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

Chaudhry Arslan[1,2,¶], Asma Sattar[1,¶], Ji Changying,[1,*], Saba Sattar[3], Khurram Yousaf[1], Sarfraz Hashim[4]

[1] College of Engineering, Nanjing Agricultural University, Nanjing, China
[2] Department of Structures and Environmental Engineering, University of Agriculture, Faisalabad, Pakistan
[3] Environmental Sciences and Engineering, GC University Faisalabad, Pakistan
[4] Department of Hydrology and Water Resources, Hohai University, Nanjing, China
[¶] Equal contribution
*For Correspondence: Professor Ji Changying, Department of Agricultural Mechanization, Engineering College, Nanjing Agricultural University, Nanjing, China, Email: chyji@njau.edu.cn, arslanakrampk@hotmail.com, Ph:0086-25-5860657

Abstract:

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 thermophilic temperature condition. Most of the production was observed during first 48 hours of incubation, that continued till 72 hours of incubation and 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 hours of incubation and the maximum consumption was observed during the first 24 hours, which was the same duration where the maximum pH drop occurred. The maximum hydrogen yields of 82.47 mL/VS, 131.38 mL/COD, and 44.90 mL/glucose were obtained from thermophilic food waste, thermophilic noodle waste and mesophilic rice waste, respectively. The production of volatile fatty acids increased with an increase in time and temperature in food waste and noodle waste reactors whereas they 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 and 3-D response surface plots developed by using models developed. These plots developed better understanding regarding the impact of temperature and incubation time on bio-hydrogen production trend, glucose consumption during incubation and Volatile fatty acids production.

1 Introduction

Anaerobic digestion , as a waste management approach, has been in practice for more than a 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 30-50 % reduction in waste volume as well as production of valuable by products such as methane and hydrogen (Lin et al., 2011). A large amount of organic fraction of municipal solid waste or food waste 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). The food waste contains more than 80% 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 represent an increase in bio-hydrogen production from food waste due to addition of 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 that made food waste more 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. Although sewerage sludge is a good source of *Clostridium* mix culture, but at the same time, it contains hydrogen consumers like methanogens. Heat treatment is mostly opted to deactivate hydrogen consumers. The traditional method of placing sewage sludge in boiling water is now no longer in practice and replaced by microwave heating that provide more uniform heating as compared to the boiling water method (Luo et al., 2010;Wang et al., 2011;Duangmanee et al., 2007). The temperature and time for heat treatment varied from 75°C to 121°C for 15 minutes to 2 hours, but 100°C for 15 minutes was mostly reported (Li and Fang, 2007;Fang et al., 2006).

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-carbohydrate 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 and noodle waste has 40% share in the total food waste produced in China (Shiwei, 2005). The noodle waste is also rich in carbohydrates, but still there is no research reported on bio-hydrogen production from noodle waste.

Temperature and pH have great impact on the smooth running of AD (Saraphirom and Reungsang, 2010). Most of the studies reported bio-hydrogen production under mesophilic as well as thermophilic conditions and few were reported under psychrophilic conditions. Lu et al. (2011) developed a microbial electrolysis cells (MECs) that could be operated at 9˚C by using *Geobacter psychrophilus* as dominating population and achieved hydrogen yield of $0.62m^3H_2m^{-3}d^{-1}$. Heidrich et al. (2013) further modified MECs to a pilot-scale MEC and achieved bio-hydrogen production of 0.015 $LH_2L^{-1}d^{-1}$ at 25˚C. On the other end, under mesophilic and thermophilic conditions, there is no need of such sophisticated technology and a better bio-hydrogen yield can be achieved by simple reactors or by lab scale batch experiments. The temperature shift from mesophilic to thermophilic conditions can change the rate of hydrogen production during anaerobic digestion (Li and Liu, 2012;Saripan and Reungsang, 2014). Whereas, hyper-thermophilic provide better pathogenic destruction but it also decreased the bio-hydrogen production (Sahlström, 2003;Yokoyama et al., 2007). Keeping the same temperature but changing the initial pH from 7 to 8, the bio-hydrogen yield was changed from 64.48 mL/VS to 55 ml/VS under no pH control conditions (Lin et al., 2013b;Nathao et al., 2013). The same yield was increased to 70 mL/VS when pH was manually controlled for food waste under thermophilic conditions, which represents the impact of pH management (Shin et al., 2004). The hydrogen production by anaerobic digestion will be further improved if pH lies in the range of 5 to 6 (Radjaram and Saravanane, 2011). The pH of food waste lies in the range of 4-5, which further decreases by the production of volatile fatty acids (VFA) to such a level that can inhibit the bacterial growth. The pH can be controlled by automatic pH controllers, addition of nutrients and buffers, manual monitoring and control (Yasin et al., 2011;Zhu et al., 2008;Kim et al., 2004). But all these methods increased the cost of operation. Along with cost, maintaining pH at specific point is not suitable especially when mix culture is used as the response of different microbial stream could be different to same pH level. So, by co-digestion, the pH of the anaerobic digestion process can be improved and it can be further adjusted to a desired initial value by adding HCl or NaOH. After adjusting the desired initial pH under co-digested conditions, the bio-hydrogen production can be achieved under no pH control conditions, which can reduce the cost of operation (Fang et al., 2006).

The yield of bio-hydrogen is calculated by dividing the cumulative hydrogen produced by VS, chemical oxygen demand (COD) or glucose (Chen et al., 2006;Dong et al., 2009;Fang et al., 2006). The yields are misleading if calculated in term of added or start up values of VS, COD and glucose as it seems quite impossible that the whole of added material is converted into hydrogen. In this regard the removal quantities of such parameters are the best option to calculate the yield.

The optimization played an important role in bio-hydrogen production and its application with respect to incubation time in combination with temperature is an important factor to get the maximum output with minimum intake of energy. In order to achieve 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 pH during incubation was not controlled and the drop of 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 were removed and left over waste was treated as food waste. The food waste was then 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 minutes, 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 experiments were conducted in duplicate in 550 mL digesters with working volume of 400 mL (Hu et al., 2014). In order to achieve 10% initial TS concentration, water was added along with feedstock and sewerage sludge in the digesters. The feedstock and sewerage sludge were added in equal proportion. As the pH of food waste was not so high and even after co-digestion with sewage sludge, the initial pH within reactor was less than 7 that was carefully raised to 7 with the help of 3M 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 sulphuric 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)

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

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), 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 (2010 a).

