

Optimizing the
impact of
temperature on
bio-hydrogen
production

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Optimizing the impact of temperature on bio-hydrogen production from food waste and its derivatives under no pH control using statistical modelling

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The effect of temperature on bio-hydrogen production by co-digestion of sewerage sludge with food waste and its two derivatives, i.e. noodle waste and rice waste, was investigated by statistical modelling. Experimental results showed that increasing temperature from mesophilic (37 °C) to thermophilic (55 °C) was an effective mean for increasing bio-hydrogen production from food waste and noodle waste, but it caused a negative impact on bio-hydrogen production from rice waste. The maximum cumulative bio-hydrogen production of 650 mL was obtained from noodle waste under mesophilic temperature condition. Most of the production was observed during 48 h of incubation that continued till 72 h of incubation, and a decline in pH during this interval was 4.3 and 4.4 from a starting value of 7 under mesophilic and thermophilic conditions, respectively. Most of glucose consumption was also observed during 72 h of incubation and the maximum consumption was observed during the first 24 h, which was the same duration where the maximum pH drop occurred. The maximum hydrogen yields of 82.47 mL VS⁻¹, 131.38 mL COD⁻¹, and 44.90 mL glucose⁻¹ were obtained from mesophilic food waste, thermophilic noodle waste and mesophilic rice waste respectively. The production of volatile fatty acids increased with an increase in time and temperature from food waste and noodle waste reactors whereas it decreased with temperature in rice waste reactors. The statistical modelling returned good results with high values of coefficient of determination (R^2) for each waste type when it was opted for the study of cumulative hydrogen production, glucose consumption and volatile fatty acid production. The 3-D response surface plots developed by the statistical models helped a lot in developing better understanding of the impact of temperature and incubation time.

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1 Introduction

Anaerobic digestion, as a waste management approach, has been in practice for more than one century (McCarty, 1981). It is widely used to treat a variety of solid wastes and wastewater on a small scale as well as on an industrial scale. It has multiple advantages like reduction of 30–50 % of waste volume as well as production of valuable by products like methane and hydrogen (Lin et al., 2011). A large amount of organic fraction of municipal solid waste or food waste (FW) is produced every year in the world. During 2010, production of food waste in China reached to 352 Mt and the major contributor was canteens and restaurants (Tai et al., 2011; Wang Lingen et al., 2013). More than 80 % of food waste consists of the volatile solids (VS) that are biodegradable solids and can be converted into hydrogen or methane easily (Shin et al., 2004; Zhang et al., 2007; Zhu et al., 2008). Several studies on bio-hydrogen production from food waste have shown good results, especially after adding buffers and minerals. Although such addition can maintain a specific pH and nutrimental required for optimum bio-hydrogen production, but it also increases the cost of production (Nielsen AT, 2001; Han HK, 2004). The cost of production can be reduced by adding sewage sludge as a source of *Clostridium* mix culture (Fang et al., 2006). Nutritional deficiency in food waste was also balanced by adding sewage sludge and made food waste suitable for bio-hydrogen production (Shin et al., 2004). It means that integrated waste management can be done at wastewater treatment plant by co-digestion of sewage sludge and food waste.

Carbohydrate-rich wastes like food waste are suitable for *Clostridium* species as stoichiometrically it can produce two mole of hydrogen from one mole of hexose (Payot R, 1998). Theoretically, 553 mL hydrogen can be produced by one gram of polysaccharides if it is totally converted into acetate. The highest practical yield of 346 mL g⁻¹ carbohydrate⁻¹ was achieved by Fang (2006) by using rice as a source of carbohydrate (78 %), pre-treated sewage sludge as a source of *Clostridium* and adding a variety of nutrients. Rice waste (RW) and noodle waste (NW) has 40 % share in food waste produced in China (Shiwei, 2005). The noodle waste is also rich in carbohy-

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this purpose, statistical modelling is an important tool to study the impact within the experimental range and can be further used for the development of response surface plots (Jo et al., 2008).

This study was designed to investigate the impact of temperature on bio-hydrogen production from co-digestion of sewerage sludge with food waste and its carbohydrate-rich derivatives i.e. rice waste and noodle waste with the help of statistical modelling. The response surface methodology was employed to study the impact of time and temperature on bio-hydrogen production, glucose consumption and VFA production. The drop in pH during anaerobic digestion was also studied to find an optimum pH range of bio-hydrogen production from food waste derivatives.

