Bioresource Technology 102 (2011) 9985–9990

Contents lists available at SciVerse ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Optimization of butanol production from tropical maize stalk juice by fermentation with *Clostridium beijerinckii* NCIMB 8052

Yi Wang [a,b], Hans P. Blaschek [b,c,d,*]

[a] Department of Agricultural and Biological Engineering, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA
[b] Institute for Genomic Biology, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA
[c] Department of Food Science and Human Nutrition, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA
[d] Center for Advanced Bioenergy Research (CABER), University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA

ARTICLE INFO

Article history:
Received 18 May 2011
Received in revised form 7 August 2011
Accepted 9 August 2011
Available online 17 August 2011

Keywords:
Butanol production
Clostridium beijerinckii
Optimization
Response surface methodology (RSM)
Tropical maize stalk juice

ABSTRACT

Mixed sugars from tropical maize stalk juice were used to carry out butanol fermentation with *Clostridium beijerinckii* NCIMB 8052. Batch experiments employing central composite design (CCD) and response surface methodology (RSM) optimization were performed to evaluate effects of three factors, i.e. pH, initial total sugar concentration, and agitation rate on butanol production. Optimum conditions of pH 6.7, sugar concentration 42.2 g/L and agitation rate 48 rpm were predicted, under which a maximum butanol yield of 0.27 g/g-sugar was estimated. Further experiments demonstrated that higher agitation facilitated acetone production, leading to lower butanol selectivity in total acetone–butanol–ethanol (ABE). While glucose and fructose are more preferable by *C. beijerinckii*, sucrose can also be easily degraded by the microorganism. This study indicated that RSM is a useful approach for optimizing operational conditions for butanol production, and demonstrated that tropical maize, with high yield of biomass and stalk sugars, is a promising biofuel crop.

1. Introduction

Bio-butanol produced from renewable resources through microbial fermentation is of great interest, because it not only can be used as an important renewable fuel that has various advantages over ethanol, but also has vast application as a chemical feedstock in many industries (Dürre, 2007). Bio-butanol has been produced through the acetone–butanol–ethanol (ABE) fermentation using the solventogenic clostridia. The cost of substrate is an important part for the overall cost of bio-butanol production (Qureshi and Blaschek, 2001a). Inexpensive and easily-degradable feedstocks are desirable for the ABE fermentation. Tropical maize, a hybrid corn variety made by crossing temperate by tropical adapted parents, is a high energy crop yielding large amounts of biomass and stalk sugar with potentially valuable use as a biofuel crop. The mixed sugars obtained from tropical maize stalk juice are composed of high concentrations of sucrose, glucose and fructose, which are all easily degraded during the microbial fermentation processes.

On the other hand, ABE fermentation is a very complex process that is influenced by many factors. Proper pH control is essential for the fermentation to shift to solventogenesis and produce a high yield of butanol (Jones and Woods, 1986; Maddox et al., 2000); while a low sugar concentration in the feedstock may result in reduced cell growth and unfavorable solvent production, a high sugar concentration can result in substrate inhibition, which may inhibit cell growth and cause failure of fermentation (Ezeji et al., 2003, 2005); suitable agitation rate can facilitate mixing of the substrates and products, enhancing substrate accessibility and products distribution, but high agitation may adversely impact the fermentation, and lead to unnecessary waste of energy and poor industrial economics. A number of studies have been carried out to investigate the effects of different factors on butanol production (Geng and Park, 1993; Nishio et al., 1983; Salleh et al., 2008; Soni et al., 1992; Welsh and Veliky, 1984). However, these studies examined only one or two factors at a time. Since biological butanol production is affected by two or more factors simultaneously, a multi-factorial experimental design approach is required.

Response surface methodology (RSM) is a statistical method useful for evaluating the relative significance of several independent variables, understanding the interactions of the various parameters affecting the process, and hence determining optimal conditions for desirable responses. RSM has the advantage of reducing the number of experiments required and making it easy for overall data analysis (Bezerra et al., 2008). RSM has demonstrated its effectiveness for the optimization of many complex processes in chemical engineering (dos Santos et al., 2005), food

* Corresponding author at: Center for Advanced Bioenergy Research (CABER), University of Illinois at Urbana-Champaign, 1207 W Gregory Drive, Urbana, IL 61801, USA. Tel.: +1 217 333 8224; fax: +1 217 244 2517.
E-mail address: blaschek@illinois.edu (H.P. Blaschek).

0960-8524/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biortech.2011.08.038

sciences (Li and Fu, 2005), wastewater treatment (Wang et al., 2007), biological fermentation (Vishwanatha et al., 2010), etc. Therefore, the main objective of this study was to investigate the effects of pH, initial total sugar concentration and agitation rate on ABE fermentation using RSM and thereby try to determine the optimum conditions for maximizing butanol yield. In addition, the potential use of mixed sugars obtained from tropical maize stalk juice as a biofuel feedstock was evaluated.

2. Methods

2.1. Raw materials and reagents

Tropical maize was bred at the Dudley Smith Farm at University of Illinois at Urbana-Champaign (UIUC). The stalks were harvested and squeezed to obtain fresh juice. The juice was autoclaved at 0.15 MPa and 121 °C for 15 min and stored at 4 °C before it was used. The sugar content of the fresh juice was analyzed as (in g/L): sucrose, 95.4; glucose, 23.8; fructose, 7.5. There were also many other mineral chemicals presented in the juice which were supplementary to the bacterial culture growth (in g/L, N: 0.64; P: 0.13; K: 1.01; Na: 0.0013; Ca: 0.16; Mg: 0.27; Fe: 0.042; Mn: 0.0011; S: 0.034).

