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Full Length Research Paper

Enhancement of organic acids production from model kitchen waste via anaerobic digestion

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The aim of this study was to obtain the optimal conditions for organic acids production from anaerobic digestion of kitchen waste using response surface methodology (RSM). Fermentation was carried out using 250 ml shake flask which was incubated using an orbital shaker set at 200 rpm. Fermented kitchen wastes were used as inoculums sources. The individual and interactive effects of pH, temperature and inoculum size (%) on organic acids production from kitchen waste were investigated. The highest level of organic acid produced was 77 g/L at optimum pH, temperature, inoculum size of 6.02, 35.37°C and 20% inoculum, respectively. The results indicate that the most significant parameters affecting the bioconversion of kitchen waste to organic acids were temperature and inoculum size. Verification experiment of the estimated optimal conditions confirmed that RSM was useful for optimizing organic acids production from fermented kitchen waste.

Key words: Bioconversion, model kitchen waste, anaerobic fermentation, organic acids, optimization, response surface methodology.

INTRODUCTION

Kitchen waste and other organic solid waste discharged from households, restaurant, and residue from the food industry make up about 70% of total municipal solid waste (MSW) in Malaysia (Hassan et al., 2001). Due to its large volume, the disposal of kitchen and organic waste will be a big problem. At present, dumping waste in landfill is the most common practice. Kitchen waste has high organic content such as soluble sugars, starch, proteins, cellulose and etc. (Wang et al., 2005). Since kitchen waste has high organic compound and moisture contents, anaerobic process is the most suitable method for its treatment in comparison with alternative treatments such as incineration, landfill and composting (Zinatizadeh et al., 2006). Anaerobic digestion is a complex process that involves hydrolysis, acidogenesis, acetogenesis and methanogenesis (Bo et al., 2007). The advantages of anaerobic digestion are volume reduction, waste stabilization and biogas recovery (Wang et al., 2008). Acidogenesis of kitchen waste produces biogas and soluble organic products such as organic acids (Lim et al., 2008). Various organic acids produced from organic wastes especially kitchen waste has been utilized as energy and carbon sources in the production of bio-degradable plastic (Horiuchi et al., 2002). Organic acids such as lactic acid followed by acetic and propionic acids were found to be the main products of kitchen waste fermentation (Bo et al., 2007). Those organic acids are also widely used in pharmaceutical, food and beverage industries.

Apart from that this study also highlights on the variation problem of the kitchen waste during anaerobic digestion process and aims to develop a model of kitchen waste. The development of model kitchen waste was important to overcome the variation and fluctuation problem of real kitchen waste that later will affect the optimization study of the effect of culture conditions on organic acids production. In anaerobic digestion, it has been reported that the organic acids production was influenced by pH, temperature and inoculums size (Zhang et al., 2008). In acidogenesis process, pH plays a significant role because some acid producing bacteria prefer pH ranging from 5.5 to 6.5, to produce acid. The disturbance of equilibrium will become toxic to bacteria.

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Besides pH, the temperature at which anaerobic digestion occurs also can significantly affect the conversion, kinetics, stability, effluent quality and net energy of the biological conversion process. Koutsomanis and Sofos (2005) suggested the importance of inoculum size for microbial growth initiation involved in synthesizing the desired product. Although, the inoculum size is rarely used as a factor in studies of microbial growth, but there is evidence that showed it may affect the microbial growth; as the cell number in the inoculum increase, the lag time reduces. That is the important factor to accelerate the biochemical reactions.

Therefore, in this study, the effects of pH, temperature, inoculum size and the interactions of the factors on organic acids production from kitchen waste were investigated. In a previous study, the conventional 'change-one-variable-at-a-time' method was applied for optimization purposes (Wang et al., 2008). However, the major disadvantage of this technique is that it could not show the interactive effects among the variables tested. Furthermore, it will increase the number of experiments needed and consequently increase the time and consumption of reagents (Bezerra et al., 2008). In order to overcome this problem, the optimization of culture conditions was carried out using response surface methodology (RSM). RSM is a statistical technique for analyzing the effects of several independent variables on the responses (Zinatizadeh et al., 2006). RSM is important in process design and optimization as well as for improving the performance of the system. With RSM, the interaction of possible affecting parameters on organic acids production from food waste could be evaluated. Therefore, the aim of this study was to investigate the effects of pH, temperature and inoculum size as well as their interactive effects on organic acids production from model kitchen waste using RSM.

