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Original Article

Response surface optimization of ethanol production from banana peels by organic acid hydrolysis and fermentation

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Abstract

The production of ethanol from banana peels was optimized by response surface methodology in a two-step process. The steps were vinegar hydrolysis of banana peels using microwave heating, and fermentation of the peel hydrolysate by commercial baker's yeast. The sugar (glucose) content in the hydrolysate was maximized over ranges of vinegar concentration, microwave power and hydrolysis time. The maximal 15.3 g/L glucose content was reached using 1.47 %w/w vinegar and 465 W microwave power for 10 min, and was used in maximizing the ethanol content from the second step. The maximal 9.2 %v/v ethanol was obtained with 4 %w/w yeast, an initial pH of 4.8, at 28°C for 192 hrs. The results suggest that a combination of microwave application and organic acid hydrolysis might contribute cost-efficiently in the production of ethanol from biological waste.

Keywords: banana peel, microwave hydrolysis, vinegar, baker's yeast, ethanol

1. Introduction

The productivity and cost effectiveness of ethanol production can be enhanced by the use of agricultural residues or wastes as feedstock (Ko *et al.*, 2009; Voca *et al.*, 2009). The feedstock has to be pretreated by inorganic alkali (Ruangmee *et al.*, 2013) or acid (Mohan *et al.*, 2013), and then be hydrolyzed to fermentable sugars by enzymes (Yan *et al.*, 2011) or inorganic acids (Avci *et al.*, 2013; Scholz *et al.*, 2013). After that the sugars are fermented to ethanol by yeast (*Saccharomyces cerevisiae*) (Kim *et al.*, 2013; Laopaiboon *et al.*, 2009; Le *et al.*, 2014; Louhichi *et al.*, 2013). However, both enzymatic and inorganic alkali/acid processes have some disadvantages. Enzymes are expensive while the by-products with inorganic acids inhibit yeast growth.

In the current work I seek for economic advantages in ethanol production cost. Banana peels are an abundant agricultural waste in Thailand, and were employed as the raw material. The aims were to investigate the hydrolysis of these peels using a solution with vinegar, an organic acid that does not inhibit yeast growth. Microwave heating that is energy efficient (Biond *et al.*, 2012) was applied to assist the hydrolysis, in order to reduce energy consumption (Datta, 2001; Gabriel *et al.*, 1998;. Microwave irradiation can increase the rate of hydrolysis (Caddick, 1995). In addition, the fermentation step producing bio-ethanol from the acid peel hydrolysate was evaluated using commercial baker's yeast, which is a low-cost affordable microorganism (Arifin *et al.*, 2011).

2. Materials and Methods

2.1 Materials

Baker's yeast, *Saccharomyces cerevisiae*, having 1.2x 10^{10} CFU/g was used as the microorganism for ethanol fermentation. The CFU count was determined by the Department of Microbiology as a routine service. It was purchased from the Bakery Center Shop (Hat Yai, Songkhla, Thailand)

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under trade name Fermipan brown. Sodium chloride (iodized salt under trade name Royal) and 5% distilled-vinegar as a source of acetic acid (trade name PFO: Preserved Food Organization) were purchased from a local market (Hat Yai, Songkhla, Thailand). These materials were of commercial grade.

Unripe cultivated bananas harvested 2-3 days earlier are easily available mainly in southern Thailand, and were obtained from a local market. The composition characteristics of their peels were determined according to AOAC methods (James, 1995), showing 65% total carbohydrate and 16% crude fiber on a dry weight basis. The bananas were pretreated by soaking in 0.5 M sodium chloride solution for 5 hrs at ambient temperature of about 28°C, after which the solid peels were separated before use.

2.2 Microwave-assisted organic acid hydrolysis

The hydrolysis conditions were varied as shown in Table 1. Thirty grams of the pre-soaked peel was cut to nominally 1 cm lengths, and hydrolyzed in a 100 ml vinegar solution, in a 250 ml beaker. The beaker was placed at the center of a rotating circular glass plate in a household microwave oven (Samsung MW71C, 2,450 MHz). The hydrolysate was passed through a fabric filter to obtain a clear liquid for the analysis of reducing sugar content by UV-Vis spectrophotometer (UV) before fermentation. A near optimal set of conditions was determined to maximize the sugar content, and these conditions were used as the starting point for optimizing the second fermentation step.

