

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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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/V_S, 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.

35 1 Introduction

36 Anaerobic digestion, as a waste management approach, has been in practice for more than a
37 century (McCarty, 1981). It is widely used to treat a variety of solid wastes and wastewater
38 on a small scale as well as on an industrial scale. It has multiple advantages like 30-50 %
39 reduction in waste volume as well as production of valuable by products such as methane and
40 hydrogen (Lin et al., 2011). A large amount of organic fraction of municipal solid waste or
41 food waste is produced every year in the world. During 2010, production of food waste in
42 China reached to 352 Mt and the major contributor was canteens and restaurants (Tai et al.,
43 2011; Wang Lingen et al., 2013). The food waste contains more than 80% volatile solids
44 (VS) that are biodegradable solids and can be converted into hydrogen or methane easily
45 (Shin et al., 2004; Zhang et al., 2007; Zhu et al., 2008). Several studies represent an increase in
46 bio-hydrogen production from food waste due to addition of buffers and minerals. Although
47 such addition can maintain a specific pH and nutritional required for optimum bio-hydrogen
48 production, but it also increases the cost of production (Nielsen AT, 2001; Han HK, 2004).
49 The cost of production can be reduced by adding sewage sludge as a source of *Clostridium*
50 mix culture (Fang et al., 2006). Nutritional deficiency in food waste was also balanced by
51 adding sewage sludge that made food waste more suitable for bio-hydrogen production (Shin
52 et al., 2004). It means that integrated waste management can be done at wastewater treatment
53 plant by co-digestion of sewage sludge and food waste. Although sewerage sludge is a good
54 source of *Clostridium* mix culture, but at the same time, it contains hydrogen consumers like
55 methanogens. Heat treatment is mostly opted to deactivate hydrogen consumers. The
56 traditional method of placing sewage sludge in boiling water is now no longer in practice and
57 replaced by microwave heating that provide more uniform heating as compared to the boiling
58 water method (Luo et al., 2010; Wang et al., 2011; Duangmanee et al., 2007). The
59 temperature and time for heat treatment varied from 75°C to 121°C for 15 minutes to 2 hours,
60 but 100°C for 15 minutes was mostly reported (Li and Fang, 2007; Fang et al., 2006).

61 Carbohydrate-rich wastes like food waste are suitable for *Clostridium* species as
62 stoichiometrically it can produce two mole of hydrogen from one mole of hexose (Payot R,
63 1998). Theoretically, 553 mL hydrogen can be produced by one gram of polysaccharides if it
64 is totally converted into acetate. The highest practical yield of 346 mL /g-carbohydrate was
65 achieved by Fang (2006) by using rice as a source of carbohydrate (78%), pre-treated sewage
66 sludge as a source of *Clostridium* and adding a variety of nutrients. Rice waste and noodle
67 waste has 40% share in the total food waste produced in China (Shiwei, 2005). The noodle

68 waste is also rich in carbohydrates, but still there is no research reported on bio-hydrogen
69 production from noodle waste.

70 Temperature and pH have great impact on the smooth running of AD (Saraphirom
71 and Reungsang, 2010). Most of the studies reported bio-hydrogen production under
72 mesophilic as well as thermophilic conditions and few were reported under psychrophilic
73 conditions. Lu et al. (2011) developed a microbial electrolysis cells (MECs) that could be
74 operated at 9°C by using *Geobacter psychrophilus* as dominating population and achieved
75 hydrogen yield of 0.62m³H₂m⁻³d⁻¹. Heidrich et al. (2013) further modified MECs to a pilot-
76 scale MEC and achieved bio-hydrogen production of 0.015 LH₂L⁻¹d⁻¹ at 25°C. On the other
77 end, under mesophilic and thermophilic conditions, there is no need of such sophisticated
78 technology and a better bio-hydrogen yield can be achieved by simple reactors or by lab scale
79 batch experiments. The temperature shift from mesophilic to thermophilic conditions can
80 change the rate of hydrogen production during anaerobic digestion (Li and Liu, 2012; Saripan
81 and Reungsang, 2014). Whereas, hyper-thermophilic provide better pathogenic destruction
82 but it also decreased the bio-hydrogen production (Sahlström, 2003; Yokoyama et al., 2007).
83 Keeping the same temperature but changing the initial pH from 7 to 8, the bio-hydrogen yield
84 was changed from 64.48 mL/V_S to 55 ml/V_S under no pH control conditions (Lin et al.,
85 2013b; Nathao et al., 2013). The same yield was increased to 70 mL/V_S when pH was
86 manually controlled for food waste under thermophilic conditions, which represents the
87 impact of pH management (Shin et al., 2004). The hydrogen production by anaerobic
88 digestion will be further improved if pH lies in the range of 5 to 6 (Radjaram and Saravanane,
89 2011). The pH of food waste lies in the range of 4-5, which further decreases by the
90 production of volatile fatty acids (VFA) to such a level that can inhibit the bacterial growth.
91 The pH can be controlled by automatic pH controllers, addition of nutrients and buffers,
92 manual monitoring and control (Yasin et al., 2011; Zhu et al., 2008; Kim et al., 2004). But all
93 these methods increased the cost of operation. Along with cost, maintaining pH at specific
94 point is not suitable especially when mix culture is used as the response of different microbial
95 stream could be different to same pH level. So, by co-digestion, the pH of the anaerobic
96 digestion process can be improved and it can be further adjusted to a desired initial value by
97 adding HCl or NaOH. After adjusting the desired initial pH under co-digested conditions,
98 the bio-hydrogen production can be achieved under no pH control conditions, which can
99 reduce the cost of operation (Fang et al., 2006).

100 The yield of bio-hydrogen is calculated by dividing the cumulative hydrogen
101 produced by VS, chemical oxygen demand (COD) or glucose (Chen et al., 2006; Dong et al.,

102 2009;Fang et al., 2006). The yields are misleading if calculated in term of added or start up
103 values of VS, COD and glucose as it seems quite impossible that the whole of added material
104 is converted into hydrogen. In this regard the removal quantities of such parameters are the
105 best option to calculate the yield.