MATERIALS AND METHODS

Kitchen waste and culture conditions

Model kitchen waste was developed based on the composition and characteristics (carbohydrate, lipids content, crude protein, fat, fiber and moisture content) of the real kitchen waste (Hafid et al., 2010). For model kitchen waste, the fresh rice was prepared at 120 g and after that meats, vegetables, salt, oil, water, sauce and ketchup were added. The fresh meats and vegetables were boiled for 5 min before adding in the rice. This is to ensure the characteristics of the model kitchen waste mimic the real one. The model kitchen waste was blended with 1:1 ratio of water using a Waring blender. The inocula employed in this study were obtained from fermented kitchen waste.

For preparation of inoculum, 500 g of collected waste with ratio (rice : meat : vegetable) of 3:1:1 was fermented in a closed beaker 1 L un-der static condition and incubated at 30°C for 15 days until all solid form changed to slurry form. The number of colonies in the broth was 1×10^9. The chemical and physical characteristics of the model kitchen waste (substrate) and inoculum used are shown in Table 1. The batch experiments were conducted in 250 ml shake flasks with final volumes of 100 ml each. The effects of different pH, inoculum sizes and temperatures on organic acids production were tested. Total sugars were determined using the Phenol-sulphuric method. The medium was incubated and agitated at 2900 rpm using an orgabital shaker. Sodium hydroxide (5 M) was added to adjust the pH intermittently every 3 h for 5 days fermentation. pH was adjusted manually depending on the level of pH drop. The lowest pH value dropped during this interval was pH 3. Without pH adjustment, the pH in the broth will drop at a very low level; thus, making it not suitable for growth of acid producing bacteria. Verification experiment of the estimated optimal conditions was done in a bioreactor for confirming the results of organic acids produced. The fermentation was conducted in duplicate experiment.

Analytical methods

Organic acids

Samples of 5 ml each were taken at interval time of 24 h and centrifuged at 10000 rpm for 10 min. The supernatant was filtered through 0.45 µm pore membrane and analyzed for organic acids. The organic acids were determined by using high performance liquid chromatography (HPLC) column packed with ion exclusion HPLC organic acids analysis column (Aminex®HPX-87H) 300 x 7.8 mm, Biorad Laboratories using 4 mM H_2SO_4 as the mobile phase at a flow rate of 0.6 ml/min and detected with UV detector at 215 nm (Hurok et al., 2005).

Total solid (TS) and total suspended solid (TSS)

For the determination of TS, 10 ml of sample was added in the weighed porcelain crucible. The sample was heated at 105°C for 24 h. After cooling in the desiccator, the sample was weighed and TS can be calculated by the difference in weight. For the determination of TSS, a dry weighed standard glass fiber filter (GF/C) with 47 mm diameter was inserted in the filtration apparatus. 10 ml of diluted sample was poured into the glass fiber filter. Vacuum and washing was applied with continuous suction to allow complete drainage. The filter was removed and placed in the aluminium foil. The filter and aluminium foil was dried in the oven at 105°C for 24 h before cooling in the desiccator.

Chemical oxygen demand (COD)

COD analysis was done according to Standard Method (APHA, 1985). The COD heating block was preheated to 150°C. Sample was diluted into appropriate concentration. 2 ml of sample was pipetted into the high range COD (HACH) reagent kit vial. The vial containing sample and COD reagent was heated at 150°C for 2 h. The COD is measured for both sample and blank using spectrophotometer DR 2800 (HACH Direct Reading Spectrophotometer Arachem) at 620 nm.

