

Optimization of operational parameters for ethanol production from Korean food waste leachate

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ABSTRACT: Recently, research on the production of ethanol from waste has been accelerating for both ecological and economical reasons, primarily for its use as an alternative to petroleum based fuels. In this study, response surface methodology based 2^3 -full factorial central composite design was employed to optimize the parameters of ethanol production from Korean food waste leachate. The reducing sugar concentration of the food waste leachate determined by the dinitrosalicylic acid method was 75 g/L. A second order polynomial model was developed to evaluate the quantitative effects of temperature, pH and reducing sugar concentration in order to find an optimum condition for the ethanol production from food waste leachate. From the experimental result, maximum ethanol concentration of 24.17 g/L was obtained at the optimum condition of temperature (38 °C), pH (5.45) and reducing sugar concentration (75 g/L). The experimental value (24.17 g/L) agreed very well with the predicted one (23.66 g/L), indicating the suitability of the model employed and the success of response surface methodology in optimizing the conditions of ethanol production from food waste leachate. Canonical analysis indicated that the stationary point was a saddle point for the ethanol yield. Despite being a waste, an ethanol yield of 0.32 g ethanol/g reducing sugar demonstrated the potential of food waste leachate as a promising biomass resource for the production of ethanol.

Keywords: Fermentation; pH; Reducing sugar concentration; Response surface methodology; *Saccharomyces cerevisiae*; Temperature

INTRODUCTION

The amount of food waste generated in Korea is approximately 13000 tons, accounting for 27 % of total municipal solid waste (Lee *et al.*, 2009). The disposal of food waste assumed significance and became a major concern in Korea since 2005 when the environmental law prohibited the sanitary landfilling of such wastes (Kim *et al.*, 2008). Therefore, food waste recycling facilities were installed in the country to mitigate the food waste disposal problem. However, 70-90% of the food waste treated in the food waste recycling facilities takes the form of a dense, malodorous liquid called food waste leachate (FWL). The FWL with a volatile solid (VS) concentration of ~15% is currently being discharged to the ocean that has serious adverse effect on the marine ecosystem.

Furthermore, the Korean government is heading for prohibiting the marine disposal of such type of organic wastes by the year 2012 (Lee *et al.*, 2009). Thus, there

is an urgent need to alleviate this problem causing serious concern recently.

Bioethanol is one of the promising future energy alternatives contributing to the reduction of negative environmental impacts generated by the use of fossil fuels (McMillan, 1997). It can be produced from a variety of raw materials containing fermentable sugars. However, the utilization of energy-rich crops such as corn and sugar cane, as feedstock for generating ethanol, may jeopardize the food security in any country and is a debated issue at the moment. Besides, the utilization of virgin resources enhances the total cost of ethanol production to a large extent. Therefore, biomass wastes such as corn fiber, waste wood, waste cardboard and paper sludge, molasses, bread residues, bagasse (Teixeira *et al.*, 1999; Iranmahboob *et al.*, 2001; Kádár *et al.*, 2004; Cazetta *et al.*, 2007; Ebrahimi *et al.*, 2007; Gaspar *et al.*, 2007) and FWL are far more attractive as cheap feedstocks for ethanol production than virgin resources. For instance, Kádár *et al.*, (2004)

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examined the simultaneous saccharification and fermentation of waste cardboard and paper sludge produces ethanol in the range of 0.31-0.34 g/g waste. The bread residues can be fermented to get the ethanol yield 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 β -limonene and subsequently can be consumed by the *Saccharomyces cerevisiae* to get ethanol yield of ~ 0.33 % (v/v).

Being enormously generated in the recycling facilities and very rich in sugar content (75 g/L, Table 1) FWL can be utilized as a potential substrate for the production of ethanol. Reports exist on the production of ethanol by fermentation of fresh kitchen garbage using *Saccharomyces cerevisiae* as inoculum (Tang et al., 2008; Wang et al., 2008). However, FWL is different from fresh kitchen garbage being contaminated in nature. Therefore, optimal production of ethanol from FWL is challenging and is a key subject pertaining to a variety of fermentation conditions.

