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COMPARING THE EFFECT OF TOTAL SOLIDS CONCENTRATION ON BIO-HYDROGEN PRODUCTION POTENTIAL OF FOOD WASTE AND ITS DERIVATIVES UNDER MESOPHILIC THERMOPHILIC CONDITIONS

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The effect of three total solid (TS) concentrations of 7.5, 10 and 12.5% on bio-hydrogen production potential of food waste (FW) in comparison with noodle waste (NW) and rice waste (RW) derived from FW were studied after co-digesting the wastes with heat shocked sludge under mesophilic (37°C) and thermophilic (55°C) conditions. The increase in TS concentration from 7.5% to 10% found an effective way to improve cumulative bio-hydrogen production from all tested wastes. As a whole, 7.5% TS concentration represented higher conversion efficiency of volatile solids into bio-hydrogen and the highest experimental yield of 95.8mL/VS_{fed} was observed in 7.5%NW digester under thermophilic conditions. The increase in temperature within experimental range found an effective way to increase bio-hydrogen production potential of FW and NW only, whereas the same increase in temperature caused negative impact on bio-hydrogen production potential of RW. The optimum time period for active bio-hydrogen production found to be 24-72h of incubation. After 72h of incubation, the bio-hydrogen production reduced considerably in all reactors due to an abrupt increase in volatile fatty acid, and due to inhibition of bio-hydrogen production the drop in pH was also decreased.

Keywords: Bio-hydrogen yield, total solids concentration, Clostridium, rice waste, noodles waste, volatile fatty acids.

INTRODUCTION

Hydrogen is a promising source of energy (122kJ/g) with zero pollutant emission as no greenhouse gases are produced during combustion of hydrogen which make it a suitable alternate to fossil fuels (Pisutpaisal and Hoasagul, 2012). Conventionally the hydrogen is produced by electrochemical or thermochemical processes that consume a lot of energy and are not environment friendly (Chen *et al.*, 2006) . On the other end, the most common biological mean of bio-hydrogen production is anaerobic digestion and the hydrogen produced by anaerobic digestion is often termed as bio-hydrogen. The anaerobic digestion can utilize a mixed consortia of *Clostridium* in the form of sludge as an optimum source of microbial culture because it contains 64 to 70% Clostridium as well as it has some nutrients which are important for bio-hydrogen production processes (Fang *et al.*, 2006; Liaquat *et al.*, 2015). Along with *Clostridium*, sludge also contains hydrogen consumers like methanogens which can be initial deactivated by pretreatment like heat shock etc.

The microorganisms require some source of carbohydrates as feedstock to produce bio-hydrogen during anaerobic digestion. Although a variety of feedstocks like lignocellulosic and carbohydrate based wastes can be utilized for this purpose but carbohydrate based wastes are more preferable because they do not need intensive pretreatment to release sugar content entangled by lignin binding (Sattar *et al.*, 2016). In this regard, food waste (FW) or organic fraction of municipal solid waste (OFMSW) is getting now widely tested for bio-hydrogen production as it is abundantly available on daily basis and the managerial issues are also resolved by anaerobic digestion (Fountoulakis and Manios, 2009). The FW has 50 to 70% share in municipal solid waste (MSW) generated in China where major contributor to food waste are canteens as 28.3% of the ordered food ends up in the waste bin (Tai *et al.*, 2011). About 40% of FW is composed of noodle waste (NW) and rice waste (RW) (Shiwei, 2005; Wang Lingen *et al.*, 2013). In order to digest FW under anaerobic conditions, microorganisms required optimum operational conditions like pH and temperature (Saraphirom and Reungsang, 2010). Normally, FW has a low pH of 4-5 which would be further reduced during anaerobic digestion due to production of volatile fatty acids (VFA). If the process pH is lowered than pH 4, it can affect metabolic pathways which ultimately inhibit the activity of bio-hydrogen producing bacteria (Morimoto *et al.*, 2004; Fang *et al.*, 2006). On the other end, increasing process pH from pH 7 to pH 8 also decreased the bio-hydrogen production (Lin *et al.*, 2013; Nathao *et al.*, 2013). Keeping in view these facts, the optimum pH range for bio-hydrogen production was found to be between pH 4.5 to pH 8.5 (Mizuno O, 2000). Apart pH, process temperature is also an important operational parameter. The bio-hydrogen production can be done under psychrophilic conditions but it required specific chamber like microbial electrolysis cells which make the process complication Lu *et al.* (2011). So most of the research on bio-hydrogen production was conducted under mesophilic and thermophilic conditions (Li and Liu, 2012; Saripan and Reungsang, 2014).

