Optimization of hydrolysis and acidogenesis of food waste for production of organic acid

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Abstract. The rising of human population in the world leads to the increasing number of food waste annually. To counter this problem, the food waste is suggested to be recycled into value-added product. Anaerobic digestion is one of the ways to recycle the food waste. The main product of a complete anaerobic digestion is methane (biogas). However, methane is very hard to handle since it is in gas form. Hence, this paper has drawn the attention toward the intermediate product of anaerobic digestion, the lactic acid. This paper presents the effect of 3 different variables: (a) pH values, (b) temperature and (c) amount of inoculum, on the hydrolysis and acidogenesis stages of anaerobic digestion of food waste. Besides, the optimal condition was determined. Experimental results show that the optimum, 0.0845 g/mL of lactic acid was obtained at pH value of 7, with 125 g of volatile solid food waste and 100 g of inoculum (Ragi Tapai), and was left in a pre-heated oven at 45°C for four days. This value was higher than the other previous study, and so showing promise in producing green acid from food waste that can be used as an intermediate substance for making other materials.

1. Introduction
Waste is an inevitable by-product of most human activity. Since the population of the world is growing rapidly and uncontrollably, which is estimated to increase from 7.3 billion in 2015 to 9.7 billion in 2050 and 11.2 billion in 2100 [1], it has created serious problems regarding the energy requirement and the disposal of the solid waste. Besides that, the economy that is booming and the rise of the standard living of the people also can lead to the amount of waste to accelerate considerably [2]. The disposal of the waste must be managed properly, so that it would not bring any harm to the future generation. Otherwise, the mismanagement of the waste would bring negative effect to the human being, such as health problem, and also living environment.

Generally, there are three type of waste, namely; municipal solid waste, industrial solid waste, and agricultural waste. Between these three, municipal solid waste contributes the largest portion of the waste in the world. Municipal solid waste came mainly from households, offices, hotels, shops and institutions, which the major components are paper, plastic, metals, glass, and food waste. Among these, 60% of the municipal solid waste is constituted of food waste [3]. A statistic done by Food and Agriculture Organization of the United Nations (FAO) shows that approximately one third of the food produced for human consumption is wasted globally [4]. In Malaysia alone, a recent statistic unveiled by Solid Waste Management and Public Cleansing Corporation (SWCorp Malaysia) stated that around
16,688 tonnes of food are thrown away as waste everyday [5]. Hence, it is a must to manage these food waste. Besides, there is a huge potential to recover the energy and nutrient from the food waste. Other than helping to develop a sustainable human society, this step is also very important as it provides substantial economic opportunity.

By considering the negative environmental impacts of landfilling, incineration, and composting of food waste, anaerobic digestion has been proposed as a relatively cost-effective technology for renewable energy production and waste treatment of this high moisture and energy-rich material, the food waste [6,7]. Anaerobic digestion is a conversion process of biomass and organic wastes into biogas (60-70% methane) through microbes in the absence of oxygen. Anaerobic digestion consists of superior advantages over the other bioenergy technologies as it can be performed at any locations in both large and small scale [8,9].

In order to fully convert the biomass into methane (biogas), it must go through four main stages, which are hydrolysis, acidogenesis, acetogenesis and methanogenesis. Since biogas is in the gas form, it is difficult to handle. So, in this paper, the conversion process was stopped at the second stage, the acidogenesis, where the food will be broken down into organic acid, such as lactic acid, which has various application in the pharmaceutical, leather and textile industries [10], and is much easier to handle as it is in liquid form. Although anaerobic digestion is a widely-used and mature bioenergy technology, however, its potential in producing organic acid has not been fully explored. Hence, in this paper, the effect of pH value, temperature and amount of inoculum (Ragi Tapai) on hydrolysis and acidogenesis stage of anaerobic digestion was investigated. Besides, the optimal condition for the lactic acid production based on the parameters studied was determined.

