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A Comparative Study of Organic Acids Production from Kitchen Wastes and Simulated Kitchen Waste

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Abstract: Simulated kitchen waste was developed in this study to overcome the problem of kitchen waste variation composition. The performance of organic acids production was compared between the simulated kitchen waste and original kitchen waste. Both substrates were subjected to anaerobic digestion by indigenous mixed microflora from fermented kitchen waste in a 250 mL shake flasks. The condition used were mixing at 200 rpm, adjusted pH 5 and 7 and temperature of 30°C, 37°C and 40°C. The highest organic acids produced in the both kitchen waste and model kitchen waste were 48.64g/L and 37.49g/L, respectively at pH 5 and 37°C. For both kitchen waste and simulated kitchen waste fermentation, lactic acid was dominant, 37g/L (76.2%) followed by acetic acid (17.7%) and butyric acid (6.1%).

Key words: Kitchen waste, model kitchen waste, anaerobic digestion, organic acid.

INTRODUCTION

Currently, about 15 268 ton/day of municipal solid waste (MSW) has been collected everyday in Malaysia and the average amount of waste generated is 0.5-0.8 kg/person/day (Karthivale *et al.,* 2003). About 71.6% of collected MSW consist of food and organic waste followed by plastics (13.3%), paper (5.8%), textile (2.4%), glass (2.1%), metal (2.0%), rubber and leather (0.5%), wood (0.5%) and others (1.8%). Food and organic waste has contributed to the high moisture content approximately 52.6% (Hassan *et al.,* 2001). On account of its high moisture content, these will be a challenge in order to appropriately provide waste disposal system since the organic waste will easily putrefies, resulting in groundwater contamination and odour generation (Wang *et al.,* 2005). So far, there are number of waste management technologies that have been adopted in Malaysia including landfill, and incineration. However, due to the new developments and space constrain, Malaysia has turning to incineration process especially in the cities (Hassan *et al.,* 2001). However, the incineration process is not suitable for treatment of food and organic waste due to combustion energy loss and undesirable byproduct dioxin related compounds are formed (Sakai *et al.,* 2001). Therefore, another appropriate method for stabilization and disposal of the food and organic waste need to be developed.

In MSW, food and organic waste mostly consist of uneaten food and food preparation waste especially from residences, restaurants and cafeteria known as kitchen waste (Village, 1998). Kitchen waste is characterized by a high organic content containing soluble sugars, starch, lipids, proteins, cellulose, and other compounds that are readily biodegradable, and generally contain few compounds that inhibit bacteria (Wang *et al.,* 2003b). There will be an advantage if the kitchen waste with high fraction of organic content can be utilized as a high value of carbon resource. So far, the recovery energy from methane fermentation and the production of organic fertilizers by composting using kitchen waste has been implemented (Wang *et al.,* 2001). These organic wastes also have been used for organic acids production. Production of organic acids from kitchen waste, not only can eliminate waste pollution problem but also reduce the production cost of organic acids (Wang *et al.,* 2003b). Lactic acid could be stably accumulated during kitchen waste fermentation by controlling some fermentation parameters such as temperature, pH etc (Sakai *et al.,* 2000; Wang *et al.,* 2002). Lactic acid was found to be the main fermentation products for kitchen wastes (Wang *et al.,* 2001; Wang *et al.,* 2003b). Recently, there is a need for lactic acids as it can be utilized as a raw material for polylactic acid, a polymer used as medical disposable and environmental friendly biodegradable plastics, which can replace synthetic plastics from petroleum feedstocks (Niju *et al.,* 2004).

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Organic acids fermentation of kitchen waste has widely been studied in certain countries (Wang *et al.,* 2005; Sakai *et al.,* 2001). It has been reported that organic acids fermentation is highly affected by aeration, pH, temperature, inocula and substrate characteristics (Zhang *et al.,* 2008). The fluctuation and variation of kitchen waste composition would affect the performance of organic acids production. In this study, the model kitchen waste was developed according to the characteristics of the real kitchen waste collected from local restaurants. Therefore, the aim of this study is to investigate the performance of organic acids production from developed model kitchen waste as compared to the real kitchen waste. Effect of pH and temperature on the organic acids production from both type of waste was also investigated.