Biological oxygen demand (BOD)

BOD reagents were prepared according to Standard Method (APHA, 1985). The sample was diluted appropriately and mixed with aerated distilled water containing 1 ml of each reagent per L (phosphate buffer solution pH 7.2, $MgSO_4$ solution, $CaCl_2$ solution, and $FeCl_3$ solution), while for control; sample was substituted by aerated distilled water. The sample was kept at 20°C in the dark for 5 days and after that the DO was measured again. The difference between the final and initial DO was calculated and known as BOD.

Moisture content

Determination of moisture and ash was based on Standard Method (APHA, 1985). A total of 2 to 5 g of sample was weighed and placed in the weighed porcelain. Then, the sample was dried in an oven at 105°C for 24 h. The sample was weighed again after cooling and the moisture content was calculated by the difference in weight.

Colony forming unit

Viable bacteria counts in kitchen waste and model kitchen waste were determined by colony forming method. Initially, serial dilutions of the sample were done aseptically. 100 µl of the diluted sample was spread on nutrient agar plate using a hockey stick. The plate was then incubated at 30°C for 24 h.

Central composite design

The central composite design (CCD) used in the optimization of organic acids production was generated by using the Stat-Ease software with Design Expert v.6 (Altaf et al., 2007). The CCD experiment was applied to develop an empirical model of the optimization process and to obtain optimum operating conditions for the factors involved. In this study, three key variables with five concentration levels each were applied (Table 2) using a full factorial design. According to this design, 20 runs of experiments were conducted containing six replications at the centre point to get good estimations of pure experimental errors (Table 3). The axial distance α was chosen to be 2 to make this design rotatable and orthogonal. The prediction of optimal point was fitted using a polynomial model function and the quality of the model expressed by the coefficient of determination R^2 and the significance of statistical analysis checked by F-test (Rodrigues et al., 2006). For predicting the optimal point, a second order polynomial function was fitted to the experimental results.

RESULTS AND DISCUSSION

Characteristics of model kitchen waste and inoculums

Organic acids fermentation utilizing model kitchen waste as substrates and fermented kitchen waste as inoculum was carried out in shake flasks without any nutrient supplementations. Table 1 shows the characteristics of model kitchen waste and inoculum. The results show that the pH value for model kitchen waste was slightly acidic (pH 4.9). This might be due to the activity of indigenous microbes and storage time after preparation and handling (Wang et al., 2002). For inoculum, the pH was close to neutral (pH 6.2) and a total of 1×10^9 cfu/ml bacteria were detected after 15 days of fermentation. The inoculum and the indigenous bacteria in fresh model kitchen waste were possibly involved in the degradation of the substrate and its conversion into organic acids. The model kitchen waste was high in organic matter as shown by the values of BOD and COD at 36864 and 110000 mg/L, respectively. This was advantageous for organic acids production as the model food waste contained large amounts of carbohydrate and other nutrients that were suitable for lactic acid bacteria proliferation. Moreover, the moisture content of the waste was high (more than 65%) which was favorable for the growth of most bacteria.

Optimization of the key culture conditions for organic acids production

The objective of this study was to investigate the effects of pH, temperature and inoculum size on organic acids production from kitchen waste using indigenous microbes. The optimal levels for the key culture conditions (pH, temperature and inoculum size) and the effect of their interaction on organic acids production were further explored using the central composite design of RSM. By applying multiple regression analysis on the experimental data, the second-order polynomial equation (Equation 1) was derived to explain the organic acids production.

$$Y = 69.03 + 0.63A + 1.55B + 8.36C - 9.09AA - 9.97BB - 1.89CC - 4.12AB + 0.38AC - 0.57BC \quad (1)$$

Where, Y is the predicted organic acids production and A, B, C are the coded values for pH, temperature and inoculum size, respectively.