2.3 Baker's yeast fermentation

The fermentation conditions were varied as shown in Table 3. The pretreated near optimally hydrolyzed peels were transferred into 250 ml air-locked flasks. The initial medium pH was adjusted to a designated value with acetic acid or ammonia solution, and an incubator shaker was used for temperature control during incubation for a designated time. The fermentation broth was then pressed through a syringe filter to obtain a clear liquid for the analysis of ethanol content by gas chromatograph (GC).

2.4 Response surface methodology

The effects of main factors were investigated by response surface methodology (RSM) to find near optimal conditions, separately for the hydrolysis and for the fermentation. The hydrolysis had vinegar concentrations in the range 0.74-1.47 %w/w (g of vinegar in 100 g blend with water), and a fixed 100 mL volume of vinegar solution was used in each trial, with microwave power setting in the range 100-800 Watt (W), and reaction time in the range 1-10 min. A central composite design (CCD) was used with these controlled variables, giving the 17 cases shown in Table 1. Note, that these cases include one in triplicate replicated point for estimating the variance in measured output, namely the glucose content to be maximized with RSM. For the fermentation, the ranges were: yeast addition ratio 2-6 %w/w (g of commercial baker's yeast to 100 g of peel), initial pH 4.5-6.5, temperature 28-40°C, and time 24-192 hrs. The CCD

Table 1. Experimental cases used in RSM, with determined reducing sugar content in the banana peel hydrolysates along with their predictions from a fitted regression model.

Experimental Run No.	CCD experimental conditions			Experimental	RSM model output
	Vinegar amount (%w/w)	Microwave power (Watt)	Time (min)	Reducing sugar content (g/L)	
1	1.1	100	6	6.8	6.1
2	1.3	300	8	10.1	10.9
3	0.9	300	3	7.3	7.6
4	0.9	300	8	9.1	9.7
5	1.3	300	3	7.6	7.9
6	1.1	450	6	11.2	11.3
7	1.1	450	10	14.5	13.4
8	1.1	450	6	11.3	11.3
9	1.1	450	1	8.6	8.3
10	1.5	450	6	12.2	11.4
11	0.7	450	6	10.9	10.3
12	1.1	450	6	11.3	11.3
13	1.3	700	8	11.2	11.8
14	0.9	700	8	10.1	10.8
15	0.9	658	3	7.6	7.7
16	1.3	658	3	7.3	7.7
17	1.1	800	6	7.5	6.9

Table 2. Analysis of variance for the regression equation of the reducing sugar content.

Source	Degree of Freedom (DF)	Coefficient	*P*-value	Standard error	Sum of Squares (SS)	Mean Squares (MS)
Regression	9	-4.198	0.653	8.951	70.11	7.790
A	1	7.914	0.565	13.120		
B	1	0.036	0.008	0.010		
C	1	0.157	0.844	0.767		
A^2	1	-3.700	0.529	5.588		
B^2	1	-0.00004	0.00032	0.00001		
C^2	1	-0.025	0.524	0.037		
AB	1	-0.001	0.841	0.007		
AC	1	0.432	0.449	0.538		
BC	1	0.00045	0.449	0.001		
Residual	7				5.467	0.781
LOF Error	5				5.466	1.093
Pure Error	2				0.001	0.0005
$R^2 = 0.928$, R^2 adjusted = 0.835, ***F***-value = 9.975, Probe > ***F*** = 0.0031						

with these controlled variables had 24 cases, as shown in Table 3. The ethanol content from fermentation was the output to be maximized with RSM.

2.5 Analytical methods

The reducing sugar content in the hydrolysate was estimated using 3, 5-dinitrosalicylic acid (DNS method) (Miller, 1959). The glucose was determined as a reducing sugar. A standard curve was determined from five known glucose concentrations (0-2 g/L). The DNS reagent was added in each sample tube at 1:1 volume ratio. The solution was then boiled at 80°C for 30 min. Then it was cooled to ambient temperature and diluted to 10 ml final volume with distilled water. The transmittance was measured at 520 nm using a UV-Vis spectrophotometer (Model HP8453).

The ethanol content in the fermentation broth was quantified by gas chromatography (GC 6890 flame ionization detector, Hewlett Packard, USA). The HP-FFAP column was 3.0 m length and had 0.32 mm internal diameter. The oven temperature was fixed at 150°C, while the detector and the injection port were kept at 250°C. Nitrogen as carrier gas was fed at 20 mL/min, and a mixture of air and hydrogen was the combustion gas. A standard curve from six known ethanol solutions (0-15% v/v) was prepared for the determination of ethanol contents in the fermentation broths.

