Advances in Food Science and Technology Vol. 2 (6), pp. 225-231, June, 2014. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

The use of ethanol product from food waste hydrolysate by co-culture of (*Zymomonas mobilis)* and (*Candida shehata)* under non-sterile situation

Buttowski E., Tawongsa W. and Adran Abdelmoula

Department of Biology, Faculty of Science, Thaksin University of Bangkok Metropolis. Bangkok 10300, Thailand

Accepted 20 May, 2014

A complete conversion of the hexose and pentose sugars in the food wastes hydrolysates (FWH) to ethanol is a prerequisite for maximizing the profitability of an industrial process for bioethanol production. Response surface methodology (RSM) was employed to optimize the effects of nitrogen source [$(NH_4)_2SO_4$], phosphorus source (KH_2PO_4), yeast extract and inoculum size on ethanol production from FWH by co-culture of *Zymomonas mobilis* and *Candida shehatae* under non-sterile condition. The optimal conditions for ethanol production were 1.15 g/L of $(NH_4)_2SO_4$, 0.95 g/L of KH_2PO_4, 1.38 g/L of yeast extract and 14.75%v/v of inoculum. The results indicate that the most significant parameters affecting ethanol production from FWH by co-culture under non-sterile condition was yeast extract. Ethanol production of 77.6 g/L obtained under optimized condition was 56% increased as compared with the use of raw FWH (34 g/L) and was in good agreement with the value predicted by quadratic model (79.98 g/L), thereby confirming its validity. Ethanol yield of FWH in batch fermentation by co-culture was 0.15 g-ethanol/g-food waste (77.6 g/L), which was 94.6% of the theoretical yield while *Z. mobilis* alone yielded 0.11 g-ethanol/g-food waste (54.2 g/L) and *C. shehatae* alone yielded 0.09 g-ethanol/g-food waste (48 g/L). Ethanol production from FWH in 1-L fermentor by co-culture also gave ethanol yield of 0.16 g-ethanol/g-food waste (78.8 g/L) which was 96% of the theoretical yield. Despite of being a waste, an ethanol yield of 0.16 g-ethanol/g-food waste demonstrated the potential of food waste as a promising biomass resource for ethanol production.

Key words: Co-culture, food waste hydrolysates, non-sterile fermentation, response surface methodology, optimization.

INTRODUCTION

Food waste is a kind of organic solid waste with higher percentage of moisture, and it is usually discharged from restaurants, kitchens and cafeterias (Wang et al., 2004). Generally, municipal solid waste includes approximately 35 to 40% organic waste, of which the dominating fraction is kitchen waste (Uncu and Cekmecelioglu, 2011) whereas, the amount of food waste generated in Thailand is approximately 600,000 kg/day, accounting for 80 to 90% of total municipal solid waste (PCD, 1994). The disposal of food waste became a major concern in Thailand when the direct animal feeding of food wastes was banned completely by Thailand government in 2005

*Corresponding author. E-mail: Buttowski78@yahoo.com.

due to the uncertainty with regard to the safety of its utilization as animal feed (Moon et al., 2009). Currently, these food wastes are disposed of by various methods such as land-filling, incineration, and recovery or recycle. Most of the food wastes are land-filled, causing ground water contamination. In addition, landfill space is limited and uncontrolled fermentation of organic wastes in landfill causes emission of greenhouse gases, such as methane and carbon dioxide (Camobreco et al., 1999), moreover, it is difficult to find new sites and the leachate generated by these materials require secondary wastewater treatments (Masaaki et al., 2008). Hence, food waste management has been an important issue for protecting the environment as well as for conserving natural resources.

Starch and cellulose materials are the major components of the food waste. It also contains some protein materials. The starch and cellulosic components of the food waste can be hydrolyzed to monomeric sugars. The sugars then can be used as substrates in the ethanol fermentative production (McMillan, 1997). But there is little information on the research of the utilization of food wastes for ethanol production. The bioethanol industry has developed rapidly in recent years to cope with the depletion of fossil fuel. Because of its environmental benefits, bioethanol is regarded as a promising biofuel substitute for gasoline in the transportation sector. It can be produced from a variety of raw materials containing fermentable sugars. The utilizations of edible starch material, such as corn and cassava for bioethanol production have caused undue pressure on the global food supply (Kim and Dale, 2004; Katz, 2008). Therefore, it is essential to research alternative and inexpensive substrate for ethanol production at a reduced cost (Hahn-Hägerdal et al., 2006).