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 analysed by full quadratic model as shown below (Kim et al., 2008;Jo et al., 2008)

$$\mathrm{Y} = a_0 + \sum_{i=1}^{n} a_i X_i + \sum_{i=1}^{n} a_{ii} X_i^2 + \sum_{i=1}^{n}\sum_{i<j=2}^{n} a_{ij}\, X_i X_j \qquad (2)$$

Where $X_i$and X_j are the controlled parameters, which influence Y and a_0, a_{ii}, a_{ij}, are the offset term, linear and quadratic coefficients respectively. As the waste types are different, so the above model is used, including waste type (n=3) and excluding waste type (n=2).

3 Results and Discussion

3.1 Effect of Temperature and time on Bio-hydrogen Production

A comparison of actual and MGE modelled bio-hydrogen production under mesophilic and thermophilic conditions is shown in Fig.1, which shows an early start of bio-hydrogen production in food waste and noodle waste as compared to rice waste. This early production was quantified with the help of MGE as λ shown in Table 2, which clearly shows that the highest lag phase belongs to rice waste under both temperatures. The bio-hydrogen production in food waste continued till 72 hours of incubation and this time period for food waste was higher than that observed for noodle waste, but still the cumulative bio-hydrogen production of food waste was the lowest as compared to other two waste types. Looking at the R_m values, it is clear that food waste has the lowest mesophilic R_m value of 6.688 mL/h among all three wastes that ultimately caused the cumulative bio-hydrogen production to decrease. Only the R_m value is not responsible for higher yield as the highest mesophilic R_m of 21.05 mL/h belongs to noodle waste, but the cumulative bio-hydrogen production of noodle waste was found smaller than rice waste having R_m value of 16.52 mL/h. The rice waste has higher production because it produced bio-hydrogen for 96 hours as compared to noodle waste that produced till 60 hours. In fact, mesophilic bio-hydrogen production in noodle waste decreased considerably after 24 hours of incubation as compared to thermophilic bio-hydrogen production where the decrease was observed after 36 hours of incubation. That's 12 hours 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 hours 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 mL/h 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 volume of 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, 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) was calculated 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;

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

(R^2 = 0.9858 F = 278.06)

Noodle waste: $$Y = 472.5 + 97.5x_1 + 42.5x_2 - 25x_1^2 + 62.5x_1x_2 \quad (4b)$$

(R^2 = 0.9011 F = 36.44)

Rice waste: $$Y = 167.5 + 71.5x_1 - 49.5x_2 - 43x_1^2 - 16.5x_1x_2 \quad (4c)$$

(R^2 = 0.7922 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 values; it is because of 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 and two dimensional (2-D) contours were developed within the experimental range for each waste type by taking bio-hydrogen production as a response by using above mentioned equations. The 3-D and 2-D curves of the calculated response showed the interaction of incubation time and temperature in figure 3a-c. For food waste, it is clear that the gas production increases with time and temperature from 115 mL at the starting end to 354 mL at the extreme modelled conditions. During 0-24 hours of incubation, bio-hydrogen production increased with increase in temperature for food waste, i.e. 115 mL of bio-hydrogen was produced at 37°C that increased 76.09 % and 152.17% at 46°C and 55°C, respectively. During next 24 hours of incubation, bio-hydrogen production reduced with the increase in temperature, i.e. 114.5 ml bio-hydrogen was produced at 37°C and 30.78% and 91.22% reduction was observed at 46°C and 55°C, respectively. Even after reduction in bio-hydrogen production during 24-48 hours of incubation, the cumulative bio-hydrogen production increased with an increase in temperature from food waste. The impact of temperature and time can be bettered viewed in 2-D contour (Fig. 3a), which shows that the increase in temperature increases bio-hydrogen production more at 24h as compared to 72h of incubation. It also revealed the fact that first 24 hours are important for bio-hydrogen production from food waste under thermophilic temperatures and next 24 are important for production under mesophilic temperature, which is in agreement with findings of Shin et al (2004). Although noodle waste also produced more bio-hydrogen at elevated temperature, but the time effect was opposite to that observed for food waste. The bio-hydrogen production in noodle waste during 0-24 hours was 350mL at 37°C that was 5.4% and 10.81% decreased at 46°C and 55°C, respectively. But in next 24-72 hours, 178.57% and 357.14% increase at 46°C at 55°C, respectively.

So far rice waste was concerned; temperature has a negative impact on bio-hydrogen production. The bio-hydrogen production in rice waste during 24-48h was 131 mL, 114.5 mL and 98 mL, which was 65.65% , 75.11% and 87.76% reduced during 48-72h under 37°C, 46°C and 55°C, respectively. The reduction in bio-hydrogen production for rice waste was in agreement with previous findings (Fang et al., 2006). The 2-D contours in fig. 3b and 3c differentiate the impact of temperature with time on bio-hydrogen production for noodle waste and rice waste as the contour patterns are quite opposite to each other.

3.2 Effect of temperature on Bio-hydrogen Yield

The bio-hydrogen yield was calculated by dividing the P value on table 2 with VS_{fed}, $VS_{removed}$, $COD_{removed}$ and $glucose_{removed}$. The bio-hydrogen yield calculated on the basis of VS_{fed} lay in the range achieved by Lin et al. (2013b) and temperature impact on yield was same as observed for P. The yield on the basis of $VS_{consumed}$ represented uptake efficiency of VS during anaerobic digestion. The increase in P with temperature for food waste was 23.41% whereas the yield increased by 2.86% only that indicated the efficient removal of VS at higher temperature. The bio-hydrogen yield calculated for FW on the basis of $VS_{removed}$ lay in the range used by Kim et al. (2004). Using the same scale of $VS_{removed}$, bio-hydrogen yield for rice waste decreased 47.37% with an increase in temperature, 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). As compared to 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 of temperature impact. The increase in temperature from 37°C to 55°C increased 42.41% bio-hydrogen yield calculated on the basis of $COD_{removed}$ for food waste. The increase in bio-hydrogen production due to same increase in temperature from 37°C to 55°C was 23.37%. Such difference in yield and production increment represented decrease in COD removal efficiency at elevated temperature for food waste. 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 $glucose_{removed}$. 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 calculated on glucose basis was further studied on daily basis and it was observed that the highest yield of 33 mL/ $glucose_{removed}$ for 0-24h duration belonged to noodle waste under mesophilic condition. During the next 24h duration, the highest yield 400 mL/ $glucose_{removed}$ 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. The yield for rice waste also increased under both temperatures but it was much higher at mesophilic as 184.37 mL/ $glucose_{removed}$ against 24.99 mL/ $glucose_{removed}$ at thermophilic temperature. During 24 to 72 hours of incubation, the yield in all reactors reduced except noodle waste under mesophilic conditions. As a whole, 24-48h duration of incubation was found more important for bio-hydrogen production from glucose. The production of glucose modelled by quadratic equation using previously defined notation as

$$Y = 13.504 - 0.604x_1 + 0.095x_2 - 0.831x_3 + 0.066x_1^2 - 5.469x_2^2 + 0.609x_1x_2 + 0.238x_1x_3 - 1.131x_2x_3 \quad (5)$$

