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Research paper

Enhanced butanol production using *Clostridium beijerinckii* SE-2 from the waste of corn processing

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ABSTRACT

Acetone-butanol-ethanol (ABE) fermentation from food processing waste is one way to reduce cost. In this study, corncob hydrolysate and corn steep liquor (CSL), two waste materials from corn processing industries, were used as carbon and nitrogen sources to yield butanol by using *Clostridium beijerinckii* SE-2. Media compositions favoring butanol production were investigated using statistical experimental designs. Media components were first screened using a fractional factorial experimental design. CSL and CH_3COONH_4 were found to be the significant variables among six factors, including the contents of CSL, CH_3COONH_4, K_2HPO_4-KH_2PO_4, $MnSO_4 \cdot H_2O$, $MgSO_4 \cdot 7H_2O$ and $FeSO_4 \cdot 7H_2O$. The two factors were further optimized by the steepest assent and central composite rotatable design. The validated experiments showed that the total ABE in the system was 19.22 g L^{-1} and the concentration of butanol could reach 11.65 g L^{-1} with the optimized medium, which was 42% higher than the initial medium. Scale-up fermentation with optimized medium in the 100 L bioreactor resulted in 20.29 g L^{-1} of ABE and 11.92 g L^{-1} of butanol. Moreover, *C. beijerinckii* SE-2 can use both glucose and xylose from corncob hydrolysate. In conclusion, our study suggested that the waste of the corn processing industry can be used as sources to produce butanol by *Clostridium,* and statistical experimental designs are a useful approach for optimizing media compositions for butanol production.

1. Introduction

Microbial production of butanol was one of the first large-scale industrial fermentation processes of global importance [1]. Butanol, which is superior to ethanol, has been considered a better transportation fuel than ethanol mainly due to its greater number of carbon atoms, more hydrophobic properties, and higher energy density [2]. *n*-butanol is a food-grade extractant of antibiotics, hormones, and vitamins. It is also broadly used as an intermediate of pharmaceuticals and polymers and a swelling agent in textiles. As biomass derived butanol has received great attention because of its potential as an alternative and renewable fuel, the efficient production of butanol has been widely studied through strain development and bioprocess engineering [3]. Well-known butanol fermentation strains include the anaerobic and mesophilic *Clostridium acetobutylicum* and *C. beijerinckii*, which are characterized by the synthesis of acetone–butanol–ethanol (ABE) in the ratio 3:6:1 [4].

Cheap and easily degradable feedstocks are desirable for butanol fermentation. *C. beijerinckii* P260 produced over 28 g L^{-1} ABE from wheat straw hydrolysate [5]. *C. beijerinckii* ATCC 55025 produced 11.8 g L^{-1} ABE from wheat bran, which is a by-product of the wheat milling industry [6]. Corncob is a significant lignocellulosic by-product of the corn industry. It can be used as a valuable raw material to obtain added-value products. *C. beijerinckii* produced 16.0 g L^{-1} ABE by NCIMB 8052 using corncob residue, which is a by-product of the furfural industry. *C. beijerinckii* TISTR 1461 generated ABE products at 11.64 g L^{-1} (5.29 g L^{-1} acetone, 6.26 g L^{-1} butanol, and 0.09 g L^{-1} ethanol), instantly using sugars from the hydrolyzed corncob without an overlying process [7], suggesting that corncob was a good carbon source for ABE fermentation. Corncob was also used as a carbon source for cellulase, D-lactic acid, and ethanol production [8–10]. Corn wet milling is the most energy intensive process within the food industry, and corn steep liquor (CSL) is a by-product. CSL can provide amino acids, vitamins and minerals for growth media [11]. CSL has been used as a cost-effective nutrient source in the fermentation of ethanol, succinic acid, bacterial cellulose, and cellulolytic enzymes [12–15]. CSL can be successfully applied to partially or totally replace expensive yeast extract. For example, CSL as an alternative nitrogen source with other components resulted in 30.4% higher lactic acid production than yeast extract in a lab fermenter [16].

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Most *Clostridia* prefer glucose over xylose as the carbon source. Previously, we reported the *C. beijerinckii* SE-2 strain, which could use either glucose or xylose as the substrate and has similar capabilities and rate of degradation for these two sugars [17]. To continue our butanol production efforts using cheap feedstocks, we are interested in identifying its capability to utilize corncob and CSL efficiently. In the current research, a multi-factorial experimental design was used to optimize the production of ABE and the by-products in the corn-processing industry were shown to have some good potential as the alternative substrates for butanol production.

2. Materials and methods

2.1. Strain and cell culture

C. beijerinckii SE-2 cells were routinely maintained in spore suspensions at 4 °C. For vegetative cultures, spores were heated at 80 °C for 10 min and allowed to grow in 50 mL Reinforced Clostridium Medium (RCM) in an anaerobic bottle at 37 °C for 20 h.

The corncob and CSL were kindly supplied by Longlive Bio-Technology Co. Ltd. in Shandong, China, and ground to a particle size of 1–2 mm. The ground corncob was pretreated using the wet-disk milling method as we reported previously [18]. The hydrolysate of the corncob contained 39.7 g L^{-1} sugar (28.9 g L^{-1} glucose and 10.8 g L^{-1} xylose) [18]. The components of CSL are listed in Table S1.