The conventional approach for the optimization of a multivariable system is usually one-variable-at-a-time. However, such approach needs to carry out numerous sequential experimental runs and cannot explain the interactions between the variables. 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, main objective being the determination of optimum operational conditions within the operating specifications (Ravikumar et al., 2005; Jo et al., 2008). This method has been successfully applied to optimize alcoholic fermentation process (Castillo et al., 1982; Ratnam et al., 2003). In the present study, the RSM based central composite design (CCD) was applied to identify the optimum condition of the FWL fermentation

process by evaluating the effects of temperature Laboratory, pH, and reducing sugar concentration (RSC) during ethanol production using *S. cerevisiae* (7904) as the inoculum. This research was carried out in Ecosystems Laboratory, University of Ulsan, South Korea during October 2007 to March 2008.

MATERIALS AND METHODS

Microorganism and FWL

The FWL was collected from Sung-am food waste resource recovery plant, Ulsan, South Korea. *Saccharomyces cerevisiae* obtained from the Korea Research Institute of Bioscience and Biotechnology, Daejeon, Korea was used in all experiments (Le Man et al., 2008). The strain (7904) was cultivated in the growth medium for a period of 48h. It was kept under sterile conditions, at neutral pH and 35 °C in an incubating shaker run at 150 rpm. The cell concentration in the cultivated medium was 2.3×10^8 cells/mL. The growth medium used for cultivation of inoculum culture consisted of (in g/L): glucose 10, yeast extract 3, malt extract 3 and peptone 3 (Nahvi et al., 2002).

Ethanol production

Collected FWL was thoroughly blended in a mixer and then sterilized in an autoclave (Vision Scientific Co. Ltd., South Korea) at 120 °C for 15 min. The pH was adjusted using 1N H₂SO₄ and 1N NaOH and RSC was adjusted either by diluting with water or by adding glucose. Batch experiments were carried out in a series of 500 mL Erlenmeyer flasks, containing 200 mL of FWL. 2.5 % inoculum culture was dispensed to each flask. The temperature, pH and RSC were varied between 26.6-43.4 °C, 3.31-6.68 and 34.77-85.23 g/L, respectively. The flasks were shaken at 180 rpm in a thermostat controlled incubating shaker (iNtRON Biotechnology Co. Ltd., South Korea) under the designed temperatures for 40 h. Experiments were carried out in duplicate for all the runs and the average values were subjected to model analysis. Besides, as a statistical measure, 6 experiments were conducted at the center-point to check for any error.

Analytical

Characterization of FWL

Analysis of total solid (TS) and VS of samples were carried out in accordance with standard methods (APHA, 1998). The pH of the samples was measured

Table 1: Characteristics of FWL

Test parameters	Unit	Average value
Total solids (TS)	%	16.0
Volatile solids (VS)	%	14.6
Moisture Content (MC)	%	84.0
RSC	g/L	75.0
pH	-	5.0

by a pH meter (420A, Orion 3-star series; Thermo scientific, MA, USA). RSC was determined using the dinitrosalicylic acid (DNS) method (Miller, 1959).

Ethanol concentration estimation

Ten mL of fermentation broth was centrifuged at 5000 rpm for 30 min at 10 °C and the supernatant was used to estimate the ethanol concentration. Ethanol was analyzed by gas chromatography (HP-5960, Global Medical Instrumentation Inc, MN, USA) equipped with CP-Wax column at 110 °C, flame ion detector (FID) 220°C and injector 170 °C. Nitrogen was used as carrier gas at a flow rate of 30 cm³/min and the combustion gas was a mixture of hydrogen and air.

Statistical design of experiments

Experiments with three independent variables temperature (X₁), pH (X₂) and RSC (X₃) were conducted (Table 2) using the experimental design obtained by the full factorial CCD. In this study, the full CCD consisted of (i) a complete 2³-factorial design, (ii) n₀, center point (n₀>1) and (iii) two axial points on the axis of each design variable at a distance of α= 1.682 from the design centre. Hence a total number of design points of N = 2^k +2k + n₀ was used. The center point was replicated six times to give five degrees of freedom for calculation of errors in the experiments. The optimal values of the experimental conditions were obtained by solving the regression equation and by analyzing the response surface contour plots. The variables were coded according to the Eq. 1:

$$X_i = \frac{x_i - x_0}{\Delta x} \tag{1}$$

Where, X_i is the coded value of variable i, x_i the uncoded real value of an independent variable, x₀ the value of X_i at the center point and Dx is the step change between levels 0 and 1. The behavior of the system was explained by the following second order polynomial equation:

$$Y = \beta_0 + \sum_{i=1}^3 \beta_i X_i + \sum_{i=1}^3 \beta_{ii} X_i^2 + \sum_{i,j=1(i \neq j)}^3 \beta_{ij} X_i X_j \tag{2}$$

Table 2: Independent variables of fermentation conditions

Variables	Coded levels				
	-1.682	-1	0	1	1.682
Temp. (X ₁ ,°C)	26.60	30	35	40	43.40
pH (X ₂)	3.31	4	5	6	6.68
RSC (X ₃ ,g/L)	34.77	45	60	75	85.23

Where, Y is the ethanol concentration, β₀ the offset term, β_i the coefficient of linear effect, β_{ii} the coefficient of squared effect, β_{ij} the coefficient of interaction effect and X_j is the coded value of variable j.