Along with pH and temperature, dry matter content or total solids (TS) is a crucial factor that can affect the anaerobic digestion. Increasing the TS concentration will reduced the reactor size but it can also reduce the bio-hydrogen yield due to osmotic stress, high metabolite accumulation that ultimately cause microbial inhabitation (Robledo-Narvaez *et al.*, 2013). Keeping in view these facts, most of the bio-hydrogen production through anaerobic digestion was done under liquid state conditions with TS concentration less than 15% (Nabi *et al.*, 2003) . The impact of increasing TS concentration under liquid state anaerobic digestion is also an important factor which was tested under 1 to 9% TS concentration under similar set of experiments resulting 9% TS concentration as an optimum TS for bio-hydrogen production from food waste (Ramos *et al.*, 2012). Still the TS concentration can be further increased till the variable maximum concentration achieved. Therefore, the objective of following research was to study the effect of initial total solids on bio-hydrogen production from co-digestion of food waste and its derivatives with heat shocked sludge under mesophilic and thermophilic temperature conditions.

MATERIALS AND METHODS

Feedstock preparation: The FW was collected from canteen at the Engineering College, Nanjing Agricultural University, Nanjing, China. It was mainly made up by rice, noodles, meat and vegetables. At first bones and other unwanted materials were removed and left over waste was termed as FW. Later the FW was grounded in a meat grinder by adding an equal amount of water on weight basis and resultant slurry was used for bio-hydrogen production (Reungsang *et al.*, 2013). The RW and NW were also separated manually from the waste collected from the canteen and converted into slurry in the same way opted for FW.

The sludge was obtained from a wastewater settling channel at Pukou, Nanjing. Foreign materials and coarse particles (diameter > 0.5 mm) were removed by sieving and washed it twice with tap water (Nathao *et al.*, 2013). Later it was placed in preheated oven at 100°C for 15 minutes to deactivate methanogens (Li and Fang, 2007). Some properties of waste used in the experiments are enlisted in Table 1.

Table 1. Properties of test materials.

Parameter	Unit	Sludge	Food waste	Rice waste	Noodles waste
TS	%	58.59	30.32	39.88	31.54
VS	%	2.87	26.9	39.30	28.51
Glucose	g/L	2.49	65.77	79.65	63.73
COD	g/L	50	147.5	105	132
Total Alkalinity	mg/L	3700	550	500	450
VFA (mg/L)	mg/L	13950	2475	9000	1500
pH	---	7.1	4.5	5.3	4.3

Experimental design: The FW was added in equal proportion on TS basis with heat shocked sludge in 550 mL lab scale digester after which water was added in such quantity to achieve the working volume of 400 mL. Three initial TS concentration of 7.5%, 10% and 12.5% were maintained in six digesters, two for each TS concentration. These reactors were placed in two water baths; one at mesophilic temperature (37±0.1°C) and other at thermophilic temperature (55±0.1°C). Two sets of experiments (in duplicate) were performed (Zhu *et al.*, 2008; Dong *et al.*, 2009). Similar procedure was opted for NW and RW. The schematic diagram of experimental layout is shown in Figure 1. The pH of all reactors was maintained to a value of 7 after every 24 hours.