The analysis of variance (ANOVA) is important to test the significance of the quadratic model (Table 4). The coefficient of determination (R^2) for the production of organic acids was 98%. This value showed a good correlation between the experimental and the predicted data for all regressions. The high value of R^2 indicated that at least 98% of the total variation in the response could be explained by the model (Renato and Nelson, 2009). The statistical value of the F-test probability showed that all variables were accurate in describing the data. The higher the F-value, the more better that the factors adequately explained the variation of the data around its mean (Mokhtari-Hosseini et al., 2009). The analysis of variance in this study demonstrated that the model was significant (F_{model}=124.39). There was only 0.01% chance that a "model F-value" as large would occur due to noise (John et al., 2007). P-values are important to check the significance of each variable. The smaller the P-value, the higher the significance of each variable (Guo et al., 2008). The value of the coefficient of variation (CV = 5.38) was low indicating a high precision and a good deal of reliability of the experimental value. The regression analysis of the experimental values showed that the linear model terms (B and C), quadratic model terms (A^2, B^2, and C^2) and interactive model terms (AB) were significant ($P < 0.05$).

Table 3 shows that the highest amount of organic acids was observed at run 14 which gave 77 g/L of total organic acids. The temperature and inoculum size showed a strong positive linear effect on the response ($P< 0.05$)

Table 1. Characteristics of substrate and inoculums.

Parameter	Unit	Substrate	Inoculum
pH	-	4.9	6.2
Total Solid	mg/L	100273	118000
Total Suspended Solid	mg/L	77400	84480
Chemical Oxygen Demand (COD)	mg/L	110000	112000
Biological Oxygen Demand (BOD)	mg/L	36864	42000
Moisture	%	66.5-74.5	65.9-70.1
Colony forming unit	cfu/ml	3.5×10^6	1×10^9

Table 2. Level of independent variables in the experimental plan.

Variable	Coded level				
	-2	-1	0	+1	+2
pH	4	5	6	7	8
Temperature	25	30	35	40	45
Inoculum size (%)	5	10	15	20	25

Table 3. Central composite design (CCD) for optimization of three variables (each at five levels) for the production of organic acids from model kitchen waste.

Standard order	pH		Temperature		Inoculum size (1×10^9 cfu/ml) (%)		Total organic acid (g/L)	
	A	Coded A	B	Coded B	C	Coded C	Predicted value (g/L)	Actual value (g/L)
1	5	-1	30	-1	10	-1	33.44	35.69
2	7	1	30	-1	10	-1	42.93	45.43
3	5	-1	40	1	10	-1	44.76	47.73
4	7	1	40	1	10	-1	37.79	40.66
5	5	-1	30	-1	20	1	50.15	51.81
6	7	1	30	-1	20	1	59.64	62.72
7	5	-1	40	1	20	1	61.48	61.22
8	7	1	40	1	20	1	54.50	56.01
9	4	-2	35	0	15	0	31.42	30.18
10	8	2	35	0	15	0	33.93	31.02
11	6	0	25	-2	15	0	26.06	23.39
12	6	0	45	2	15	0	32.24	30.77
13	6	0	35	0	5	-2	44.77	41.55
14	6	0	35	0	25	-2	78.20	77.28
15	6	0	35	0	15	0	69.03	67.99
16	6	0	35	0	15	0	69.03	68.41
17	6	0	35	0	15	0	69.03	69.14
18	6	0	35	0	15	0	69.03	69.85
19	6	0	35	0	15	0	69.03	66.47
20	6	0	35	0	15	0	69.03	68.18

(Table 4). Significant interaction was noted between pH (AA), temperature (BB) and inoculum size (CC). Graphical representations of the response surface are shown in Figures 1 to 3; to view the effects of pH, temperature and inoculum size on organic acids production.

The prediction of the model was that increasing the inoculum size resulted in an increase in the organic acids concentration when 25% of inoculum was applied. This might be due to the fact that the utilization of the substrate at the lower range of inoculum to substrate ratio

Table 4. Analysis of variance of the model.

