

Effect of Adding Corn Stalk Residue Pretreated by Laser on Immobilized Yeast

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The effect of immobilized yeast on bioethanol production before and after adding laser-pretreated corn stalk residue were investigated. Response surface methodology was used to optimize the conditions of adding residue. An optimum experimental condition was obtained at pH 4.5, 2.08% yeast concentration, and 0.20% corn stalk residue. Under these conditions, adding residue increased the yield of bioethanol from 53.2% to 86.5%, which matched the predicted value. The yield was relatively stable within 28 d, with a downward trend subsequently appearing.

Keywords: Immobilized yeast; Corn stalk; Adding; Bioethanol

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INTRODUCTION

The use of bioethanol has grown in many countries and has brought environmental benefits as well as established cost-competitive domestic energy resources and generated additional economic development (Meinita *et al.* 2011). Majority of bioethanol is made from terrestrial biomass, which is essentially food such as corn, sweet potato, and sugarcane (John *et al.* 2011). Corn stalk is an effective feedstock for cellulosic bioethanol production because of its high cellulose content (Liu *et al.* 2012). It is clear that biocatalytic structures composed of microorganisms entrapped in gel beads lead to the appearance of cell-growth gradients that induce a heterogeneous development of the biomass inside the structure. The maximum cell concentration is located near the gel-solution interface (Mathew *et al.* 2014).

In this study it was confirmed that the yield bioethanol would increase with immobilized *Saccharomyces cerevisiae* using laser-pretreated corn stalk. To obtain the maximum cell concentration during the yeast immobilization process, technology for the addition of residue was used in immobilizing the yeast gel beads. The process of immobilizing the yeast gel beads was conducted using the embedding method with sodium alginate and manganese alginate (Rogalski *et al.* 2006). The reducing sugars in residue hydrolysates were subsequently fermented using immobilized *S. cerevisiae*.

EXPERIMENTAL

Materials

Preparation of samples

Corn stalk was harvested from Taiping Farm in Heilongjiang, China. It was

milled and sized through a 60 Tyler mesh sieve. Corn stalk was pretreated with a laser for 68 min at 265 W and a water to solid ratio of 21:1 (mL/g). The crude cellulase was provided by Hualing Biological Technology Co., Ltd., Gansu, China (Tian *et al.* 2011).

The strains of *S. cerevisiae*, AS 2.607, which was provided by China General Microbiological Culture Collection Center (CGMCCC), were used in the fermentation experiments. Yeast cells were grown on agar plates which contained 20 g/L peptone, 20 g/L glucose, 10 g/L yeast extract, and 20 g/L agar. Bioethanol fermentation experiments were conducted in residue hydrolysates.

Methods

Preparation of alginate calcium beads

Yeast seeds were cultured in slant culture medium for 48 h. After 36 h fermentation in the batch fermenter, yeast cells were collected and blended with the sodium alginate in solution. To prepare the calcium alginate beads, 3 mL of yeast seed culture and pretreated corn stalk were added to 2% sodium alginate in 100 mL of deionized water. An alginate solution (2%) was sprayed through a thin inner nozzle (about 2 mm diameter at exit) into 150 mL of calcium chloride solution. The calcium alginate beads were stored after being washed three times with deionized water to remove residual calcium chloride. The beads were packed and stored in deionized water at 4 °C for 3 d for the preparation of manganese alginate beads.

Preparation of alginate manganese beads

Stability studies of manganese alginate beads revealed marked differences in ion binding capacity, rendering manganese alginate beads with high guluronic acid content the most stable (Mørch *et al.* 2012). According to Mørch methods, the calcium alginate beads were added to 1% manganese alginate solution and placed in the refrigerator for 24 h (4 °C) to solidify. Therefore, the manganese alginate beads were then ready for proliferation of immobilized cells. The beads were placed in the immobilization proliferation medium at 30 °C and 200 rpm. The proliferation mediums were replaced with fresh media every 12 h, a total of four times during the proliferation. The proliferated immobilized cells were then ready for bioethanol fermentation.

Concentrated hydrolysates of corn stalk residue

Concentrated hydrolysates of corn stalk residue were prepared using vacuum evaporation. Evaporation was carried out under low vacuum at 0.5 bar and 55 °C for 30 min. After evaporation, the hydrolysates were sterilized at 120 °C for 15 min and then used for bioethanol fermentation. Before evaporation, there was 0.30 mg/mL xylose and 2.62 mg/mL glucose in the hydrolysates of corn stalk pretreated by laser. After evaporation, the concentrations of xylose and glucose increased to 1.28 mg/mL and 12.07 mg/mL, respectively (Tian *et al.* 2012).