106 The optimization played an important role in bio-hydrogen production and its
107 application with respect to incubation time in combination with temperature is an important
108 factor to get the maximum output with minimum intake of energy. In order to achieve this
109 purpose, statistical modelling is an important tool to study the impact within the experimental
110 range and can be further used for the development of response surface plots (Jo et al., 2008).

111 This study was designed to investigate the impact of temperature on bio-hydrogen
112 production from co-digestion of sewerage sludge with food waste and its carbohydrate-rich
113 derivatives i.e. rice waste and noodle waste with the help of statistical modelling. The
114 response surface methodology was employed to study the impact of time and temperature on
115 bio-hydrogen production, glucose consumption and VFA production. The pH during
116 incubation was not controlled and the drop of pH during anaerobic digestion was also studied
117 to find an optimum pH range of bio-hydrogen production from food waste derivatives.

118 2 Material and methods

119 2.1 Batch Experiment for Bio-hydrogen Production

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

131 Two series of experiments were conducted in duplicate in 550 mL digesters with
132 working volume of 400 mL (Hu et al., 2014). In order to achieve 10% initial TS
133 concentration, water was added along with feedstock and sewerage sludge in the digesters.
134 The feedstock and sewerage sludge were added in equal proportion. As the pH of food waste

135 was not so high and even after co-digestion with sewage sludge, the initial pH within reactor
136 was less than 7 that was carefully raised to 7 with the help of 3M NaOH solution (Zhu et al.,
137 2008). Series I was to observe the bio-hydrogen production under mesophilic temperature
138 (37°C) and series II was to investigate the impact of thermophilic temperature (55°C) on bio-
139 hydrogen production potential of feed stock in comparison with that produced under
140 mesophilic conditions.

141 **2.2 Chemical Analysis**

142 The volume of bio-hydrogen produced was measured by displacement of 3% NaOH
143 solution. This concentration of NaOH can remove other gases and water vapours (Fang et al.,
144 2006;Lin et al., 2013a;Saraphirom and Reungsang, 2010). Other parameters like TS, VS,
145 COD, VFA and alkalinity were measured according to standard methods (APHA, 2005). For
146 glucose detection, phenol sulphuric acid method was used (Lay and Fan, 2003).

147 **2.3 Assay Methods**

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

$$150 \quad H = P \exp \left\{ -\exp \left[\frac{R_m e}{P} (\lambda - t) + 1 \right] \right\} \quad (1)$$

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

155 In this study, the effects of different types of food waste, temperature and incubation
156 time on bio-hydrogen and VFA production as well as on glucose consumption, were analysed
157 by full quadratic model as shown below (Kim et al., 2008;Jo et al., 2008)

$$158 \quad Y = a_0 + \sum_{i=1}^n a_i X_i + \sum_{i=1}^n a_{ii} X_i^2 + \sum_{i=1}^n \sum_{i < j=2}^n a_{ij} X_i X_j \quad (2)$$

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

163 **3 Results and Discussion**

164 **3.1 Effect of Temperature and time on Bio-hydrogen Production**

165 A comparison of actual and MGE modelled bio-hydrogen production under
166 mesophilic and thermophilic conditions is shown in Fig.1, which shows an early start of bio-
167 hydrogen production in food waste and noodle waste as compared to rice waste. This early

168 production was quantified with the help of MGE as λ shown in Table 2, which clearly shows
169 that the highest lag phase belongs to rice waste under both temperatures. The bio-hydrogen
170 production in food waste continued till 72 hours of incubation and this time period for food
171 waste was higher than that observed for noodle waste, but still the cumulative bio-hydrogen
172 production of food waste was the lowest as compared to other two waste types. Looking at
173 the R_m values, it is clear that food waste has the lowest mesophilic R_m value of 6.688 mL/h
174 among all three wastes that ultimately caused the cumulative bio-hydrogen production to
175 decrease. Only the R_m value is not responsible for higher yield as the highest mesophilic R_m
176 of 21.05 mL/h belongs to noodle waste, but the cumulative bio-hydrogen production of
177 noodle waste was found smaller than rice waste having R_m value of 16.52 mL/h. The rice
178 waste has higher production because it produced bio-hydrogen for 96 hours as compared to
179 noodle waste that produced till 60 hours. In fact, mesophilic bio-hydrogen production in
180 noodle waste decreased considerably after 24 hours of incubation as compared to
181 thermophilic bio-hydrogen production where the decrease was observed after 36 hours of
182 incubation. That's 12 hours active duration increased the cumulative bio-hydrogen production
183 from noodle waste under thermophilic temperature even after observing the fact that the R_m
184 values are very close to each other. It can be seen in Fig. 2 that the difference in active
185 duration was due to pH as it was dropped from 7 to 4.6 in the noodle waste reactor during the
186 first 12 hours under mesophilic temperature and it was further dropped to 4.4 whereas a pH
187 drop in thermophilic reactor was 5.3 to 4.9 in the same duration. On the other end, pH drop in
188 rice waste reactor under thermophilic conditions was higher as compared to mesophilic
189 temperature, which ultimately reduced the cumulative bio-hydrogen production that was
190 found in agreement with the finding of Fang et al.(2006). The reduction in bio-hydrogen
191 production with low pH is due to homoacetogenic bacteria, which are more active at low pH
192 (Ramos et al., 2012; Schiel-Bengelsdorf and Dürre, 2012). The trend for pH drop with
193 temperature in food waste reactors was opposite to other reactors and R_m increased to a value
194 of 26.42 mL/h when temperature was shifted from mesophilic to thermophilic. This increase
195 is due to *Thermoanaerobacterium thermosaccharolyticum* that grow at higher temperature in
196 food waste and produced more hydrogen (Shin et al., 2004). As a whole, R_m values found in
197 present study were higher than those reported in previous studies (Fang et al., 2006; Ramos et
198 al., 2012).