MATERIALS AND METHODS

Kitchen Waste:

Kitchen waste was collected from several restaurants in Sri Serdang, Selangor, Malaysia. The ratio of rice, meat and vegetables in the kitchen waste were determined at 3:1:1. The kitchen waste was added with water at 1:1 ratio and then ground using a Waring blender (Wang *et al.,* 2005). Kitchen waste with total sugars of 100g/L was used (Horiuchi *et al.,* 2002).

Proximate Analysis:

Proximate analyses of the kitchen waste were performed. Kitchen wastes and model kitchen wastes were then characterized based on carbohydrate, lipids content, crude protein, fat, fiber and moisture content. The simulated kitchen waste was developed based on the composition and characteristics of the collected kitchen wastes. The moisture content, carbohydrate, crude protein, fat, fiber and ash were analyzed for both kitchen wastes and model kitchen wastes (Wang *et al.,* 2005).

Inoculum Preparation:

500 g of the collected kitchen waste with ratio 3 rice: 1 meat: 1 vegetable was fermented at 30°C for 15 days until all the solid form changed to the slurry form. The pH, total solids (TS) and total suspended solids (TSS) were 6.2, 118 g/L and 84 g/L, respectively with the number of colonies at 1 x 10^9.

Organic Acids Production:

100 ml of smashed kitchen waste and model kitchen waste were placed separately into a 250 ml of shake flasks without adding any other nutritional sources. The simulated kitchen waste was prepared based on the composition listed in Table 1. The fermentation was performed at 30°C, 37°C and 40°C, respectively with an agitation speed of 200rpm. The pH was adjusted to pH 5 and pH 7, respectively every 6 hours with the addition of 5 M of NaOH and 1 M HCl. 10% (v/v) of inocula was added prior to the fermentation (Horiuchi *et al.,* 2002). The samples were collected at regular interval and subjected to analysis of total sugars, organic acids, colony forming unit (cfu), total solids, and total suspended solids.

Analytical Methods:

Total sugar concentration was measured by the phenol sulphuric method (Fukui, 1990) using glucose as a standard. Each sample was filtered through 0.45 µm pore membrane after centrifuged at 4000 rpm for 15 minutes. The filtrate was subjected to analysis of organic acids such as lactic acid, formic acid, acetic acid, propionic acid and iso-butyric acid. The organic acids production was measured by high performance liquid chromatography (HPLC) column packed with ion exclusion HPLC organic acids analysis column (Aminex®HPX-87H) 300mm x 7.8mm, Biorad Laboratories using 4mM H_2SO_4 as the mobile phase at a flow rate (0.6mL/min) and detected with UV detector at 215nm (Hurok *et al.,* 2005). Viable bacteria counts in kitchen waste and simulated waste were determined by colony forming method at 30°C for 24 hours. Analysis of total solid and total suspended solid were determined according to Standard Methods (APHA, 1985).

RESULTS AND DISCUSSION

Characteristics of Kitchen Waste:

Kitchen waste is available in large amount from restaurant, cafeteria, canteen and residential area in Malaysia. Most of the time, composition of kitchen waste is vary and this will affect the production of organic acids in acidogenesis stages. The development of model kitchen waste is important to overcome the variation of kitchen waste composition in the fermentation process. Table 1 shows the composition and characteristics of kitchen wastes and model kitchen waste. The main composition of the kitchen waste consisted of rice (60%) followed by fish and meat (20%) and vegetable and fruit (20%). For the model kitchen waste, the value of carbohydrate, crude protein, fat, fiber and ash were fitted within the range of kitchen waste characteristics (Table 2). Therefore, it is very important to standardize the composition of model kitchen waste for the treatment process to ensure the organic acids production was not influenced due to the variation of substrate compositions. Kitchen waste and model kitchen waste contained high carbohydrate that will be broken down to soluble sugars such as glucose, fructose, and galactose in the saccharification process. The soluble sugars will then be utilized for growth of acidogenic bacteria that commonly found in the kitchen waste for organic acids production (Wang *et al.,* 2005; Zhang *et al.,* 2006). The growth of organic acids bacteria is fast on the complex nutrient. It is more advantageous for organic acids production using kitchen waste containing carbohydrate and others nutrient for the growth of organic acids bacteria (Wang *et al.,* 2005).

