and solids concentration.

Analytical methods

Total solids, volatile solids, and biochemical oxygen demand (BOD) were measured according to the Standard Methods (APHA/AWWA/WEF, 1995). Chemical oxygen demand (COD) was measured using COD digester (DRB, 200, Hach) and a spectrophotometer (DR, 2800, Hach). Volatile fatty acids (VFA) were determined using method based on the original procedure described by (DiLallo and Albertson, 1961). Total Phosphorus was determined using EPA method (USEPA, 1978). For tap water chlorides, sulphates, and total hardness were determined according to the Standard Methods (APHA/AWWA/WEF, 1995). pH, conductivity and Total dissolved solids (TDS) were measured with a Hach pH meter (Orion, Model 370) and turbidity with a nephelometer. Fruit and vegetable feed, seeding sludge, and tap water were analyzed according to the methods mentioned above and the results are presented in Table 2 and Table 3.

Nanomaterials waste Characterization

Although the nanomaterials can be used for number of applications but their properties, effect on human health and environment are still unknown. However, nanomaterials are characterized by a very high surface area to volume which makes them highly reactive and can be used as a catalyst as demonstrated by (Ryan *et al.*, 2011). Nanomaterials are manufactured because of their benefits in various research activities or for industrial purposes. The cost of obtaining a nanomaterial in purest form (99% purity) is very high however the wastes obtained in manufacturing these

Table 1. Arrival quantity of major fruits and vegetables and their contribution in the prepared feed of FVW.

Fruit/Vegetable	Arrival quantity (Metric tons)					Annual Average	Quantity in %
	2013-14	2014-15	2015-16	2016-17	2017-18		
Apple	731610.8	669926.9	706384.8	588530.6	674290.6	674148.74	31.20
Banana	84821.7	82254.6	90289.2	60619	47700	73136.9	3.39
Mango	208085.9	272929.8	197638.5	216405.8	199114.7	218834.94	10.13
Potato	537152.2	513454.7	560792	548158.3	560752.7	544061.98	25.18
Onion	395291.5	410936.4	405592.7	419615.1	395054.3	405298	18.76
Tomato	243463.8	243981.1	244758.2	263624.9	229021.2	244969.84	11.34
Total	2200425.9	2193483.5	2205455.4	2096953.7	2105933.5	2160450.4	100

Table 2 Characteristics of fruit and vegetable feed and seeding sludge

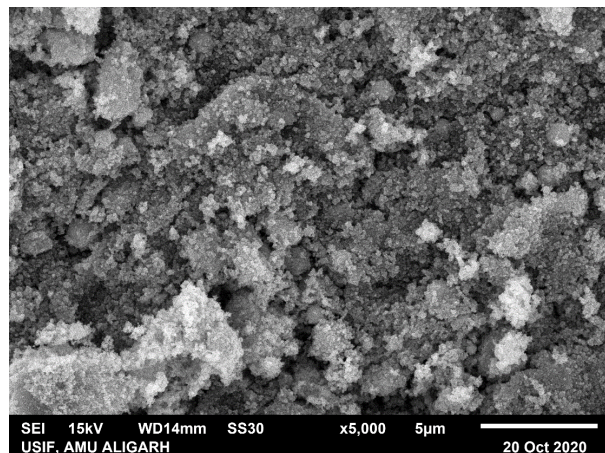
S. No.	Parameter	Fruit and vegetable feed	Seeding sludge
1	pH	5.1	4.19
2	COD (mg/L)	92,998	88,700
3	BOD (mg/L)	55,800	53,400
4	Total Solids (TS) (g/L)	147.2	40.46
5	Total Volatile Solids (TVS) (g/L)	139.52	36.67
6	Total Phosphorus (mg/L)	200	1620
7	Total Kjeldahl Nitrogen (mg/L)	2446.8	3284.5