Source	Sum of square	Degree of freedom	Mean square	*F* value	Prob > *F*	
Model	5123.63	6	853.93	124.39	< 0.0001	Significant
A	6.31	1	6.31	0.91	0.36	
B	38.22	1	38.22	5.56	0.0346	
C	1117.39	1	1117.39	162.76	< 0.0001	
A^2	2077.29	1	2077.29	302.59	< 0.0001	
B^2	2498.99	1	2498.99	364.02	< 0.0001	
C^2	89.41	1	89.41	13.02	0.0032	
AC	1.15	1	1.15	0.14	0.71	
AB	135.54	1	135.54	19.74	0.0007	
BC	2.61	1	2.61	0.33	0.58	
Residual	82.93	1	6.91			
Lack of fit	76.36	7	10.91	8.33	0.0166	
Pure error	6.57	5	1.31			
Cor Total	5212.88	19				
Std. Dev.	2.62		R^2	0.98		
Mean	52.27		Adj R^2	0.97		
C.V.	5.38		Pred R^2	0.93		
PRESS	348.77		Adeq Precision	33.64		

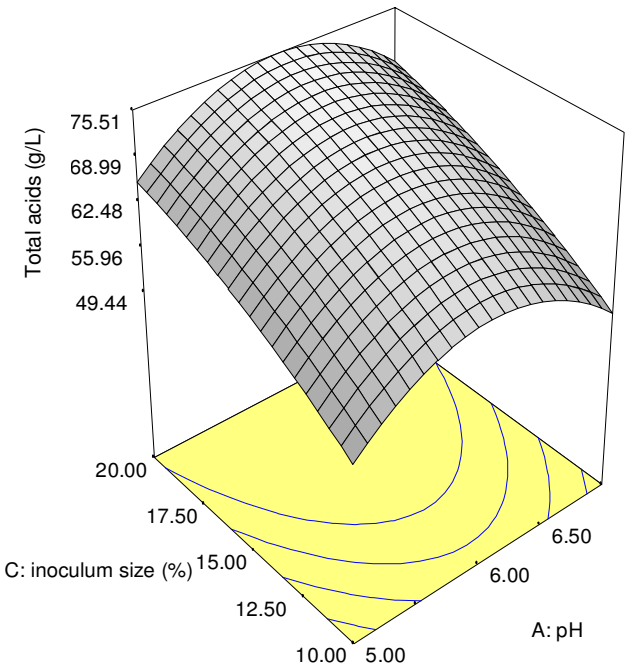

Figure 1. Interaction between inoculum size and pH on organic acids production.

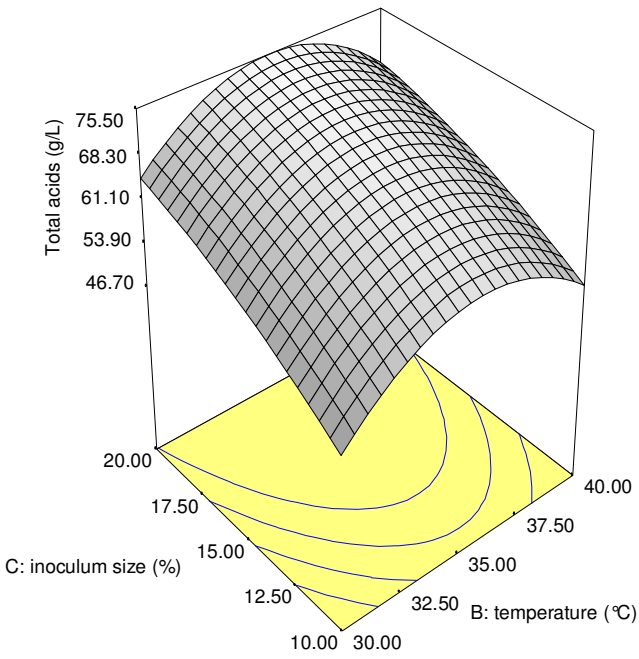

Figure 2. Interaction between inoculum size and temperature on organic acids production.

was limited by the amount of inoculum. Hence, increasing the inoculum to substrate ratio could significantly enhance the organic acids production (77.28 g/L). It is expected that by increasing the number of viable cells (approximately 1×10^9), more substrates will be utilized leading to increased product yield (John et al., 2007). However, a higher range of inoculum to substrate ratio may produce a large amount of biomass but lead to the lack of substrate and rapidly depletes the nutrient necessary for growth and thus, decrease the organic acids production (Wang et al., 2008).