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

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

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

($R^2 = 0.9713$ F = 270.81)

Noodle waste: $Y = 8.697 - 1.601x_1 + 0.055x_2 + 0.439x_1^2 - 0.307x_1x_2$ (6b)

($R^2 = 0.7994$ F = 31.89)

Rice Waste: $Y = 21.817 - 3.1x_1 - 0.938x_2 - 0.323x_1^2 - 0.354x_1x_2$ (6c)

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

The three dimensional response plots and contours for glucose removal were developed by the above models (Figure 5). It was observed that in the first 24 hours 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 for glucose utilization rate was in the rank of NW>FW>RW. During 24-48h, glucose utilization rate decreased for food waste and increased for noodle and rice waste under mesophilic and thermophilic conditions. During 48-72h, rate of utilization remained same as previous one but rank was slightly changed as FW>RW>NW. With an increase in temperature, during 24-72h, the rate of glucose utilization decreased for food waste but increased for noodle and rice waste. As a whole, the glucose consumption at the end of incubation was higher at 37°C as compared to 55°C for food waste. At the end of incubation, noodle waste and rice waste represented quite opposite picture of glucose consumption with temperature as observed for food waste. The contours represented better understanding for glucose consumption and the contour varied in different manners for each waste type as shown in figure 5.

3.3 Effect of temperature on VFA production

The VFA revealed an increase with time as ~~also~~ reported by Lin et al. (2013b), which is shown in figure 6. In the present study, it is observed that VFA in food waste and noodle waste increased with an increase in temperature from 37°C to 55°C but decreased for rice waste that lay in the range calculated by Shin et al. (2004). It can be seen in figure 6 that during 24-48h, increase in VFA was much higher in food waste under thermophilic conditions as compared to mesophilic conditions. During the same interval, bio-hydrogen production was almost ceased in thermophilic food waste reactor whereas it was continuously producing in mesophilic food waste reactor. One of the possible reasons for this reduction is the conversion of glucose to VFA at this stage 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 FW 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 for higher production of bio-hydrogen 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. $NW_{55^\circ C}$>$RW_{37^\circ C}$ >$NW_{37^\circ C}$ >$FW_{55^\circ C}$ >$FW_{37^\circ C}$ >$RW_{37^\circ C}$.

The quadratic model was tried to fit for VFA production data in the same way as opted for glucose and the resultant equation was as follows;

$$Y = 1795.82 + 686.16x_1 - 300.51x_2 + 641.13x_3 + 4.94x_1^2 + 658.51x_2^2 + 127.30x_1x_2 + 33.26x_1x_3 - 319.25x_2x_3 \quad (7)$$

(R^2 = 0.5975 F = 41.56)

Here, again the coefficient of determination is not so high due to the variability of waste type, so the model was repeated for each waste as;

Food waste: $Y = 2362.67 + 458.15x_1 + 545.05x_2 - 12.88x_1^2 + 2.76x_2^2 - 22.42x_1x_2$

(R^2 = 0.8654 F = 51.44) (8a)

Noodle waste: $Y = 4196.48 + 1464.56x_1 + 772.876x_2 - 163.19x_1^2 + 224.98x_1x_2$

(R^2 = 0.8415 F = 42.48) (8b)

Rice Waste: $Y = 3258.94 + 756.62x_1 - 1147.22x_2 - 303.1x_1^2 - 390.28x_1x_2$

(R^2 =0.9430 F = 132.37). (8c)

The three dimensional response plots and contours for VFA production on the basis of above models are shown in figure 7. The 3-D contours for food waste and noodle waste seems almost same but the contour lines for both varied in different manner. Although the production of VFA increased with time and temperature in all reactors but the intensity of change is different for each waste type as observed in figure 7. It can be observed from figure 7 that the production of VFA has increased for food waste and noodle waste when temperature was increased from 37°C to 55°C. Although with time, the VFA concentration has increased, but the rate by which VFAs produced was decreased with time, i.e. VFA production during 24-48h was greater than that produced during 48-72h and this trend continued till 120h for food waste and noodle waste. As a whole, more VFA was produced during 24-120h 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 the fact that during 0-24h 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 can be increased for FW as it stopped too early in thermophilic food waste reactor as compared to mesophilic food waste reactor. Thermophilic VFA production was higher than mesophilic VFA production in noodle waste reactor. On the other end, mesophilic VFA production was higher than that produced under thermophilic conditions for RW and VFA increased with time in the same manner as observed for food waste under 37°C to 40°C. Above 40°C to 55°C, the VFA trend for rice waste remained the same as of food waste till 96h after which it started to decrease till 120h.

4 Conclusion:

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 pH was not controlled throughout the incubation. The most effective VS removal was observed in 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. Thermophilic conditions should be preferred for bio-hydrogen production as most of the time food waste is used as feed stock. The quadratic modelling returned good results that were close to experimental ones, when it was done for each waste type of bio-hydrogen, VFA production and glucose removal. The response surface plots and contour plots within the experimental range adequately explained the effect of temperature and 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. 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.

Author contributions. C. Arslan and A. Sattar 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.

Acknowledgements. We thank K. Chen for providing lab facilities; H. Fang, Y. P. Huang and J. B. Kang for their help during lab work; I. A. Mari for field work and technical assistance; F. A. Chandio and F. Ahmad for helpful discussion and critical review. We extend our thank to Higher Education Commission, Pakistan, Chines Scholarship council and the College of Engineering, Nanjing Agricultural University, Nanjing for supporting and providing research facilities for this study.

References

Abdeshahian, P., Al-Shorgani, N. K. N., Salih, N. K., Shukor, H., Kadier, A., Hamid, A. A., and Kalil, M. S.: The production of biohydrogen by a novel strain Clostridium sp. YM1 in dark fermentation process, International Journal of Hydrogen Energy, 39, 12524-12531, 2014.

APHA: Standard Methods for the Examination of Water and Wastewater., 25 ed., American Public Health Association, Washington, DC, 94-100 pp., 2005.

Chen, W.-H., Chen, S.-Y., Kumar Khanal, S., and Sung, S.: Kinetic study of biological hydrogen production by anaerobic fermentation, International Journal of Hydrogen Energy, 31, 2170-2178, 2006.

Chu, C.-F., Li, Y.-Y., Xu, K.-Q., Ebie, Y., Inamori, Y., and Kong, H.-N.: A pH-and temperature-phased two-stage process for hydrogen and methane production from food waste, international journal of hydrogen energy, 33, 4739-4746, 2008.