2 Material and methods

2.1 Batch experiment for bio-hydrogen production

The waste was collected from student dining at the Nanjing Agricultural University. It was the food left in the plates after lunch/dinner consisted of rice, noodles, meat, bones, potato and other vegetables. At first bones and other foreign material was removed and left over waste was treated as food waste. It was grounded in a meat grinder with equal amount of water and resultant slurry was used for bio-hydrogen production (Reungsang et al., 2013). Rice and noodles were removed from collected waste and converted into slurry in the same way opted for food waste. The sludge was obtained from a settling channel and it was washed with tap water and sieved to remove foreign materials (Nathao et al., 2013). The sludge was placed in preheated oven at 100 °C for 15 min, so that hydrogenotropic methanogens could be deactivated (Li and Fang, 2007). Some important properties of feed stock and sewerage sludge are enlisted in Table 1.

Two series of experiment were conducted in duplicate by using 550 mL digesters with working volume of 400 mL (Hu et al., 2014). Feedstock and sewerage sludge were added in such an equal proportion to the digesters that the 10 % initial TS concentration

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was maintained. As the pH of food waste was not so high and even after co-digestion with SS, the initial pH within reactor was less than 7 that was carefully raised to 7 with the help of 3 M NaOH solution (Zhu et al., 2008). Series I was to observe the bio-hydrogen production under mesophilic temperature (37 °C) and series II was to investigate the impact of thermophilic temperature (55 °C) on bio-hydrogen production potential of feed stock in comparison with that produced under mesophilic conditions.

2.2 Chemical analysis

The volume of bio-hydrogen produced was measured by displacement of 3 % NaOH solution. This concentration of NaOH can remove other gases and water vapours (Fang et al., 2006; Lin et al., 2013a; Saraphirom and Reungsang, 2010). Other parameters like TS, VS, COD, VFA and alkalinity were measured according to standard methods (APHA, 2005). For glucose detection, phenol sulfuric acid method was used (Lay and Fan, 2003).

2.3 Assay methods

Modelling of hydrogen production was done by Modified Gompertz equation (MGE), which was used for cumulative bio-hydrogen measurement (Ramos et al., 2012)

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

Where H , t , P , R_m , λ and e represent cumulative bio-hydrogen production (mL), incubation time (h), bio-hydrogen production potential, maximum bio-hydrogen production rate (mL h^{-1}), lag phase duration (h) and 2.71828 respectively. The values of H , t , P , R_m were solved by using curve fitting tool in Matlab (2010a).

In this study, the effects of different types of food waste, temperature and incubation time on bio-hydrogen and VFA production as well as on glucose consumption, were

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till 60 h. In fact, mesophilic bio-hydrogen production in noodle waste decreased considerably after 24 h of incubation as compared to thermophilic bio-hydrogen production where the decrease was observed after 36 h of incubation. That 12 h active duration increased the cumulative bio-hydrogen production from noodle waste under thermophilic temperature even after observing the fact that the R_m values are very close to each other. It can be seen in Fig. 2 that the difference in active duration was due to pH as it was dropped from 7 to 4.6 in the noodle waste reactor during the first 12 h under mesophilic temperature and it was further dropped to 4.4 whereas a pH drop in thermophilic reactor was 5.3 to 4.9 in the same duration. On the other end, pH drop in rice waste reactor under thermophilic conditions was higher as compared to mesophilic temperature which ultimately reduced the cumulative bio-hydrogen production that was found in agreement with the finding of Fang et al. (2006). The reduction in bio-hydrogen production with low pH is due to homoacetogenic bacteria which are more active at low pH (Ramos et al., 2012; Schiel-Bengelsdorf and Dürre, 2012). The trend for pH drop with temperature in food waste reactors was opposite to other reactors and R_m increased to a value of 26.42 when temperature was shifted from mesophilic to thermophilic. This increase is due to *Thermoanaerobacterium thermosaccharolyticum* that grow at higher temperature in food waste and produced more hydrogen (Shin et al., 2004). As a whole, R_m values found in present study were higher than those reported in previous studies (Fang et al., 2006; Ramos et al., 2012).