Materials unsuitable for human consumption are considered ideal substrates for bioethanol production such as food wastes. For instance, bread residues can be fermented to get the ethanol yield of around 0.35 g/g substrate (Ebrahimi et al., 2007). Wilkins et al. (2007) reported that the citrus peel waste can undergo steam explosion process to remove the D-limonene and subsequently can be consumed by the *Saccharomyces cerevisiae* to get ethanol yield of around 0.33% (v/v). Reports also exist on the production of ethanol by fermentation of fresh kitchen garbage using *S. cerevisiae* as inoculum (Tang et al., 2008; Wang et al., 2008). Open fermentation of ethanol production has various merits compared with conventional sterile and closed-system fermentation. The non-sterile open fermentation of food waste could be carried out on-site at localized storage sites before collection to centralized processing plants. Furthermore, autoclave process could cause bad effect on desired product, such as degradation of substrate sugars and other nutritional elements. Some negative reactions would also take place, such as the Maillard reaction; it could cause decreases in the amounts of functionally useful sugars and amino, and increase the production of unfavorable furfural compounds, which inhibited bacterial growth (Akao et al., 2007; Sakai and Yamanami, 2006).

Research on ethanol production from starch by open fermentation had been carried out successfully (Tao et al., 2005). If the open fermentation could be done on food waste to produce ethanol, a lot of energy and cost would be saved. The starch and cellulosic components of the food waste can be hydrolyzed to monomeric sugars, which composed mainly of mixture of glucose and xylose. A complete and efficient conversion of these hexose and pentose sugars present in the food wastes hydrolysates to ethanol is a prerequisite for maximizing the profitability of an industrial process for bioethanol production (Vanmaris et al., 2006). Since there is no wild type microorganism that could efficiently accomplish this process, the utilization of two microorganisms and the construction of genetically modified biocatalysts have been two common approaches. The bacterium *Zymomonas mobilis* is known for better ethanol productivity and tolerance compared to *S. cerevisiae* (Davis et al., 2006), it has rarely been employed in such a co-culture process. A sequential culture of *Z. mobilis* and *Pachysolen tannophilus* has been previously reported (Fu and Peiris, 2008). Co-culture of *Z. mobilis* and *Pichia stipitis* for efficient ethanol production on glucose/xylose mixtures are also reported (Fu et al., 2009). Bansal and Singh (2003) reported a comparative study of ethanol production from molasses using *S. cerevisiae* and *Z. mobilis*. However, no study has been reported on the co-culture *Z. mobilis* and *Candida shehatae* on ethanol production from glucose/xylose mixtures substrates.

Recently, many statistical experimental design methods have been employed in bioprocess optimization. Response Surface Methodology (RSM) is one such scientific approach that is useful for developing, improving and optimizing processes and is used to analyze the effects of several independent variables on the system response. This method has been successfully applied to optimize alcoholic fermentation process (Castillo et al., 1982; Ratnam et al., 2003). The present study reports for the first time the new strain combination of *Z. mobilis* and *C. shehatae* for ethanol production from food wastes hydrolysates and to determine the optimum level of fermentation variables, nitrogen source [$(NH_4)_2SO_4$], phosphorus source (KH_2PO_4), yeast extract and inoculum size for ethanol production under non-sterilized condition using RSM.

MATERIALS AND METHODS

Microorganisms

Z. mobilis TISTR0548 and *C. shehatae* TISTR5843 were obtained from the Thailand Institute of Scientific and Technological Research (TISTR) culture collection. Cultures were maintained on agar plates

Table 1. Characteristics of food waste hydrolysate.

Parameter	Concentration (g/L)
Total carbohydrate	232
Total reducing sugar	164
Total nitrogen	6.5
Ammonium–nitrogen	0.45
Total phosphorus	643
Oil	10.6
Total solids	323
Volatile solid	261
Glucose	111
Xylose	23
Sucrose	21
Lactic acid	2.3
Formic acid	1.5
pH	4.2

at 4°C with subculture to fresh media every 2 weeks. Glucose agar for *Z. mobilis* consisted of 20 g/L glucose, 10 g/L yeast extract, 1 g/L KH_2PO_4, 1 g/L $MgCl_2$, 1 g/L $(NH_4)_2SO_4$, 15 g/L agar and pH 6.0. Xylose agar for *C. shehatae* was as previously described (Sreekumar et al., 1999). Inoculum medium consisted of 10 g/L yeast extract, 1 g/L $MgCl_2$, 1 g/L $(NH_4)_2SO_4$, 1 g/L KH_2PO_4, with 20 g/L glucose for *Z. mobilis* and 20 g/L xylose for *C. shehatae*. Media were sterilized at 121°C for 15 min and sugars were separately autoclaved from yeast extract and inorganic salts solution. The strains were grown at a temperature of 35°C and cultured for 48 h before being used in the fermentation.

Food waste hydrolysate (FWH)

The food waste was collected from Tong-Song municipal waste management plant, Nakhonsrithammarat Province, Southern Thailand. Food waste was fermented by fungi from Look-Pang for 24 h. It was mixed with water at ratio 1:1 (v/v) and crushed into small particles using liquidizer. It was subsequently incubated at 55°C for 12 h and then hydrolysate was generated richly in sugar content (164 g/L) (Table 1).