Dong, L., Zhenhong, Y., Yongming, S., Xiaoying, K., and Yu, Z.: Hydrogen production characteristics of the organic fraction of municipal solid wastes by anaerobic mixed culture fermentation, International Journal of Hydrogen Energy, 34, 812-820, 2009.

Duangmanee, T., Padmasiri, S., Simmons, J., Raskin, L., and Sung, S.: Hydrogen production by anaerobic microbial communities exposed to repeated heat treatments, Water Environ. Res., 79, 975-983, 2007.

Fang, H. H., Li, C., and Zhang, T.: Acidophilic biohydrogen production from rice slurry, International Journal of Hydrogen Energy, 31, 683-692, 2006.

Gadow, S., Li, Y.-Y., and Liu, Y.: Effect of temperature on continuous hydrogen production of cellulose, International Journal of Hydrogen Energy, 37, 15465-15472, 2012.

Gottschalk, G.: Bacterial metabolism, 2nd ed., Springer, New York, 1986.

Han HK, S. H.: Performance of an innovative two-stage process converting food waste to hydrogen and methane, J. Air Waste Manage. Assoc., 54, 242–249, 2004.

Heidrich, E., Dolfing, J., Scott, K., Edwards, S., Jones, C., and Curtis, T.: Production of hydrogen from domestic wastewater in a pilot-scale microbial electrolysis cell, Applied microbiology and biotechnology, 97, 6979-6989, 2013.

Hu, C. C., Giannis, A., Chen, C.-L., and Wang, J.-Y.: Evaluation of hydrogen producing cultures using pretreated food waste, International Journal of Hydrogen Energy, 39, 19337-19342, 2014.

Jo, J. H., Lee, D. S., Park, D., Choe, W.-S., and Park, J. M.: Optimization of key process variables for enhanced hydrogen production by Enterobacter aerogenes using statistical methods, Bioresource technology, 99, 2061-2066, 2008.

Kapdan, I. K., and Kargi, F.: Bio-hydrogen production from waste materials, Enzyme and microbial technology, 38, 569-582, 2006.

Kim, S.-H., Han, S.-K., and Shin, H.-S.: Feasibility of biohydrogen production by anaerobic co-digestion of food waste and sewage sludge, International Journal of Hydrogen Energy, 29, 1607-1616, 2004.

Kim, S.-H., Han, S.-K., and Shin, H.-S.: Optimization of continuous hydrogen fermentation of food waste as a function of solids retention time independent of hydraulic retention time, Process Biochem., 43, 213-218, 2008.

Lay, J.-J., and Fan, K.-S.: Influence of chemical nature of organic wastes on their conversion to hydrogen by heat-shock digested sludge, International Journal of Hydrogen Energy, 28, 1361-1367, 2003.

Li, C., and Fang, H. H.: Fermentative hydrogen production from wastewater and solid wastes by mixed cultures, Critical Reviews in Environmental Science and Technology, 37, 1-39, 2007.

Li, Q., and Liu, C.-Z.: Co-culture of Clostridium thermocellum and Clostridium thermosaccharolyticum for enhancing hydrogen production via thermophilic fermentation of cornstalk waste, International Journal of Hydrogen Energy, 37, 10648-10654, 2012.

Lin, Y., Wang, D., Li, Q., and Xiao, M.: Mesophilic batch anaerobic co-digestion of pulp and paper sludge and monosodium glutamate waste liquor for methane production in a bench-scale digester, Bioresource technology, 102, 3673-3678, 2011.

Lin, Y., Liang, J., Wu, S., and Wang, B.: Was pretreatment beneficial for more biogas in any process? Chemical pretreatment effect on hydrogen–methane co-production in a two-stage process, Journal of Industrial and Engineering Chemistry, 19, 316-321, 2013a.

Lin, Y., Wu, S., and Wang, D.: Hydrogen-methane production from pulp & paper sludge and food waste by mesophilic–thermophilic anaerobic co-digestion, International Journal of Hydrogen Energy, 38, 15055-15062, 2013b.

Lu, L., Ren, N., Zhao, X., Wang, H., Wu, D., and Xing, D.: Hydrogen production, methanogen inhibition and microbial community structures in psychrophilic single-chamber microbial electrolysis cells, Energy & Environmental Science, 4, 1329-1336, 2011.

Luo, G., Xie, L., Zou, Z., Wang, W., Zhou, Q., and Shim, H.: Anaerobic treatment of cassava stillage for hydrogen and methane production in continuously stirred tank reactor (CSTR) under high organic loading rate (OLR), International Journal of Hydrogen Energy, 35, 11733-11737, 2010.

McCarty, P. L.: One hundred years of anaerobic treatment, Anaerobic digestion, 1981.

Mizuno O, S. M., Suzuki K, Yaguchi J, Noike T. E: Effect of pH on Hydrogen Production from Noodle Manufacturing Wastewater, Environ Eng Res (in Japanese), 37, 97-106, 2000.

Nathao, C., Sirisukpoka, U., and Pisutpaisal, N.: Production of hydrogen and methane by one and two stage fermentation of food waste, International Journal of Hydrogen Energy, 38, 15764-15769, 2013.

Nielsen AT, A. H., Bjorklund R, Dannetun H, Ejlertsson J, Ekedahl LG: Hydrogen production from organic waste, Int. J. Hydrogen Energy, 26, 547–550, 2001.

Okamoto, M., Miyahara, T., Mizuno, O., and Noike, T.: Biological hydrogen potential of materials characteristic of the organic fraction of municipal solid wastes, Water Science and Technology, 41, 25-32, 2000.

Payot R, G. E., Cailliez C, Gelhage E, Petitdemange H: Metabolism of cellobiose by Clostridium cellulolyticum growing in continuous culture: evidence for decreased NADH reoxidation as a factor limiting growth, Microbiology, 144, 375–384, 1998.

Radjaram, B., and Saravanane, R.: Assessment of optimum dilution ratio for biohydrogen production by anaerobic co-digestion of press mud with sewage and water, Bioresource technology, 102, 2773-2780, 2011.

Ramos, C., Buitrón, G., Moreno-Andrade, I., and Chamy, R.: Effect of the initial total solids concentration and initial pH on the bio-hydrogen production from cafeteria food waste, International Journal of Hydrogen Energy, 37, 13288-13295, 2012.

Reungsang, A., Sreela-or, C., and Plangklang, P.: Non-sterile bio-hydrogen fermentation from food waste in a continuous stirred tank reactor (CSTR): Performance and population analysis, International Journal of Hydrogen Energy, 38, 15630-15637, 2013.