The goodness-of-fit of the regression model and the significance of parameters estimates were determined through appropriate statistical methods. Statistical calculations and analysis were done using the software MINITAB (Version 14.12, PA, USA).

RESULTS AND DISCUSSION

To evaluate the potential of ethanol yield from FWL, 20 experiments were conducted according to the CCD method (Table 3).

Six experiments (runs 15-20) were replicated at the center point to verify any change in the estimation procedure as a measure of the precision property. Generally the parameters that predominantly affect the ethanol yield in a fermentation process are temperature, pH and RSC (King and Hossain, 1982). To study the combined effects of these factors, experiments were performed at different combinations of these parameters using statistically designed experiments.

The second-order model and ANOVA

The results from this study helped to frame a second order polynomial equation (Eq. 3) (in coded units) that relates the ethanol concentration, Y (g/L)

Table 3: 2³-full factorial CCD for ethanol yield

Run no.	X ₁	X ₂	X ₃	Ethanol conc., Y (g/L)	
				Observed	Predicted
1	-1	-1	-1	10.21	8.36
2	1	-1	-1	12.93	11.25
3	-1	1	-1	10.49	11.20
4	1	1	-1	10.76	10.38
5	-1	-1	1	13.36	12.70
6	1	-1	1	23.56	21.81
7	-1	1	1	17.82	18.46
8	1	1	1	23.05	23.86
9	-1.682	0	0	10.28	10.46
10	1.682	0	0	16.15	17.43
11	0	-1.682	0	10.65	13.68
12	0	1.682	0	19.35	17.79
13	0	0	-1.682	6.24	7.64
14	0	0	1.682	22.56	22.63
15	0	0	0	18.22	17.97
16	0	0	0	18.20	17.97
17	0	0	0	18.03	17.97
18	0	0	0	17.88	17.97
19	0	0	0	17.83	17.97
20	0	0	0	17.91	17.97

Optimization of operational parameters for ethanol production

$$Y = 17.9697 + 2.0716 X_1 + 1.2222 X_2 + 4.4554 X_3 - 1.4219 X_1^2 - 0.7908 X_2^2 - 1.0029 X_3^2 - 0.9275 X_1 X_2 + 1.555 X_1 X_3 + 0.73 X_2 X_3 \quad (3)$$

to the initial conditions of temperature, pH and RSC. This equation was used to predict the ethanol concentration in Table 3. Apart from explaining the linear effects of temperature, pH and RSC on ethanol yield, the CCD approach described the quadratic and interaction effects of the parameters too.

ANOVA is important in determining the adequacy and significance of the quadratic model. The analyses were done by means of Fisher's 'F'-test. Generally the 'F' value with a low probability 'P' value indicates high significance of the regression model (Rene et al., 2007). From the ANOVA summary (Table 4), the model was found to be statistically significant (P < 0.01) at the 99% confidence level. Due to the lowest 'P' value (P = 0), the linear effects were highly significant compared to the quadratic (P = 0.017) and interaction (P = 0.047) effects. Thus, the ethanol concentration was adequately explained by the model equation (Eq.3). Alternatively, the fitting of the experimental data to the regression model was checked and suitably explained by the value of determination coefficient (R² = 0.94). It indicated that ~ 6 % of the total variations in the ethanol concentration were not satisfactorily explained by the model (Eq. 3). This unexplained value of response (~ 6 %) was presented in terms of residual error in the ANOVA table (Table 4). Though this value would be considered low in applied statistics, it can be accepted and be attributed to the complex biotransformation of waste during the fermentation process. In their work, Chauhan and Gupta (2004) have emphasized on the acceptance of any model with R² > 0.75. Besides, the relationship between the

Table 4: ANOVA of the model

Source	DF	Seq SS	Adj SS	F	P
Regression	9	425.340	425.340	17.61	0.000
Linear	3	350.109	350.109	43.48	0.000
Square	3	44.742	44.742	5.56	0.017
Interaction	3	30.489	30.489	3.79	0.047
Residual error	10	26.841	26.841		
	5	26.701	26.701	190.88	0.000
Lack-of-fit	5	0.140	0.140		
Pure error	19	452.181			
Total					

DF: degrees of freedom; Seq SS: sequential sum of squares; Adj SS: adjusted sum of squares; F: Fischer's variance ratio; P: probability value.

experimental values and predicted values (Fig.1) showed that the plotted points cluster around the diagonal line, indicating good fitness of the model.