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

$$\begin{aligned}
201 \quad Y &= 202.83 + 56.86x_1 + 73.38x_2 + 8.5x_3 - 22.5x_1^2 + 243.25x_2^2 - 113.8x_3^2 - \\
202 \quad &23.75x_1x_2 - 1.86x_1x_3 - 30.38a_9x_2x_3 \quad (3) \\
203 \quad &(R^2 = 0.5576, F=19.921)
\end{aligned}$$

204 Where Y is the predicted bio-hydrogen production; x_1, x_2 and x_3 are the coded
205 values of incubation time, waste type and temperature respectively. There is a poor
206 relationship between actual and predicted value as the coefficient of determination (R^2) was
207 calculated to be 0.5576, which can explain only 55.76% variability of the response. The
208 diversity among waste type is the main reason for such a low value and this value could be at
209 a higher level if the same waste was used in different proportions as reported in other studies
210 (Kim et al., 2008; Jo et al., 2008). To overcome this problem, quadratic model was again
211 developed for each waste type and following equations were obtained;

$$\begin{aligned}
212 \text{ Food waste: } Y &= 281.75 + 57.25x_1 + 62.25x_2 - 22x_1^2 - 25.25x_1x_2 \quad (4a) \\
213 \quad &(R^2 = 0.9858 \quad F = 278.06)
\end{aligned}$$

$$\begin{aligned}
214 \text{ Noodle waste: } Y &= 472.5 + 97.5x_1 + 42.5x_2 - 25x_1^2 + 62.5x_1x_2 \quad (4b) \\
215 \quad &(R^2 = 0.9011 \quad F = 36.44)
\end{aligned}$$

$$\begin{aligned}
216 \text{ Rice waste: } Y &= 167.5 + 71.5x_1 - 49.5x_2 - 43x_1^2 - 16.5x_1x_2 \quad (4c) \\
217 \quad &(R^2 = 0.7922 \quad F = 15.26)
\end{aligned}$$

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

224 The three dimensional (3-D) response surfaces and two dimensional (2-D) contours
225 were developed within the experimental range for each waste type by taking bio-hydrogen
226 production as a response by using above mentioned equations. The 3-D and 2-D curves of the
227 calculated response showed the interaction of incubation time and temperature in figure 3a-c.
228 For food waste, it is clear that the gas production increases with time and temperature from
229 115 mL at the starting end to 354 mL at the extreme modelled conditions. During 0-24 hours
230 of incubation, bio-hydrogen production increased with increase in temperature for food
231 waste, i.e. 115 mL of bio-hydrogen was produced at 37°C that increased 76.09 % and
232 152.17% at 46°C and 55°C, respectively. During next 24 hours of incubation, bio-hydrogen
233 production reduced with the increase in temperature, i.e. 114.5 ml bio-hydrogen was

234 produced at 37°C and 30.78% and 91.22% reduction was observed at 46°C and 55°C,
235 respectively. Even after reduction in bio-hydrogen production during 24-48 hours of
236 incubation, the cumulative bio-hydrogen production increased with an increase in
237 temperature from food waste. The impact of temperature and time can be bettered viewed in
238 2-D contour (Fig. 3a), which shows that the increase in temperature increases bio-hydrogen
239 production more at 24h as compared to 72h of incubation. It also revealed the fact that first
240 24 hours are important for bio-hydrogen production from food waste under thermophilic
241 temperatures and next 24 are important for production under mesophilic temperature, which
242 is in agreement with findings of Shin et al (2004). Although noodle waste also produced more
243 bio-hydrogen at elevated temperature, but the time effect was opposite to that observed for
244 food waste. The bio-hydrogen production in noodle waste during 0-24 hours was 350mL at
245 37°C that was 5.4% and 10.81% decreased at 46°C and 55°C, respectively. But in next 24-72
246 hours, 178.57% and 357.14% increase at 46°C at 55°C, respectively.

247 So far rice waste was concerned; temperature has a negative impact on bio-hydrogen
248 production. The bio-hydrogen production in rice waste during 24-48h was 131 mL, 114.5
249 mL and 98 mL, which was 65.65% , 75.11% and 87.76% reduced during 48-72h under 37°C,
250 46°C and 55°C, respectively. The reduction in bio-hydrogen production for rice waste was in
251 agreement with previous findings (Fang et al., 2006). The 2-D contours in fig. 3b and 3c
252 differentiate the impact of temperature with time on bio-hydrogen production for noodle
253 waste and rice waste as the contour patterns are quite opposite to each other.

254 3.2 Effect of temperature on Bio-hydrogen Yield

255 The bio-hydrogen yield was calculated by dividing the P value on table 2 with VS_{fed} ,
256 $VS_{removed}$, $COD_{removed}$ and $glucose_{removed}$. The bio-hydrogen yield calculated on the basis of
257 VS_{fed} lay in the range achieved by Lin et al. (2013b) and temperature impact on yield was
258 same as observed for P. The yield on the basis of $VS_{consumed}$ represented uptake efficiency of
259 VS during anaerobic digestion. The increase in P with temperature for food waste was
260 23.41% whereas the yield increased by 2.86% only that indicated the efficient removal of VS
261 at higher temperature. The bio-hydrogen yield calculated for FW on the basis of $VS_{removed}$ lay
262 in the range used by Kim et al. (2004). Using the same scale of $VS_{removed}$, bio-hydrogen yield
263 for rice waste decreased 47.37% with an increase in temperature, whereas the decrease in P
264 was 78.81% that represented a decrease in removal of VS at elevated temperature which was
265 in agreement with the findings of Fang et al. (2006). As compared to food waste and noodle

266 waste, the increase in P and yield calculated by VS_{removed} was close, but it revealed the fact
267 that VS removal efficiency slightly decreased at elevated temperature.