2. Methodology

2.1. Materials
The food waste was collected from the food court of College of Engineering, Universiti Tenaga Nasional (UNITEN). The samples collected were hand-sorted to eliminate the plastic and metal, if any. Then, it was blended to reduce its size into the range of 0.2-1.0 mm. After that, the well-prepared food waste sample was kept frozen for further usage. The inoculum, Ragi Tapai, however, was purchased from the local market.

2.2. Volatile solid determination
To determine the volatile solid (VS) amount in the food waste, a crucible was firstly put in a furnace at 550 °C for 15 minutes to kill any bacteria present. Then, the crucible was left to cool down in the desiccator until it reached room temperature. An analytical balance was used to measure the weight of the crucible. After that, the food waste was placed on the crucible and weighed before putting into the furnace at 550 °C for 30 minutes. Next, the food waste with the crucible was weighed again after cooling to room temperature. The experiment was conducted three times to ensures reproducibility.

2.3. Optimization of anaerobic digestion (hydrolysis and acidogenesis) of food waste for lactic acid production

2.3.1. Amount of inoculum. The anaerobic digestion was conducted in a 100 mL glass bottle, where 125 g (VS) of food waste was added into the bottle. Then, 50g of Ragi Tapai was mixed well with the food waste and the pH value of the mixture was adjusted to pH 5 using potassium chloride (KOH) or hydrochloric acid (HCl). After that, the bottle was connected to a vacuum pump to remove all the oxygen presence, and it was left in a pre-heated oven at 45 °C for four days. Lastly, the experiment was repeated with different amount of inoculum, 100 g, 150 g, 200 g, 250 g, and 300 g.
2.3.2. *Temperature control.* The procedures were similar with the previous experiments with the inoculum fixed at 100 g and digestion was conducted with the initial pH value of 5 at various temperature (25 °C, 35 °C, 40 °C, 45 °C) for 4 days.

2.3.3. *pH control.* The procedures were similar with the previous experiments with the inoculum fixed at 100 g and the digestion was conducted at 45 °C for 4 days with different initial pH value (4, 5, 6, 7, 8).

2.4. *Construction of calibration curve for lactic acid determination*

Lactic acid (1.2 g) with a known concentration (89%, ρ = 1.2 g/mL) was diluted with water in a 10 mL volumetric flask. Then, a series of lactic acid solutions was prepared using two-fold dilutions. After that, a solution of iron (III) chloride was prepared by diluting 0.3 g of iron (III) chloride with distilled water in a 100 mL volumetric flask. Next, 50 µL of lactic acid solution was added into 2 mL of iron (III) chloride and stirred. The absorbance of the mixture was measured at 390 nm using a Optizen Pop UV/Vis spectrophotometer. The procedure was repeated to all diluted lactic acid solution. Finally, a calibration curve, showing the relationship between the UV absorbance at 390 nm and the concentration of lactic acid (g/mL) was plotted.

2.5. *Lactic acid determination*

A 50 µL of the top layer of the liquid product after the acidogenesis (anaerobic digestion) was added to 2 mL of iron (III) chloride solution and mixed-well. Next, the lactic acid content (absorbance) was measured using Optizen Pop UV/Vis spectrophotometer at 390 nm. Lastly, the concentration of lactic acid was calculated based on the calibration curve (Figure 1).

3. *Results and discussion*

3.1. *Calibration curve*

After conducting the absorbance measurement for a series of lactic acid with known concentration, the calibration curve was successfully constructed. Based on the results, the absorbance of iron (III) lactate solution is directly proportional to the concentration of lactic acid in the range of 0.003125 g/mL to 0.1 g/mL. This is expected as the absorbance increases, more light is absorbed by the location. In the other words, higher absorbance value suggests higher concentration of lactic acid. Besides, a best-fit line was added into Figure 1 to determine the following equation, which was extremely useful in getting the lactic acid concentration in the later experiment:

\[
\text{Absorbance (OD}_{390nm} = 11.087 \times \text{Concentration of Lactic Acid} - 0.0085 \tag{1}
\]