The bio-hydrogen production with time was used to fit in a quadratic model by using solver function MS Excel and the resultant equation obtained was;

$$Y = 202.83 + 56.86x_1 + 73.38x_2 + 8.5x_3 - 22.5x_1^2 + 243.25x_2^2 - 113.8x_3^2 - 23.75x_1x_2 - 1.86x_1x_3 - 30.38a_9x_2x_3 \quad (3)$$

$$(R^2 = 0.5576, \quad F = 19.921)$$

Where Y is the predicted bio-hydrogen production; x_1 , x_2 and x_3 are the coded values of incubation time, waste type and temperature respectively. There is a poor relationship between actual and predicted value as the coefficient of determination (R^2) calculated

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to be 0.5576, which can explain only 55.76 % variability of the response. The diversity among waste type is the main reason for such a low value and this value could be at a higher level if the same waste was used in different proportions as reported in other studies (Kim et al., 2008; Jo et al., 2008). To overcome this problem, quadratic model was again developed for each waste type and following equations were obtained;

$$\text{Food waste: } Y = 281.75 + 57.25x_1 + 62.25x_2 - 22x_1^2 - 25.25x_1x_2 \quad (4a)$$

$$(R^2 = 0.9858 \quad F = 278.06) \quad (4b)$$

$$\text{Noodle waste: } Y = 472.5 + 97.5x_1 + 42.5x_2 - 25x_1^2 + 62.5x_1x_2$$

$$(R^2 = 0.9011 \quad F = 36.44) \quad (4c)$$

$$\text{Rice waste: } Y = 167.5 + 71.5x_1 - 49.5x_2 - 43x_1^2 - 16.5x_1x_2$$

$$(R^2 = 0.7922 \quad F = 15.26)$$

Where x_1 and x_2 are the coded values of time and temperature. All F values represented that the regression model obtained are statistically significant. The F value of food waste is much higher as compared to other F values, which is due to the coefficient of determination that explained 98.58 % variability. The quadratic model obtained from rice waste was not perfectly fit, but it can explain better results as compared to the model developed for all waste types as a whole.

The three dimensional (3-D) response surfaces were developed within the experimental range for each waste type by taking bio-hydrogen as a response by using above mentioned equations. The 3-D curves of the calculated response showed the interaction of incubation time and temperature in Fig. 3a–c. For food waste, it is clear that bio-hydrogen production increases with time and temperature from 115 mL at the starting and ends to 354 mL at the extreme modelled conditions. During 24 to 48 h of incubation of food waste, bio-hydrogen production reduced with the increase in temperature, i.e. 114.5 mL at 37 °C, 79.25 at 46 °C and 10 mL 55 °C. At 24 h of incubation, more bio-hydrogen was produced at higher temperature, i.e. 290 mL at 55 °C, 202.5 mL

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whereas the decrease in P was 78.81 % that represented a decrease in removal of VS at elevated temperature which was in agreement with the findings of Fang et al. (2006). For food waste and noodle waste, the increase in P and yield calculated by VS_{removed} was close, but it revealed the fact that VS removal efficiency slightly decreased at elevated temperature.

When the yield measuring scale was shifted from VS_{removed} to COD_{removed} , the results represent quite different picture as for food waste, the bio-hydrogen yield was increased to 42.39 % when the temperature was increased from 37 to 55 °C that represented a decrease in COD removal efficiency at elevated temperature. For rice waste, the decrease in yield was 61 %, which was close to 78 % decrease in P . Increasing temperature also increased the yield for noodle waste to 20 %, which was smaller than the increase in P , representing a higher rate of COD removal at elevated temperature. All the bio-hydrogen yields calculated on the basis of COD_{removed} lay in the range calculated by Tawfik and El-Qelish (2014).