Sahlström, L.: A review of survival of pathogenic bacteria in organic waste used in biogas plants, Bioresource technology, 87, 161-166, 2003.

Saraphirom, P., and Reungsang, A.: Optimization of biohydrogen production from sweet sorghum syrup using statistical methods, International Journal of Hydrogen Energy, 35, 13435-13444, 2010.

Saripan, A. F., and Reungsang, A.: Simultaneous saccharification and fermentation of cellulose for bio-hydrogen production by anaerobic mixed cultures in elephant dung, International Journal of Hydrogen Energy, 39, 9028-9035, 2014.

Schiel-Bengelsdorf, B., and Dürre, P.: Pathway engineering and synthetic biology using acetogens, FEBS Lett., 586, 2191-2198, 2012.

Shin, H.-S., Youn, J.-H., and Kim, S.-H.: Hydrogen production from food waste in anaerobic mesophilic and thermophilic acidogenesis, International Journal of Hydrogen Energy, 29, 1355-1363, 2004.

Shiwei, X.: Analysis of China food consumption and waste, food and nutrition in China 11, 4-8, 2005.

Switzenbaum MS, G.-G. E., Hickey RF: Monitoring of the anaerobic methane fermentation process. Enzyme Microbial Technology, Enzyme Microbial Technology 12, 722–730, 1990.

Tai, J., Zhang, W., Che, Y., and Feng, D.: Municipal solid waste source-separated collection in China: A comparative analysis, Waste management, 31, 1673-1682, 2011.

Tawfik, A., Salem, A., and El-Qelish, M.: Two stage anaerobic baffled reactors for bio-hydrogen production from municipal food waste, Bioresource technology, 102, 8723-8726, 2011.

Tawfik, A., and El-Qelish, M.: Key factors affecting on bio-hydrogen production from co-digestion of organic fraction of municipal solid waste and kitchen wastewater, Bioresource technology, 2014.

Wang Lingen , Cheng Shengkui , LI Qunji , and Zengrang, X.: Tourist Dining Behavior in Lhasa City, Resources Science, 35, 848-857, 2013.

Wang, Y.-Y., Ai, P., Hu, C.-X., and Zhang, Y.-L.: Effects of various pretreatment methods of anaerobic mixed microflora on biohydrogen production and the fermentation pathway of glucose, International Journal of Hydrogen Energy, 36, 390-396, 2011.

Wongthanate, J., and Chinnacotpong, K.: Optimal Conditons for Biological Hydrogen Production from Food Waste, Environ. Eng. Res, 2015.

Yasin, N. H. M., Man, H. C., Yusoff, M. Z. M., and Hassan, M. A.: Microbial characterization of hydrogen-producing bacteria in fermented food waste at different pH values, international journal of hydrogen energy, 36, 9571-9580, 2011.

Yokoyama, H., Waki, M., Moriya, N., Yasuda, T., Tanaka, Y., and Haga, K.: Effect of fermentation temperature on hydrogen production from cow waste slurry by using anaerobic microflora within the slurry, Applied microbiology and biotechnology, 74, 474-483, 2007.

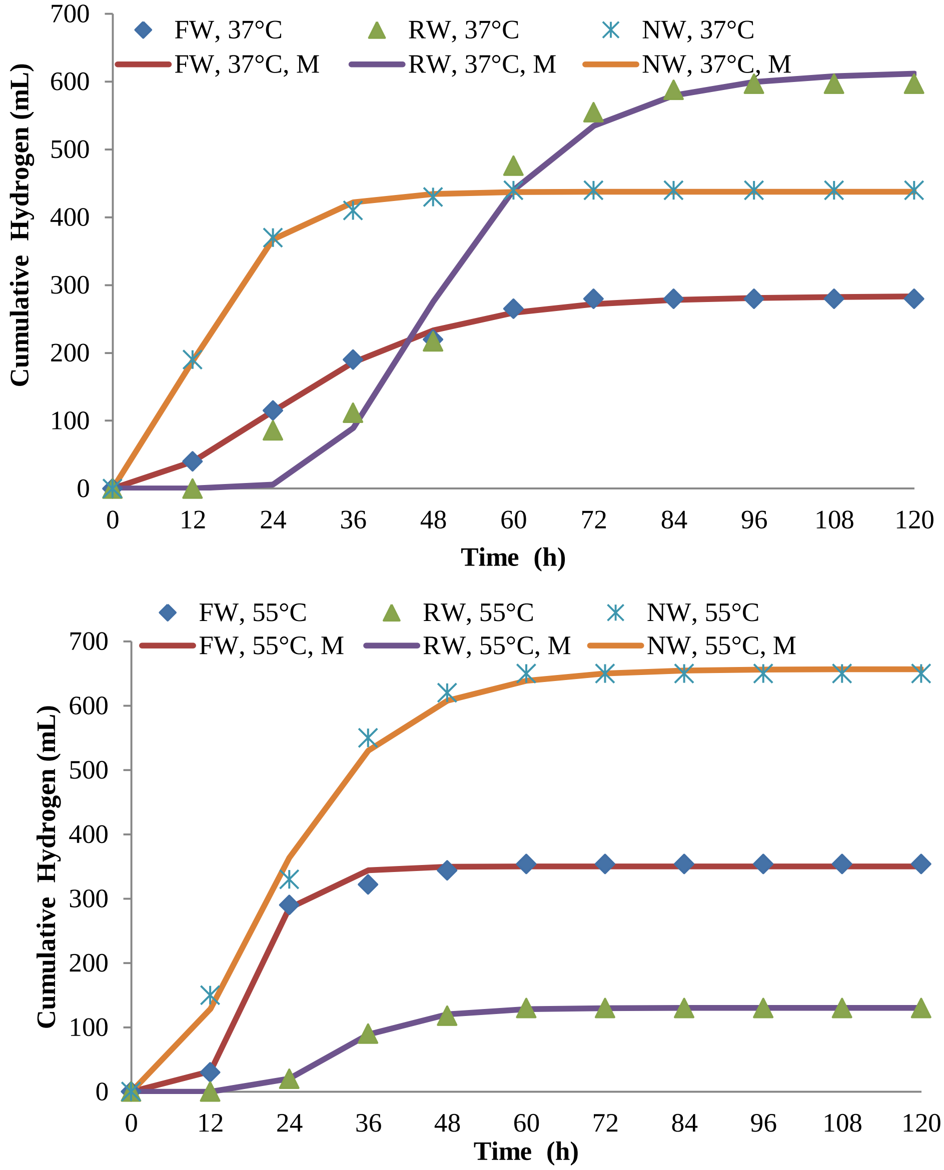
Zhang, L., Ban, Q., Li, J., and Xu, Y.: Assessment of Effects of Yeast Extract on Bio-hydrogen Production from Anaerobic Activated Sludge, Int. J. Agric. Biol, 16, 1189-1193, 2014.