268 When the yield measuring scale was shifted from VS_{removed} to COD_{removed} , the results
269 represent quite different picture of temperature impact. The increase in temperature from
270 37°C to 55°C increased 42.41% bio-hydrogen yield calculated on the basis of COD_{removed} for
271 food waste. The increase in bio-hydrogen production due to same increase in temperature
272 from 37°C to 55°C was 23.37%. Such difference in yield and production increment
273 represented decrease in COD removal efficiency at elevated temperature for food waste. For
274 rice waste, the decrease in yield was 61%, which was close to 78% decrease in P. Increasing
275 temperature also increased the yield for noodle waste to 20 %, which was smaller than the
276 increase in P, representing a higher rate of COD removal at elevated temperature. All the bio-
277 hydrogen yields calculated on the basis of COD_{removed} lay in the range calculated by Tawfik
278 and El-Qelish (2014).

279 Glucose removal efficiency for food waste decreased with an increase in temperature
280 as the increase in P was 23.41% against 42.19% when bio-hydrogen yield was calculated on
281 the basis of $glucose_{\text{removed}}$. Whereas the change in yield for noodle waste and rice waste was
282 close to the change observed for P. The decrease in glucose concentration was close to that
283 observed in previous studies (Abdeshahian et al., 2014;Kapdan and Kargi, 2006). The yield
284 calculated on glucose basis was further studied on daily basis and it was observed that the
285 highest yield of 33 mL/ $glucose_{\text{removed}}$ for 0-24h duration belonged to noodle waste under
286 mesophilic condition. During the next 24h duration, the highest yield 400 mL/ $glucose_{\text{removed}}$
287 was achieved by noodle waste under thermophilic temperature, which was close to the
288 finding of Fang et al. (2006) but still smaller than the theoretical yield of 553 mL/g-
289 carbohydrate. The yield for rice waste also increased under both temperatures but it was
290 much higher at mesophilic as 184.37 mL/ $glucose_{\text{removed}}$ against 24.99 mL/ $glucose_{\text{removed}}$ at
291 thermophilic temperature. During 24 to 72 hours of incubation, the yield in all reactors
292 reduced except noodle waste under mesophilic conditions. As a whole, 24-48h duration of
293 incubation was found more important for bio-hydrogen production from glucose. The
294 production of glucose modelled by quadratic equation using previously defined notation as

$$\begin{aligned} 295 \quad Y &= 13.504 - 0.604x_1 + 0.095x_2 - 0.831x_3 + 0.066x_1^2 - 5.469x_2^2 + \\ 296 \quad &0.609x_1x_2 + 0.238x_1x_3 - 1.131x_2x_3 \quad (5) \\ 297 \quad &(R^2 = 0.6959 \quad F = 64.07) \end{aligned}$$

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

300 Food waste: $Y = 7.820 - 3.561x_1 + 0.412x_2 + 1.554x_1^2 + 1.094x_1x_2$ (6a)
301 $(R^2 = 0.9713 \quad F = 270.81)$

302 Noodle waste: $Y = 8.697 - 1.601x_1 + 0.055x_2 + 0.439x_1^2 - 0.307x_1x_2$ (6b)
303 $(R^2 = 0.7994 \quad F = 31.89)$

304 Rice Waste: $Y = 21.817 - 3.1x_1 - 0.938x_2 - 0.323x_1^2 - 0.354x_1x_2$ (6c)
305 $(R^2 = 0.715 \quad F = 20.07)$

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

320 3.3 Effect of temperature on VFA production

321 The VFA revealed an increase with time as **also** reported by Lin et al. (2013b), which is
322 shown in figure 6. In the present study, it is observed that VFA in food waste and noodle waste
323 increased with an increase in temperature from 37°C to 55°C but decreased for rice waste that lay in
324 the range calculated by Shin et al. (2004). **It can be seen in figure 6 that during 24-48h, increase**
325 **in VFA was much higher in food waste under thermophilic conditions as compared to**
326 **mesophilic conditions. During the same interval, bio-hydrogen production was almost ceased**
327 **in thermophilic food waste reactor whereas it was continuously producing in mesophilic food**
328 **waste reactor.** One of the possible reasons for this reduction is the conversion of glucose **to VFA** at
329 this stage by homoacetogenic bacteria that reached up to such level where bio-hydrogen
330 production was not feasible under thermophilic conditions, whereas the VFA production in
331 the mesophilic FW reactor was much smaller than that observed under thermophilic reactor,
332 because of which production **continued** in the mesophilic reactor (Zhang et al., 2014). **The**

333 higher concentration of VFA together with low pH can be inhibitory to bacteria that can
 334 cause unfavourable physical changes in the cell. By such physical changes, excessive energy
 335 is required to pump ions and that energy can be available at higher temperature. So it
 336 increased the yield at elevated temperatures, as observed in case of food waste and noodle
 337 waste (Gottschalk, 1986; Zoetemeyer RJ, 1982; Switzenbaum MS, 1990). The higher
 338 concentration of VFA can also be used as an indicator for higher production of bio-hydrogen
 339 as observed by Dong et al. (2009). In the present study, the order to VFA production and
 340 cumulative bio-hydrogen production was same i.e. $NW_{55^{\circ}C} > RW_{37^{\circ}C} > NW_{37^{\circ}C} > FW_{55^{\circ}C}$
 341 $> FW_{37^{\circ}C} > RW_{37^{\circ}C}$.

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

$$344 \quad Y = 1795.82 + 686.16x_1 - 300.51x_2 + 641.13x_3 + 4.94x_1^2 + 658.51x_2^2 +$$

$$345 \quad 127.30x_1x_2 + 33.26x_1x_3 - 319.25x_2x_3 \quad (7)$$

$$346 \quad (R^2 = 0.5975 \quad F = 41.56)$$

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

$$349 \quad \text{Food waste: } Y = 2362.67 + 458.15x_1 + 545.05x_2 - 12.88x_1^2 + 2.76x_2^2 - 22.42x_1x_2$$

$$350 \quad (R^2 = 0.8654 \quad F = 51.44) \quad (8a)$$

$$351 \quad \text{Noodle waste: } Y = 4196.48 + 1464.56x_1 + 772.876x_2 - 163.19x_1^2 + 224.98x_1x_2$$