Glucose removal efficiency for food waste decreased with an increase in temperature as the increase in P was 23.41 % against 42.19 % when bio-hydrogen yield was calculated on the basis of $glucose_{\text{removed}}$. Whereas the change in yield for noodle waste and rice waste was close to the change observed for P . The decrease in glucose concentration was close to that observed in previous studies (Abdeshahian et al., 2014; Kapdan and Kargi, 2006). The yield was further studied on a daily basis and it was observed that the highest yield of 33 mL $glucose_{\text{removed}}^{-1}$ for 0–24 h duration belonged to noodle waste under mesophilic condition. In the next 12 h duration, the highest yield 400 mL $glucose_{\text{removed}}^{-1}$ was achieved by noodle waste under thermophilic temperature, which was close to the finding of Fang et al. (2006) but still smaller than the theoretical yield of 553 mL g – carbohydrate⁻¹. The yield for rice waste also increased under both temperatures but it was much higher at mesophilic as 184.37 mL $glucose_{\text{removed}}^{-1}$ against 24.99 mL $glucose_{\text{removed}}^{-1}$ at thermophilic temperature. During 28 to 72 h of incubation, the yield in all reactors reduced except noodle waste under mesophilic conditions. As a whole, 24–48 h duration of incubation was found more important for bio-hydrogen

production from glucose. The production of glucose was modelled by quadratic equation using previously defined notation as

$$Y = 13.504 - 0.604x_1 + 0.095x_2 - 0.831x_3 + 0.066x_1^2 - 5.469x_2^2 + 0.609x_1x_2 + \quad (5)$$

$$0.238x_1x_3 - 1.131x_2x_3$$

$$5 \quad (R^2 = 0.6959 \quad F = 64.07)$$

As the coefficient of determination is not so high, so quadratic modelling was done for each waste type as

$$\text{Food waste: } Y = 7.820 - 3.561x_1 + 0.412x_2 + 1.554x_1^2 + 1.094x_1x_2 \quad (6a)$$

$$(R^2 = 0.9713 \quad F = 270.81) \quad (6b)$$

$$10 \quad \text{Noodle waste: } Y = 8.697 - 1.601x_1 + 0.055x_2 + 0.439x_1^2 - 0.307x_2$$

$$(R^2 = 0.7994 \quad F = 31.89) \quad (6c)$$

$$\text{Rice Waste: } Y = 21.817 - 3.1x_1 - 0.938x_2 - 0.323x_1^2 - 0.354x_2$$

$$(R^2 = 0.715 \quad F = 20.07)$$

15 The three dimensional response plots for glucose removal were developed by the above models (Fig. 5). It was observed that in the first 24h of incubation, the rate of utilization of glucose was increased with an increase in temperature up to 55°C for food waste, decreased for noodle waste and remained almost unaffected for rice waste. The sequence rate of utilization of glucose was in the rank of NW > FW > RW. During 24–48 h, glucose utilization rate decreased for food waste, increased for noodle waste and rice waste under 37 to 55°C and it was ranked as FW > NW > RW. During 20 48–72 h, the rate of utilization remained the same as before one, but order changed to FW > RW > NW. With an increase in temperature, during 24–72 h, the rate of glucose utilization decreased for food waste, but increased for noodle waste and for rice waste.

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3.3 Effect of temperature on VFA production

The VFA revealed an increase with time as reported by Lin. et al. (2013b), which is shown in Fig. 6. In present study, it is observed that VFA in food waste and noodle waste increased with an increase in temperature from 37 to 55 °C but decreased for rice waste that lay in the range calculated by Shin et al. (2004). It can be seen in Fig. 6 that during 24–48 h, increase in VFA was much higher in food waste under thermophilic conditions as compared to mesophilic condition. During the same interval, bio-hydrogen production was almost ceased in thermophilic food waste reactor whereas it was continuously produced in mesophilic food waste reactor. One of the possible reasons for this reduction is the conversion of glucose at this stage to VFA by homoacetogenic bacteria that reached up to such level where bio-hydrogen production was not feasible under thermophilic conditions, whereas the VFA production in the mesophilic food waste reactor was much smaller than that observed under thermophilic reactor, because of which production continued in the mesophilic reactor (Zhang et al., 2014). The higher concentration of VFA together with low pH can be inhibitory to bacteria that can cause unfavourable physical changes in the cell. 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 food waste and noodle waste (Gottschalk, 1986; Zoetemeyer RJ, 1982; Switzenbaum MS, 1990). The higher concentration of VFA can also be used as an indicator of higher production of bio-hydrogen production as observed by Dong et al. (2009). In the present study, the order to VFA production and cumulative bio-hydrogen production was same i.e. $NW_{55^{\circ}\text{C}} > RW_{37^{\circ}\text{C}} > NW_{37^{\circ}\text{C}} > FW_{55^{\circ}\text{C}} > FW_{37^{\circ}\text{C}} > RW_{37^{\circ}\text{C}}$.