Inoculum preparation

Inoculum size was expressed as volume of inoculum medium that the cells were from to volume of fermentation medium that the cells were inoculated into. Cells from the corresponding volume of inoculum medium were firstly centrifuged to exclude the inoculum medium and concentrate the cells, and then the cell pellets were re-suspended with the sterilized yeast extract and inorganic salts solution to be inoculated into each flask. All inocula were incubated in 250 ml conical flasks with 25 ml of inoculum medium at 30°C, with a 24 h stationary incubation for *Z. mobilis* and a 36 to 48 h shaking incubation at 150 rpm for *C. shehatae*. Multiple flasks were simultaneously cultured to get the desirable volume of inoculum medium and subculture up to three times was carried out to ensure that all inoculum flasks contained an identical culture.

Ethanol production

RSM batch fermentation

FWH was neutralized with 1 N NaOH adjusting the pH to 5.0, and then FWH without sterilization was used as a fermentation medium. The nitrogen source $[(NH_4)_2SO_4]$, phosphorus source (KH_2PO_4) and yeast extract were added into FWH according to Table 2 varying between 0.5 to 2.0, 0.5 to 2.0 and 0.3 to 2.0 g/L, respectively. Batch experiments were carried out in a series of 250 ml Erlenmeyer flasks, containing 100 ml of FWH. 5 to 25% inoculum culture was dispensed to each flask. The flasks were shaken at 180 rpm in a thermostat controlled incubating shaker at temperature of 35°C for 72 h. Experiments were carried out in triplicate for all the runs and the average values were subjected to model analysis. Besides, as a statistical measure, six experiments were conducted at the center point to check for any error. For confirmation of optimization conditions, fermentations were done in 1-L fermentor (BIOFLO 3000, New Brunswick Scientific, Edison, NJ, USA) with pH monitoring. Fermentation parameters and FWH components were carried out with the optimized conditions. Fermentation studies were done with *Z. mobilis* alone, *C. shehatae* alone and a mixed culture of both. Time course experiment was also done. Reproducibility of the process was checked in repeat runs with the aforementioned conditions.

Experimental design and data analysis

A central composite experimental design was used to optimize the nitrogen source (X_1), phosphorus source (X_2), yeast extract (X_3) and inoculum size (X_4) on ethanol production from FWH. Ethanol production was used as dependent output variables. 21 experiments were performed in triplicate according to Table 2 to optimize the parameters. A quadratic model (Box et al., 1978) was used to evaluate the optimization of environmental factors as the following equation (equation 1):

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{14} X_1 X_4 + \beta_{23} X_2 X_3 + \beta_{24} X_2 X_4 + \beta_{34} X_3 X_4 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{44} X_4^2 \quad (1)$$

Where, Y = predicted response; X_1, X_2, X_3 and X_4 = parameters; $_0$ = offset term; β_1, β_2, β_3 and β_4 = linear coefficients; β_{11}, $\beta 22$, β_{33} and β_{44} = squared coefficients; and β_{12}, β_{23}, β_{13}, β_{14}, β_{24} and β_{34} = interaction coefficients.

The response variable (Y) was fitted using a predictive polynomial quadratic equation in order to correlate the response variable to the independent variables (Lay, 2000). The Y values were regressed with respect to nitrogen source, phosphorus source, yeast extract and inoculum size. Design expert software version 6.0 (Stat-Ease. Inc., MN, USA) was used for regression and graphical analysis of the experimental data obtained. The optimum levels of the selected variables were obtained by solving the regression equation and by analyzing the response surface contour and surface plots. The quality of the fit of quadratic model was expressed by the coefficient of determination R^2, and its statistically significance was checked by the *t*-test in the same program.

Analytical methods

Ten milliliters of fermentation broth was centrifuged at 5000 rpm for 30 min at 4°C and the supernatant was used to determine the ethanol, reducing sugars concentration and soluble end products. Fermentation end products (volatile fatty acids and ethanol), lactic acid, formic acid, xylose, fructose, sucrose and glucose were analyzed with a high performance liquid chromatograph (HPLC; Agilent 1200 series), equipped with Aminex® HPX-87H ion exclusion column (Hniman et al., 2011). Oil concentration and pH were determined in accordance with the standard methods (Clescerl et al., 1998). Total nitrogen, ammonium-nitrogen, total

Table 2. Experimental design for ethanol production from food waste hydrolysate using RSM.

