Food wastes as potential substrates for lactic acid production in open fermentation

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ABSTRACT

Chemical compositions of food wastes collected in Universiti Sains Malaysia were compared against Japanese food wastes. Food waste slurry was fermented in open condition without any sterilization and neither inoculums nor nutrients were added. The relation of glucose to lactic acid with parameters of fermentation time, temperature and initial pH were observed. Highest lactic acid yield for all three parameters were 18.06±4.61, 15.91±1.23 and 24.09±2.34 g/L respectively. When initial pH was set at pH 9, bioconversion of initial glucose to lactic acid was 38.04 % compared to only 30.30 % when initial pH was 7. Lactic acid production showed an increasing trend as initial pH increased from 7 to 9.

1. Introduction

Food leftover and kitchen wastes are food waste rich in nutrients collected from homes, food eateries and restaurants. In Malaysia alone, fraction of this waste from municipal solid waste in 2005 was 4.404 million tons and was estimated to increase to 6.54 million tons in 2020 (Kathrivale et al. 2003). Currently, food waste was disposed in landfills but the rising pollution of greenhouse gases and foul odor emission, toxic leachate and vermin attraction as well as area limitation had initiated better ways of handling these organic wastes (Kim and Kim, 2010). Developed countries are already taking steps to reduce amount of food wastes generated. One of the steps is by using food wastes to produce beneficial products such as hydrogen and methane (Liu, et al. 2006) as well as lactic acid (Yang et al. 2006; Ohkouchi and Inoue, 2007).

Lactic acid or 2-hydroxypropionic acid is naturally produced from glucose metabolism during fermentation. Production of lactic acid commercially is done either by chemical synthesis or natural synthesis by applying carbohydrate fermentative technology (John et al. 2007; Wee, et al. 2006). Alternatively, lactic acid also has been reported being produced from agriculture resources (Oh et al. 2005) and food waste by utilizing specific lactic acid-producing bacteria (Ohkouchi and Inoue, 2006). The factor that makes food wastes suitable for lactic acid production is the high content of carbohydrates mainly starch. Food wastes are aplenty thus costs of obtaining this substrate for large scale production would not be an issue. There were hardly any researches done to study the effect of existing glucose in food waste in relation to lactic acid content during open fermentation.
Table 1 Chemical components of food waste in this experiment compared to Japanese food wastes

<table>
<thead>
<tr>
<th></th>
<th>Food leftover</th>
<th>Japanese food wastes (Okouchi &amp; Inoue, 2006 and 2007; Sakai et al. 2000)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ash</td>
<td>1.54±0.36</td>
<td>0.8-1.68</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>5.12±1.11</td>
<td>6.37-10.2</td>
</tr>
<tr>
<td>Cellulose</td>
<td>3.64±0.69</td>
<td>0.3-1.87</td>
</tr>
<tr>
<td>Moisture</td>
<td>86.20±2.28</td>
<td>77.5-80</td>
</tr>
<tr>
<td>Lipids</td>
<td>1.15±0.19</td>
<td>1.8-5.74</td>
</tr>
<tr>
<td>Proteins</td>
<td>3.44±0.44</td>
<td>3.75-5.6</td>
</tr>
<tr>
<td>Glucose</td>
<td>11.76±1.08</td>
<td>9.85-12</td>
</tr>
</tbody>
</table>

Numerous kinetics studies have been done on food waste to produce hydrogen but no reports were available for lactic acid production by endogenous microbes. Sakai et al. (2000) studied lactic acid production from kitchen refuse by continuously and intermittently adjusting pH throughout the experiment. However, adjustment of initial pH alone has been proven as effective to produce better lactic acid yield (Okouchi and Inoue, 2007). Nevertheless, comparison was not possible due to the different fermentation conditions. This study has attempted to observe the effect of initial pH on lactic acid production and glucose levels of food wastes. Temperature is also considered the most important factor to increase lactic acid production. Previous studies utilizing endogenous microbes of food wastes have been using cultivation temperature of 35-37°C (Sakai et al., 2000) and no other reports of using temperature of 25°C and below. Current study also tested the effect of temperatures on lactic acid and glucose levels in the culture media. Multiple regression analysis was conducted in all experiments to determine the relation among variables and yield.

2. Materials and methods

2.1 Food waste samples

Three different batches of food leftover were collected from different cafeterias in Universiti Sains Malaysia. The next day, 50 g of each batch were taken for chemical composition analysis while 100 g of food leftovers were added with 200 ml distilled deionized and grinded to form slurry. Initial pH was adjusted to 7 before placing the slurry in 500 ml conical flasks (fermenter) and sealed tightly and ready for fermentation at 25°C statically. All experiments were done in triplicates.