Table 3. Dilution water characteristics

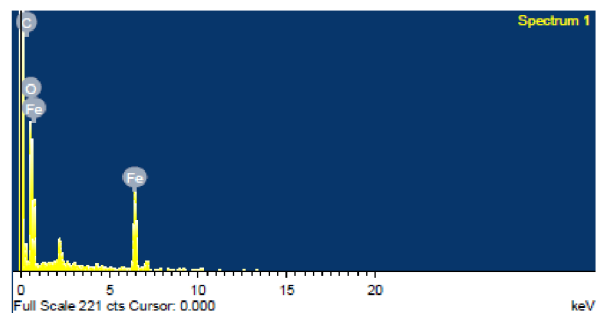
S. No.	PARAMETER	Quantity
1	pH	7.8
2	Total Hardness (mg/L as Ca CO ₃)	284.32
3	Turbidity (NTU)	2.79
4	TDS (mg/L)	656.68
5	Conductivity (µS/cm)	995.6
6	Sulphates (mg/L)	37.5
7	Chlorides (mg/L)	154.456
8	Total Nitrogen (mg/L)	22.435
9	Total Phosphorus (mg/L)	2.174

nanomaterials is very cheap with purity in the range of 70% to 80% and can also be used as a catalyst for enhancing the anaerobic digestion of various organic wastes.

In the present study Grade II nanomaterials waste in the size range of 100 nm obtained from an industry named "Saveer Matrix Nano Pvt. Ltd." Greater Noida, India was utilized to study their impact on VFA production. Nanomaterial waste was hydrothermally pretreated at 150°C to activate the Fe₃O₄ particles for its synthesis. Nanomaterials wastes were characterized by Energy Dispersive X-Ray Spectroscopy (EDS) in conjunction with scanning electron microscopy (SEM) done at USIF,

**Fig. 3.** SEM image of pretreated Fe₃O₄ nanomaterial waste

AMU, Aligarh. SEM image and EDS spectra of nanomaterials wastes are shown in Figure 3 and 4 respectively. In the Figure 3 darker portions represent the Fe₃O₄ particles and the lighter portions represents the impurities present in the waste. In figure 4 individual peaks of carbon, iron and oxygen represent their unique electromagnetic emission property when passed through an X-ray. The elemental composition of the waste is presented in Table 4.

**Fig. 4.** EDS spectra of pretreated Fe₃O₄ nanomaterial waste

Design parameters

Factorial design approach was used to optimize the design parameters namely OLR, hydraulic retention time (HRT), temperature and catalyst Iron oxide (Fe₃O₄) for the present study. Design experiments were conducted in four cycles by varying one parameter while keeping the other parameters at optimum ranges as reported in literature. To determine the optimum condition in acetogenesis of the fruit and vegetable wastes OLR, hydraulic

Table 4. Elements and their weight (%) in the nanomaterials waste.

Element	Weight%
Carbon (C)	16.71
Oxygen (O)	45.16
Iron (Fe)	38.13
Total	100

retention time (HRT) and temperature were varied from 0.6gVS/l.d to 1.4gVS/l.d, 2d to 7d, and 35 pC to 55 pC respectively in batch reactors. The catalyst chosen for the present study is 100nm size grade II Iron oxide (Fe_3O_4) and was varied from 25 to 100 mg/l.

RESULTS AND DISCUSSION

The batch experiments were performed in four cycles. The wastes were diluted with tap water according to the required organic loading rate (OLR). Tap water was chosen since it represents the prevailing practice at the biogas plants treating different organic wastes and would be an economical option. After dilution prepared FVW and the seed sludge were mixed in the proportion of 80:20 before being fed to the reactor. The reactors were sealed, covered with aluminum foil and incubated according to the required temperature as shown in Figure 5. In each of the four cycles 5 replicates of each experimental run were performed and mixing was achieved daily when a volume equal to the volume of the digestate divided by HRT of the digester was removed and fresh waste of the same volume was added. Initially for start-up reactors were operated at very low OLR of 0.2 gVS/l.d for a period of 3 days which was increased by 0.2 gVS/l.d after every 3 days until it reaches a value of 1 gVS/l.d, after which the 1st cycle was performed as explained below.



Fig. 5. Acetogenesis batch reactor and incubation

1st Cycle

In the first cycle 25 experiments were performed concurrently to find the optimum temperature by observing the volatile fatty acids (VFA) concentration while keeping the OLR and HRT constant at 1 g VS/l.d and 5 days respectively, since for batch digestion optimum OLR is close to 1 gVS/

l.d (Shen *et al.*, 2013) and the optimum HRT for acidogenic reactor is close to 5 (Dinsdale *et al.*, 2000). Effect of temperature on VFA concentration and pH are shown in Figure 6 and 7.