$$352 \quad (R^2 = 0.8415 \quad F = 42.48) \quad (8b)$$

$$353 \quad \text{Rice Waste: } Y = 3258.94 + 756.62x_1 - 1147.22x_2 - 303.1x_1^2 - 390.28x_1x_2$$

$$354 \quad (R^2 = 0.9430 \quad F = 132.37). \quad (8c)$$

355 The three dimensional response plots and contours for VFA production on the basis of above
 356 models are shown in figure 7. The 3-D contours for food waste and noodle waste seems almost same
 357 but the contour lines for both varied in different manner. Although the production of VFA increased
 358 with time and temperature in all reactors but the intensity of change is different for each waste type as
 359 observed in figure 7. It can be observed from figure 7 that the production of VFA has
 360 increased for food waste and noodle waste when temperature was increased from 37°C to
 361 55°C. Although with time, the VFA concentration has increased, but the rate by which VFAs
 362 produced was decreased with time, i.e. VFA production during 24-48h was greater than that produced
 363 during 48-72h and this trend continued till 120h for food waste and noodle waste. As a whole, more
 364 VFA was produced during 24-120h under mesophilic temperature as compared to thermophilic
 365 temperature in food waste reactor, but as a whole, mesophilic VFA production was found less than
 366 that of thermophilic as reported by Gadow et al. (2012). It is because of the fact that during 0-24h

367 duration, thermophilic VFA production was much higher than that produced under mesophilic
368 conditions in food waste. By controlling the VFA production during this interval, the yield of bio-
369 hydrogen can be increased for FW as it stopped too early in thermophilic food waste reactor as
370 compared to mesophilic food waste reactor. Thermophilic VFA production was higher than
371 mesophilic VFA production in noodle waste reactor. On the other end, mesophilic VFA production
372 was higher than that produced under thermophilic conditions for RW and VFA increased with time in
373 the same manner as observed for food waste under 37°C to 40°C. Above 40°C to 55°C, the VFA trend
374 for rice waste remained the same as of food waste till 96h after which it started to decrease till 120h.

375 **4 Conclusion:**

376 Food waste and its two major derivatives, i.e. noodle waste and rice waste, were co-
377 digested with sewerage sludge to produce bio-hydrogen with an initial pH of 7 under
378 mesophilic and thermophilic conditions. **The pH was not controlled throughout the**
379 **incubation.** The most effective VS removal was observed in noodle waste reactor that
380 produced the highest experimental cumulative bio-hydrogen of 656.5 mL under thermophilic
381 conditions. The food waste possessed the highest bio-hydrogen yield calculated on the basis
382 of VS_{removed} that represents an efficient conversion of VS into bio-hydrogen. The increase in
383 temperature within the studied range increased the bio-hydrogen production in food waste
384 and noodle waste reactors. The rice waste reactor represented the negative impact of
385 increasing temperature on bio-hydrogen and VFA production. **Thermophilic conditions**
386 **should be preferred for bio-hydrogen production as most of the time food waste is used as**
387 **feed stock.** The quadratic modelling returned good results that were close to experimental
388 ones, when it was done for each waste type of bio-hydrogen, VFA production and glucose
389 removal. The response surface plots and contour plots within the experimental range
390 adequately explained the effect of temperature and time on studying parameters and helped to
391 develop better understanding regarding the variation among the studied parameters especially
392 when the different treatments represented similar trends. VFA production in rice waste
393 reactor changes the trend after 40°C that was identified only due to quadratic modelling. The
394 lowest limit of pH for bio-hydrogen production was identified as 4.3 and 4.4 for mesophilic
395 and thermophilic temperatures respectively.

396 *Author contributions.* A. Sattar and C. Arslan designed the research and performed all the lab
397 works. C. Ji provided the financial and technical support for designing and conducting
398 research as well as supervised the whole research process. S. Sattar developed and
399 customized 3-D surface plots and assisted in manuscript preparation. K. Yousaf assisted the
400 lab works and analysis. S. Hashim performed the statistical analysis. C. Arslan wrote the

401 manuscript with comments from all authors and A. Sattar finalized the manuscript under the
402 supervision and guidelines of C. Ji.

