Bioresource Technology 205 (2016) 82–89

Contents lists available at ScienceDirect

Bioresource Technology

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

Effective multiple stages continuous acetone–butanol–ethanol fermentation by immobilized bioreactors: Making full use of fresh corn stalk

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HIGHLIGHTS

- Three-stage immobilized fermentation process was established for ABE production.
- 18–22 g/L of ABE solvents with 0.61–0.92 g/L h of productivity was achieved using corn stalk juice as substrate.
- The process showed good performances in ~400 h of operation.

ARTICLE INFO

Article history:
Received 31 October 2015
Received in revised form 7 January 2016
Accepted 8 January 2016
Available online 21 January 2016

Keywords:
ABE fermentation
Corn stalk juice
Corn stalk bagasse
Cell immobilization

ABSTRACT

In order to make full use of the fresh corn stalk, the sugar containing juice was used as the sole substrate for acetone–butanol–ethanol production without any nutrients supplement, and the bagasse after squeezing the juice was used as the immobilized carrier. A total 21.34 g/L of ABE was produced in batch cells immobilization system with ABE yield of 0.35 g/g. A continuous fermentation containing three stages with immobilized cells was conducted and the effect of dilution rate on fermentation was investigated. As a result, the productivity and ABE solvents concentration reached 0.80 g/L h and 19.93 g/L, respectively, when the dilution rate in each stage was 0.12/h (corresponding to a dilution rate of 0.04/h in the whole system). And the long-term operation indicated the continuous multiple stages ABE fermentation process had good stability and showed the great potential in future industrial applications.

1. Introduction

N-butanol (butanol) is an important bulk chemical as well as a candidate of potential biofuels (Jiang et al., 2014). However, the low productivity and poor yield of butanol were regarded as bottlenecks in acetone–butanol–ethanol (ABE) fermentation. Typically, the theoretical yield of butanol based on sugar is only 40–60% of bioethanol, resulting in a significant impact of the substrate cost on final butanol price (accounting for over 56% of production cost) (Qureshi and Blaschek, 2001). Thus, it is essential to decrease the substrate cost and find out a potential low-cost feedstock for ABE fermentation.

In recent years, the exploration of biofuels converted from lignocelluloses biomass is in the ascendant (Sun et al., 2013; Liu et al., 2012). Corn is widely cultivated in China as a major food crop. Consequently, a lot of corn stalks as agriculture residual are co-generated with the grain every year. However, most of the corn stalks residuals are not properly used, which results in severely environmental problems, e.g. the haze pollution (Zhang et al., 2013). Currently, efforts have been made to convert the lignocellulosic biomass resource into valuable chemicals based on the concept of biorefinery. Unfortunately, due to the recalcitrance of strong inter-chain hydrogen-bonding network and high-order structure, the high cost of pretreatment and saccharification make the schemes non-economical competitiveness (Luo et al., 2009; Tao et al., 2014; Xue et al., 2013; Yu et al., 2012). In addition, the toxic by-products from pretreated lignocelluloses also have negative effects on the metabolic pathways of the strains (Qureshi et al., 2012; Ezeji et al., 2007).

In contrast, the stalk juice rich in sugars is considered to be a relatively cheaper substrate for ABE fermentation (Jiang et al., 2014; Ni et al., 2013). In previous researches, the tropical maize

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http://dx.doi.org/10.1016/j.biortech.2016.01.034
0960-8524/

stalk juice has been use as substrate for ABE fermentation. It demonstrated that a variety of sugar in the stalk juice can be metabolized by the butanol producing strains (Wang and Blaschek, 2011; Bankar et al., 2013). Similarly, corn stalks cultivated in the northeast region of China could also output considerable amount of fermentable juice after grains harvest. And it is possible to apply the juice for ABE fermentation.

In order to further reduce solvents producing cost and enhance the productivity, it is necessary to improve the ABE fermentation processes. Previous studies showed continuous ABE fermentation containing multiple stages has advantages in high solvents output as well as high productivity. And this strategy could significantly overcome the bottlenecks of ABE fermentation (Van Hecke et al., 2013; Chiao and Sun, 2007; Bankar et al., 2013). Besides, the fermentation efficiency could also be improved markedly by using the technique of immobilized cells (Chang et al., 2014; Bankar et al., 2012). Owing to the higher cell density and the better butanol tolerant, ABE fermentation by cells immobilization could provide better stability than that of the traditional ones (Jiang et al., 2014). However, to our knowledge, there are limited researches focusing on multiple stages continuous ABE fermentation by immobilized cells.