With the increase in temperature from 35 to 55 pC VFA concentration increased from 1859.38 ± 197.19 mg/l to 2316.88 ± 185.19 mg/l and pH decreased from 4.60 ± 0.09 to 4.03 ± 0.05 . The results were in contrast to the results obtained by (Jiang *et al.*, 2013) for food wastes where VFA at 55 pC were lower than VFA reported at 35 pC and 45 pC. Increase in temperature resulted in enhanced hydrolysis and faster utilization of FVW which resulted in higher VFA production thus decreasing pH. pH is an important factor which affects the rate of degradation at higher pH higher rate was observed as explained by (Infantes *et al.*, 2011). The permeability of un-dissociated acids through the cell membranes is reduced at lower pH which inhibits anaerobic digestion (Infantes *et al.*, 2011) since transportation of un-dissociated acid require more energy at lower pH (Rodríguez *et al.*, 2006).

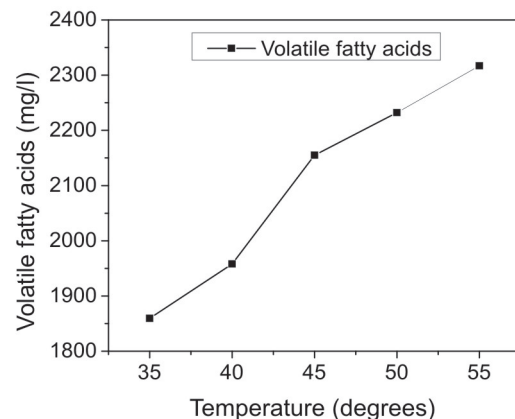


Fig. 6. Effect of temperature on VFA

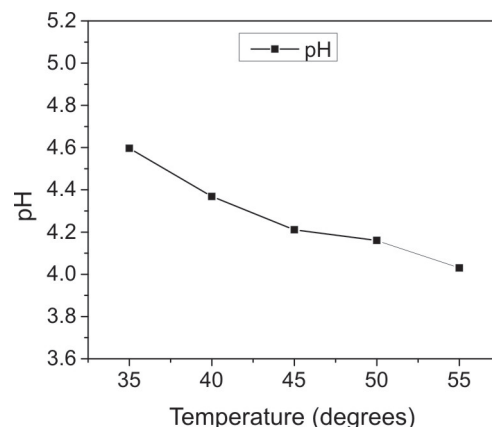


Fig. 7. Effect of temperature on pH

Thus, a balance of pH should be maintained to achieve higher degradation rate as well as higher methane yield.

2nd Cycle

In the second cycle 20 experiments were performed concurrently to find the optimum HRT, by varying the HRT from 2 day to 7 days and observing the volatile fatty acids concentration (VFA) while keeping the OLR and optimum temperature obtained above constant at 1gVS/l.d and 55°C respectively. Effect of HRT on VFA concentration and pH are shown in Figure 8 and 9.

VFA concentration increased from 1484.06±29.65 mg/l at 2-day HRT and reached maximum of 2413.13±145.83 mg/l at 7-day HRT. The pH varied from 3.93±0.12 at 7-day HRT to 4.60±0.06 at 2-day HRT. Increase in HRT resulted in increased VFA due to the improved hydrolysis and acetogenesis of FVW which resulted in decrease of pH. However, increase in HRT above 5 day resulted in the