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

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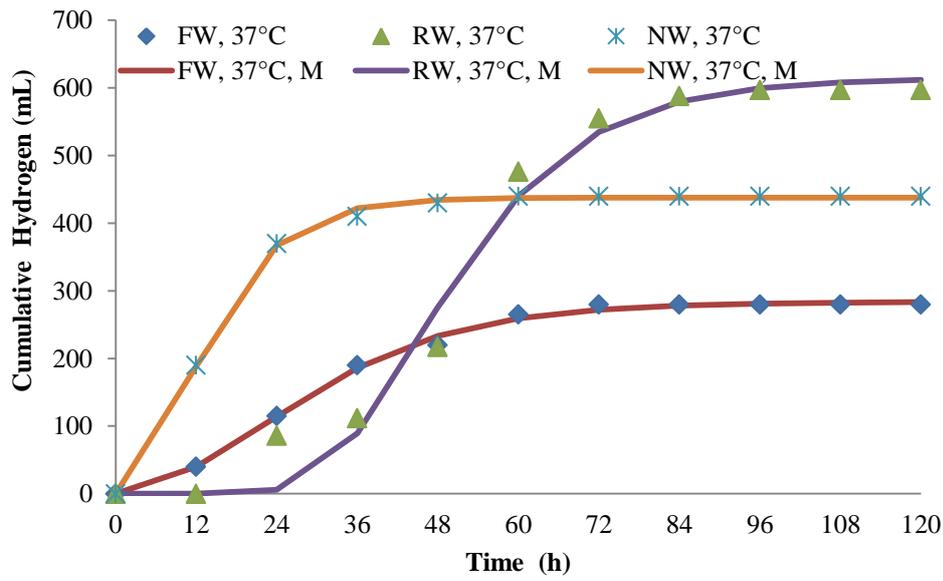
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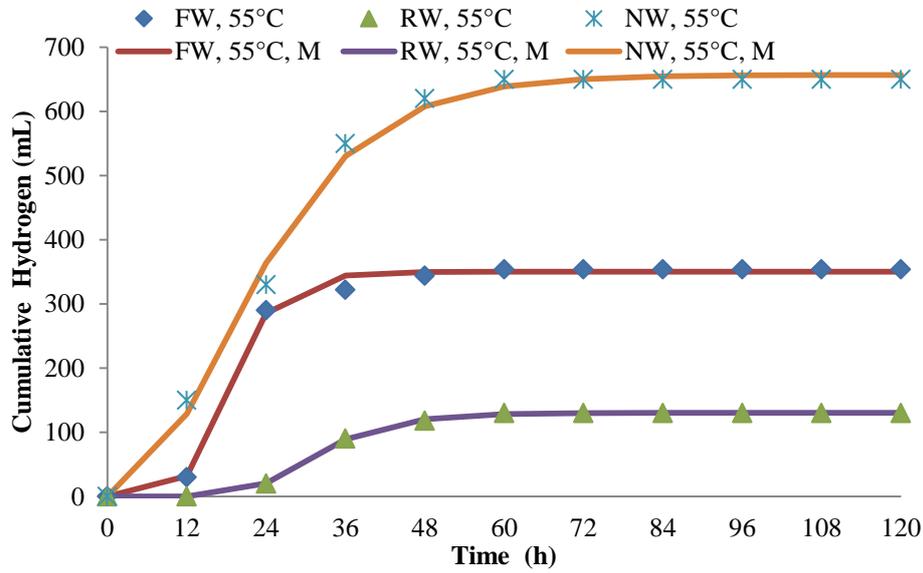
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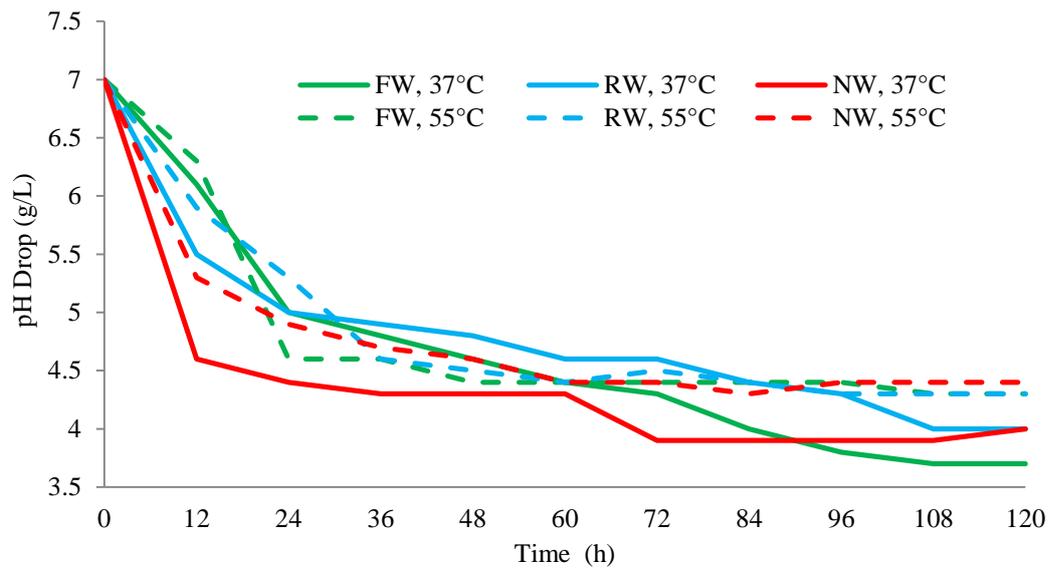
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*M stands for modelled bio-hydrogen production on the basis of Modified Gompertz Equation

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

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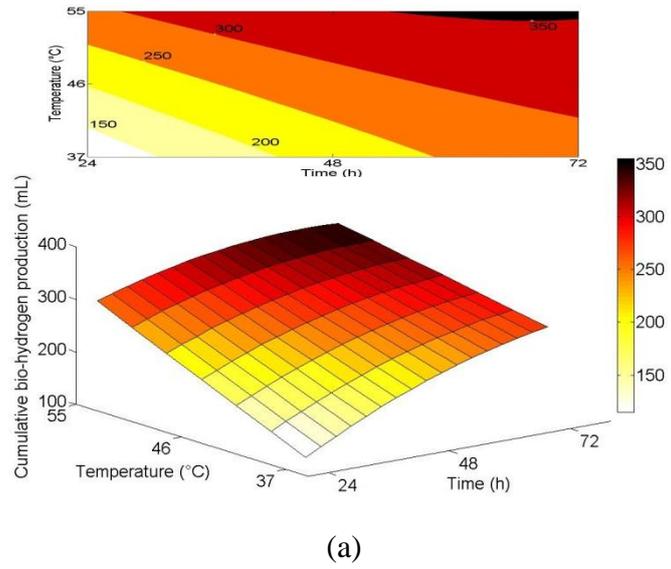


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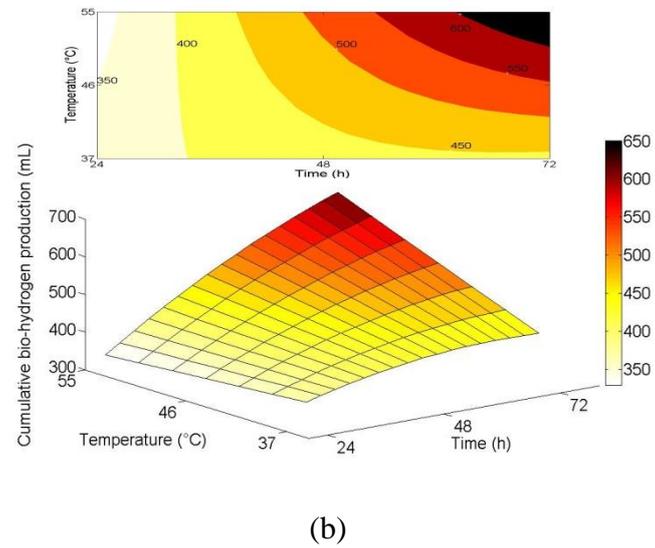
561 **Figure 2.** Effect of temperature on pH drop during incumation