Each item in the regression model (Eq. 3) has an identified effect on the ethanol concentration. Students 't'-test can be used to quantify the intensity of parameters (temperature, pH and RSC) on the ethanol concentration, while 'P' values signify the pattern of interaction among the parameters. The larger the value of 't' and the smaller the value of 'P', the more significant is the corresponding coefficient term (Douglas, 2001). The regression coefficients and 't' and 'P' values for all the linear, quadratic and interaction effects of the parameters are given in Table 5. A positive sign in the 't' value indicated a synergistic effect, while a negative sign represented an antagonistic effect of the parameters on the ethanol concentration. Ethanol concentration was significantly affected by the synergistic effect of the linear terms of RSC, with a 'P'-

Table 5: Estimated regression coefficients, 't'- and 'P'-values of the model

Relationship	Factor	Coefficient	't'-Value	'P'-Value	
Main effects	Linear	X ₁	2.0716	4.673	0.001***
	Linear	X ₂	1.2222	2.757	0.020**
	Linear	X ₃	4.4554	10.050	0.000***
Interactions	Pure quadratic	X ₁ ²	-1.4219	-3.295	0.008***
		X ₂ ²	-0.7908	-1.832	0.097*
		X ₃ ²	-1.0029	-2.324	0.042**
	Cross product	X ₁ X ₂	-0.9275	-1.601	0.140
		X ₁ X ₃	1.5550	2.685	0.023*
		X ₂ X ₃	0.7300	1.260	0.236

*Significant at 10% ('P'-Value); ** Significant at 5% ('P'-Value); and *** Significant at 1% ('P'-Value). (+): synergistic effect ('t'-Value) (-): antagonistic effect ('t'-Value)

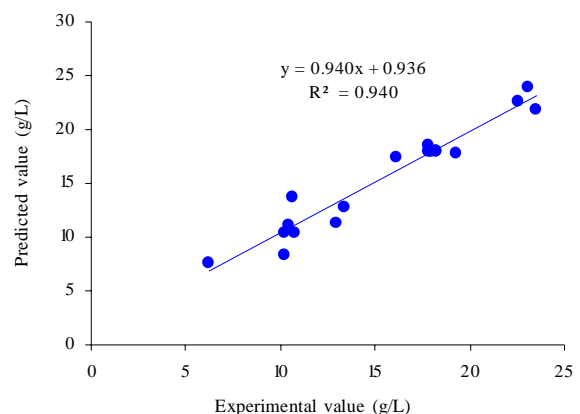


Fig. 1: Parity plot showing the distribution of experimental and predicted values of ethanol concentration

This result suggested that the RSC had a direct relationship with the production of ethanol from FWL, i.e. any minor change in this variable may cause a great positive or negative change in ethanol concentration. Besides, the quadratic terms (X_1^2 , X_2^2 and X_3^2) showed the antagonistic effects on the ethanol concentration, typically X_1^2 ($P = 0.008$).

Response surface plots and optimization conditions

The ultimate objective of CCD method used in this study was to find out the significant effects of the parameters viz., temperature, pH and RSC and trace the optimum condition for ethanol production from FWL. Surface and contour plots demonstrating the effects of different process parameters, two parameters varied at a time while keeping the third at middle level, on the ethanol concentration were shown in Figs. 2-4. 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).

Fig. 2 showed the effect of temperature and pH on the ethanol concentration. The convex response surface suggested well-defined optimum variables (temperature and pH) and that the ethanol concentration increased to the peak with the increase of temperature and pH up to 38 °C and 5.45, respectively; then declined with the further increase of these two parameters. This result demonstrated that the response surface had a maximum point for ethanol yield. Similar results have been obtained by Wilkins *et al.*, (2007) who reported that ethanol production from simultaneous saccharification and fermentation of citrus peel waste by *S. cerevisiae* was greatest when the fermentation temperature and pH were adjusted to 37 °C and 6.0, respectively. Besides, higher ethanol productivity from the kitchen waste using *S. cerevisiae* KF-7 as inoculum has been reported at the fermentation conditions of temperature (37 °C) and pH (5.85) (Tang *et al.*, 2008).