This calibration method was developed by Borschchevskaya et al. [11]. They proved that this method is an efficient and inexpensive method as compared to High Performance Liquid Chromatography (HPLC) method, as the results obtained from both methods were almost similar (< 3% difference).
The anaerobic digestion was conducted in a 100 mL glass bottle, where 125 g (VS) of food waste was added into the bottle. Then, 50g of Ragi Tapai was mixed well with the food waste and the pH value of the mixture was adjusted to pH 5 using potassium chloride (KOH) or hydrochloric acid (HCl). After that, the bottle was connected to a vacuum pump to remove all the oxygen presence, and it was left in a pre-heated oven at 45 °C for four days. Lastly, the experiment was repeated with different amount of inoculum, 100 g, 150 g, 200 g, 250 g, and 300 g.

3.2. Volatile solid determination
The determination of volatile solid in the food waste is an important step in biological treatment because it predicts the amount of organic matter present in the waste. Other than providing a first approach of the organic matter available to be biodegraded, the amount of volatile solid determined is also used as a process control parameter. Besides, the product of biological treatment is usually expressed in term of volatile solid. Hence, the determination of volatile solid is an analysis that is widely-used not only in the research area [12,13], but also in the plant operation. The result of this study shows that the food waste collected consists of 94.8% of volatile solid. In the other words, it has huge potential to be biodegraded and produce lactic acid after second stage of anaerobic digestion, the acidogenesis.

3.3. Optimization of anaerobic digestion (hydrolysis and acidogenesis) of food waste for lactic acid production

3.3.1. Amount of inoculum. To investigate the effect of inoculum (Ragi Tapai) amount on the anaerobic digestion for lactic acid production, different amount of inoculum (50 g, 100 g, 150 g, 200 g, 250 g, and 300 g) was mixed well with the food waste. After the second stage of anaerobic digestion, the experiment was terminated, and a brownish solution (lactic acid) produced was collected and measured using UV/VIs spectrophotometer. However, the absorbance value of the brownish solution is in between 2 to 3, which is way above the range of absorbance plotted in the calibration curve (Figure 1). Hence, in order to get the absorbance in between the range (OD390nm = 0 to 1.2), five times dilution was conducted, and its absorbance was measured again at 390nm using spectrophotometer. After that, the lactic acid concentration was determined using the calibration curve constructed previously. The concentration of the diluted product sample and the actual product sample were recorded in Table 1.
Table 1. The summary result for the studied parameter: amount of inoculum (Ragi Tapai).

| Amount of Inoculum (g) | OD\textsubscript{390nm} before dilution | OD\textsubscript{390nm} after dilution | Concentration of the lactic acid after dilution (g/mL) | Actual concentration of the lactic acid (g/mL) |
|------------------------|----------------------------------------|--------------------------------------|------------------------------------------------------|-----------------------------------------------|
| 50                     | 2.090                                  | 0.022                                | 2.751x10\textsuperscript{-3}                         | 0.013755                                      |
| 100                    | 2.427                                  | 0.111                                | 0.0108                                              | 0.054                                         |
| 150                    | 2.531                                  | 0.137                                | 0.0131                                              | 0.0655                                        |
| 200                    | 2.776                                  | 0.154                                | 0.0147                                              | 0.0735                                        |
| 250                    | 2.893                                  | 0.173                                | 0.0164                                              | 0.082                                         |
| 300                    | 2.895                                  | 0.175                                | 0.0166                                              | 0.083                                         |

From the results obtained, it can be observed that as the amount of inoculum increases, the concentration of the lactic acid present in the product solution also increases, from 0.014 g/mL with 50 g of inoculum to 0.083 g/mL with 300 g of inoculum. The experiment was terminated at 300 g of inoculum because the concentration of the lactic acid obtained was almost similar with the one using 250 g of inoculum. Hence, it can be concluded that, 250 g of inoculum is the optimal parameters for the lactic acid production with the advantages of lower cost but comparable lactic acid yield.