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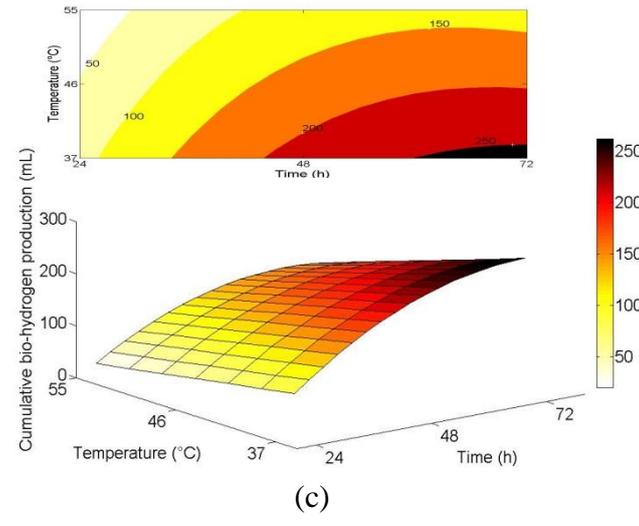
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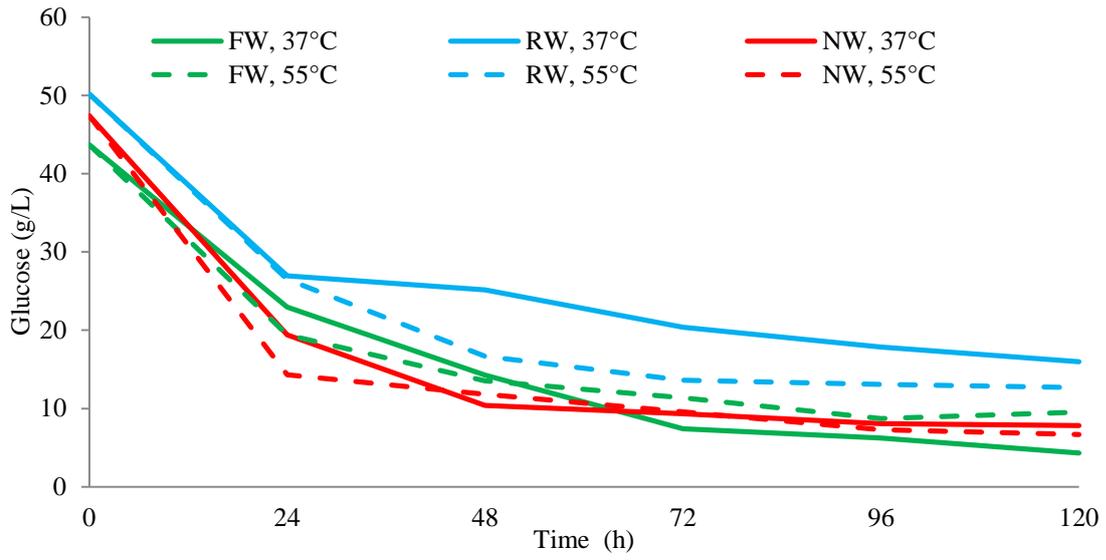


(b)



(c)

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



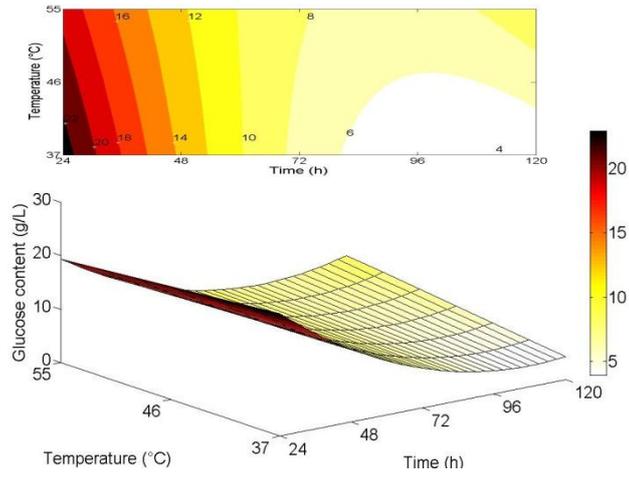
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598 **Figure 4.** Glucose consumption during incubation under mesophilic and thermophilic

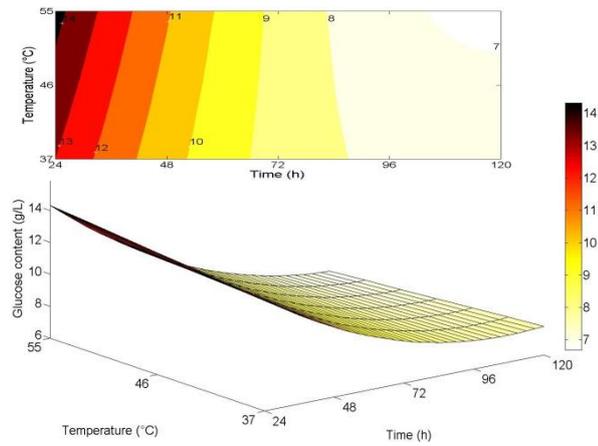
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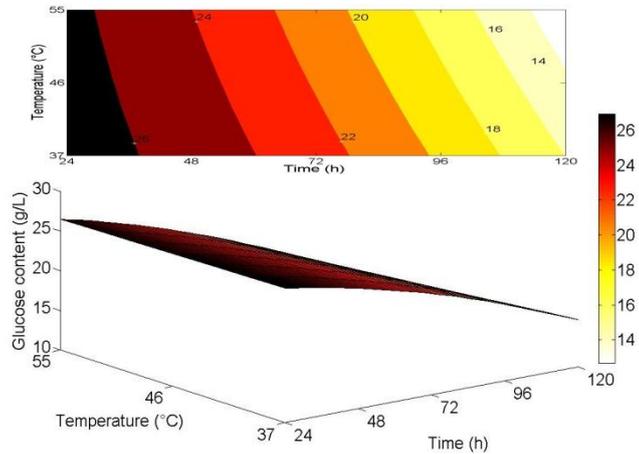
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(a)