Determination bioethanol concentration

The concentration of bioethanol was detected using a GS chromatograph (GC-8A Shimadzu, Tokyo, Japan) equipped with a 20% PEG column. Isopropyl alcohol was used for an internal standard to detect bioethanol from each bioethanol fermented sample. The column temperatures at the injector and detector were 130 °C and 110 °C, respectively. The yield of bioethanol was calculated using the following equation:

$$X = \frac{C_1}{C_2 \times 0.51} \times 100\% \quad (1)$$

where X is the concentration of bioethanol (%), C_1 is the concentration of bioethanol in fermentation broth, and C_2 is the concentration of total reducing sugar (glucose and xylose) before fermentation.

Experimental design and data analysis

Triplicate experiments were carried out for all fermentation experiments. Bioethanol production yields were designed using a Box-Behnken design (BBD) of response surface methodology (RSM) to examine the relationship during three response variables (Box and Behnken 1960). Three fermentation factors were selected: corn stalk concentration (X_1), yeast concentration (X_2), and pH (X_3). Minitab (Version 17.0, Minitab Inc., USA) statistical software was used to determine the optimum conditions for bioethanol production. The designed matrix, with 17 experimental sets in two blocks and five repetitions of the midpoint, is shown in Table 1.

Design-Expert Version 8.5 (Stat-Ease Inc., USA) was used to obtain the correlation coefficients of the quadratic polynomial equation and predict the response of the dependent variables.

Table 1. Box–Behnken Design Matrix for the Predicted Values and Experimental Values for the Bioethanol Yield

Number	Coded variable levels			Yield (%) values
	X_1 (%)	X_2 (%)	X_3	
1	0.20	2.0	4.5	84.2
2	0.20	2.5	5.0	72.5
3	0.20	2.0	4.5	84.1
4	0.20	1.5	4.0	65.3
5	0.25	1.5	4.5	68.3
6	0.20	2.0	4.5	84.1
7	0.20	1.5	5.0	74.1
8	0.25	2.5	4.5	75.8
9	0.15	2.5	4.5	76.2
10	0.20	2.0	4.5	81.6
11	0.15	2.0	5.0	73.1
12	0.15	1.5	4.5	71.4
13	0.15	2.0	4.0	66.9
14	0.20	2.0	4.5	82.9
15	0.20	2.5	4.0	71.0
16	0.25	2.0	5.0	73.0
17	0.25	2.0	4.0	69.5

Repeated-batch fermentation

Repeated-batch fermentation tests were carried out to evaluate the immobilized cell biotreatability in the same bioreactor (Watanabe *et al.* 2012). Manganese alginate beads were washed with pH 3 deionized water in the concentrated saccharification liquid medium at 35 °C and 150 rpm. After 24 h, all fermented broth was taken out without removing the immobilized manganese alginate beads. The immobilized beads were

washed with pH 3 deionized water, and the same amount of the concentrated saccharification liquid medium was immediately added to begin the next batch fermentation. Five repeated-batch fermentations were carried out, after which the samples were gathered for analysis.

RESULTS AND DISCUSSION

Effect of Yeast Concentration on Bioethanol Fermentation

Different cell concentrations (0.50%, 1%, 1.50%, 2.00%, 2.50%, and 3.00%) of immobilized yeast were studied to observe the effect of this parameter on bioethanol fermentation. The effect of yeast concentration on bioethanol fermentation was examined with an initial pH of 5.0 and a 0.21% residue concentration. The results clearly demonstrated that higher yeast concentrations resulted in higher ethanol yield values, as can be seen in Fig. 1a. The yield of bioethanol increased from 63.2% to 75.0%. However, when yeast concentrations exceeded 2%, the yield of bioethanol declined. This illustrates that increasing the concentration of yeast cells could significantly increase the yield of bioethanol, which may result from the cells density over the immobilized cells ($p < 0.05$). Ariyajaroenwong *et al.* (2012) reported that increasing the immobilized yeast cells' concentration for fermentation resulted in higher bioethanol production efficiencies.