However, a lower inoculum level may cause insufficient

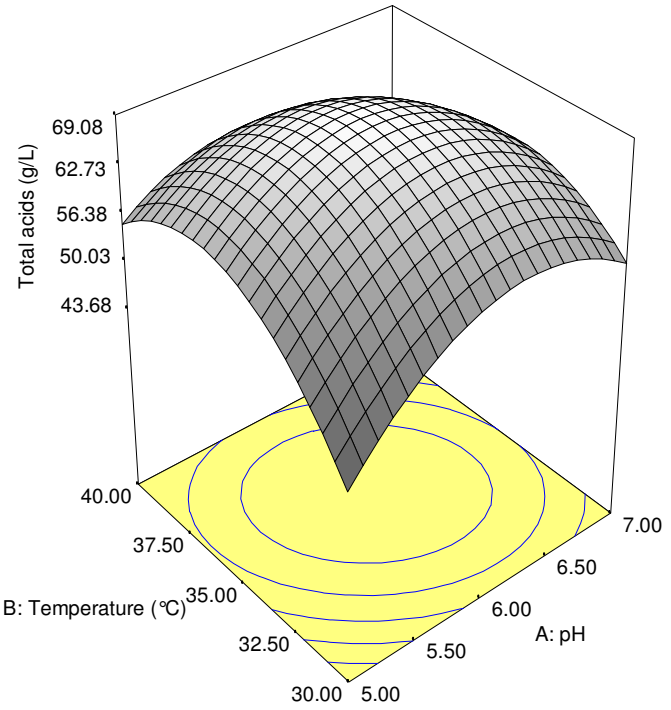

Figure 3. Interaction between temperature and pH on organic acids production.

cell biomass and later will allow the growth of undesirable microorganisms in the medium. As a result, it will increase the time needed for the microbes to grow and consume the substrate and synthesis of the desired product (Renato and Nelson, 2009). The production of organic acids with high inoculum size was also limited by temperature and pH (Figures 1 and 2). According to Koutsomanis and Sofos (2009), inoculum size gave a greater effect if an inhibitory factor (pH and temperature) was present in the environment. High production of organic acids (77.28 g/L) was observed at a higher level of inoculum size and an intermediate value of pH. In this study, the optimal pH for organic acids production (77.28 g/L) was at pH 6. Some of the organic acids producer or acidogens species have an optimal pH of 7 to 8 for cell growth, while pH 6 is known to be optimal pH for many other bacteria in acids reactor (Horiuchi et al., 2002). An inhibitory effect could also be seen when high pH was applied (Figure 1). This is because in acidic or alkali condition, the metabolisms of some microorganisms are reduced resulting in low organic acids production (Zhang et al., 2008). The production capacity also decreased probably as a consequence of a reduction in the metabolisms of the microbes (Renato and Nelson, 2009). Acidogenic bacteria would not be able to convert starch and polysaccharides to organic acids at above pH 6.5 and below pH 4.5 (Ohkouchi and Inoue, 2006).

Consequently, the organic acids production decreased; although, high inoculum size was applied. An acidogenic bacterium plays an important role in the conversion of kitchen waste to organic acids. Therefore, there is a need to increase the growth rate of these microbes in order to increase the organic acids production. Temperature is another factor that influences the organic acids production. In this experiment, the optimal temperature obtained was 35°C. Figure 2 shows the interaction between temperature and inoculum size on organic acids production. An intermediate level (35°C) of temperature and high inoculum size favored organic acids production. As reported by Demirci et al. (1993), 37°C was found to be the best temperature for growth and lactic acid production of *Lactobacillus casei* ATTC 11443. According to Zhang et al. (2008), neutral and mesophilic conditions were favorable for the growth of all types of microorganisms including hydrolytic microorganisms and acidogenic bacteria. At a high temperature, reaction rates proceed significantly faster, but as a consequence, it might inhibit certain bacteria. At higher temperatures, products like acetate, CO_2, H_2, propionate and long-chained fatty acids and other organic acids are oxidized to acetate by the activity of acetogenic associated with methanogenic bacteria (Horiuchi et al., 2002). Methanogenic bacteria will later utilize acetate and carbon dioxide for methane generation. As a result, organic acids production decreased due to their degradation into other by products.