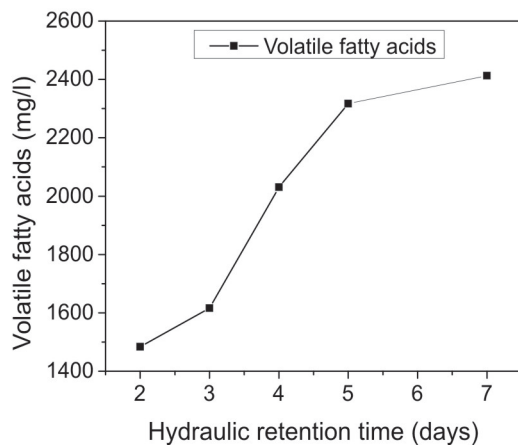


Fig. 8. Effect of HRT on VFA

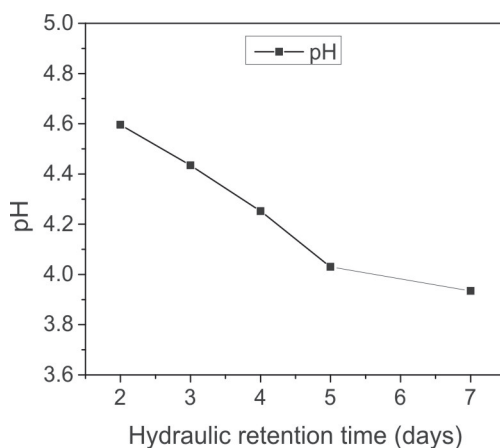


Fig. 9. Effect of HRT on pH

decreasing rate of VFA production and decrease of pH which could ultimately result in lower methane yields. The present results are similar to the one obtained by (Lim *et al.*, 2008) for food wastes where the VFA increased with the increase in HRT and had an optimum HRT of 8 days in a laboratory reactor, although the OLR was comparably higher at 5gTS/l.d which resulted in low methane yield.

3rd Cycle

In the third cycle 20 experiments were performed concurrently to find the optimum OLR by varying the OLR from 0.6gVS/l.d to 1.4gVS/l.d and observing the VFA while keeping the temperature and HRT constant at optimum values that is at 55°C and 5 days respectively as obtained in cycle 1 and cycle 2. Effect of OLR on VFA concentration and pH are presented in Figure 10 and 11.

VFA concentration increased with the increase in organic loading rate from 1504.06±87.33 mg/l at 0.6 gVS/l.d to 2951.67±137.12 mg/l at 1.4 gVS/l.d. The present results are similar to the ones obtained by (Lim *et al.*, 2008) for food wastes where the VFA increased with the increase in the OLR although the OLR chosen was much higher in the range of 5gTS/

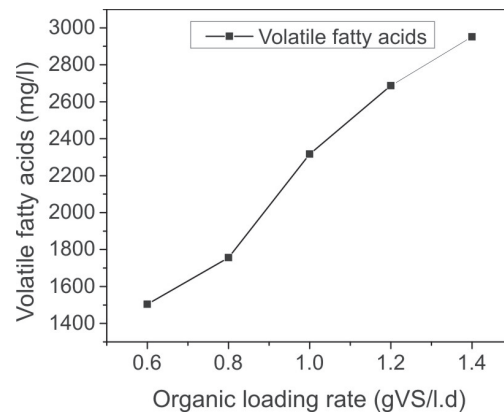


Fig. 10. Effect of OLR on VFA

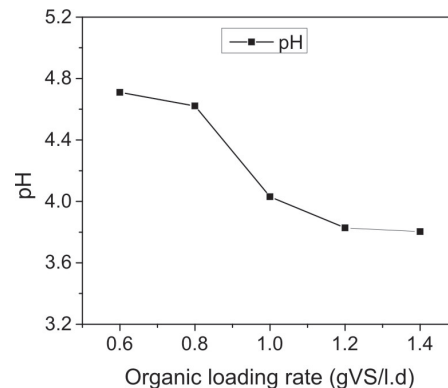


Fig. 11. Effect of OLR on pH

l.d to 13 gTS/l.d which resulted in the low yields due to higher VFA concentration and unutilized solid food wastes. Increase in OLR had a direct impact on the pH due to increased production of VFA at higher OLR. The pH varied from 4.83 ± 0.07 at 0.6 gVS/l.d to 3.80 ± 0.02 at 1.4 g VS/l.d . pH values of less than 4.0 was observed at 1.2 gVS/l.d and 1.4 gVS/l.d respectively which could inhibit the anaerobic digestion reducing the overall methane yield. Thus, for a higher methane yield OLR should be kept in the range of 1 gVS/l.d for FVW.