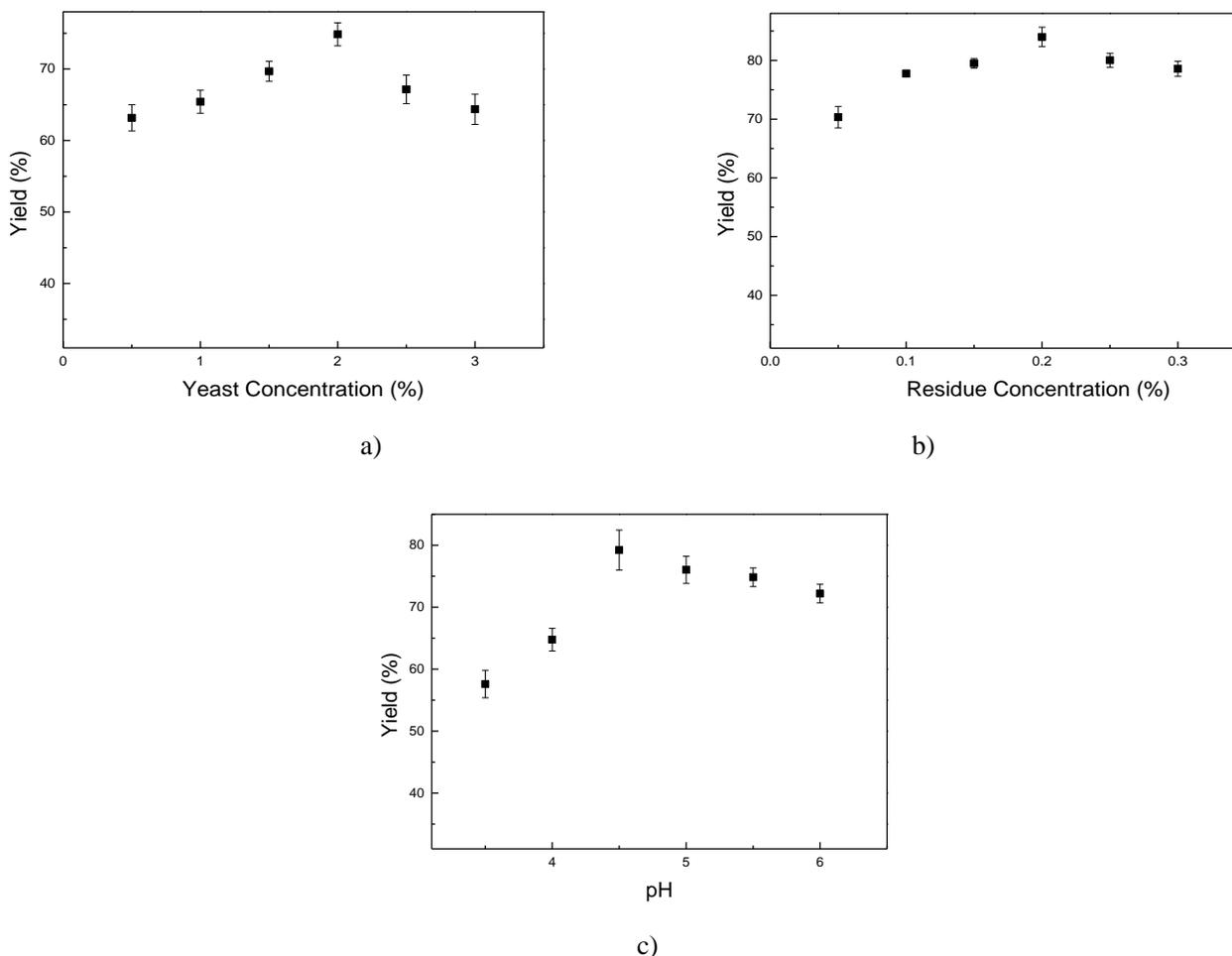


Fig. 1. Effect on bioethanol yield of various levels of variables

Effect of Pretreated Residue on Bioethanol Fermentation

Different residue concentrations (0.05%, 0.10%, 0.15%, 0.20%, 0.25%, and 0.30%) of pretreated residue were studied to observe the effect of this parameter on bioethanol fermentation. The effect of different substrate concentrations on bioethanol fermentation was examined with an initial pH of 5.0 and a 2% concentration of immobilized yeast. Consequently, Fig. 1b shows that the 0.20% pretreated residue concentration was the best for the ethanol yield (84.1%).