3. Results and Discussion

3.1 Hydrolysis condition optimization

The soaking in a sodium chloride solution gave the fresh banana peels a lower gum content, making slicing easy. The slicing increased the surface area thus promoting hydrolysis. The performance measure targeted for maximization was the reducing sugar content. Table 1 shows the experimental and fitted model results of glucose (reducing sugar) contents for all cases. Regression analysis produced the following second-order polynomial fit with a satisfactory coefficient of determination ($R^2 = 0.928$):

$$\text{Reducing sugar (g/L)} = -4.198 + 7.914A + 0.036B + 0.157C - 3.700A^2 - 0.00004B^2 - 0.025C^2 - 0.001AB + 0.432AC + 0.00045BC \quad (1)$$

where, A, B and C represent vinegar amount (% w/w), microwave power (Watt) and hydrolysis time (min), respectively. AB, AC and BC are the interactions, and A^2, B^2 and C^2 are the quadratic terms.

Response surface plots of the reducing sugar content are shown in Figure 1 (a)-(c). The reducing sugar depends on all the controlled variables, namely vinegar amount, microwave power and hydrolysis time. As illustrated in Table 2, the analysis of variance for a P-value < 0.05 indicates a significant effect on the response. The model F-value of 9.975 and value of probability >F indicate significant model terms. The linear term B and the quadratic term B^2 were significant factors indicating that near optimal B-value should be pursued. The rate of hydrolysis increased consistently with vinegar amount and time (Figure 1 (b)), because an increased hydrogen ion activity was beneficial (Tasic *et al.*, 2009). Figure 1 (a) and (c) show that microwave-assisted hydrolysis should be performed at medium microwave power setting around 400-500 W, with comparatively high reducing sugar contents. The approximate optimum, based on the fitted quadratic model, was at 1.47 %w/w vinegar and 465 W for 10 min, and a validation experiment was conducted. The experimental reducing sugar content was 15.3 g/L while the model predicted content was 15.1 g/L at these settings. The close match of experimental and predicted values suggests

Table 3. Experimental and RSM predicted results of ethanol contents in the fermentation broths.

Experimental Run No.	CCD experimental conditions				Experimental	RSM model output
	Initial pH (%w/w)	Yeast amount (%w/w)	Temperature (°C)	Time (min)	Ethanol content (%v/v)	
1	5.5	4	28	120	8.1	8.2
2	6	3	31	144	7.2	7.0
3	5	5	31	72	7.2	7.1
4	5	3	31	72	7.0	7.1
5	5	5	31	144	7.1	7.0
6	6	5	31	144	7.3	7.3
7	5	3	31	144	7.8	7.5
8	6	3	31	72	6.9	7.2
9	6	5	31	72	7.7	8.0
10	5.5	4	34	120	8.1	7.4
11	4.5	4	34	120	6.2	6.5
12	6.5	4	34	120	7.0	6.6
13	5.5	4	34	120	6.9	7.4
14	5.5	2	34	120	6.7	6.8
15	5.5	4	34	192	7.4	7.9
16	5.5	6	34	120	7.1	7.0
17	5.5	4	34	120	7.2	7.4
18	5.5	4	34	24	5.4	4.8
19	5	3	37	144	8.5	8.0
20	6	3	37	72	4.8	4.7
21	5	5	37	72	4.6	4.7
22	6	3	37	144	7.0	7.4
23	5	3	37	72	4.3	4.6
24	6	5	37	144	7.9	7.8
25	5	5	37	144	7.7	7.6
26	6	5	37	72	5.2	5.6
27	5.5	4	40	120	7.4	7.3

that the RSM approach was successful and this operating point is indeed near optimal, within the ranges of settings tested.

3.2 Optimization of fermentation conditions

The hydrolysate obtained at near optimal hydrolysis conditions, as described above, was used as substrate for bio-ethanol production. The only microbe used was *S. cerevisiae* or common baker's yeast, producing ethanol from the total carbohydrates. Table 3 shows the experiments used in the RSM approach, along with experimental ethanol contents and outputs from the fitted regression model (R^2 = 0.913). The best fit with a second order polynomial is given by:

$$\text{Ethanol content (\%v/v)} = 17.350 - 1.523D - 0.124E - 0.933F + 9.254G + 0.010D^2 - 0.00012E^2 - 0.133F^2 - 0.849G^2 + 0.007DE + 0.009DF - 0.010DG - 0.003EF - 0.010EG + 0.395FG \quad (2)$$

where D, E, F and G represent yeast amount (% w/w), initial pH, temperature (°C) and time (h), respectively. DE, DF, DG, EF, EG and FG are the interactions, and D^2, E^2, F^2 and G^2 are the quadratic terms.