Run	$X_1(NH_4)_2SO_4$ (g/L)	$X_2KH_2PO_4$(g/L)	X_3yeast extract (g/L)	x_4Inoculum size (%)	Ethanol production (g/L)
1	0.5	2	2	20	31
2	2	0.5	2	20	48
3	1.25	1.25	1.15	12.5	79
4	2	2	2	5	31.78
5	1.25	1.25	1.15	20	69.94
6	2	0.5	0.3	20	56.75
7	0.5	0.5	0.3	5	33.56
8	1.25	1.25	1.15	12.5	79.45
9	0.5	2	0.3	20	38
10	1.25	1.25	1.15	12.5	79.5
11	1.25	1.25	1.15	12.5	79.2
12	1.25	0.5	1.15	12.5	76.02
13	1.25	1.25	1.15	12.5	79.6
14	2	2	0.3	5	22.7
15	2	1.25	1.15	12.5	64.97
16	1.25	1.25	2	12.5	67.67
17	0.5	0.5	2	5	20.43
18	1.25	1.25	1.15	5	60.86
19	1.25	1.25	0.3	12.5	74.48
20	0.5	1.25	1.15	12.5	65.4
21	1.25	2	1.15	12.5	74.48

phosphorus and phosphate concentration were analyzed using commercial test kits from Spectroquant (Merck Ltd., Germany). The total carbohydrate in FWH was analyzed using anthrone method (Morris, 1948). The reducing sugars concentration in FWH and fermentation broth was assayed by the Somogyi-Nelson method (Somogyi, 1952).

RESULTS AND DISCUSSION

Optimization conditions for ethanol production

Food waste is an important municipal waste and mainly composed of carbohydrate. Hydrolysis of food waste by microbial digestion (Look-Pang) generated hydrolysates with high concentration of reducing sugar (164 g/L). Food waste hydrolysate (FWH) characterized abundance in nutrition (Table 1). Ethanol production from food waste was analyzed as low-cost feedstock by co-culture of *Z. mobilis* and *C. shehatae* fermentation. In the present paper, a central composite design (CCD) of response surface methodology (RSM) has been used to optimize conditions for transforming FWH to ethanol by co-culture of *Z. mobilis* and *C. shehatae* under non sterilized condition. Sreekumar et al. (1999) has proved that the most important chemical factors, which affected the ethanol production were the nitrogen source, phosphorus source, yeast extract and inoculums size. To evaluate the effect of each parameter on ethanol production from FWH by co-culture, 21 experiments were conducted according to the CCD method. Table 2 shows the actual parameters and concentration of ethanol. The maximum ethanol concentration was 79.5 g/L, corresponding to ethanol yield of 0.158 g-ethanol/g-food waste revealed 96.8% of the theoretical yield (theoretical yield of ethanol was calculated by the equations used by other researchers (Keating et al., 2004). The results from this study helped to frame a second order polynomial equation (Equation 2) that relates the ethanol concentration (Y) to the concentrations of $(NH_4)_2SO_4$ (X_1), KH_2PO_4 (X_2), yeast extract (X_3) and inoculums size (X_4).

The regression coefficients and significance levels are given in Table 3. This equation was used to predict the ethanol concentration at optimum condition. Although, the model showed a satisfactory explanation ($R^2 = 0.99$), not all the effects of factors and their interactions on ethanol concentration were significant ($P < 0.01$). Thus, the ethanol concentration was adequately explained by the model equation (Equation 2). Table 3 illustrates the main effect of each variable upon ethanol production (Y). Yeast extract (X_3) and inoculum size (X_4) showed a significant positive effect for ethanol production; whereas, K_2HPO_4 (X_2) and $(NH_4)_2SO_4$ (X_1) had only positive effect on ethanol production. The significant positive variables (X3 and X4) with confidence levels at 99% ($P < 0.001$) were considered as significant variables in model for ethanol prediction. X_1 and X_2 variables should not be used in equation 2 for individual variable prediction but could be used for interaction variables:

Table 3. Summary of model coefficient estimate by multiples linear regression.

Relationship	Factor	Coefficient	F value	Prob >F
Model	-	-	-	<0.0001
	Intercept	-48.2	-	-
Main effects (linear)	X_1	62.5	-0.34	0.7445
	X_2	34.9	-1.22	0.2674
	X_3	18.4	-9.45	<0.0001
	X_4	7.8	7.21	<0.0001
Interactions (pure quadratic)	X_1^2	-27	-27.21	<0.0001
	X_2^2	-9.1	-9.16	<0.0001
	X_3^2	-12.9	-16.65	<0.0001
	X_4^2	-0.3	-26.8	<0.0001
Interactions (cross product)	X_1X_2	-6.4	-5.14	0.0021
	X_1X_3	4	8.12	<0.0001
	X_1X_4	0.7	5.17	0.0021
	X_2X_3	4.7	9.51	<0.0001
	X_2X_4	-0.8	-6.74	0.0005
	X_3X_4	0.2	-4.64	<0.0001
Lack of fit	-	-	1.26	0.4039