S. cerevisiae is reported to grow well within the temperature range 28-40 °C (Rivera *et al.*, 2006). The increase in temperature accelerates the inhibition effect of the RSC and ethanol on the cell activities, thereby lowering both cell and ethanol yields (Phisalaphong *et al.*, 2005). The deleterious effect of higher temperatures on ethanol yield can be attributed to the denaturation of ribosomes and enzymes and problems associated with the fluidity of membranes (McMeekin *et al.*, 2002).

The inhibitory effect of pH (at the high level) on the ethanol yield could be due to the lower ATP production during the metabolic changes in *S. cerevisiae* (Pena *et al.*, 1972). During the investigation for ethanol production from various waste resources viz., bread residue, citrus peel, kitchen garbage and pineapple cannery waste, *S. cerevisiae* was found to grow well within the range of pH 4.18 - 6 (Nigam, 2000; Ebrahimi *et al.*, 2007; Wilkins *et al.*, 2007; Wang *et al.*, 2008).

The effects of other two pairs viz. temperature-RSC and pH-RSC on the ethanol concentration formed the stationary ridge systems as shown in Fig. 3 and Fig. 4, respectively. It can be observed from these Figs that, within the range of experiment (Temperature: 26.59 - 43.41 °C, pH: 3.31 - 6.68), any changes in temperature and pH had a small difference in the ethanol concentration, while the change in RSC had a significant effect on the ethanol concentration. With increase in RSC from 34.77 to 85.23 g/L, the ethanol concentration increased from 7.64 to 22.63 g/L (Table 3). This result indicated that there was no inhibition to the ethanol yield by the increase in RSC. While producing ethanol from cheese-whey powder, Ozmihci and Kargi (2007) reportedly kept the sugar concentration below 75 g/L to avoid substrate inhibition due to osmotic stress on the *Kluyveromyces marxianus* (DSMZ-7239). During the investigation for ethanol production by *S. cerevisiae* M30 using cane molasses as the substrate, the significant effect of RSC on ethanol yield was observed at a concentration of 22 % (w/v) (Phisalaphong *et al.*, 2005).

The stationary ridge systems observed in Figs. 3 and 4 were analyzed based on the canonical form of the second-order model. The nature of the response surfaces were described by the following equation:

$$y = 23.659 - 3.329w_1^2 - 0.248w_2^2 + 0.362w_3^2 \quad (4)$$

Where, w_1 , w_2 and w_3 are the axes of the response surface. The eigen values λ_1 (-3.329) is negative; λ_2 (-0.2484) and λ_3 (0.3619) were closed to zero that makes the surface a rising ridge system and the stationary point was a saddle point for the response. Also, the two eigen values closed to zero (λ_2 and λ_3) indicate that the plane in the (w_2 , w_3) coordinates will be closed to the optimal operating conditions. The maximum point in the ethanol yield can be achieved by increasing the RSC under the

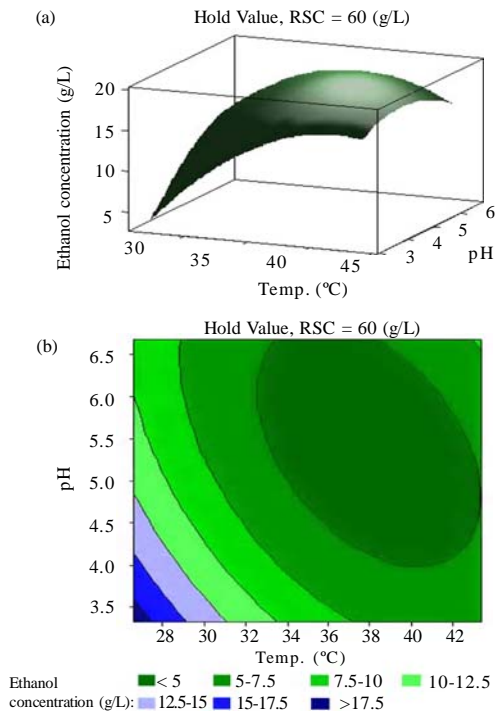


Fig. 2: Interaction effects of temperature and pH on ethanol concentration: (a) surface plot (b) contour plot