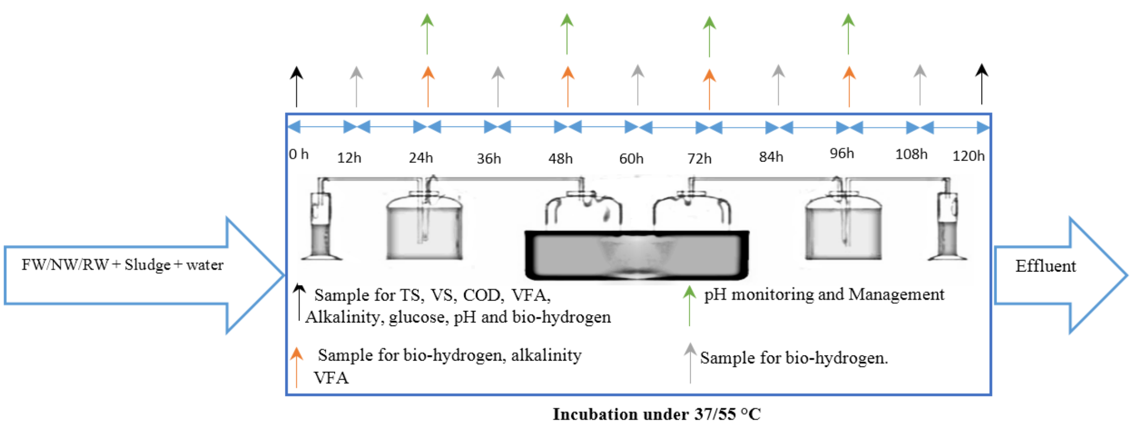


Figure 1. Schematic diagram of experimental setup.

Samples collected before and during the experiment were stored in the refrigerator (0-4°C).

***Analytic methods and kinetic modeling*:** The bio-hydrogen production volume was measured by connecting each reactor with a measuring bottle containing 3% NaOH solution as followed in our previous work (Chaudhry *et al.*, 2015; Sattar *et al.*, 2016). The TS, volatile solids (VS), Chemical oxygen demand (COD), alkalinity and volatile fatty acids were measured according to standard methods (APHA, 2005) . Glucose was measured by the Phenol Sulfuric acid method (Lay and Fan, 2003). For TS, VS and COD, samples were taken before and after incubation, whereas for VFA and glucose, samples were taken with glass syringe after every twenty-four hours (Sreela-Or *et al.*, 2011).

The kinetic parameters of bio-hydrogen production were calculated by the modified Gompertz equation using cumulative bio-hydrogen data (Dong *et al.*, 2009; Ramos *et al.*, 2012).

$$H = P\exp\left\{-\exp\left[\frac{R_m e}{P}\ (\lambda - t) + 1\right]\right\} \qquad (1)$$

Where H, t, P, Rm, and e represent cumulative hydrogen production (mL), incubation time (h), hydrogen production potential, maximum hydrogen production rate (mL/h), lag phase duration (h) and 2.72, respectively. The equation was solved by using Matlab (ver. 2010 a).

In order to assess the effect of TS concentration, the yield was calculated by dividing the P value with volatile solids added (Liu *et al.*, 2013).

RESULTS AND DISCUSSION

***Effect of TS concentration on bio-hydrogen production*:** The effect of increase in TS concentration and shifting the temperature from mesophilic to thermophilic was different on different type of waste as shown in Figure 2. It can be observed from Figure 2 that the FW digester with 7.5% TS concentration has the higher cumulative bio-hydrogen production as compared to other two FW digester under mesophilic conditions. It is due to shorter lag phase (λ) of 7.5% FW (Table 2) as compared to other two digesters under mesophilic conditions, which caused an early start in bio-hydrogen production (Fig. 2a). The increase in TS concertation of FW decreased the cumulative bio hydrogen production under mesophilic condition due late start in production of bio-hydrogen due to high λ value (Table 2, Fig. 2a). Although the λ for 12.5%FW was smaller than λ for 10%FW i.e., 24.1h at 10% and 21.54h at 12.5%, but still the cumulative bio-hydrogen production of 12.5%FW TS was smaller than 10%FW under mesophilic conditions. It was because of higher hydrogen production rate (R_m) of 10%FW as compared to 12.5%FW under mesophilic conditions (Table 2). The increase in temperature from 37 to 55°C also increased the bio-hydrogen production. At the same time, the increase in FW TS concentration also increased cumulative bio-hydrogen production under thermophilic conditions, which was quit opposite to the effect observed under mesophilic conditions. This increase is due to *Thermoanaerobacterium thermosaccharolyticum* that grows at higher temperature in food waste and produced more hydrogen (Shin *et al.*, 2004).