*Coefficient of determination (R^2) of this model was 0.97.

$$Y_{ethanol} = -41.2 + 62.5X_1 + 34.9X_2 + 18.4X_3 + 7.8X_4 - 6.4X_1X_2 + 4X_1X_3 + 0.7X_1X_4 + 4.7X_2X_3 - 0.8X_2X_4 - 0.2X_3X_4 - 27X_1^2 - 9.1X_2^2 - 12.9X_3^2 - 0.3X_4^2 \quad (2)$$

Each item in the regression model (Equation 2) has an identified effect on the ethanol concentration. F value can be used to quantify the intensity of parameters on the ethanol concentration, while P values signify the pattern of interaction among the parameters. The larger the value of F value and the smaller the value of P, the more significant is the corresponding coefficient term (Douglas, 2001). The regression coefficients and F value and P values for all the linear, quadratic and interaction effects of the parameters are given in Table 3. A positive sign in the F value indicated a synergistic effect, while a negative sign represented an antagonistic effect of the parameters on the ethanol concentration. The significant of the regression coefficients of the model, indicating that yeast extract (X_3) and inoculum size (X_4) had highly positive effect on ethanol production ($P<0.001$). The effect of the interaction of nitrogen source and yeast extract, interaction of phosphorus source and yeast extract, and interaction of inoculum size and yeast extract were significant ($P<0.001$). Besides, the quadratic terms (X_1X_2, X_2X_2, X_1X_3, X_1X_4, X_2X_3 and X_3X_4) showed the synergistic effects on the ethanol concentration, typically (X_1X_3, X_3X_4 and X_2X_3) ($P < 0.001$). Surface and contour plots demonstrating the effects of different parameters, two parameters varied while keeping the third parameters at middle level, on the ethanol concentration were shown in Figure 1. The stationary points were examined by analyzing these plots. Generally, circular contour plots indicate that the interactions between parameters are negligible. On the contrary, elliptical ones indicate the evidence of the interactions (Muralidhar et al., 2003). From the plots, it was easy and convenient to understand the interactions between two nutrients and also to locate the optimum levels. Figure 1A showed the effect of $(NH_4)_2SO_4$ (X_1) and yeast extract (X_3) on the ethanol concentration. The convex response surface suggested well-defined optimum variables [$(NH_4)_2SO_4$ and yeast extract].

The ethanol concentration increased to the peak with the increasing of yeast extract and $(NH_4)_2SO_4$ to 0.9 and 1.44 g/L, respectively; then declined with the further increase of these two parameters. Figure 1B shows the effect of yeast extract (X_3) and K_2HPO_4 (X_2) on ethanol production. The equation demonstrated that interaction between yeast extract and KH_2PO_4 showed highly significance. At the middle concentration of yeast extract (0.9 g/L) and middle concentration of K_2HPO_4 (0.93 g/L) gave maximum ethanol production (79.5 g/L), a further increase in concentration of yeast extract and K_2HPO_4, the trend was reversed. In a relative low concentration yeast extract and K_2HPO_4, optimum ethanol production could be attained. Figure 1C showed the effect of inoculum size (X_4) and yeast extract (X_3) on the ethanol concentration. The convex response surface suggested