Zhang, R., El-Mashad, H. M., Hartman, K., Wang, F., Liu, G., Choate, C., and Gamble, P.: Characterization of food waste as feedstock for anaerobic digestion, Bioresource technology, 98, 929-935, 2007.

Zhu, H., Parker, W., Basnar, R., Proracki, A., Falletta, P., Béland, M., and Seto, P.: Biohydrogen production by anaerobic co-digestion of municipal food waste and sewage sludges, International Journal of Hydrogen Energy, 33, 3651-3659, 2008.

Zoetemeyer RJ, M. A., Cohen A, Boelhouwer C: Product inhibition in the acid forming stage of the anaerobic digestion process, Water Research 16, 633-639, 1982.

FIGURES



*M stands for modelled bio-hydrogen production on the basis of Modified Gompertz Equation

Figure 1. Mesophilic and thermophilic Bio-Hydrogen Production with time

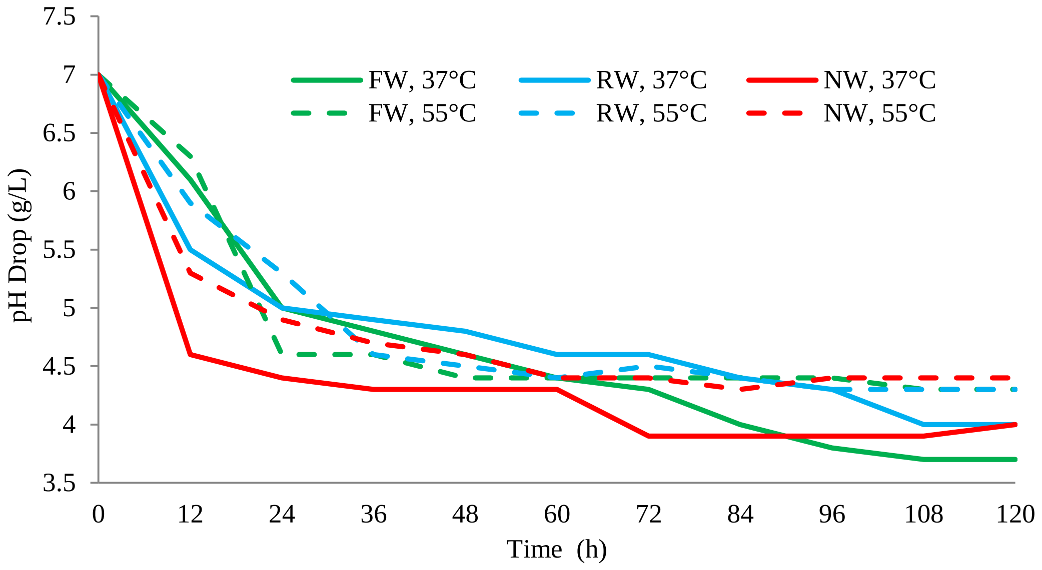
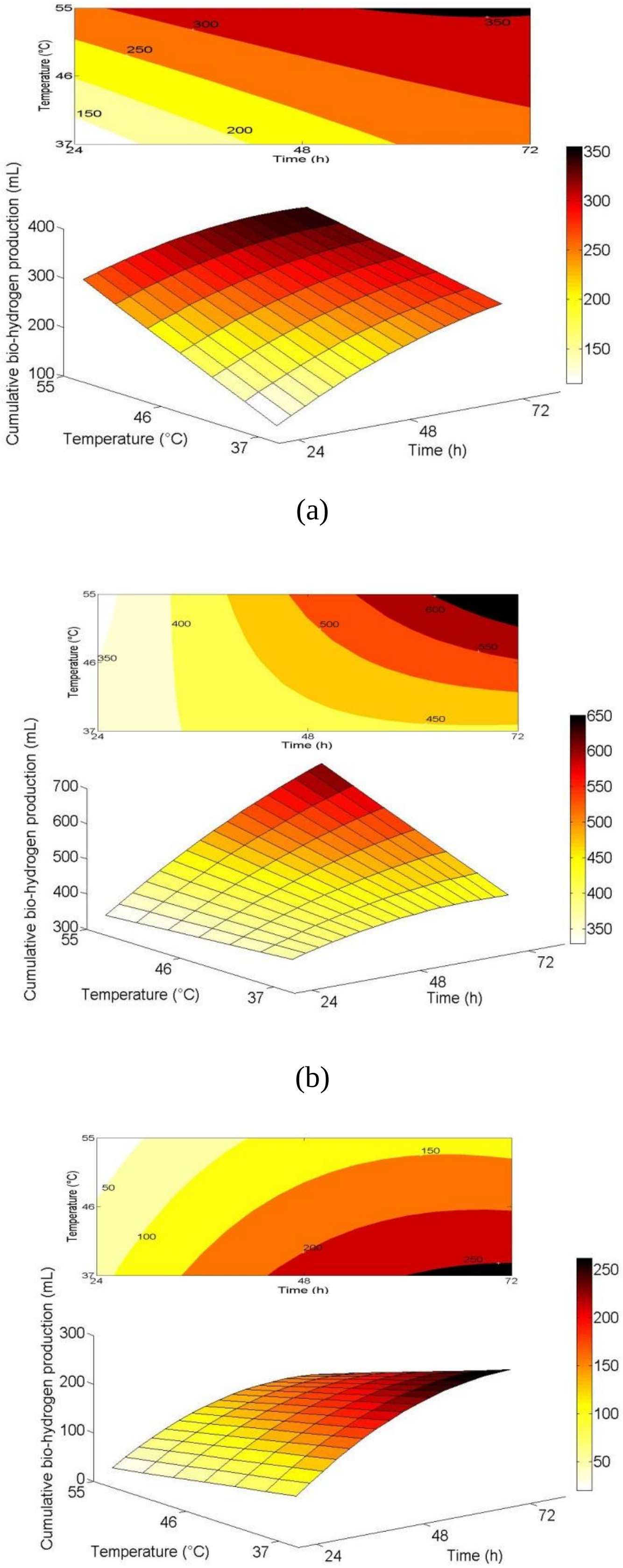


Figure 2. Effect of temperature on pH drop during incubation



(a)

(b)

(c)

