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Short communication

Statistical optimization of enzymatic saccharification and ethanol fermentation using food waste

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ABSTRACT

Response surface methodology (RSM) based on the 2^3 factorial central composite design (CCD) was applied to optimize the conditions of enzymatic saccharification and ethanol fermentation using food waste. Optimum conditions were found to be saccharification pH of 5.20, enzyme reaction temperature of 46.3 °C, enzyme concentration of 0.16% (v/v), fermentation pH of 6.85, fermentation temperature of 35.3 °C, and fermentation time of 14 h. The model predicted that maximum concentration of reducing sugar and ethanol under the above optimum conditions were 117.0 g reducing sugar/L and 57.6 g EtOH/L, respectively. Experimental results were in close agreement with model prediction with 120.1 g reducing sugar/L and 57.5 g EtOH/L, respectively.

1. Introduction

Around 60% of the total ethanol is produced by fermentation [1]. Many research and development efforts aimed at commercial production of ethanol by fermentation from renewable resources such as crop residues and biomass waste [2–7], municipal solid wastes (MSW) [8–11], municipal sludge [12], and dairy/cattle manures [13] have increased. Considering energy producing cost and nutrient amounts available, however, food waste might be suitable for bio-ethanol production. In Korea, food waste accounts for about 30% of total MSW generation, and disposal of food waste becomes a serious social problem because sanitary landfill of organic waste was prohibited by environmental law from the year of 2005.

High ethanol yield and low production cost need optimization of saccharification and fermentation processes. The traditional 'one-factor at a time' optimization method is simple, but this one often fails to seek the optimum region because the joint effects of factors are not considered. Response surface methodology (RSM) is a statistical model widely used to study an aggregate effect of several variables and to seek optimum conditions for a multi-variable system. A combination of factors generating a certain optimum response can be identified though factorial design as well as RSM [14]. In this study, RSM based on central composite design (CCD) was used for the optimization of enzymatic saccharification and ethanol production from Korean food waste.

2. Materials and methods

2.1. Substrate

Food waste was collected from a cafeteria of the Chosun University. It was mixed with water at a ratio of 1:1 (v/v) and crushed into small particles (average size of 1–2 mm) using a liquidizer. The characteristics of the food waste mixture used in this study were pH 5.12, total solid 12.9%, volatile solid per total solid 89.5%, total chemical oxygen demand 85.1 g/L, and total nitrogen 5.4 g/L. The average elemental composition of food waste mixture was carbon 47.8%, hydrogen 6.1%, oxygen 40.9%, and nitrogen 5.2%. This characteristic was very similar to others that have been reported [15–19].

2.2. Enzyme and microorganism

Spirizyme Plus FG (*Aspergillus niger* glucoamylase, Novozymes, Denmark) was purchased for food waste saccharification. The specific activity was 400 AGU/g (one unit is defined as the amount of enzyme which hydrolyses 1 μmol of maltose per minute under specified conditions). *Saccharomyces cerevisiae* KA4, which was isolated from a secondary acidogenic digester of the three-stage methane fermentation system developed in this lab, was used for ethanol fermentation [20]. The strain was grown on YM medium containing 0.3% (w/v) yeast extract, 0.3% (w/v) malt extract, 0.5% (w/v) peptone, and 1% (w/v) glucose. The cells were cultivated at 35 °C and pH 6.0.

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2.3. Enzymatic saccharification and ethanol fermentation

All experiments were carried out in 500 mL Erlenmeyer flasks with a working volume of 100 mL. One hundred milliliter of food waste mixture, which was mixed with water at a ratio of 1:1 (v/v), was mixed with Spirizyme Plus FG for 4 h to produce reducing sugar. Liquid phase of food waste hydrolyzate obtained by the enzymatic saccharification was stored in the −20 °C freezer before ethanol fermentation. Ethanol fermentation was conducted by *S. cerevisiae* KA4 under anaerobic condition using stored food waste hydrolyzate without adding of any nutrient components. To ensure anaerobic condition, all inoculations and manipulations were performed in the vacuum anaerobic chamber (SK-G002-A1, Three-Shine, Seoul, Korea) and flasks were sealed by rubber stopper after inoculation of 2% (v/v) precultured inocula. For experimental design, different conditions of enzymatic saccharification and ethanol fermentation such as saccharification pH, enzyme reaction temperature, enzyme concentration, fermentation pH, fermentation temperature and fermentation time were used. Table 1 shows coded and actual values of the experimental variables.