3.3.2. Temperature control. Similar with the previous section, the absorbance value for the brownish solution produced in this section was exceed the range of the calibration curve. Hence, a five times dilution was conduction and the results determined were reported in Table 2.

Table 2. The summary result for the studied parameter: anaerobic temperature.

| Temperature (°C) | OD\textsubscript{390nm} before dilution | OD\textsubscript{390nm} after dilution | Concentration of the lactic acid after dilution (g/mL) | Actual concentration of the lactic acid (g/mL) |
|----------------|----------------------------------------|--------------------------------------|------------------------------------------------------|-----------------------------------------------|
| 45            | 2.394                                  | 0.155                                | 0.0147                                              | 0.0735                                        |
| 40            | 2.201                                  | 0.112                                | 0.0109                                              | 0.0545                                        |
| 35            | 2.515                                  | 0.108                                | 0.0105                                              | 0.0525                                        |
| 25 (room temperature) | 2.332                                  | 0.087                                | 8.613x10\textsuperscript{-3}                         | 0.0431                                        |

As shown in Table 2, the concentration of the lactic acid present in the solution decreases as the temperature decreases. The optimal temperature for lactic acid production is at 45 °C, since it has the highest concentration, which is 0.0735 g/mL, compared to other temperatures. The concentration at 25°C is 41.4% lower than the optimal condition. Meanwhile, the concentration at 40°C and 35°C is almost the same, which might due to the small temperature difference.

3.3.3. pH control. Table 3 below is the results of lactic acid production using pH values as the manipulated variable. This study has the same case with the amount of inoculum experiment, where the results obtained exceeded the range mentioned in the curve. A five times dilution was performed on the brown solution, and the diluted solution was then measured using the spectrophotometer.
From the results, it is observed that as the pH value increases, the lactic acid concentration increases until pH 7. This shows that the anaerobic digestion for lactic acid production is suitable to be conducted in the alkaline condition. However, after pH 7, the lactic acid concentration decreases. Hence, it can be concluded that the optimal pH range for lactic acid production is at 7. This matches the results obtained from the previous study [14].

3.3.4. Summary of optimization study. Based on the results for every parameters studied above, the optimal conditions for the lactic acid production is the one under pH control experiment, which has the pH value of 7, 100 g of inoculum at 45°C with 125 g of volatile solid as its lactic acid produced has a concentration of 0.0845 g/mL. This is in contrast with the previous study done by Raymon and Abigail, in which their optimal condition was occurred at 150 g of volatile solid, pH value of 5.5, temperature of 41°C and it has 0.0408 g/mL [15]. Comparatively, the concentration obtained in the current work at pH value of 5, and temperature of 40°C is 0.0545 g/mL, which is still slightly higher with a lower amount of volatile solid used. This might due to shorter digestion time was applied in Raymon’s work, which was 16 hours only, instead of 96 hours, the digestion time allowed in the current study. Moreover, based on another study that had been done by Zhang et al., the optimal condition was at pH value of 7, 58.5 g of volatile solid and temperature of 35°C, with 0.064 g/mL as the maximum concentration of lactic acid [16]. In this case, their digestion was left for 120 hours, which is longer than the digestion time of current work but producing 24.2% lower concentration of lactic acid. This might because no inoculum was used in their work. Hence, it can be concluded that the addition of inoculum can further enhance the lactic acid production using anaerobic digestion.

4. Conclusion
As a conclusion, this work suggested that the production of organic acid, in this case lactic acid, from food waste can be done by using low level of technology, with the presence of inoculum, and no electricity consumption. The digestion of food waste offers both the ability to treat them (waste management) and produce value-added product for industrial usage. Out of all chemicals produced in digestion process, the chemical that has high value and high production potential under the appropriate condition is lactic acid. The optimal condition for lactic acid production in this work was at 45°C with pH value of 7 from 125 g(VS) of food waste and 100 g of inoculum for 96 hours. The lactic acid concentration obtained under the optimal condition was 0.0845 g/mL, which is higher than the other previous works. Besides, this study concluded that as the amount of inoculum increased, the lactic acid concentration increased. Similar trend was observed for pH value until pH 7. In contrast, the concentration lactic acid decreases with temperature. Even though a further economic analysis is needed to prove that food waste can be beneficial to the economy, the results obtained in these experiments show that lactic acid production from anaerobic digestion of food waste can be considered as an encouraging way of food waste management. This paper can be act as a good starting point to produce green acid from food waste.