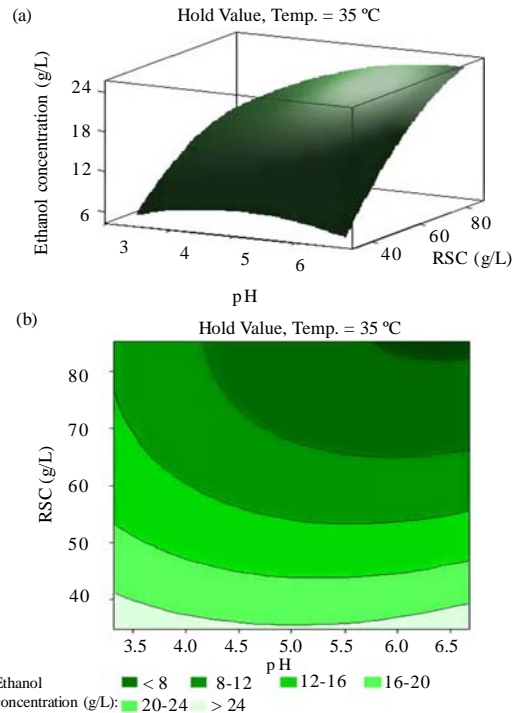


Fig. 4: Interaction effects of pH and RSC on ethanol concentration: (a) surface plot and (b) contour plot

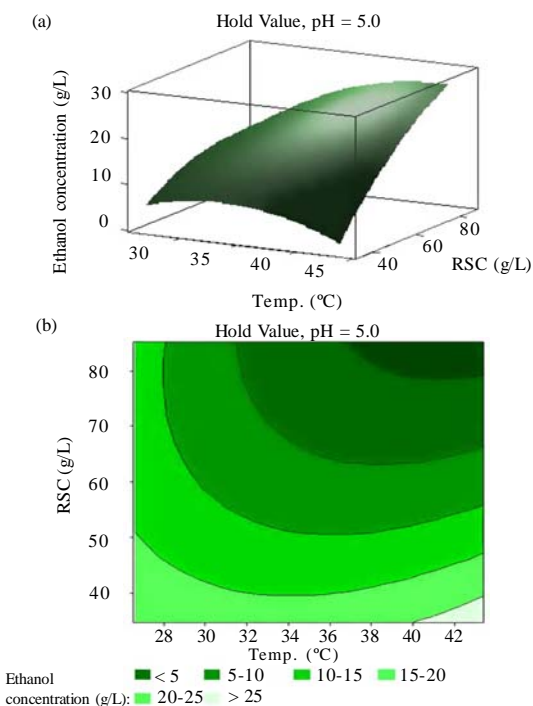


Fig. 3: Interaction effects of temperature and RSC on ethanol concentration: (a) surface plot and (b) contour plot

steepest ascent method (Douglas, 2001). However, the addition of sugar (glucose) to the contaminated medium for ethanol fermentation is meaningless in real practice. Therefore, the RSC of FWL (+1 level, Table 2) can be chosen as the optimum value in this study.

Model validation and confirmation

To confirm the validity of the statistical experimental strategies and gain a better understanding of ethanol yield from FWL, a confirmation experiment with duplicate set was performed at the specified optimum condition. Experiments conducted at the optimum condition (Temperature: 38 °C, pH: 5.45 and RSC of 75 g/L) demonstrated that the ethanol concentration (24.17 g/L) was closer to the predicted value (23.66 g/L). Corresponding to the ethanol concentration of 24.17 g/L, the ethanol yield was calculated as 0.32 g ethanol/g reducing sugar accounting for as high as 63 % of the stoichiometric value. This showed that the model was useful to predict the ethanol concentration as well as optimize the experimental conditions.

CONCLUSION

The present study using RSM based CCD established an efficient second order polynomial model to describe the ethanol yield by FWL fermentation. The high similarity between the experimental value and the predicted ones ($R^2 = 0.94$) suggested that the model was a good fit. Maximum ethanol concentration of 24.17 g/L was obtained at the optimum condition of temperature (38 °C), pH (5.45) and RSC (75 g/L). The ethanol concentration at the optimum experimental condition (24.17 g/L) agreed well with the predicted one (23.66 g/L). This indicated the suitability of the model employed and the success of RSM to optimize the conditions of ethanol production from FWL. Corresponding to the ethanol concentration of 24.17 g/L, the ethanol yield from the FWL was obtained as 0.32 g ethanol/g reducing sugar. This research lays down the basis for ethanol production from the FWL despite being a contaminated substrate. The results from the investigation showed that FWL 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. Further research is, however, warranted to examine the economical viability of the process.

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