In case of NW, the increase in TS from 7.5 to 10% increased the bio-hydrogen production but further increase in TS concentration to 12.5% decreased the cumulative bio-hydrogen production under both tested temperatures (Fig. 2b). Although, increase in NW TS concentration reduced λ but it also affects the R_m which was higher for 10% (Table 2).

Table 2. Kinetic parameters and bio-hydrogen yield.

Waste Type	TS (%)	Temperature	P (mL)	R_m (mL/h)	λ (h)	R^2	Bio-hydrogen yield (mL/VS fed)
Food waste	7.5	37°C	517.80	21.77	10.38	0.9960	36.90
		55°C	845.00	14.49	6.48	0.9890	60.22
	10	37°C	490.10	13.34	24.10	0.9969	27.05
		55°C	981.20	10.09	3.23	0.9885	54.15
	12.5	37°C	280.40	6.18	21.54	0.9911	12.20
		55°C	1124.00	17.98	1.10	0.9833	48.91
Noodles waste	7.5	37°C	663.80	18.84	11.74	0.9890	46.42
		55°C	1370.00	21.38	13.16	0.9870	95.80
	10	37°C	1193.00	43.08	9.32	0.9910	62.75
		55°C	1723.00	25.56	8.56	0.9820	90.64
	12.5	37°C	832.70	13.13	5.65	0.9920	34.82
		55°C	599.20	54.58	0.46	0.9725	25.07
Rice waste	7.5	37°C	660.00	15.02	1.24	0.9889	41.22
		55°C	82.02	2.47	28.99	0.9924	5.46
	10	37°C	876.00	23.62	31.31	0.9819	42.52
		55°C	122.90	4.25	35.02	0.9897	5.97
	12.5	37°C	270.90	5.36	21.60	0.9496	10.46
		55°C	357.60	11.70	24.27	0.9905	13.81

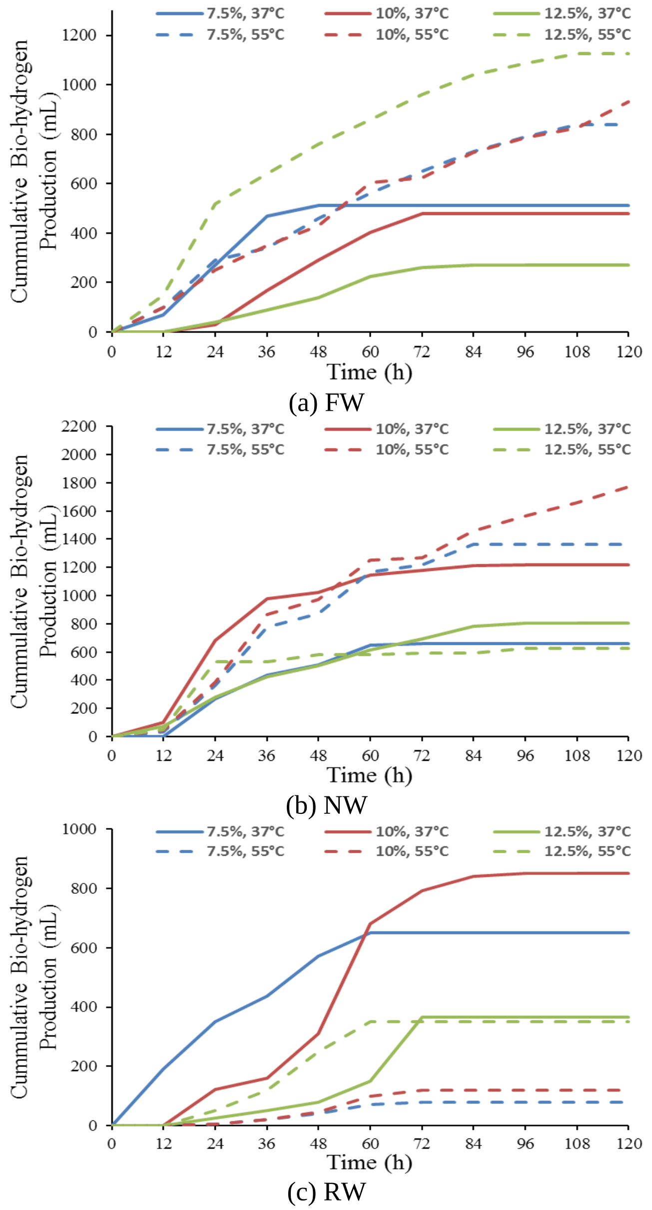


Figure 2. Cumulative Bio-hydrogen production from different waste under different TS concentration.