Anaerobic Digestion:

Anaerobic digestion of kitchen waste consists of the following stages which are hydrolysis of kitchen waste by various bacteria followed by acidogenic phase (where complex organic matter will be converted to volatile fatty acids and organic acids and other low molecular weight compound) and methanogenesis (Banerjee *et al.,* 1999). In the anaerobic acidogenesis process, total solids (TS) and total suspended solids (TSS) observed were 118 000 mg/L and 84 480 mg/L in kitchen waste while in model kitchen waste were 100 273 mg/L and 77 400 mg/L, respectively (Table 3). The extent of organic substrate utilization can be viewed from value of both TS and TSS (Banerjee *et al.,* 1999). Along the fermentation, TS and TSS decreased gradually with time. The decreasing pattern might be due to the hydrolysis of the complex compounds in the kitchen waste by the existence of indigenous microbes (Wang *et al.,* 2005). All the solids were degraded and converted to soluble sugars and then fermented to produce organic acids, water, simple sugars methane, hydrogen and carbon dioxide (Claassen *et al.,* 1999). The degradation of TS and TSS in model kitchen waste showed lower than in kitchen waste.

To investigate the performance of model kitchen waste, anaerobic digestion of model kitchen waste was conducted and compared with kitchen waste. Table 4 shows the total organic acids produced during anaerobic digestion of kitchen waste and simulated kitchen waste under various temperatures and pH adjustment. Kitchen waste produced the highest acids (48.64 g/L) at 37°C while model kitchen waste produced organic acids at 37.49 g/L at the same condition. In this study, the suitable pH was found to occur between 5.0-5.5. In general, the fermentation of sugars can take place at relatively low pH values of 5 to 6. Acidogenesis bacteria would not be able to convert all the starch to organic acids above pH 6.0 or below pH 4.5. For rapid and complete fermentation, the optimal pH ranges between pH 5.5 - 6.0 (Ohkouchi and Inoue, 2006). Even though most bacteria grown optimal at or near pH 7 (neutral), but the majority could not grow under strongly acidic or strongly alkaline condition resulted in net organic acids production is decreases (Min *et al.,* 2005). In acidic or alkali condition, bioactivity of some microorganism is reduced resulted in low organic acids yield even though the microbial culture are less susceptible to microbial contamination (Zhang *et al.,* 2008). Hydrogen ion concentration (pH) affects the activity of enzymes and therefore also affects the microbial growth rate. According to (Ferguson *et al.,* 1984), acid producing bacteria is a key role in the conversion of kitchen waste to volatile fatty acids (VFA) in anaerobic acidogenesis. The pH is expressed in relation to the VFA concentration (Mata-Alvarez, 1986). Control of pH is an existing and very common way to stabilize stressed anaerobic system which suffer from poor buffer capacity when high loading is applied (Yan *et al.,* 1993).

The growth rate of microorganisms in the fermentation process is highly influenced by the cultivation temperature. The suitable temperature for organic acids production was reported in mesophilic range (30°C to 40°C) (Ong, 2001). In this experiment, the optimum temperature obtained was 37°C. This temperature is suitable to increase reaction rates of the bacteria, specifically for lactic acid bacteria. It has been reported by (Demirci *et al.,* 1993), that 37°C was the best temperature for the growth of *L.casei* ATTC 11443. The neutral and mesophilic condition was favorable to the growth of all type of microorganism including hydrolytic microorganism and organic acids bacteria (Zhang *et al.,* 2008). The organic acids production was relatively low at 40°C (34.1 g/L). At high temperature, reaction rates proceed faster, but might inhibit certain bacteria. The products like acetate, CO_2, H_2, Jimeno *et al* (1990) reported that propionate and long-chained fatty acids and other organic acids are oxidized to acetate (Bryant, 1979). There are some benefits can be obtained at high temperature such as higher rates of digestion, faster solid-liquid separation, less threat of microbial pathogens but greater conversion of waste to gas (methane) which later might decreased the organic acids production. In this case, operation at lower temperature is an attractive alternative particularly at room temperature (Jimeno *et al.,* 1990) but the reaction among bacteria is quite slow and organic acids production will take a longer time.