Other chemicals and reagents were obtained from either Sigma (St. Louis, MO) or Fisher Scientific, Inc. (Hanover Park, IL) and were of analytical grade.

2.2. Bacterial culture and fermentation experiment

Laboratory stocks of *Clostridium beijerinckii* 8052 spores were stored in sterile H_2O at 4 °C. Spores were heat-shocked at 80 °C for 10 min, followed by cooling on ice for 5 min. The heat-shocked spores were inoculated into tryptone–glucose–yeast extract (TGY) medium containing 30 g/L tryptone, 20 g/L glucose, 10 g/L yeast extract and 1 g/L L-cysteine at a 1% inoculum level. The TGY culture was incubated at 35 ± 1 °C for 12–14 h in an anaerobic chamber under N_2:CO_2:H_2 (volume ratio of 85:10:5) atmosphere. Subsequently, actively growing culture was inoculated into the broth containing various concentrations of autoclaved tropical maize stalk juice, 1 g/L yeast extract, and filter-sterilized P2 medium (Qureshi et al., 2001) in a Sixfors bioreactor system (Infors AG, Bottmingen, Switzerland). Oxygen-free nitrogen was flushed through the broth to initiate anaerobiosis until the culture initiated its own gas production (CO_2 and H_2). Initial pH of the fermentation broth was adjusted using 2 M NaOH or HCl. Temperature was controlled at 35 ± 1 °C. Various agitation rates were employed for mixing. During the course of fermentation, 2 ml culture aliquots were collected for product concentration quantification.

2.3. Experimental design

RSM with a full factorial central composite design (CCD) was employed in this study, as shown in Table 1.The variables were coded according to Eq. (1):

$$x_i = \frac{X_i - X_0}{\Delta X_i} (i = 1, 2, 3, \cdots, k) \quad (1)$$

where x_i is the coded value of the *i*th test variable; X_i is the uncoded value (real value) of the *i*th test variable; X_0 is the value of X_i at the central point of the investigated range, and ΔX_i is the step size of the *i*th test variable. pH (X_1), initial total sugar concentration (X_2) and agitation rate (X_3) were chosen as the three independent factors, while final butanol yield (Y) as the response variable. The central values in the experimental design were selected as pH 6.5, sugar concentration 60 g/L and agitation 100 rpm. OriginPro 8.1 (OriginLab Corporation, Northampton, MA) and Matlab 7.10 (The MathWorks, Inc., Natick, MA) were used for the data analysis.

2.4. Analytical procedures

The mineral composition of the fresh juice was analyzed by the Internal Analytical Services Lab at Illinois State Water Survey (Champaign, IL). The sugar concentration was determined using a Shimadzu (Columbia, MD) high-pressure liquid chromatography system. A Bio-Rad HPX-87P column (Bio-Rad Laboratories, Inc., Hercules, CA) equipped with a guard column (30 × 4.6 mm) was used with a mobile phase of ultrapure water at a flow rate of 0.6 ml/min. The column temperature was kept at 85 °C. A refractive index (RI) detector (Waters Corporation, Milford, MA) set at 35 °C was used for signal detection. ABE, acetic acid, and butyric acid concentrations were quantified using a gas chromatography (GC) (Hewlett Packard, Avondale, PA, USA) equipped with a flame ionization detector (FID), a 1829 × 2 mm glass column (10% CW-20M, 0.01% H_3PO_4, support 80/100 Chromosorb WAW) and an Agilent 7683 series automatic liquid sampler (Agilent Technologies, Inc., Palo Alto, CA). The butanol yield was calculated as grams of butanol produced per gram of sugar utilized, while the butanol productivity was calculated as the butanol produced in g/L of broth divided by the fermentation time in h.

3. Results and discussion

3.1. Optimization of butanol production employing response surface methodology (RSM)

CCD was employed to determine the individual and interactive effects of three parameters on butanol yield. The following response equation was used to correlate the dependent and independent variables.

$$Y_i = a_0 + \sum_{i=1}^{k} a_i x_i + \sum_{i=1}^{k} a_{ii} x_i^2 + \sum_{i}^{k} \sum_{j}^{k} a_{ij} x_i x_j \quad (2)$$

where Y_i is the response; x_i, x_j are the input variables, which influence the response variable Y_i; a_0 is the offset term; a_i is the *i*th linear coefficient; a_{ii} is the quadratic coefficient and a_{ij} is the *ij*th interaction coefficient.

The RSM experimental design matrix with three factors at five levels and the experimental results are presented in Table 1. The regression coefficient, standard error, student's test *t* values, and significance level are summarized in Table 2. The *t*-test value indicates the significance of the regression coefficient. From Table 2, the linear coefficients a_1 and a_2, the quadratic coefficient a_{11}, as well as the interaction coefficient a_{12} are all significant at a 5% significance level. Therefore, the linear effect of pH (a_1) and sugar concentration (a_2), the quadratic effect of pH (a_{11}) and the interaction effect between pH and sugar concentration (a_{12}) are the most influential factors. In addition, a_2 and a_{11} were less than zero, indicating negative effects of these parameters on butanol yield. On the other hand, the linear and quadratic effects of agitation (a_3 and a_{33}), as well as the interactive effects of agitation with pH and sugar concentration (a_{13} and a_{23}) on butanol yield were all slight, as indicated by the large *P*-values.

Fisher's statistical test for analysis of variance (ANOVA) was used to evaluate the quality of the regression (Table 3). The regression statistics showed that the model represented an accurate representation of the experimental data, as the computed $F_{statistic}$ (10.29) is much larger than $F_{0.05,9,10}$ (3.02). In addition, the small *P*-value for the regression in Table 3 also indicated the adequacy of the model.