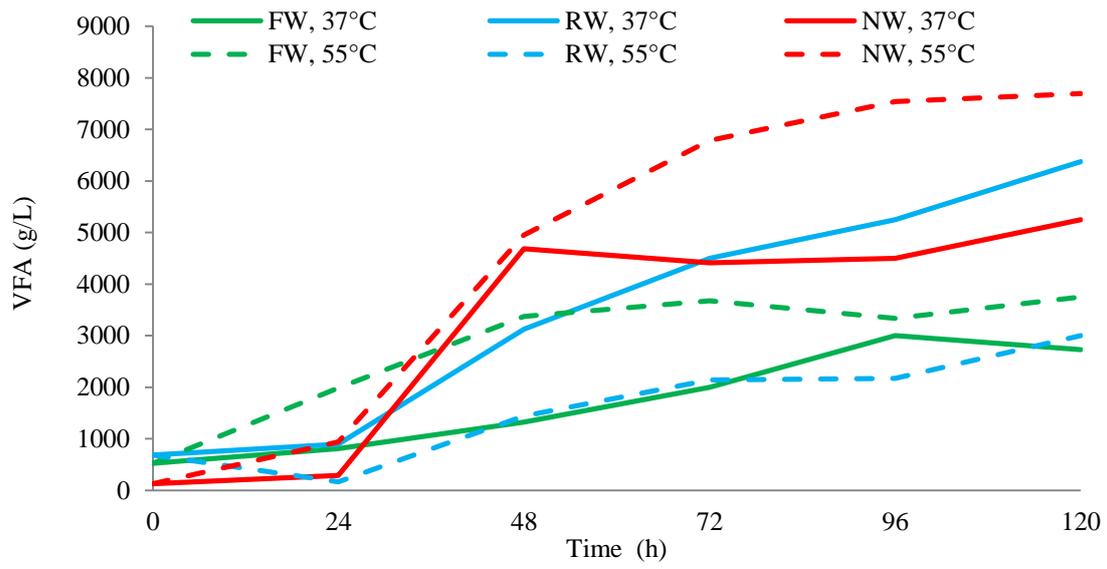


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(c)

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

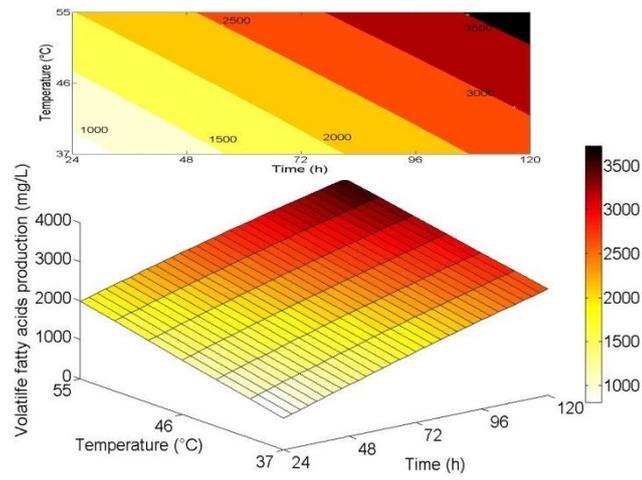


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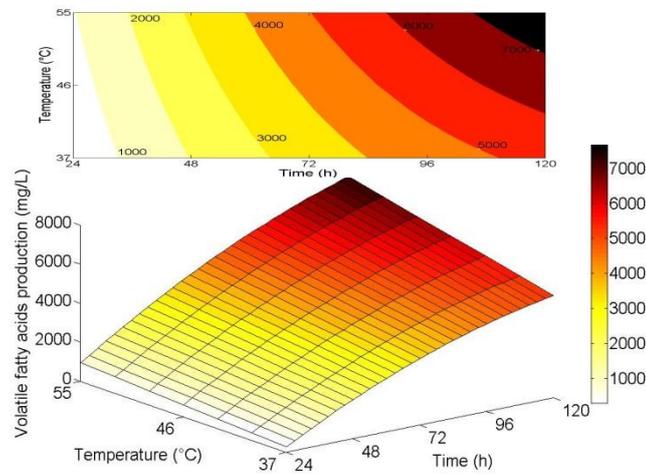
633 **Figure 6.** Increase in VFA concentration with time under mesophilic and thermophilic

634 temperatures

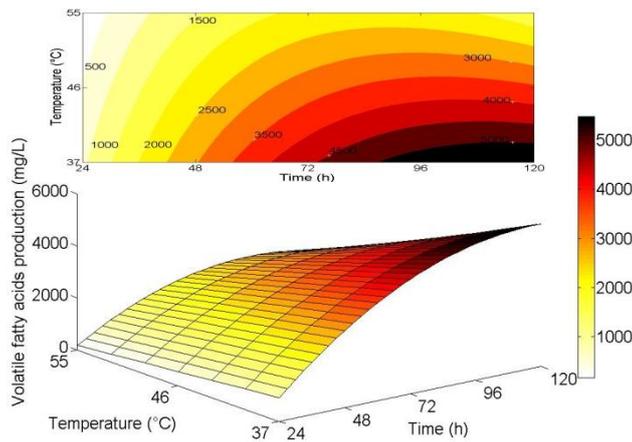
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(a)



(b)



(c)

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

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670 **Table 1.** Properties of test materials

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TABLE CONTENT

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

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674 **Table 2.** Kinetic parameters and bio-hydrogen yield
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Waste Type	Temperature	P (mL)	R _m (mL/h)	λ (h)	R ²	Hydrogen Yield			mL/g glucose Removed
						mL/g VS Fed	mL/g VS Removed	mL/g COD 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

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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