Effect of pH on Bioethanol Fermentation

Lee *et al.* (2011) reported that regulated pH conditions are better than unregulated, in terms of bioethanol fermentation. Among regulated saccharification liquids, a pH of 5.0 provided the best conditions, with > 90% of glucose consumed in 19.5 h. To study the effect of pH on bioethanol fermentation from residue hydrolysate, the pH values of saccharification liquids were fixed at pH 3.5, 4.0, 4.5, 5.0, 5.5, and 6.0, with 4 M NaOH. Changes in pH values in this range were found to have a significant effect on bioethanol fermentation at this early stage ($p < 0.05$), but Fig. 1c showed a pH of 4.5 had the best effect on the yield of biomass ethanol (79.7%). However, the yield of biomass ethanol was slow to subside.

RSM Analysis and Optimization of Bioethanol Fermentation

The regression coefficients of the intercept, linear, quadratic, and interaction terms of RSM model were calculated using the least square technique and shown in Table 2. The data, after regression analysis, fit a second-order polynomial equation. The determination coefficient (R^2) of the BBD experimental design was 0.965, which showed that the current model does not contradict the observed data points. However, a coefficient of variation of less than 5% showed that the experimental model could be reproduced (Wanasundara and Shahidi 1996). The model F-value, 21.48, illustrated that the experimental model was very significant. The predicted second-order polynomial model yielded the following model curve,

$$Y = 83.95 + 2.04\chi_2 + 2.49\chi_3 - 1.82\chi_2\chi_3 - 5.55\chi_1^2 - 5.48\chi_2^2 - 7.77\chi_3^2 \quad (4)$$

where Y is the yield of bioethanol.

Table 2. Analysis of Variance for Regression Equation in RSM Model

Source	Estimated coefficients	df	Mean Square	F Value	Prob. > F
Model	83.95	9	74.18	21.48	0.0003
X ₁ -residue	-0.12	1	0.11	0.03	0.865
X ₂ -yeast concentration	2.04	1	33.29	9.64	0.0172
X ₃ -pH	2.49	1	49.51	14.33	0.0068
X ₁ X ₂	0.67	1	1.76	0.51	0.50
X ₁ X ₃	-0.70	1	1.94	0.57	0.48
X ₂ X ₃	-1.82	1	13.31	3.85	0.041
X ₁ ²	-5.55	1	129.51	37.50	0.0005
X ₂ ²	-5.48	1	126.43	36.60	0.0005
X ₃ ²	-7.77	1	254.11	73.57	< 0.0001
Lack of Fit		3	423	1.47	0.35
R-Squared	0.965				

RSM was used to determine the optimum conditions for fermentation using immobilized yeasts. The optimal values for this fermentation process were found to be 2.08% yeast concentration, adding 0.20% residue, and pH 4.5. Under these conditions, the yield of bioethanol was 86.5% (n =3).

Repeated-Batch Fermentation

To study the stability of immobilized yeast fermentation with added residue as an immobilizing carrier and the fermentation ability in long-term bioethanol operations, we investigated cell-recycling repeated-batch SSF of pretreated corn stalk saccharification liquid. As can be seen from Fig. 2, after adding residue, the yield of biomass bioethanol was higher than that using non-added manganese alginate gel beads, and fermentation stability was more reliable and stable. The yield of biomass bioethanol fermentation was relatively stable within 28 d, with a subsequent downward trend appearing. However, for non-added manganese alginate gel beads, the yield of biomass ethanol presented a dramatic decline after 20 d.

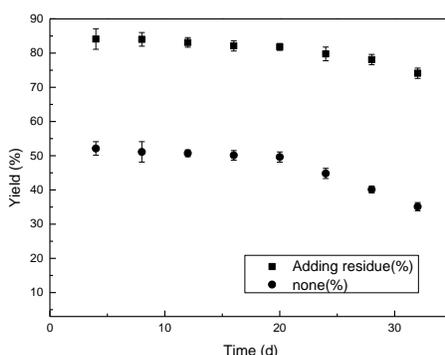


Fig. 2. Effect of immobilized yeast cell fermentation cycles on bioethanol yield

CONCLUSIONS

1. Adding corn stalk residue caused structural change to the manganese alginate gel beads and increased the yield of biomass bioethanol from 53.2% to 86.5%.
2. The yield of bioethanol fermentation using adding corn stalk pretreated by laser was relatively stable within 28 d. However, the yield of common bioethanol fermentation was relatively stable within 20 d, and the yield of bioethanol was keeping low level.

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