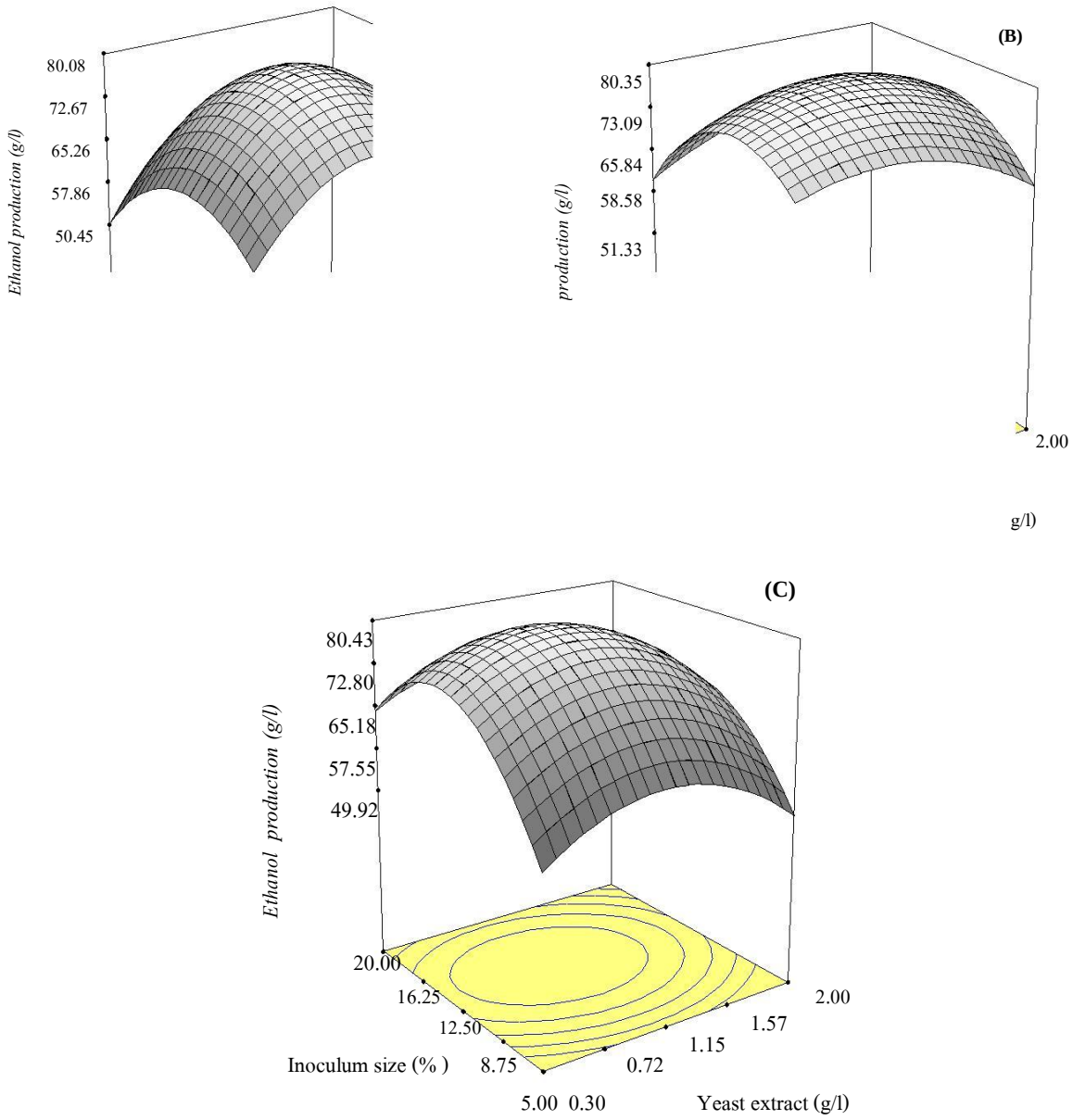

Figure 1. Response surface plot of ethanol production (Y) from FWH under non sterile condition. **A)** The effect of nitrogen source and yeast extract. **B)** The effect of phosphorus source and yeast extract. **C)** The effect of inoculum size and yeast extract on ethanol production.

well-defined optimum variables (inoculum size and yeast extract) and that the ethanol concentration increased to the peak with the increase of inoculum size and yeast extract up to 15% and 0.9 g/L, respectively; then declined with the further increase of these two parameters. This result demonstrated that the response surface had a maximum point for ethanol production. The contour plot described by the model Y is represented in Figure 1, which shows that the maximum concentration of ethanol was 79.5 g/L. The optimal concentration obtained from the maximum point of the model was 1.15 g/L $(NH_4)_2SO_4$, 0.95 g/L KH_2PO_4, 1.38 g/ yeast extract and 14.75%v/v inoculums size.

Ethanol yield in batch fermentation using co-culture was predicted at 0.16 g-ethanol/g-food waste (79.98 g/L), which was 97% of the theoretical yield. The ethanol production increased by 57.2% as compared with the use of raw food waste hydrolysates (34 g/L).

Model validation and confirmation

To confirm the validity of the statistical experimental strategies and gain a better understanding of ethanol production from FWH, a confirmation experiment with triplicate set was performed at the specified optimum

Table 4. Comparison of ethanol fermentation among individual and mixed strains fermentation in 250 flask and 1-L fermentor.

Parameter	250 ml flask fermentation			1-L fermentor
	Z. mobilis	*C. shehatae*	*Z. mobilis + C. shehatae*	*Z. mobilis + C. shehatae*
Ethanol production (g/L)	54.2	48	77.6	78.8
Ethanol yield (g-ethanol/ g-food waste)	0.11	0.09	0.15	0.16
Ethanol yield (g-ethanol/ g-reducing sugar)	0.33	0.29	0.47	0.48
Theoretical ethanol yield (%)	65	58.6	94.6	96

condition representing the maximum point of the concentration of ethanol. Experiments conducted at the optimum condition [1.15 g/L $(NH_4)_2SO_4$, 0.95 g/L KH_2PO_4, 1.38 g/ yeast extract and 14.75%v/v inoculums size] demonstrated that the ethanol concentration (77.6 g/L) was closer to the predicted value (79.98 g/L). This is improvement of concentration by 57.2% relative to that obtained from raw food waste hydrolysate. The good correlation between predicted and experimental values after optimization justified the validity of the response model and the existence of an optimum point. Corresponding to the ethanol concentration of 77.6 g/L, the ethanol yield was calculated as 0.15 g-ethanol/g-food waste. This showed that the model was useful to predict the ethanol concentration as well as optimize the experimental conditions. *Z. mobilis* alone yielded 0.11 g-ethanol/g-food waste (54.2 g/L), which is 65% of the theoretical yield and *C. shehatae* alone yielded 0.09 g-ethanol/g-food waste (48 g/L), reaching more than the published value for *C. tropicalis* with starch (Nellaiah et al., 1988).