The decrease in R_m is because of the decrease in the amount of soluble compounds due to less available water as well as increase in adsorption of nutrients to solid surface (Robledo-Narváez *et al.*, 2013). On the other hand, the reduction in cumulative bio-hydrogen production at higher TS of 12.5% was likely to associate due to shifting of acidogenic phase to solventogenic phase because of consumption of hydrogen to acids to alcohols (Ginkel *et al.*, 2001). The increase in temperature from mesophilic to thermophilic also increased the NW cumulative bio-hydrogen production (Chaudhry *et al.*, 2015). As a whole, the highest experimental cumulative bio-hydrogen production of 1723 mL was observed in 10%NW digester under thermophilic conditions.

Similar effect of TS was also observed for RW under mesophilic conditions (Fig. 2c). The increase in temperature within experimental range decreased the cumulative bio-hydrogen production from RW digesters except 12.5% TS concentration. The RW has higher carbohydrate content (Table 1) as compared to NW and FW, which caused a negative impact of increase in temperature on bio-hydrogen production from RW as reported by Fang *et al.* (2006).

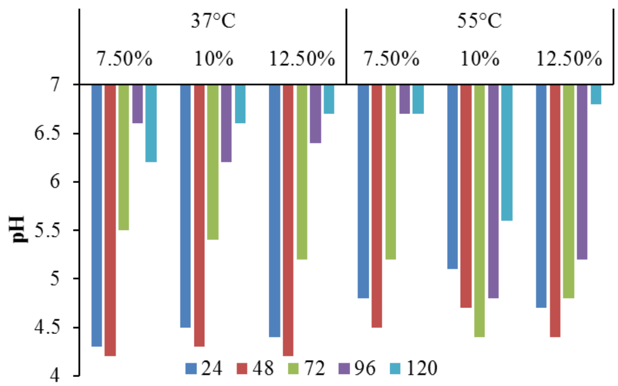
Most of the bio-hydrogen was produced during 24 to 60 hours of incubation as reported in previous studies (Dong *et al.*, 2009; Liu *et al.*, 2013). After 72 hours, hydrogen production reduced considerably to zero under mesophilic as well as thermophilic temperatures. That might be due to consumption of hydrogen consumers like homoacetogens as methanogens were deactivated initially due to thermal treatment (Oh *et al.*, 2003).

***Hydrogen yield comparison*:** The bio-hydrogen yield is shown in Table 2. It can be observed that the 7.5%FW produced 5% more bio-hydrogen under mesophilic conditions, but the bio-hydrogen yield was increased by 26.8% which represented the efficient conversion of organic matter to bio-hydrogen. On the other hand, the highest cumulative bio-hydrogen of 1124mL was observed from 12.5%FW under thermophilic conditions but the highest bio-hydrogen yield of 60.31mL/VS_{fed} from FW was observed at 7.5% TS concentration under thermophilic conditions. Similarly, the 10%FW also has higher bio-hydrogen yield as compared to 12.5%FW under thermophilic conditions (Table 2). This represented that the efficiency of converting organic matter in term of volatile solids decreased with an increased in TS concentration within experimental range. The negative impact of increase in TS was also reported by other researchers (Motte *et al.*, 2013; Paula N. Robledo-Narváez, 2013). At the same time, the effect of increase in temperature with in experimental increased with an increase in TS concentration of FW.