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24–120 h under mesophilic temperature as compared to thermophilic temperature in food waste reactor, but as a whole, mesophilic VFA production was found less than that of thermophilic as reported by Gadow et al. (2012). It is because of fact that during 0–24 h duration, thermophilic VFA production was much higher than that produced under mesophilic conditions in food waste. By controlling the VFA production during this interval, the yield of bio-hydrogen production can be increased for food waste as it stopped too early in thermophilic food waste reactor as compared to mesophilic reactor. Thermophilic VFA production was higher than mesophilic VFA production in the noodle waste reactor. On the other end, mesophilic VFA production was higher than that produced under thermophilic conditions for rice waste and VFA increased with time in the same manner as observed for food waste under 37 to 40 °C. Above 40 to 55 °C, the VFA trend for rice waste remained the same as of food waste till 96 h after which it started to decrease till 120 h.

4 Conclusions

Food waste and its two major derivatives, i.e. noodle waste and rice waste, were co-digested with sewerage sludge to produce bio-hydrogen with an initial pH of 7 under mesophilic and thermophilic conditions. The most effective VS removal was observed in a noodle waste reactor that produced the highest experimental cumulative bio-hydrogen of 656.5 mL under thermophilic conditions. The food waste possessed the highest bio-hydrogen yield calculated on the basis of VS_{removed} , that represents an efficient conversion of VS into bio-hydrogen. The increase in temperature within the studied range, increased the bio-hydrogen production in food waste and noodle waste reactors. The rice waste reactor represented the negative impact of increasing temperature on bio-hydrogen and VFA production. The quadratic modelling returned good results that were close to experimental ones, when it was done for each waste type of bio-hydrogen and VFA production and glucose removal. The response surface plots within the experimental range adequately explained the effect of temperature and

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time on studying parameters and helped to develop better understanding regarding the variation among the studied parameters especially when the different treatments represented similar trends. The VFA production in rice waste reactor changes the trend after 40 °C that was identified only due to quadratic modelling. The lowest limit of pH for bio-hydrogen production was identified as 4.3 and 4.4 for mesophilic and thermophilic temperatures respectively.

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Author contributions. A. Sattar and C. Arslan designed the research and performed all the lab works. C. Ji provided the financial and technical support for designing and conducting research as well as supervised the whole research process. S. Sattar developed and customized 3-D surface plots and assisted in manuscript preparation. K. Yousaf assisted the lab works and analysis. S. Hashim performed the statistical analysis. C. Arslan wrote the manuscript with comments from all authors and A. Sattar finalized the manuscript under the supervision and guidelines of C. Ji.

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Table 1. Properties of test materials.

Parameter	Unit	Sludge	Food waste	Rice waste	Noodles waste
Total Solids	%	58.59	30.32	39.88	31.54
Volatile Solids	%	2.87	26.9	39.30	28.51
Glucose	gL ⁻¹	2.49	65.77	79.65	63.73
Chemical Oxygen Demand	gL ⁻¹	50	147.5	105	132
Total Alkalinity	mgL ⁻¹	3700	550	500	450
Volatile Fatty Acids	mgL ⁻¹	13 950	2475	9000	1500
pH	–	7.1	4.5	5.3	4.3

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Table 2. Kinetic parameters and bio-hydrogen yield.