To prepare the initial fermentation medium, 1 g yeast extract, 2.2 g CH_3COONH_4, 0.5 g KH_2PO_4, 0.5 g K_2HPO_4, 0.01 g $MnSO_4 \cdot H_2O$, 0.2 g $MgSO_4 \cdot 7H_2O$, 0.01 g $FeSO_4 \cdot 7H_2O$, 0.01 g NaCl and vitamins (para-amino-benzoic acid, 0.1 g L^{-1}; thiamin, 0.1 g L^{-1}; biotin, 0.001 g L^{-1}) were added to 1-L of corncob hydrolysate. For media optimization, the indicated amount of CSL was used as a nitrogen source to replace the yeast extract and vitamins. The pH was adjusted to 6.8 using 1 M NaOH or HCl. The medium was sterilized at 121 °C for 15 min, cooled to 37 °C and purged with O_2-free N_2 for 5 min to remove the dissolved oxygen [19]. The fermentation studies were conducted in a 100 mL serum bottle (sealed with a septum and an aluminum crimp seal) containing 50 mL of medium. The medium was subsequently inoculated with 5% highly motile *C. beijerinckii* SE-2 and cultured for 72 h with a rotation speed of 50 rpm. 1 mL of the reaction system was removed at predetermined intervals for analysis.

2.2. Analytical procedures

Sugars were analyzed by high-performance liquid chromatography (HPLC). For sugar analyses, the corncob hydrolysates and fermentation broth were centrifuged at 5000 × *g* for 10 min to remove the insoluble particles. The supernatant was analyzed using an HPLC system equipped with a refractive index detector (RI-2031Plus; JASCO, Tokyo, Japan) and an Aminex HPX-87 P column (7.8 mm I.D. × 30 cm; BioRad, Richmond, CA, USA) at 65 °C with distilled water as the mobile phase at 0.8 mL min^{-1}.

Acetone, butanol, ethanol, acetic acid, and butyric acid in the fermentation solutions were analyzed by gas chromatography (GC) using an Agilent 7890 gas chromatography system (Agilent Technologies, Inc., CA, USA) with an HP-5 column (30 m × 0.25 mm × 0.25 μm) and flame ionization detector (FID) detector. *n*-pentanol was used as the internal standard. The injector temperature was 100 °C and detector temperature was 250 °C. The injection volume was 2 μL. The GC oven temperature was initially held at 40 °C for 2 min. The temperature was raised at 2.5 °C per minute until 60 °C. The temperature was then raised at 5 °C min^{-1} until 80 °C. The temperature was finally increased to 220 °C at 2 °C min^{-1}. The column flow rate was 0.5 mL min^{-1}.

2.3. Experimental design using response surface methodology (RSM)

2.3.1. Fractional factorial design (FFD)

Six factors, including CH_3COONH_4, CSL, K_2HPO_4-KH_2PO_4, $MnSO_4 \cdot H_2O$, $MgSO_4 \cdot 7H_2O$ and $FeSO_4 \cdot 7H_2O$ were estimated and selected for an FFD analysis, and the main effects of the factors could be evaluated in the tests. The first order equation was established to fit the results of the FFD and was represented as:

$$Y = \beta_0 + \sum \beta_i X_i \quad (1)$$

where Y is the predicted value; β_0 is the intercept; β_i is the linear coefficient and X_i is the coded independent factor.

2.3.2. Steepest assent (SA) design

The direction of the SA was the direction in which the Y response value increased most rapidly. The direction of the tests was parallel to the normal contour line of the response curve of the model and passed through the center point of the FFD. The steps along the path were according to the regression coefficients β_i. Experiments were conducted along the path until the response did not increase any longer. Therefore, this point should be near the optimum point and could be selected as the center point for optimization by RSM.

2.3.3. Central composite rotatable design (CCRD)

CCRD and RSM were further used for the optimization of the two factors (CH_3COONH_4 and CSL) screened from the FFD for enhancing butanol production. The two independent factors, such as X_1 and X_2, were studied at five different levels, such as −1.414, −1, 0, 1, and +1.414. The factors were coded according to the following equation:

$$X_i = \frac{(Xi - X0)}{\Delta X} \; i = 1,2,3, \; k \quad (2)$$

where X_i is the independent factor, X_0 is the value of X_i at the center point, ΔX is the step change value in the tests.

The behavior of the system could be explained by the following second order polynomial equation:

$$Y = \beta 0 + \sum \beta_i X_i + \sum \beta_{ii} X_i^2 + \sum \beta_{ij} X_i X_{ji} = 1,2,3, \; k \quad (3)$$

where Y is the predicted response, β_0 is the intercept, X_i and X_j are the coded independent variables, β_i is the linear coefficient, β_{ii} is the quadratic coefficient and β_{ij} is the interaction coefficient.

2.4. Scale-up of fermentation

Scale-up of ABE production was performed in a 100 L bioreactor (Dongfang Bioengineering Equipment Co. Ltd., Zhenjiang, China). 5% (v/v) seed culture was inoculated into 50 L initial medium or optimized medium, respectively. The culture medium was purged with aseptic nitrogen gas to remove oxygen. The fermentation was carried out at 37 ± 0.5 °C with agitation at 50 rpm for 72 h. Aliquots were collected periodically to estimate sugar concentrations and product concentrations.

2.5. Statistical analysis

Design Expert (version 7.0, STATEASE Inc., Minneapolis, USA) was used for the experimental design and regression analyses of the data obtained from the tests in this study. Statistical analysis of the model was carried out to estimate the analysis of variance (ANOVA). The quality of the polynomial model equation was determined by the determination coefficient R^2 and the statistical significance was estimated by an *F*-test. The significance of the regression coefficients was evaluated by a *t*-test.