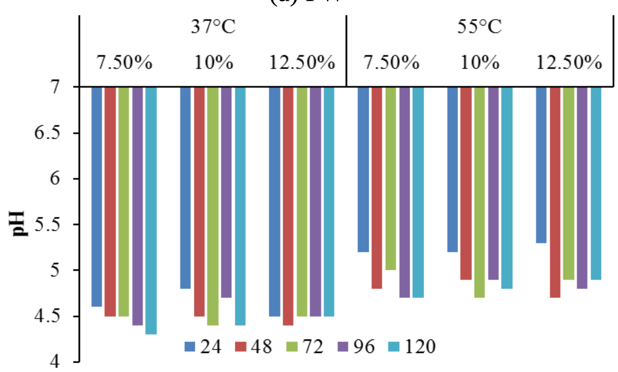
The NW also represented the similar effect of increasing TS and thermophilic 7.5%NW represented the highest experimental yield of 95.8, whereas the highest experimental cumulative bio-hydrogen production of 1723mL was observed by thermophilic 10%NW digester. The increase in TS concentration in NW digester from 10 to 12.5% decreased the bio-hydrogen yield and cumulative bio-hydrogen production both.

The increase in TS concentration of RW from 7.5 to 10% increased the bio-hydrogen yield, but further increase in TS to 12.5% decreased the yield under mesophilic conditions. The similar impact of increasing TS was observed under thermophilic conditions except that the 12.5%RW has higher bio-hydrogen yield under thermophilic conditions. As a whole, the bio-hydrogen yields obtained in the present study are in agreement with the finding of Lin *et al.* (2013).

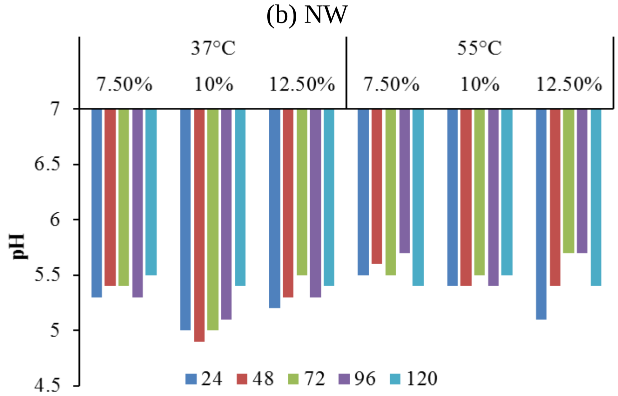
Fermentation process stability: There are many factors like pH, alkalinity and VFA etc. that can affect the anaerobic digestion. The production of fatty acids with time caused the pH to reduce as well as decrease the buffering capacity of the reactor that was measured as alkalinity. The pH of all reactors was daily maintained at pH 7, which was found an optimum pH in our previous study (Sattar *et al.*, 2016) and the drop in pH is presented in Figure 3.



(a) FW



(b) NW



(c) RW

Figure 3. Effect of TS concentration and waste type on drop in pH.

It can be observed that the drop in pH was high during bio-hydrogen production (24-48h) which was reduced as the bio-hydrogen production was started to decline (48-72h) in FW digesters (Fig. 2a, 3a). The intensity of drop in pH was further decreased when bio-hydrogen production from FW ceased as in all FW reactors. On the other end, the drop in pH remained higher in 10%FW digester under thermophilic conditions, where the bio-hydrogen production was not ceased. As a whole, It is clear from the Figure 3a and b that the drop in pH under thermophilic conditions was lower than that observed under mesophilic conditions, which was also reported by Gadow *et al.* (2012). On the other hand, the drop in pH was higher in NW digesters as compared to FW digesters (Fig. 3a, 3b) as a whole due to which the bio-hydrogen production was also higher in NW digesters as observed in Table 2. The effect of temperature opposite on drop in pH in case of NW as compared to FW.

Similarly, the drop in pH due to increase in temperature was same in RW digesters as observed for NW digester as both are rich in carbohydrates as compared to FW. The drop in pH during incubation was less in RW as a whole as compared to other two tested wastes, which was in agreement with the findings of Fang *et al.* (2006). Alkalinity can be considered as another process stability parameter along with pH, as pH cannot represent buffering capacity of the digester during incubation. The effect of TS, waste type, temperature and time on Alkalinity is presented in Figure 4. It was observed that the alkalinity increased with time as reported by Lin *et al.* (2013). Increase in FW alkalinity was more than under mesophilic conditions as compared to thermophilic conditions, which was in agreement with the findings of Shin *et al.* (2004). An abrupt increase in FW alkalinity was observed after 60 hours during which most of the bio-hydrogen was produced (Fig. 2a, 4a). It was also observed that the alkalinity of 10% FW digester was smaller at the end of fermentation as compared to other FW TS concentrations.