The statistical significances are tabulated in Table 4. The significance of the overall model is implied by an *F*-value of 9.012 corresponding to probability $P<0.00025$. The linear term E, the quadratic terms E^2, G^2 and the interaction term DE are significant with *P*-values better than 0.05. The response surfaces from this fit (Figure 2 (a)-(f)) indicate the rank order of the factors affecting ethanol concentration as: initial pH > time > yeast amount > temperature.

The time traces of ethanol content are similarly shaped and increase with time (Figure 2 (c), (e) and (f)). This suggests that *S. cerevisiae* from baker's yeast, having $1.2x10^{10}$ CFU/g, needs more time for partial hydrolysis and fermentation than laboratory type of *S. cerevisiae* having $2x10^{13}$ CFU/g (Customs Department, 2014). In Figure 2 (a), (b) and (c), pH 5.5 appears subjectively close to optimal, and ethanol contents in prior work have slightly increased with pH in

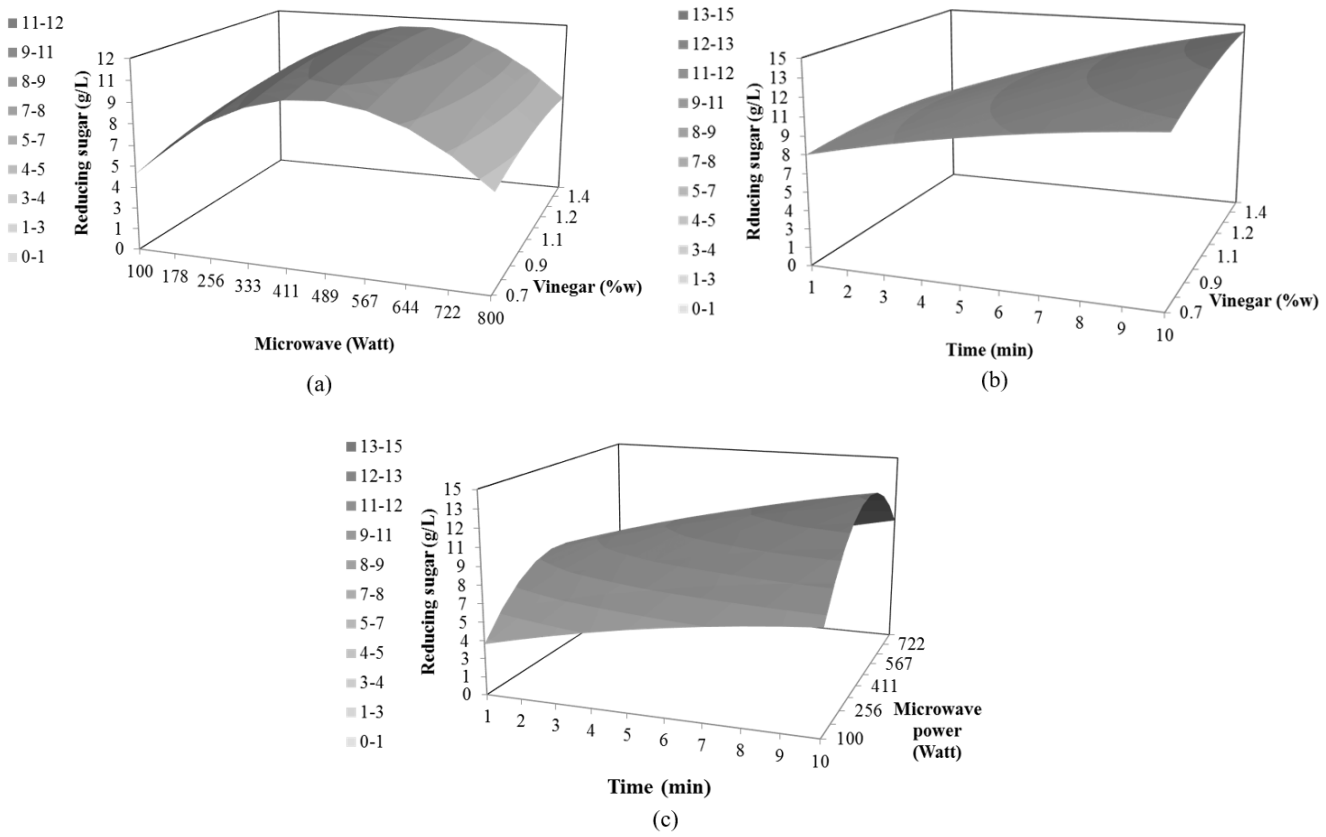

Figure 1. Response surface plots of reducing sugar content based on model equation fit to experimental results, given in Equation 1; (a) interaction of vinegar amount and microwave power for 5.5 min, (b) interaction of vinegar amount and time at 450 watt microwave power, and (c) interaction of microwave power and time at 1.1 %w/w vinegar amount.

Table 4. Analysis of variance for the regression equation of the ethanol content.

Source	Degree of Freedom (DF)	Coefficient	***P***-value	Standard error	Sum of Squares (SS)	Mean Squares (MS)
Regression	14	17.350	0.562	29.090	9.012	0.00025
D	1	-1.523	0.132	0.943		
E	1	-0.124	0.04469	0.055		
F	1	-0.933	0.669	2.127		
G	1	9.254	0.123	5.576		
D^2	1	0.010	0.438	0.012		
E^2	1	-0.00012	0.084	0.00006		
F^2	1	-0.133	0.235	0.107		
G^2	1	-0.849	0.070	0.427		
DE	1	0.007	0.00004	0.001		
DF	1	0.009	0.836	0.040		
DG	1	-0.010	0.898	0.081		
EF	1	-0.003	0.320	0.003		
EG	1	-0.010	0.189	0.007		
FG	1	0.395	0.128	0.242		
Residual	12				2.807	0.234
LOF Error	10				2.042	0.204
Pure Error	2				0.765	0.382

$R^2 = 0.913$, R^2 adjusted = 0.812, ***F***-value = 9.012, Probe > ***F*** = 0.00025