| pH value | \( \text{OD}_{390\text{nm}} \) before dilution | \( \text{OD}_{390\text{nm}} \) after dilution | Concentration of the lactic acid after dilution (g/mL) | Actual concentration of the lactic acid (g/mL) |
|----------|-----------------------------------------------|-----------------------------------------------|------------------------------------------------------|-----------------------------------------------|
| 4        | 2.251                                         | 0.122                                         | 0.0118                                               | 0.059                                         |
| 5        | 2.369                                         | 0.073                                         | 7.35x10\(^{-3}\)                                     | 0.037                                         |
| 6        | 2.550                                         | 0.165                                         | 0.0156                                               | 0.077                                         |
| 7        | 2.493                                         | 0.179                                         | 0.0169                                               | 0.085                                         |
| 8        | 2.474                                         | 0.160                                         | 0.0152                                               | 0.076                                         |
Acknowledgments
The work was funded by AAIBE Chair of Renewable Energy (Grant code: 201801 KETTHA). Besides, the authors would also like to thank UNITEN for the research facilities.

References
[1] United Nations, Department of Economic and Social Affairs and Population Division 2015 *World Population Prospects: The 2015 Revision, Key Findings and Advance Tables* 53 (New York)
[2] De S and Debnath B 2016 *Procedia Environ. Sci.* 35 (20) 1-8
[3] Yukalang N Clarke B and Ross K 2018 *Int. J. Environ. Res. Public Health* 15
[4] Gustavsson J Cederberg C Sonesson U Van Otterdijk R and Meybeck A 2011 *Global Food Losses and Food Waste: Extent, Causes and Prevention* (Rome)
[5] Sharif N A M 2018 Amount of food wasted by Malaysians enough to feed 12 million people a day *New Straits Times*
[6] Posmanik R Labatut R A Kim A H Usaek J G Tester J W and Angenen L T 2017 *Bioresour. Technol.* 233 134–43
[7] Romero-Güiza M S Vila J Mata-Alvarez J Chimenos J M and Astals S 2016 *Renew. Sustain. Energy Rev.* 58(14) 86–99
[8] Sivasankari R, Kumaran P, Normanbhay S and Shamsuddin A H 2013 *IOP Conf. Ser. Earth Environ. Sci.* 16 012009
[9] Kumaran P Hephzibah D Sivasankari R Saifuddin N and Shamsuddin A H 2016 *Renew. Sustain. Energy Rev.* 56 929–40
[10] Ghadiri A Pourkhalili S Taravati A and Veysi S 2018 Lactic Acid Production by *LactobacillusDelbrueckii* from Agricultural Waste: Role of C:N Ratio and Different Carbon and Nitrogen Sources 19th International Iranian Congress of Microbiology (Tehran, Iran)
[11] Borschchevskaya L N Gordeeva T L Kalinina A N and Sineokii S P 2016 *J. Anal. Chem.* 71(75) 5–8
[12] Kelly Orhorhoro E 2017 *Am. J. Mod. Energy* 3(1) 31
[13] Peces M Astals S and Mata-Alvarez J 2014 *Environ. Technol.* 35(304) 1–6
[14] Loh C W Fakhrul-Razi A Hassan M A and Karim M I *Artif. Cells. Blood Substit. Immobil. Biotechnol.* 27(45) 5–9
[15] RedCorn R and Engelberth A S 2016 *Biochem. Eng. J.* 105 205–13
[16] Zhang B He P Jing Ye N Fang and Shao L Ming 2008 *Bioresour. Technol.* 99 855–62