Waste Type	Temperature	P (mL)	R_m (mL h ⁻¹)	λ (h)	R^2	Hydrogen Yield			
						mL g ⁻¹ VS Fed	mL g ⁻¹ VS Removed	mL g ⁻¹ COD Removed	mL g ⁻¹ glucose Removed
Food waste	37°C	283.7	6.688	6.949	0.9971	17.59	82.47	47.28	18.02
	55°C	350.1	26.42	11.39	0.9965	21.57	80.11	67.33	25.63
Rice waste	37°C	614.3	16.52	31.29	0.9819	32.76	56.36	66.77	44.90
	55°C	130.2	6.325	21.1	0.9997	6.94	29.66	26.04	8.68
Noodle waste	37°C	437.9	21.05	3.047	0.9987	15.26	23.40	109.48	27.65
	55°C	656.9	20.41	5.935	0.9955	22.89	38.15	131.38	40.33

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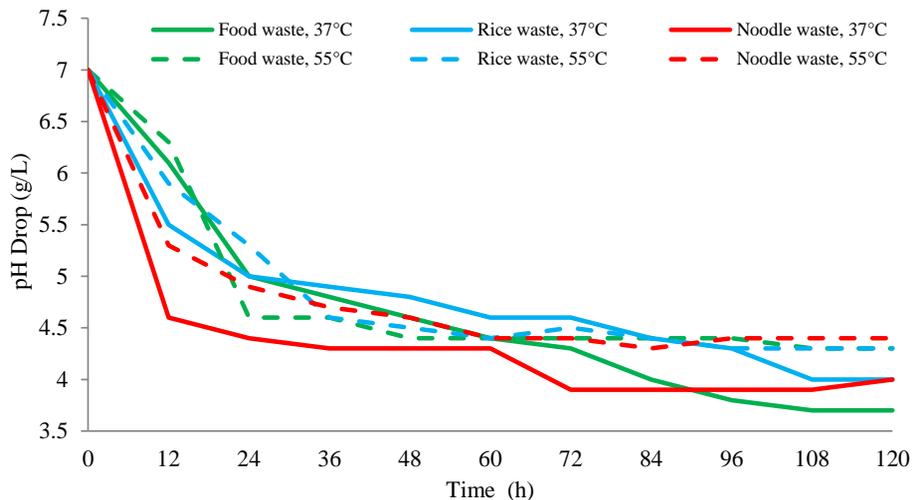


Figure 2. Effect of temperature on pH drop during incubation.

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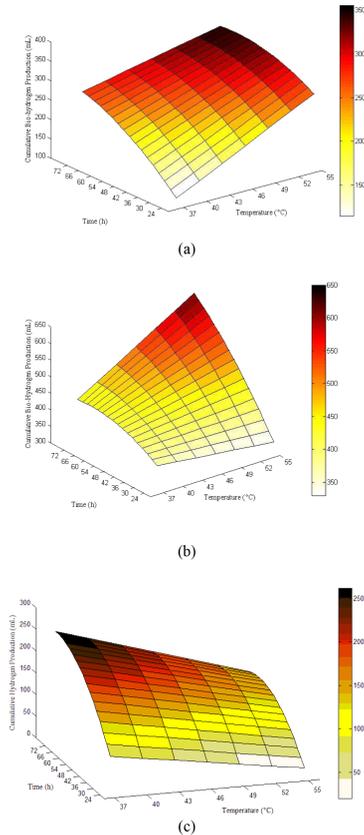


Figure 3. Three dimensional response plots for bio-hydrogen production **(a)** Food waste, **(b)** Noodle waste, **(c)** Rice waste.

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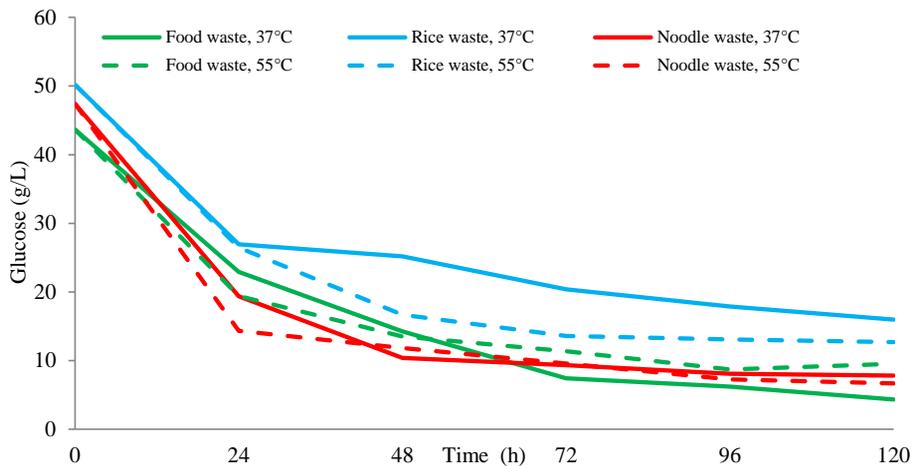


Figure 4. Glucose consumption during incubation under mesophilic and thermophilic temperature.