The alkalinity of NW and RW was smaller than FW and remained less than 5000 mg/L, representing a fermentation process as an optimum process (Ren and Wang, 2004). The increase in TS concentration in NW digesters affect the alkalinity in the same way observed for cumulative bio-hydrogen production i.e., increase in TS from 7.5 to 10% increased the alkalinity and further increase in TS decreased the alkalinity as observed in Figure 4b and table 2. In case of RW, the alkalinity increased with an increase in TS till 72h of incubation during which most of the bio-hydrogen was produced. The increase in temperature decreased the alkalinity of RW digesters and as a whole 12.5%TS have the high alkalinity as compared to other RW TS concentrations. An abrupt increase and decrease in alkalinity was observed during 60 to 96 hours of incubation, the same duration where bio-hydrogen production from RW ceased or reached to its minimum value. As a whole the increase in temperature decreased the alkalinity of FW and RW only. There was an abrupt change in alkalinity in all digesters after 48 to 72 h of incubation.

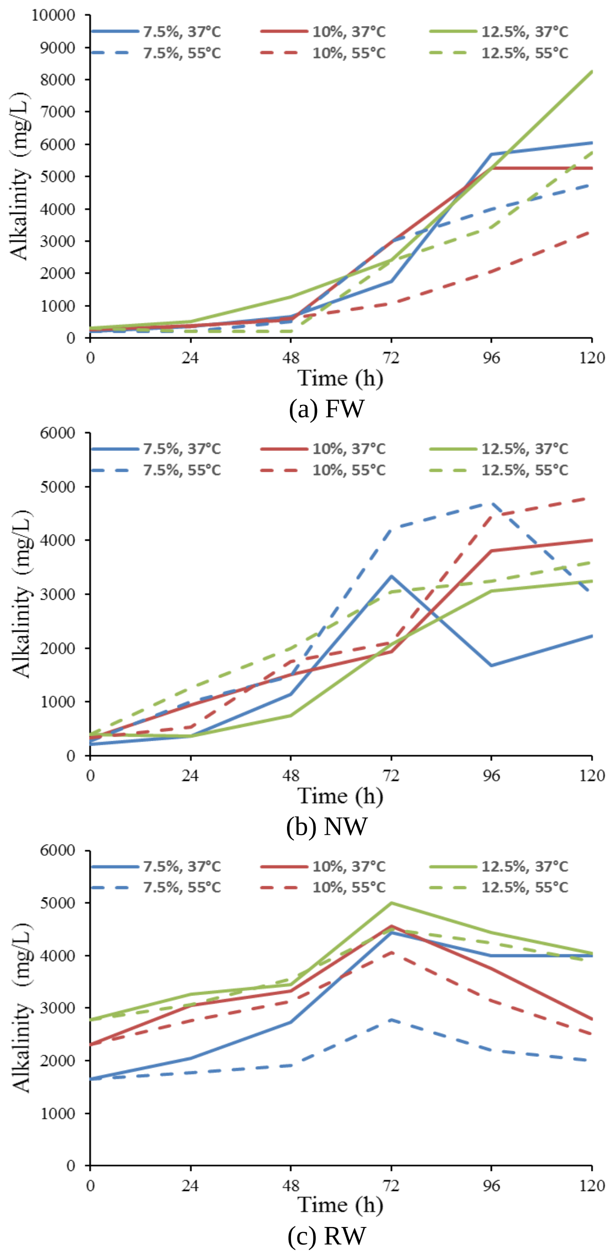


Figure 4. Effect of TS and temperature on Alkalinity.