Therefore, by increasing the inoculum size did not much affect organic acids production at slightly lower or higher temperature operation (John et al., 2007). The three-dimensional response surface of organic acids with the interaction between pH and temperature is shown in Figure 3. By analyzing the pH-temperature plot, a significant synergistic interaction effect to enhance total organic acids production was studied when pH was combined with temperature. From Equation 1, the maximum point of the model can be obtained, with the best temperature of 35°C and pH 6. The model predicted a maximum response for organic acids production at 77.58 g/L at this point. From the result obtained, it could be seen that during the fermentation, sugar was utilized rapidly (more than 80%) by the indigenous microbes. It could be inferred that when organic acids concentration was around 60 g/L, almost 50% (50 g/L) of sugar had been consumed and converted into organic acids (Figure 4). The results are in agreement with Wang et al. (2005) where the author successfully converted almost 60% of total sugar into lactic acid. The concentration of organic acids increase gradually until day 4 might be due to the acidogenesis process. At that time, the degradation of the substrate by acidogens was high, thus, increasing the organic acids production. After day 4 of fermentation, the concentration of organic acids start to decrease probably linked to the activity of the methanogens. The production of biogas that consumes acid as carbon substrate is favored by mesophilic temperature (Min et al., 2005).

Since the fermentation broth was a complex media due

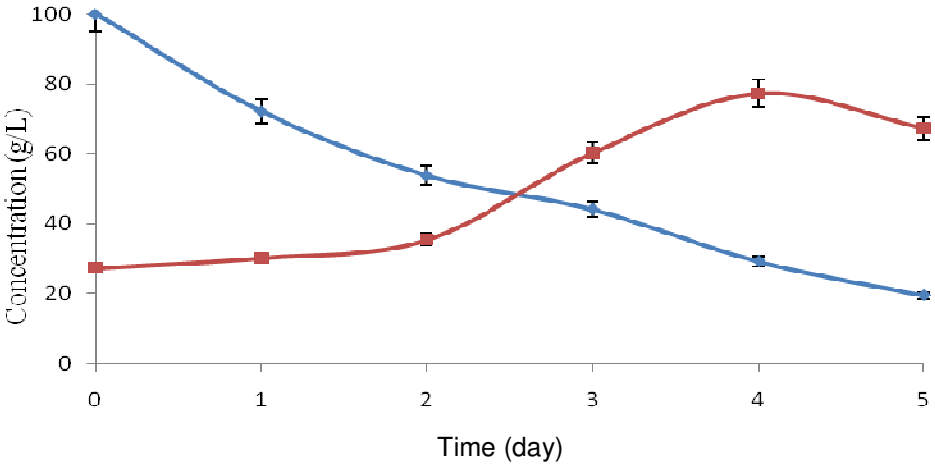

Figure 4. Profile of organic acids production and total sugar reduction in the fermentation of kitchen waste incubated at 35°C, adjusted pH 6 and inoculum size of 20% (■ Total organic acids; ♦ Total sugars) (Results are mean of duplicate experiments).