2.4. Analytical methods

During saccharification and ethanol fermentation, the total chemical oxygen demand (tCOD) was measured by Kim et al. [21] and the concentration of reducing sugars was determined by Miller's method [22]. To determine ethanol concentration, aqueous phase samples were centrifuged at 1000 × *g* for 10 min and then analyzed by gas chromatography (Flame Ionization Detector, M600D, YoungLin, Seoul, Korea) by Kim et al. [20].

2.5. Response surface methodology

The series of experiments designed and conducted are shown in Table 1. To produce reducing sugar and ethanol from food waste, enzymatic saccharification and ethanol fermentation conditions were optimized by RSM based on the 2^3 factorial central composite design. Two series of 20 experiments were carried out with three variables, and each variable varied at five levels ($\alpha = 2$) for enzymatic saccharification and ethanol fermentation. The value of the dependent response was the mean of three replications. Reducing sugar and ethanol production were the response (dependent) variables. The second order polynomial model was fitted for reducing sugar production (Y_1) and reduced cubic polynomial model was fitted for the ethanol production (Y_2), using the following Eqs. (1) and (2) in Table 1. The statistical analysis of the data was performed using "Design Expert" software (version 7.1.1, Stat-Ease, Inc., Minneapolis, USA).

3. Results and discussion

3.1. Statistical analysis

RSM is generally used to investigate a combined effect of several variables and to find optimum conditions for a multivariable system [14]. The most common experimental design used in RSM is CCD which has equal predictability in all directions from the center. In addition, CCDs are optimized designs for fitting quadratic models [23].

Statistical significance of respective model equation was checked using *F*-test analysis of variance (ANOVA) (Table 2). The fitness of the models was also expressed by the coefficient of determination, R^2, which was found to be 0.9713 and 0.9987 on the production of reducing sugar and ethanol, respectively. These values indicate 97.13% of the response variability in enzymatic saccharification and 99.87% of the response variability in ethanol production. The closer the R^2 is to 1, the stronger the model and the better it predicts the response [24]. The lower the value of the coefficient of the variation (CV) (2.17% for enzymatic saccharification and 3.88% for ethanol production), the greater is the precision and reliability of the experiments carried out. The probability *p*-value for models of less than 0.0001 also indicated that the models were highly significant and insignificant *p*-value of lack of fit for models indicated that

Table 1
Experimental design (conditions, responses and polynomial models) for enzymatic saccharification (A) and ethanol production (B)

(A) Enzymatic saccharification

Run[a]	Initial pH[b], A_1		Temp.[c] (°C), A_2		Enzyme inoculation[d] (%), A_3		Reducing sugar concentration[e] (g/L), Y_1
1	5	−1.00	35	−1.00	0.10	−1.00	95
2	7	+1.00	35	−1.00	0.10	−1.00	98.3
3	5	−1.00	55	+1.00	0.10	−1.00	98.5
4	7	+1.00	55	+1.00	0.10	−1.00	101
5	5	−1.00	35	−1.00	0.20	+1.00	97.1
6	7	+1.00	35	−1.00	0.20	+1.00	102.3
7	5	−1.00	55	+1.00	0.20	+1.00	102.5
8	7	+1.00	55	+1.00	0.20	+1.00	105.1
9	4	−2.00	45	0.00	0.15	0.00	94.4
10	8	+2.00	45	0.00	0.15	0.00	103.1
11	6	0.00	25	−2.00	0.15	0.00	92.4
12	6	0.00	65	+2.00	0.15	0.00	104.1
13	6	0.00	45	0.00	0.05	−2.00	89.2
14	6	0.00	45	0.00	0.025	+2.00	92.5
15	6	0.00	45	0.00	0.15	0.00	114
16	6	0.00	45	0.00	0.15	0.00	117.3
17	6	0.00	45	0.00	0.15	0.00	117.5
18	6	0.00	45	0.00	0.15	0.00	115.9
19	6	0.00	45	0.00	0.15	0.00	113.9
20	6	0.00	45	0.00	0.15	0.00	121