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

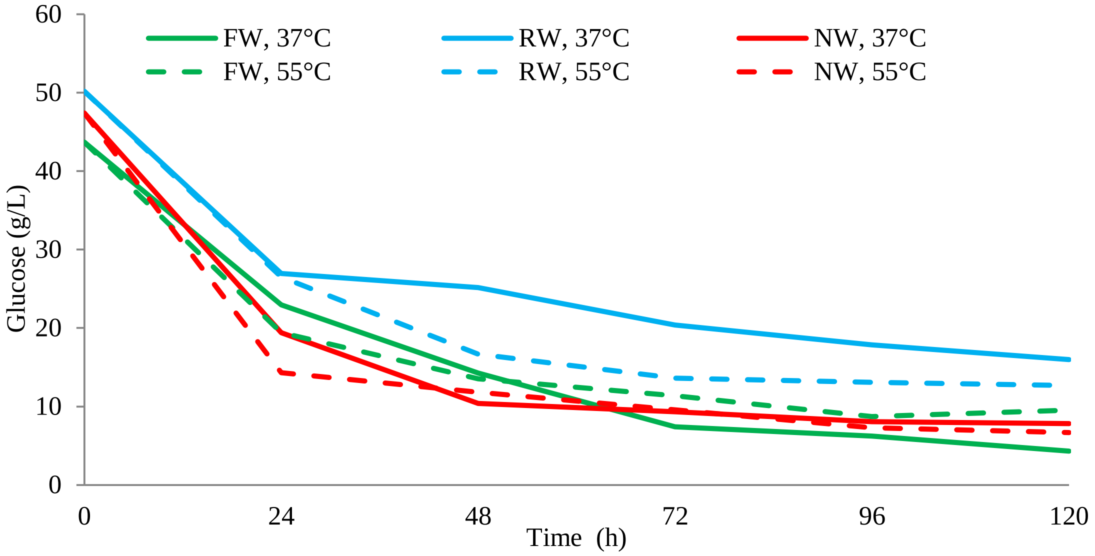
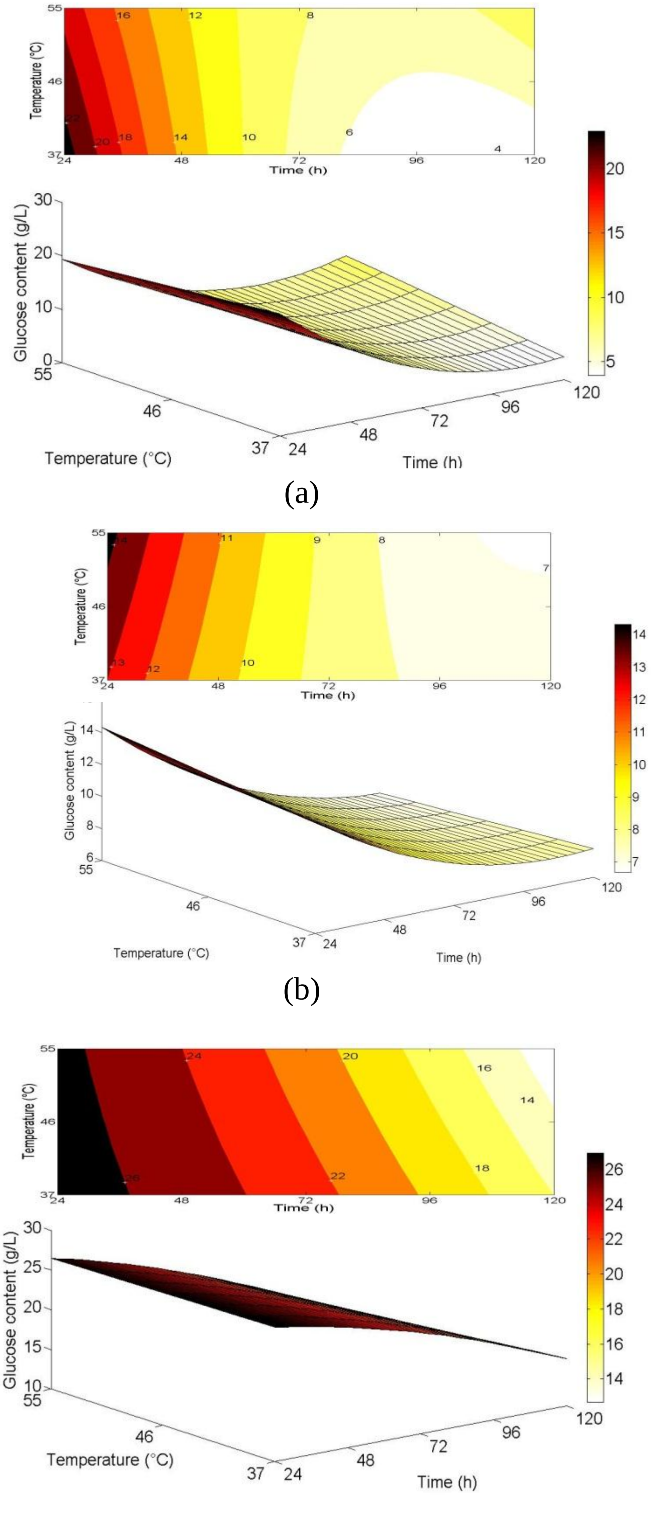


Figure 4. Glucose consumption during incubation under mesophilic and thermophilic temperature



(a)

(b)

(c)

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

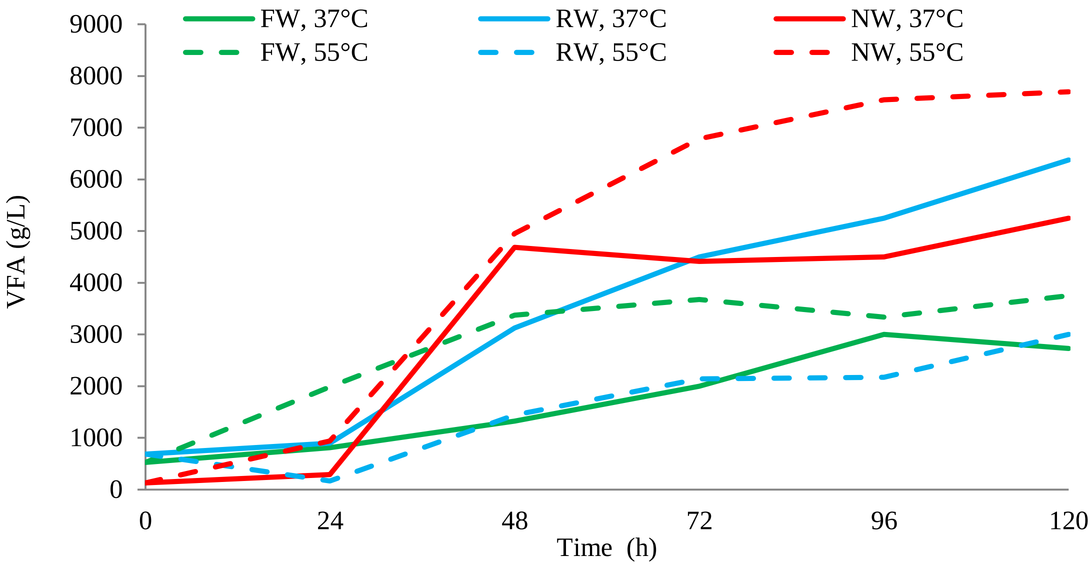


Figure 6. Increase in VFA concentration with time under mesophilic and thermophilic temperatures