Scale up experiment

The process scaled up with FWH had shown higher ethanol yield than batch fermentation (Table 4). Ethanol yield in batch fermentation using mixed culture was 0.15 g-ethanol/g-food waste in 72 h, which was 94.6% of the theoretical yield. Ethanol yield by *Z. mobilis* alone was 0.11 g-ethanol/g-food waste (54.2 g/L), which is 65% of the theoretical yield and *C. shehatae* alone yielded 0.09 g-ethanol/g-food waste (48 g/L) which was 58.6% of theoretical yield in 72 h (Table 4). However, in the 1 L fermentor using mixed culture, the ethanol yield was 0.16 g-ethanol/g-food waste (78.8 g/L) which was 96% of the theoretical yield. Reproducibility of the process was checked in repeat runs with the aforementioned conditions.

Conclusions

A significant improvement in ethanol yield (0.48 g/g-reducing sugar) was demonstrated, resulting in very low sugar and fewer by-products. CCD design have shown that yeast extract and inoculum size are the key parameters that influence ethanol production from FWH, while $(NH_4)_2SO_4$ and KH_2PO_4 showing a little effect. Maximum ethanol concentration of 79.98 g/L was obtained at the optimum condition of 1.15 g/L $(NH_4)_2SO_4$, 0.95 g/L KH_2PO_4, 1.38 g/L yeast extract and 14.75 %v/v inoculums size. The ethanol concentration at the opti-mum experimental condition (77.6 g/L) agreed well with the predicted one (79.5 g/L). This indicated the suitability of the model employed and the success of RSM to optimize the conditions of ethanol production from FWH. The ethanol yield was reproduced in 1 L fermentor with 0.16 g-ethanol/g-food waste (78.8 g/L) which was 96% of the theoretical yield. The results from the investigation showed that FWH can be used as an alternative substrate for ethanol production, in comparison to virgin biomass resources such as energy-rich crops, if sterilized suitably prior to fermentation by some low cost energy sources such as excess heat or waste heat from some industrial processes adjacent to ethanol production facility.

REFERENCES

Akao S, Tsuno H, Cheon J (2007). Semi-continuous l-lactate fermentation of garbage without sterile condition and analysis of the microbial structure. Water Res. 41:1774-1780.

Bansal R, Singh RS (2003). A comparative study on ethanol production from molasses using *Saccharomyces cerevisiae* and *Zymomonas mobilis*. Indian J. Microbiol. 43:261-264.

Box GEP, Hunter WG, Hunter JS (1978). Statistics for experimenters. John Wiley & Sons. p. 672.

Camobreco V, Ham R, Barlaz M, Repa E, Felker M, Rousseau C, Rathle J (1999). Life-cycle inventory of a modern municipal solid waste landfill. Waste Manage. Res. 17:394-408.

Castillo FJ, Izaguirre ME, Michelena V, Moreno B (1982). Optimization of fermentation conditions for ethanol production from whey. Biotechnol. Lett. 4(9):567-572

Clescerl L, Greenberg AE, Eaton AD (1998). Standard Methods for the Examination of Water and Wastewater. 20th ed. Washington DC, American Public Health Association. p.1000

Davis L, Rogers P, Pearce J, Peiris P (2006). Evaluation of *Zymomonas*-based ethanol production from a hydrolysed waste starch stream. Biomass Bioenergy 30:809-14. .

Douglas CM (2001). Design and analysis of experiments. 5th. Ed. John Wiley and Sons, Arizona, USA. p. 672.

Ebrahimi F, Khanahmadi M, Roodpeyma S, Taherzadeh MJ (2007). Ethanol production from bread residues. Biomass Bioenergy 32(4):333-337.

Fu N, Peiris P (2008). Co-fermentation of a mixture of glucose and xylose to ethanol by *Zymomonas mobilis* and *Pachysolen*

tannophilus. World J. Microbiol. Biotechnol. 24:1091-1097.

Fu N, Peiris P, Markham J, Bavor J (2009). A novel co-culture process with Zymomonas mobilis and Pichia stipitis for efficient ethanol production on glucose/xylose mixtures. Enzyme Microb. Techol. 45(3):210-217.