A summary of organic acids generated from both kitchen waste and simulated kitchen waste is presented in Fig. 1 and 2. Lactic, acetic and butyric acid were the most prevalent acids being produced. For kitchen waste fermentation, lactic acid was dominant, 37 g/L (76.2%). Acetic acid obtained was 8.64 g/L (17.7%) and the rest was butyric acid, 3 g/L (6.1%). For the model kitchen waste fermentation, lactic acid was found at 27.4 g/L (72.6%) followed by acetic acids 6.64 g/L (18.8%) and butyric acid 1.2 g/L (3.4%). Lactic acid was predominant acid being produced throughout the fermentation. This might be due to the lactate producing bacteria are resistant to low pH (pH 4.5 - 6.5) and able to produce lactic acid compared to other acids-producing bacteria. Based on the nucleotide sequences of microbial community, there were variety of lactic acid bacteria and *Clostridium* sp. in the original food waste (Zhang *et al.,* 2008). In all food waste samples, about 58.5–81.8% of carbohydrate existed as starch. Then, the starch is broken down to sugars of low molecule weight such as maltose, glucose, fructose and galactose with the sacharification process (Zhang *et al.,* 2008).

Table 1: Composition of kitchen wastes and model kitchen waste used in this work

Composition	Kitchen Wastes	model Kitchen Waste
Rice (g)	120	120
Meat (g)	20	10 -15
Fish (g)	20	15 - 20
Vegetables and fruits (g)	40	45 - 60
Oil (ml)	-	7 - 12
Distilled water (ml)	-	4 - 10
Salt (g)	-	1 - 2
Sauces and ketchup (ml)	-	1 - 3

Table 2: Proximate analysis of kitchen wastes and model kitchen waste

	Kitchen wastes	model kitchen waste
Moisture (%)	65.98 - 70.01	66.59 - 74.54
Carbohydrate (%)	54.04 - 62.01	56.21 - 61.13
Crude Protein (%)	20.10 - 23.30	18.74 - 24.30
Fat (%)	11.6 – 15.4	8.38 – 13.35
Fiber (%)	1.25 – 1.78	1.06 – 1.83
Ash (%)	1.60 – 2.13	1.40 – 2.11

Table 3: Effect of anaerobic digestion process on total solid and total suspended solid.

	Kitchen waste			model kitchen waste		
	Initial	Final	% reduction	Initial	Final	% reduction
Total Solid (mg/L)	118000	48537	58.8	100273	67509	32.6
Total Suspended Solid (mg/L)	84480	63235	25.1	77400	69157	10.6

Table 4: Highest total organic acids produced from kitchen waste and simulated waste during fermentation at different temperature and pH.

	30°C		37°C		40°C	
Total Acid (g/L)	5	7	5	7	5	7
Kitchen Waste	29.13	23.82	48.64	35.24	40.56	32.23
Model Waste	16.38	17.68	37.49	29.87	34.1	28.24

Table 5: Performance of kitchen waste and simulated waste fermentation incubated at 37°C, pH 5

	Kitchen Waste	Simulated Waste
Total Acid (g/L)	48.64	35.24
Productivity (g/L/h)	0.41	0.29
Product yield coefficient (g acid/g carbohydrate)	0.57	0.47
cfu/ml	4.56×10^{8}	3.5×10^{6}
Total Sugar reduction (%) / Substrate conversion	85.65	75.21