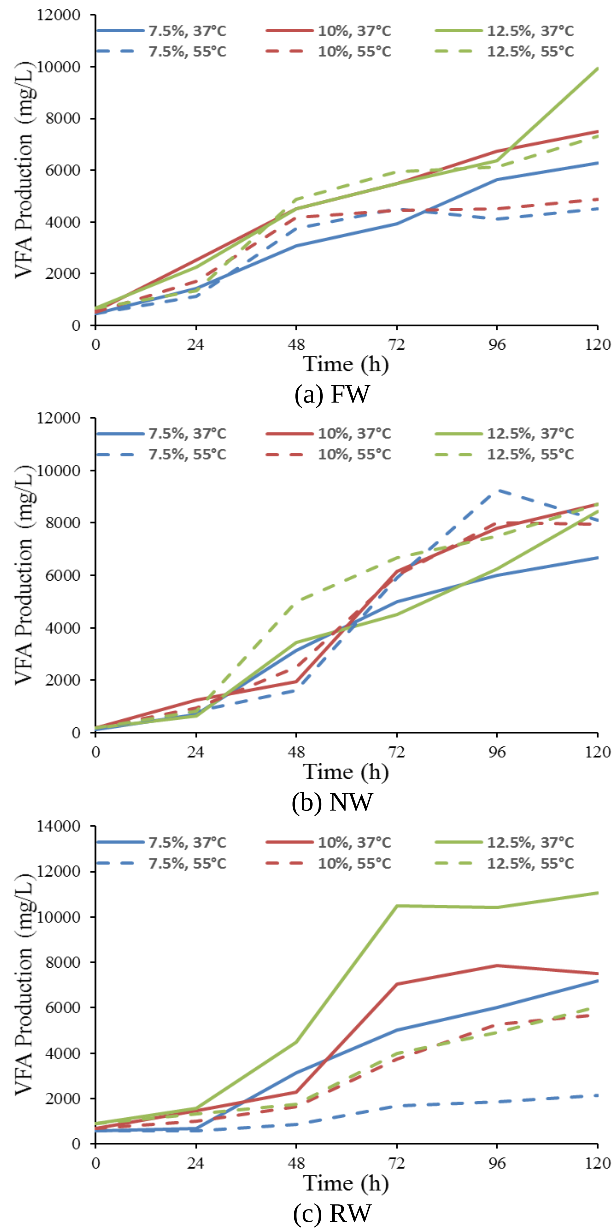


Figure 5. Effect of TS concentration and waste type on VFA production.

The VFA is used as an indicator of biodegradable organics during the fermentation process. The effect of time and temperature on VFA is shown in Figure 5. It was observed that VFA increased with time in all digester as Clostridium mix consortia was used as seed for bio-hydrogen production (Lin *et al.*, 2013). The VFA of FW under mesophilic conditions was higher than those at thermophilic conditions and TS 10% FW produced the least amount of VFA as compared to other two TS levels under both temperatures (Gadow *et al.*, 2012).

The same trend was observed for RW but the increase in VFA of RW with time and temperature was less than FW. In case of NW, the increase in temperature from mesophilic to thermophilic conditions caused an increase in VFA. The increase in VFA during active bio-hydrogen duration (24-48h) was not so high that represent bio-hydrogen as main product of anaerobic digestion. The decrease in bio-hydrogen production after 72h (Fig. 2) is might be conversion of glucose to VFA by homoacetogenic bacteria that reached up to such level where bio-hydrogen production was not feasible (Zhang *et al.*, 2014). The higher concentration of VFA together with low pH can also be inhibitory to bacteria that can cause unfavourable physical changes in the cell that reduce the bio-hydrogen production. By such physical changes, excessive energy is required to pump ions and that energy can be available at higher temperature. So it increased the yield at elevated temperatures, as observed in case of FW and NW (Zoetemeyer RJ, 1982; Gottschalk, 1986; Switzenbaum MS, 1990).

***Conclusion*:** The effect of initial TS concentration (7.5 to 12.5%) on bio-hydrogen production potential on food waste (FW), noodle waste (NW)and rice waste (RW) co-digested with heat shocked sludge under mesophilic and thermophilic conditions were studied. As a whole, the increase in TS concentration from 7.5 to 10% found effective to increase the bio-hydrogen production as a whole. The 7.5% TS concentration represented the higher conversion efficiency of volatile solids to bio-hydrogen as compared to other TS concentrations as the highest experimental bio-hydrogen yield of 95.8mL/VS_{fed} were obtained by 7.5%NW digester under thermophilic conditions. Increase in temperature from mesophilic to thermophilic conditions was increased the bio-hydrogen production from FW and NW, whereas the same increase in temperature found effective mean to reduce VFA production from FW and NW. As a whole, the bio-hydrogen was mostly produce during 24-72h of incubation from all waste under both tested temperatures.

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