Hahn-Hagerdal B, Galbe M, Gorwa-Grauslund M-F, Liden G, Zacchi G (2006). Bio-ethanol-the fuel of tomorrow from the residues of today. Trends Biotechnol. 24(12):549-556.

Hniman A, O-Thong S, Prasertsan P (2011). Developing a thermophilic hydrogen producing microbial consortia from geothermal spring for efficient utilization of xylose and glucose mixed substrates. Int. J. Hydrogen Energy 36(14):8785-8793.

Katz SH (2008). Food to fuel and the world food crisis. Anthropol. Today 24:1-3.

Keating JD, Robinson J, Cotta MA, Saddler JN, Mansfield SD (2004). An ethanologenic yeast exhibiting unusual metabolism in the fermentation of lignocellulosic hexose sugars. J. Ind. Microbiol. Biotechnol. 31:235-44.

Kim S, Dale BE (2004). Global potential bioethanol production from wasted crops and crop residues. Biomass Bioenergy 26:361-375.

Lay JJ (2000). Modeling and optimization of anaerobic digested sludge converting starch to hydrogen. Biotechnol. Bioeng. 68(3):269-278.

Masaaki TK, Hitoshi O, Akira A, Shin JK, Hiroshi MI (2008). Basic characteristics of food waste and food ash on steam gasification. Ind. Eng. Chem. Res. 47:2414-2419.

Mcmillan JD (1997). Bioethanol production: Status and prospects. Renew. Energy 10(2):295-302.

Moon HC, Song IS, Kim JC, Shirai Y, Lee DH, Kim JK, Chung SO, Kim DH, Oh KK, Cho YS (2009). Enzymatic hydrolysis of food waste and ethanol fermentation. Int. J. Energy Res. 33:164-172.

Morris DL (1948). Quantitative determination of carbohydrates with Dreywood's anthrone reagent. Science 107(278):254-255.

Muralidhar R, Gummadi SN, Dasu VV, Panda T (2003). Statistical analysis on some critical parameters affecting the formation of protoplasts from the mycelium of *Penicillium griseofulvum*. Biochem. Eng. J. 16(3):229-235.

Nellaiah H, Karunakaran T, Gunasekaran P (1988). Ethanol fermentation of cassava starch by Zymomonas mobilis NRRL B-4286. Biomass Bioenergy 15:201-207.

PCD (1994). Pollution Control Department, Laws and standards on pollution control in Thailand. (3rd ed.), Ministry of Science, Technology and Environment, Bangkok, Thailand.

Ratnam BVV, Rao MN, Rao MD, Rao SS, Ayyanna C (2003). Optimization of fermentation conditions for the production of ethanol from sago starch using response methodology. World J. Microbiol. Biotechnol. 19(5):523-526.

Sakai K, Yamanami T (2006). Thermotolerant *Bacillus licheniformis* TY7 produces optically active l-lactic acid from kitchen refuse under open condition. J. Biosci. Bioeng. 102:132-134.

Somogyi M (1952). Notes on sugar determination. J. Biol. Chem.195:19-23.

Sreekumar O, Chand N, Basappa (1999). Optimization and interaction of media components in ethanol production using Zymomonas mobilis by response surface methodology. J. Biosci. Bioeng. 88(3): 334-338.

Tang YQ, Koikem Y, Liu K, An M. Z, Morimura S, Wu X. L, Kida K (2008). Ethanol production from kitchen waste using the flocculating yeast *Saccharomyces cerevisiae* strain KF-7. Biomass Bioenergy 32(11):1037-1045.

Tao F, Miao JY, Shi GY, Zhang K.C (2005). Ethanol fermentation by an acid-tolerant *Zymomonas mobilis* under non-sterilized condition. Process Biochem. 40:183-187.

Uncu ON, Cekmecelioglu D (2011). Cost-effective approach to ethanol production and optimization by response surface methodology. Waste Manage. 31:636-643.

Vanmaris A, Abbott DA, Bellissimi E, Van den brink J, Kuyper M, Luttik MAH (2006). Alcoholic fermentation of carbon sources in biomass hydrolysates by *Saccharomyces cerevisiae*: current status. A van leeuw J. Microb. 90:391-418.

Wang Q, Ma H, Xu W, Gong L, Zhang W, Zou D (2008). Ethanol production from kitchen garbage using response surface methodology. Biochem. Eng. J. 39(3):604-610.

Wang QH, Ma HZ, Wang XM, Ji YZ (2004). Resource recycling technology of food wastes. Modern Chemical Industry 24:56-59.

Wilkins MR, Widmer WW, Grohmann K (2007). Simultaneous saccharification and fermentation of citrus peel waste by *Saccharomyces cerevisiae* to produce ethanol. Process Biochem. 42(12):1614-1619.