4th Cycle

In the fourth cycle, 20 experiments were performed concurrently to find the optimum catalyst concentration, by observing the volatile fatty acids concentration (VFA) while keeping the Temperature, HRT and OLR as constant at 55°C , 5 days and 1 gVS/l.d , respectively. The catalyst chosen was Grade-II Iron Oxide (Fe_3O_4) and the Fe_3O_4 concentration was varied from 25 mg/l to 100 mg/l as similar concentration were reported by (Ali *et al.*, 2017) for enhanced anaerobic digestion for municipal solid waste. Effect of grade II iron oxide (Fe_3O_4) nanomaterial waste on VFA concentration and pH are demonstrated in Figure 12 and 13.

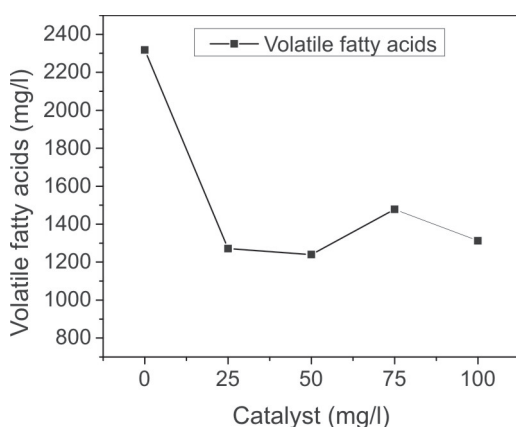


Fig. 12. Effect of grade II Fe_3O_4 on VFA

Highest VFA concentration obtained with Fe_3O_4 nanomaterials wastes was $1478.25 \pm 15.55 \text{ mg/l}$ at 75 mg/l which is only 64% of the VFA ($2316.88 \pm 185.19 \text{ mg/l}$) when Fe_3O_4 was not used. Addition of grade II Fe_3O_4 nanomaterials did not have any significant impact on the pH and the pH of the reactors with Fe_3O_4 was close to the pH of 4.03 ± 0.05 in the reactor with no Fe_3O_4 . This shows that grade II Fe_3O_4 nanomaterials wastes had a negative impact on the VFA production which resulted in process inhibition

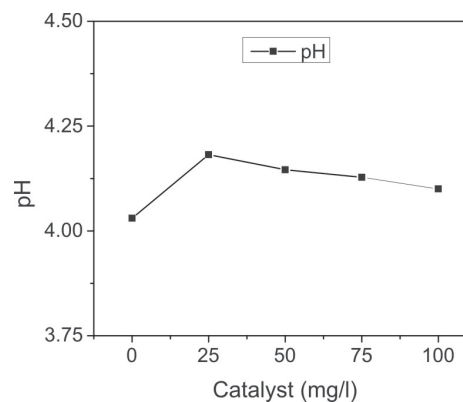


Fig. 13. Effect of grade II Fe_3O_4 on pH

and reduced acetogenesis microbial population. Thus, 100 nm grade II Fe_3O_4 nanomaterials waste could not be used for enhancing the acetogenesis of FVW.

CONCLUSION

Temperature, OLR and HRT had a significant impact on the VFA production during acetogenesis of FVW. Increase in temperature resulted in increase of VFA concentration with maximum VFA of $2316.88 \pm 185.19 \text{ mg/l}$ obtained at 55°C . Increase in HRT resulted in the increase of VFA concentration with maximum VFA of $2413.13 \pm 145.83 \text{ mg/l}$ obtained at 7-day HRT, but the degradation rate was considerably reduced after 5 days which indicates process inhibition after 5-day HRT. Similarly, VFA increased with the increase in OLR with maximum VFA concentration of $2951.67 \pm 137.12 \text{ mg/l}$ obtained at an OLR of 1.4 gVS/l.d . However at OLR of 1.2 gVS/l.d and 1.4 gVS/l.d the pH was reduced below 4.0 which could result in process inhibition. Thus the optimum OLR of 1 gVS/l.d is suggested for anaerobic digestion of FVW. Use of 100 nm size Grade II iron oxide (Fe_3O_4) nanomaterial waste as a catalyst with FVW resulted in decrease of VFA concentration and was ineffective in enhancing the acetogenesis process.

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