(B) Ethanol production

Run[f]	Initial pH[g], B_1		Temp.[h] (°C), B_2		Reaction Time[i] (h), B_3		Ethanol concentration[j] (g/L), Y_2
1	5	−1.00	30	−1.00	10	−1.00	8.9
2	7	+1.00	30	−1.00	10	−1.00	13.6
3	5	−1.00	40	+1.00	10	−1.00	15.9
4	7	+1.00	40	+1.00	10	−1.00	19.8
5	5	−1.00	30	−1.00	14	+1.00	32
6	7	+1.00	30	−1.00	14	+1.00	35.3
7	5	−1.00	40	+1.00	14	+1.00	43.6
8	7	+1.00	40	+1.00	14	+1.00	41.8
9	4	−2.00	35	0.00	12	0.00	1
10	8	+2.00	35	0.00	12	0.00	41.1
11	6	0.00	25	−2.00	12	0.00	3.9
12	6	0.00	45	+2.00	12	0.00	1.1
13	6	0.00	35	0.00	8	−2.00	9.9
14	6	0.00	35	0.00	16	+2.00	55.7
15	6	0.00	35	0.00	12	0.00	45.4
16	6	0.00	35	0.00	12	0.00	46.5
17	6	0.00	35	0.00	12	0.00	45.2
18	6	0.00	35	0.00	12	0.00	44.7
19	6	0.00	35	0.00	12	0.00	45
20	6	0.00	35	0.00	12	0.00	43.9

$$Y_1 = \alpha_0 + \alpha_1 A_1 + \alpha_2 A_2 + \alpha_3 A_3 + \alpha_{11} A_1^2 + \alpha_{22} A_{22} + \alpha_{33} A_3^2 + \alpha_{12} A_1 A_2 + \alpha_{13} A_1 A_3 + \alpha_{23} A_2 A_3 \quad (1)$$

$$Y_2 = \beta_0 + \beta_1 B_1 + \beta_2 B_2 + \beta_3 B_3 + \beta_{11} B_1^2 + \beta_{22} B_2^2 + \beta_{33} B_3^2 + \beta_{12} B_1 B_2 + \beta_{13} B_1 B_3 + \beta_{23} B_2 B_3 + \beta'_{12} B_1^2 B_2 + \beta'_{13} B_1^2 B_3 + \beta''_{12} B_1 B_2^2 + \beta_{123} B_1 B_2 B_3 \quad (2)$$

where A_1, A_2, A_3, B_1, B_2 and B_3 represent coded levels of the independent variables; α_0 and β_0 are intercept terms; $\alpha_1, \alpha_2, \alpha_3, \beta_1, \beta_2$ and β_3 are linear terms; $\alpha_{11}, \alpha_{22}, \alpha_{33}, \beta_{11}, \beta_{22}$ and β_{33} are quadric terms; $\alpha_{12}, \alpha_{13}, \alpha_{23}, \beta_{12}, \beta_{13}, \beta_{23}, \beta'_{12}, \beta'_{13}, \beta''_{12}$ and β_{123} are interaction terms.

[a,f]Run order, [b,g]left col.; actual pH, right col.; level (coded unit*), [c,h]left col.; actual temperature (°C), right col.; level (coded unit), [d]left col.; actual enzyme inoculation (%), right col.; level (coded unit), [e]actual reducing sugar concentration (g/L), [i]left col.; actual fermentation time (h), right col.; level (coded unit), [j]actual ethanol concentration (g/L).
*For statistical calculations, the relationship between the coded values and actual values are described as the following equation: $x_i = (X_i - X_0)/\Delta X_i$, $i = 1, 2, 3, \ldots, k$, where; x_i is the dimensionless value of an independent variable; X_i is the real value of an independent variable; X_0 is the real value of an independent variable at the center point and ΔX_i is the step change of variable.