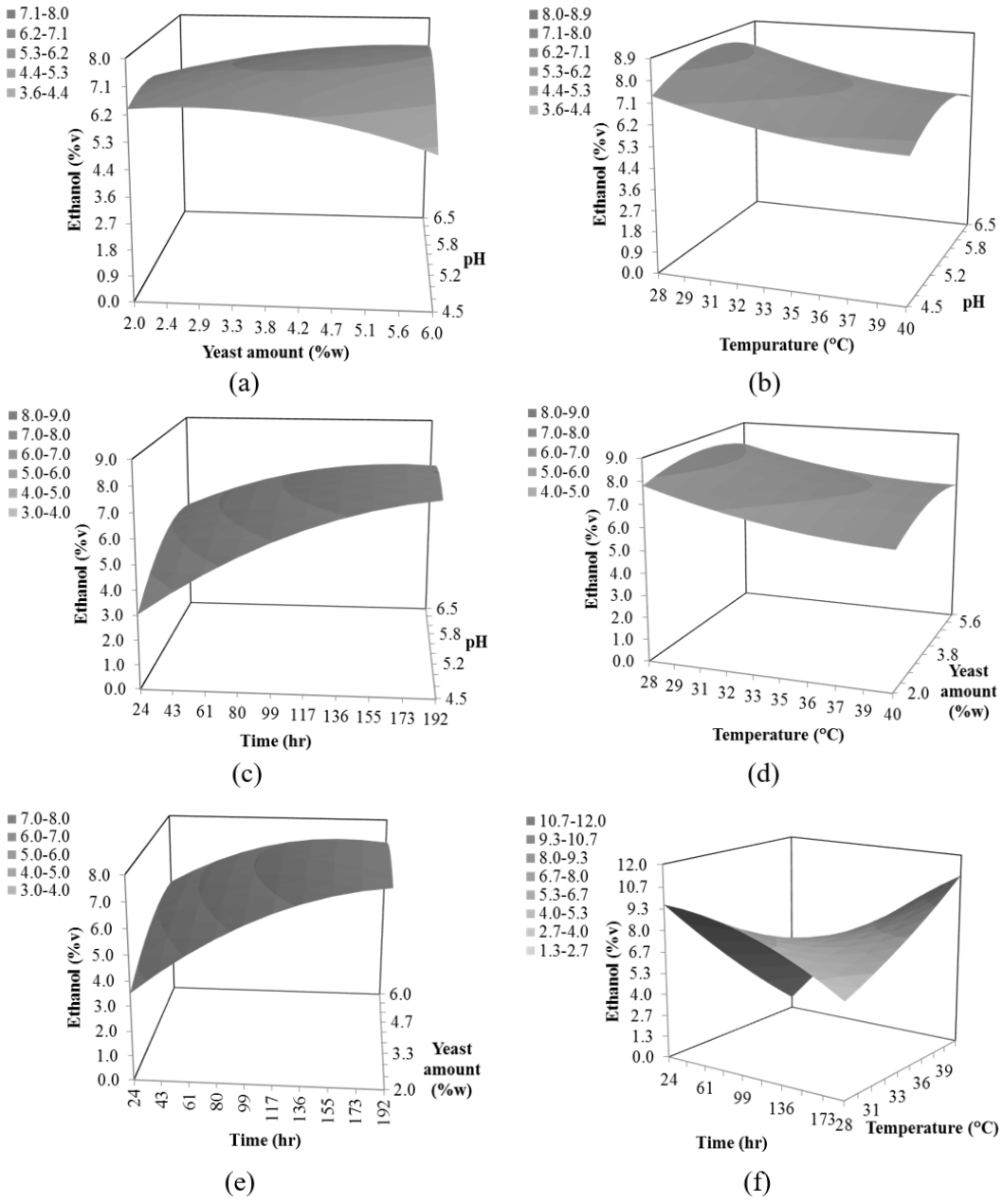

Figure 2. Response surface plots of ethanol content based on the quadratic polynomial fit to experimental results, given in Equation 2; (a) interaction of initial pH and yeast amount at 34°C for 108 hrs, (b) interaction of initial pH and temperature using 4 %w/w yeast for 108 hrs, (c) interaction of initial pH and time using 4 %w/w yeast at 34 °C, (d) interaction of yeast amount and temperature at pH 5.5 for 108 hrs, (e) interaction of yeast amount and time at pH 5.5 and 34°C, and (f) interaction of temperature and time at pH 5.5 with 4 %w/w yeast.

acidic conditions (pH < 5.5) (Lin *et al.*, 2012). At a lower temperature the metabolism of microorganisms from baker's yeast can produce more ethanol (Figure 2 (b), (d) and (f)) (Lin *et al.*, 2012). The appropriate amount of yeast was in the range of 3-4% w/w, while a further increase did not improve ethanol content (Figure 2 (a), (d) and (e)).

The model based optimal conditions are: an initial pH of 4.8, 3% w/w yeast at 28°C for 192 hrs. The experimentally achieved ethanol content was 9.2% v/v (or 72.6 g/L) which is higher than typical values in prior work, such as 26.8 g/L (Xu, 2011) and 34.3 g/L (Zhu, 2006) ethanol contents from wheat straw, and 32.9 g/L from potato (Tasic, 2009).