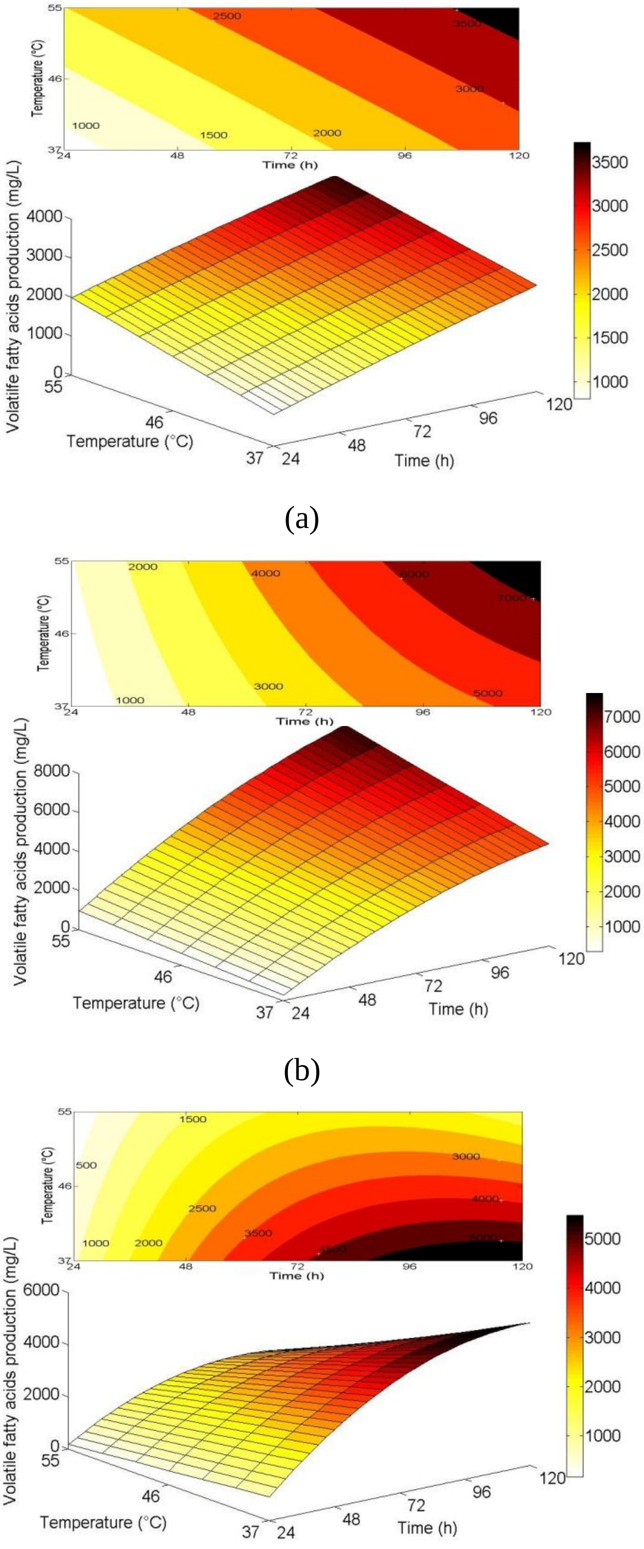


Figure 7. Three dimensional response plots for VFA production a) Food waste, (b) Noodle waste, (c) Rice waste

TABLE CONTENT

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

Table 2. Kinetic parameters and bio-hydrogen yield

						Hydrogen Yield			
Waste Type	Temperature	P (mL)	R_m (mL/h)	λ (h)	R^2	mL/g VS Fed	mL/g VS Removed	mL/g COD Removed	mL/g glucose Removed
FW	37 ˚C	283.7	6.688	6.949	0.9971	17.59	80.11	47.28	18.02
	55 ˚C	350.1	26.42	11.39	0.9965	21.57	82.47	67.33	25.63
RW	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
NW	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

Table 3. Comparison of Bio-hydrogen yield

Feed stock	Inoculum	Yield	Initial pH	Optimum pH	pH Management	Temperature (°C)	References
Food waste	Sludge	64.48 mL H_2/ VS_{fed}	7	4.8-6.4	Not controlled	37	(Lin et al., 2013b)
Food waste	Sludge	250 ml H_2 /$VS_{removed}$	6.5	6.5-5.2	Not controlled	26	(Tawfik et al., 2011)
Food waste	Kitchen wastewater	148 ± 42 ml H_2/$COD_{removed}$	5±03	5±0.3	Manually Controlled	40	(Tawfik and El-Qelish, 2014)
Food waste	Sludge	70 mL H_2/VS	5.5	5.5	Manually Controlled	55	(Shin et al., 2004)
Rice waste	Sludge	71mL H_2/VS	7	7	Not controlled	37	(Okamoto et al., 2000)
Rice waste	Sludge	134 mL H_2/VS	5.5	5.5	Manually Controlled	37	(Dong et al., 2009)
Food waste	Sludge	55 mL H_2/VS	7	6	Not controlled	55	(Nathao et al., 2013)
Rice waste	Sludge	346 mL H_2/g carbohydrates	4.5	4.5	Manually Controlled	37	(Fang et al., 2006)
Noodle Industry wastewater	Anaerobic microflora	1.47 mol H_2/mol hexose	4.5-8.5	5.2	Controlled	35	(Mizuno O, 2000)
Food waste	Sludge	44.83 mL H_2/g COD	8	8-4.5	Not Controlled	55	(Wongthanate and Chinnacotpong, 2015)
OFMSW	Sludge	205 ml H_2/g VS added	5.5	5.5	Automatic pH controller	55	(Chu et al., 2008)
Food waste	Sludge	82.47 mL/g VS Removed	7	7-4.4	Not Controlled	55	This study
Noodle waste	Sludge	131.38 mL/g COD Removed	7	7-4.4	Not Controlled	55	This study
Rice waste	Sludge	44.90 mL/g glucose Removed	7	7-4.3	Not Controlled	37	This study