2.2 Effect of fermentation time, temperature and initial pH on lactic acid yield

To test the effect of fermentation time, the three different batches of food waste was fermented from day one until day five. Reaction was stopped at the end of each day by diluting 10 g sample with distilled deionized water, shaken vigorously for 30 seconds, added tricholoacetic acid (v/v) and centrifuged (3000 rpm, 10 minutes). Supernatant was kept for lactic acid and glucose analysis. After the best duration was obtained, test on temperature of 5°C to 55°C with 10°C increment was conducted with initial pH 7. The best temperature with the highest yield and the best fermentation time were used to test the effect of initial pH of pH 1, pH 3, pH 5, pH 7 and pH 9.

2.3 Food waste analysis

Lactic acid content was determined spectrophotometrically (UV-VIS 1900, Hitachi) by methods of Taylor (1996). Glucose was determined by phenol-sulphuric acid test of Dubois et al. (1956). Moisture was determined by the loss of weight after drying for 24 hours in 70°C while ash was determined by combustion in 600°C for 2 hours. The carbohydrate content of food waste was determined by methods of Crampton and Maynard (1937). Protein and lipid were determined using the Kjeldahl and Soxhlet method respectively. The carbohydrate contents were obtained by subtracting the total organic fraction with 100.
Glucose is highly important as this is the main substrate to be used to produce lactic acid. Cellulose content (3.30±1.19 %) was higher than the amount found in Japanese kitchen wastes. Cellulose, a complex carbohydrate is also utilized by some lactic acid-producing microorganisms endogenous of food waste (Sakai et al. 2000). The authors stated other microbes might contribute to hydrolysis of polysaccharides and form commensalism relationship with the lactic acid bacteria. Other research utilized agriculture sources or waste from food processing industries that contain high amount of cellulose by digesting it and obtaining supernatant rich in glucose for lactic acid production (Musatto et al. 2007).

It has been demonstrated that protein or nitrogen content is very important for bacterial survival (Xiaodong et al. 2007; Bulut et al. 2004). Amount of protein (3.10±0.16 %) was relatively in the same range found in the kitchen waste (Ohkouchi and Inoue, 2007). The authors confirmed that nitrogen sources equivalent to carbohydrate amount could be a key factor for efficient conversion of initial glucose to lactic acid or bioconversions by lactic acid bacteria. This further supports the potential of food waste leftover as substrate in lactic acid production.

### 3.2 Effect of fermentation time

Highest lactic acid production (18.05±4.61 g/L) was on the third day of fermentation with bioconversion of 28.50 % (P<0.05) from initial glucose (63.33 g/L) (Figure 1). Rate of production was the most rapid (10.52 g/L d⁻¹) from initial amount (3.49±0.26) g/L to day one (13.91±6.84 g/L).

Sakai et al. (2000) that showed lactic acid production reached 22 g/L after 60 hours of incubation and remained at 27 g/L even after 120 hours with 6 hr interval adjustment of pH 7 and statically at 37°C. They also demonstrated that highest productivity was within 12-48 hours and number of bacterial cells increased quickly after 12 hours and number of lactic acid bacteria increased to reach similar cell number of total bacteria after 24 hours. This explains the abrupt drop in glucose amount in the present study from 63.33±19.54 to 48.19±19.93 g/L within the first day indicating high consumption of glucose. On the second day as lactic acid bacteria population increased, more glucose (50.63±4.03 g/L) was released into the media indicating degradation of simple and complex carbohydrates. Lactic acid yield on the third day of fermentation with initial pH 1, 3, 5, 7 and 9 for three days at 25°C statically.

### 3.3 Effect of temperature

From Figure 1, it is obvious that lactic acid concentrations were much lower than glucose concentrations. On average glucose content was 43.30 g/L while lactic acid was 15.23 g/L that is about half the amount of glucose present. Theoretically, 1
molecule of glucose would give 2 molecules of lactic acid. Nevertheless, in the natural environment some of the glucose is used for metabolism of endogenous microorganisms (Ohkouchi and Inoue, 2007). Other studies showed a crossing of glucose level curve with lactic acid curve indicating depletion of glucose as lactic acid production increases (Anuradha et al. 1999).

Zhang et al. (2008) and Sakai et al. (2000) showed that intermittently adjusting pH improved lactic acid yield. Nevertheless, Zhang et al. (2008) obtained highest lactic acid concentration (64 g/L) higher than Sakai et al. (2000) (45 g/L). This was due to the initial sugar (28.3 %) and protein content (17.8 %) of Zhang’s work was much higher than Sakai’s sugar (12 %) and protein (5.6 %). It had been reported that total sugar content to protein content was important to determine lactic acid yield (Ohkouchi and Inoue, 2007). Current work contained sugar (11.76±1.08 %) and protein (3.44±0.44 %) of similar range to Sakai et al. (2000). Furthermore, Sakai and his team showed when initial pH 7 was set and no pH adjustment was done on food waste fermented at 37°C statically for 3 to 5 days, 13 g/L of lactic acid was obtained. This result was lower than current research (18.06±4.61 g/L lactic acid) when initial pH 7 was set and fermented at 25°C statically for 3 days.