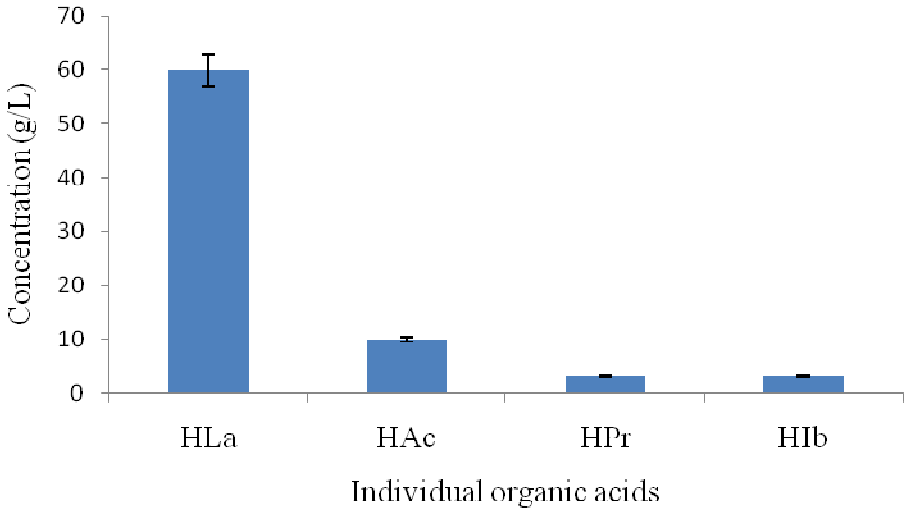

Figure 5. Individual organic acids produced during fermentation of kitchen waste incubated at 35°C, adjusted pH 6 and inoculum size of 20%. HLa – lactic acid, HAc- acetic acid, HPr-Propionic acid, HIb- Butyric acid (Results are mean of duplicate experiments).

to the variable composition of kitchen waste containing a lot of indigenous microbes, the fermentation process is considered as heterotypic of organic acids fermentation instead of homotypic fermentation. This type of fermentation will produce considerable amounts of carbon dioxide, lactic acid, acetic acid and or ethanol (Hammes and Whiley, 1993). Therefore, in the fermentation of kitchen waste, organic acids such as lactic acid, acetic acid, iso-butyric acid and propionic acid were detected in the fermentation broth. Lactic acid was found to be the main product in kitchen waste fermentation (Bo et al., 2007). Since carbohydrate is the main component of kitchen waste, the carbohydrate is broken down to low molecular weight sugars such as maltose, glucose, galactose and fructose in the saccharification process. The soluble sugars will serve as substrates available for the growth of most lactic acid bacteria. Therefore, a high concentration of lactic acid was detected. High accumulation of lactic acid led to the production of propionic acid (Zhang et al., 2008). As demonstrated by Bo et al. (2007), lactic acid was easily converted to propionic acid and acetic acid under hydrogen partial pressure above 100 Pa during the anaerobic digestion process.

In this study, the concentration of acetic acid was about 10 g/L whereas the lactic acid production was up to 60 g/L (Figure 5). The results obtained was relatively higher

compared to Wang et al. (2005) that produced 30 g/L of total organic acids from fermentation of kitchen waste in a non-sterile condition. The action of the mixed population of acetogenic bacteria degraded the lactic acid, protein and amino acids, fatty acids and other products from the hydrolysis stages into acetate. In all experiments, the proionic acid and the iso-butyric acid concentration remained low about 3 g/L. A similar result was obtained by Bo et al. (2007), where the lactic acid produced was higher than the acetic, butyric and propionic acids produced. Organic acids produced from kitchen waste could be recovered using freezing and thawing, centrifugation, filtration and evaporation method (Omar et al., 2009). Using this integrated process, about 224 g/L of organic acids could be recovered from the initial 26 g/L acids produced during the anaerobic digestion process.

Verification of the predicted results in the optimal conditions

Based on the surface plot shown in Figures 1 to 3, there were regions where the organic acids produced could be more than the 78.20 g/L predicted value. Therefore, the value optimized for temperature, initial pH and inoculum size (%) were used for confirming the results of organic acids output. Based on the calculated optimal values, experiment was conducted using the optimum point. The organic acids produced were 77 g/L which was close to the predicted value (Figure 4). Hence, the model developed can be considered to be suitable for forecasting the organic acids production level.

Conclusions

During the fermentation of kitchen waste, lactic acid was the most predominant acid produced followed by acetic acid, propionic acid and iso-butyric acid. The pH, temperature, inoculum size and the interaction among the parameters had significant effect on organic acids production. The optimal conditions that favored organic acids production were pH 6.02, temperature of 35.37°C and 20% of inoculum. The model validation experiment confirmed that the optimum organic acids produced were close to the value estimated by RSM analysis. It can be concluded that RSM is useful for the prediction of organic acids production level from kitchen waste through anaerobic digestion.

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