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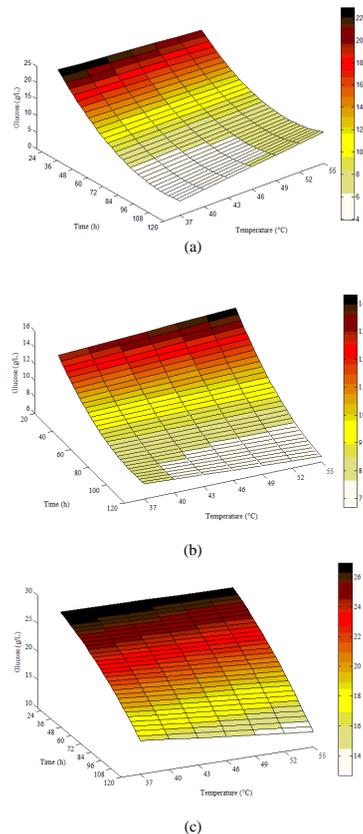


Figure 5. Three dimensional response plots for glucose consumption **(a)** Food waste, **(b)** Noodle waste, **(c)** Rice waste.

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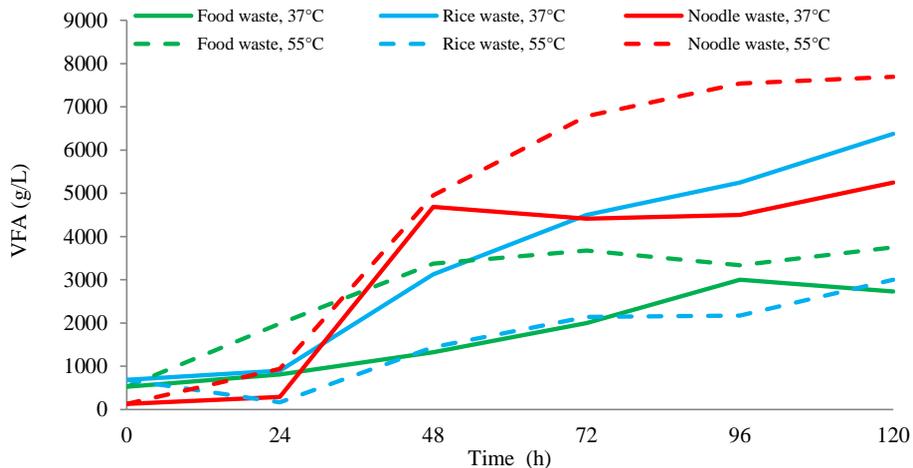


Figure 6. Increase in VFA concentration with time under mesophilic and thermophilic temperatures.

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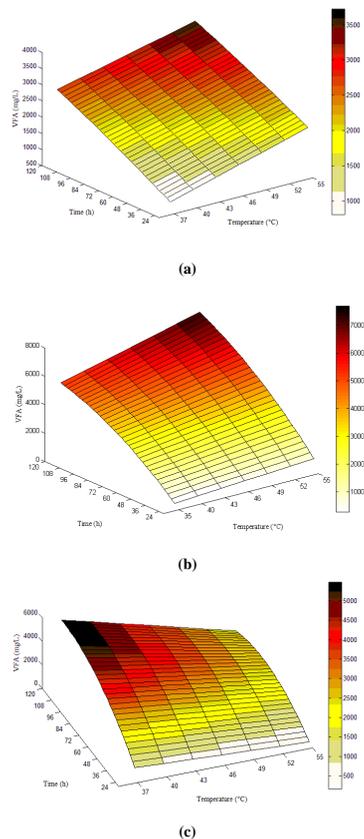


Figure 7. Three dimensional response plots for VFA production (a) Food waste, (b) Noodle waste, (c) Rice waste.