3.2.1 Regression analysis

The $R^2$ square value to show fitness of data to the linear regression line is 0.3355 and indicated clearly by the largely scattered points. ANOVA tests on variables also showed $P$>0.05 except for intercept. Residuals calculated from actual and predicted data did not indicate present of outliers but the regression equation formed showed a weak interaction among time, glucose and lactic acid levels in food leftover.

3.3 Effect of temperature

Temperature difference affected glucose content more than lactic acid content ($P$<0.05) (Figure 2). Lactic acid concentration was highest at 25°C (15.91±1.23g/L) with lowest glucose amount (25.42±2.18 g/L) suggesting consumption of glucose by endogenous lactic acid bacteria to produce lactic acid was more efficient at this temperature. As temperature increased from 25°C, lactic acid yield decreased signifying production was not improved by higher temperature. Zhang et al (2008) discovered total lactic acid concentration was reduced by 60 % when food wastes fermentation temperature increased from 35°C to 45°C although isomer purity was more at 45°C. Alternatively, as temperature increased from 45°C to 55°C, glucose amount in the current study shot up from 38.79±2.24 to 99.38±3.74 g/L. This signified that saccharification of starch to glucose occurred at this temperature as proposed by Ye et al. (2008) that α-amylase was active at temperature of 55-75°C but no lactic acid bacterial growth was observed.

3.3.1 Regression analysis

A multiple linear regression analysis was conducted to relate the effects of temperature, glucose and lactic acid. The regression model obtained showed that $R$ squares value was 0.06765 which was very far from 1 as well as the adjusted $R$ squares. The more further apart those two values, the lesser the reliability of the model. The $P$-value significance of the variables and intercept was also more than 0.05. Residual values calculated based on predicted values and actual values were also larger than ±3.0. Any residuals that have larger values than ±3.0 were the outliers that could signify a problem in data collection. In this case, it was due to the response of variable glucose to temperature that has no effect on lactic acid yield.

3.4 Effect of initial pH

Lactic acid production increased as initial pH increased from 7 to 9 ($P$<0.05) (Figure 3). Initial pH 9 showed the highest lactic acid production at 24.09±2.34 g/L suggesting that when initial alkaline state was set, the media stayed at near neutral range longer thus promoting more lactic acid production. Bioconversions from initial glucose to lactic acid increased from 30.30 % (pH 7) to 38.04 % (pH 9). Sakai et al. showed lactic acid yield was highest (45 g/L) when intermittent pH adjustments were done at pH 7 and 10 at 37°C statically for 3 to 5 days. Zhang et al. (2008) showed total lactic acid was highest at pH 7 (64 g/L) compared to pH 8 but L(+) lactic acid isomer was higher at pH 8 when incubated (shaken) at 35°C for 120 h. Lactic acid bacteria prefer range of pH 5 to 7 (Hofvendahl & Hahn-Hagedal, 2000). Moreover, several lactic acid bacteria also have good growth over pH 4.5 to pH 9.5 such as L.plantarum KY-1 and L.brevis KY-2 (Sakai et al. 2000). Alternatively, glucose concentration was not affected by initial pH ($P$>0.05).

3.4.1 Regression analysis

Based on the multiple regression analysis the $R$ square is 0.9797 and adjusted $R$ square is 0.9594. ANOVA test resulted in $P$-value more than 0.05 for intercept and variable $x_2$ but less than 0.05 for variable $x_1$ which is initial pH. Calculated residuals plotted against variables are also within ±3 and no outliers are observed. From the regression modeling conducted, it can be concluded that initial pH has a significant effect on lactic acid production and glucose concentration.

4. Conclusion

Lactic acid production in open fermentation was feasible and highest lactic acid was produced with fermentation time of three days, initial pH 9 at 25°C.
Multiple linear regression analysis showed that interaction and response among initial pH, glucose and lactic acid content was the best compared to other parameters and their interaction with glucose and lactic acid. Nevertheless, residual glucose after fermentation was twice the amount of lactic acid accumulated. Unlike other research that show as substrate depleted and product increased, levels of glucose and lactic acid in open fermentation of food leftover did not show similar trends thus indicating inefficiency of glucose utilization in the food waste. Bioconversion of initial glucose to lactic acid was only 38.04 % but better lactic acid yield could be obtained if nitrogen sources were supplemented (Ohkouchi and Inoue, 2007). Furthermore, food leftover was only compared against other food wastes with similar chemical characteristics and nutrient of similar proportions (Sakai et al. 2000 and Zhang et al. 2008). There should be a comparison to other food wastes with different nutrient proportions such as vegetable and fruits peels that contain high complex carbohydrates or fish waste that contains high protein. The production of lactic acid from such substrates would provide better insights on possible lactic acid yield predictions through modeling. Optimization of this process should also be done to increase bioconversion of glucose into lactic acid. Such experiments will be reported elsewhere.

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References