In a bench-scale 5 L fermenter, these near optimal fermentation parameters increased ethanol yield by about 2%. Differing effects at different production scales may relate to control of pH, temperature and mixing. The combined organic acid hydrolysis using microwave heating and baker's yeast fermentation has the following advantages. The

materials are of low cost and no high-pressure equipment is required, which gives savings in investment and production costs. Microwave application could increase production per unit time, and save energy. Moreover, an organic degradable acid is friendly to the environment.

4. Conclusions

An organic acid (acetic acid in vinegar) was used in the microwave-assisted hydrolysis of biological waste, and baker's yeast was used for the fermentation on producing ethanol from banana peels in a two-step process. The near optimal conditions for the first hydrolysis step of banana peels were 1.47% w/w vinegar and 465 Watt microwave power for 10 min, for 30 g peel samples in 100 g of vinegar solution. These conditions gave a 15.3 g/L reducing sugar content that matched well the prediction from a quadratic response surface model. The near optimal conditions for the second fermentation step were 3% w/w yeast at 4.8 pH and 40°C for 192 hrs, providing the maximal 9.2% v/v ethanol content and yielding 400 L/ton (L of ethanol to ton of raw peel). The production of bio-ethanol from banana peels by such two-step process appears technically feasible, and might provide economic advantages.

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