Organic acids production in kitchen waste was relatively higher than that of simulated kitchen waste. This might be due to the different number of natural microflora present in the kitchen waste and simulated kitchen waste as shown in Table 5. A high numbers of microorganisms implying high microbial activities thus increase the conversion rate of kitchen waste to organic acids. Since the low number of microorganism in the simulated kitchen waste at the end of fermentation indicating the ability to ferment polysaccharides was lower compared to kitchen waste. Moreover, it was found that the substrate consumption for kitchen waste was higher than that in the simulated kitchen waste due to the decreasing pattern of total sugars. The decreased of sugar to the lower level suggested that the larger amount of sugar has been converted into organic acids (Wang *et al.,* 2005). The viable numbers of acid-producing bacteria implying the rate of substrates conversion to organic acids (Wang *et al.,* 2005). This explained the low organic acids productivity and yield in the simulated kitchen (0.29 g/L/h and 0.47 g acid/g carbohydrate, respectively) compared to kitchen waste (0.41 g/L/h and 0.57 g acid/g carbohydrate, respectively).

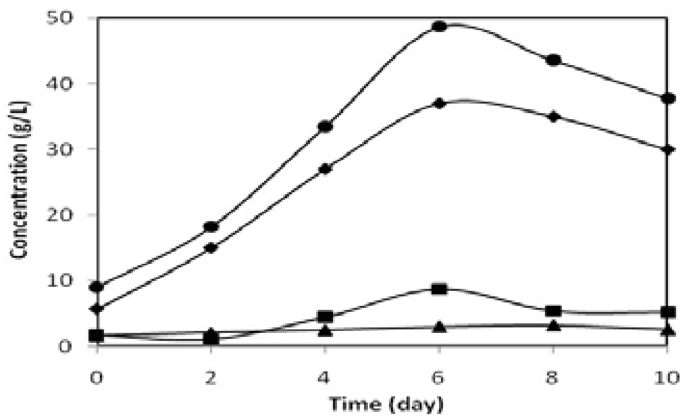

Fig. 1: Profile of organic acids production in the fermentation of kitchen waste incubated at 37°C, adjusted pH 5, 200 rpm (◆ Lactic acid ■ Acetic acid ▲ Iso-butyric acid ● Total acid). Results are means for duplicate experiment.

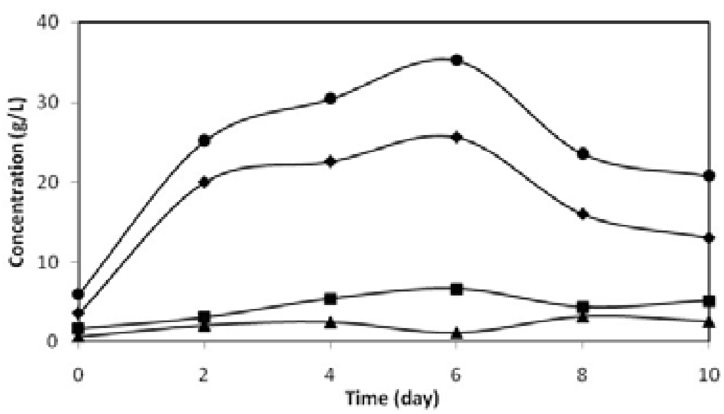

Fig. 2: Profile of organic acids production in the fermentation of model kitchen waste incubated at 37°C, adjusted pH 5, 200 rpm. (◆ Lactic acid ■ Acetic acid ▲ Iso-butyric acid ● Total acid). Results are means for duplicate experiment.

Conclusion:

Model kitchen waste was successfully developed in this study. The suitable temperature for organic acids production was 37°C while pH 5 gave a better result as compared to pH 7. The yields of organic acid from kitchen waste and model kitchen waste were 0.57 g acid/g carbohydrate and 0.47g acid/g carbohydrate, respectively. Even though the organic acids concentration in model kitchen waste was slightly lower compared to kitchen waste, several modifications such as seeding strategy using fermented kitchen waste could be applied.

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