In this study, in order to make full use of fresh corn stalk, the raw material was squeezed, and the corn stalk bagasse (CSB) was used as the carrier for multiple-stage ABE fermentation, while the corn stalk juice (CSJ) was used as the substrate for ABE fermentation directly without adding any extra nutrition. Three-stage continuous bioreactors with immobilized cells were connected in series and the influence of dilution rate on fermentation was evaluated. Generally, balanced advantages of the ABE productivity and concentration were achieved in the present work. The results showed the feasibility in raising the economic benefit of the corn based on biorefinery concept.

2. Methods

2.1. Raw materials

The fresh corn stalks (Jingke 968) from local farm in Tongliao of Inner Mongolia were harvested in September of 2014 after 120 days of cultivation. The grains were picked for sale, and the withered leaves were stripped from the stem by hand. Then, the stems were squeezed by a 3-rollermill (SY-20, Guangzhou Fukang Co. Ltd, China) to collect the corn stalk juice (CSJ) within 24 h after harvest. The CSJ obtained was boiled at 100 °C for 5 min firstly. Then, it was filtered through a 60 mesh screen in order to remove the chippings of the lignocelluloses and the heteropolymeric proteins. And the clarified CSJ was stored at −20 °C before use.

The nutrients constitutions were determined via Inductively Coupled Plasma Mass Spectrometry (ICP–MS) (Agilent 7500, USA). Generally, the CSJ contained 362.48 mg/L of K^+, 11.04 mg/L of Na^+, 238.68 mg/L of Ca^{2+}, 468.10 mg/L of Fe^{2+}, 1.14 mg/L of Mn^{2+}, 154.86 mg/L of phosphorus source, and 1128.2 mg/L of nitrogen source. In addition, the CSJ contained 13.15 g/L of sucrose, 30.34 g/L of glucose, and 30.22 g/L of fructose via HPLC (Shimadzu LC-10A, Japan) analysis.

The nutrients constitutions of CSJ indicated it could satisfy the need of the metabolism of the strain for ABE solvents production. No additional nutrients were added into the CSJ before it was used in ABE fermentation. Generally, the overall free sugars content of the fresh stalk was 6.2 g/100 g of fresh corn stalk.

2.2. Microorganism and media

The mutant strain *Clostridium acetobutylicum* ABE 1201 was derived from ATCC 824 through evolutionary engineering and laboratory stored. The previous study indicated that this mutant strain had a well stability in long-term immobilized fermentation (Chang et al., 2014). Before cultivation, the spores were heat shocked for 10 min at 80 °C and then cooled in cold water. At last the spores were inoculated into the seed medium as described previously (Cai et al., 2013). For the fermentation using CSJ, 10% (v/v) hyper strains were inoculated into the sterilized (121 °C for 20 min) CSJ at 37 °C without pH control and stirring.

2.3. Fermentation

The free-cell and immobilized-cell fermentations were carried out in a 1-L bioreactor (Baoxing bio-engineering equipment Co. Ltd., Shanghai, China) with 600 ml working volume at 37 °C without pH control and stirring. For free-cell batch fermentation, the bioreactor was purged with N_2 for 1 h to construct an anaerobic environment after sterilization. Then, 60 ml active strains were inoculated into 540 ml medium (CSJ).

For the immobilized fermentation, the CSB were chopped into small particles (1–2 cm in length and 0.5–1 cm in width) and dried as the immobilized carriers according to the method of Yu et al. (2010). The CSB was washed by water in order to eliminate the interruption by residual sugars, then put into the bioreactor with a solid to liquid ratio of 1:20 (w/v) in the immobilized-cell bioreactor and then inoculated with the same condition of the free-cell one. For three-stage continuous immobilized fermentation, to ensure more active cells were maintained in the first stage, after 12 h of the inoculation with 10% (v/v) fresh seeds of third stage bioreactor, the second stage was inoculated with 10% (v/v) seed. Then, 10% (v/v) seed were inoculated into the first stage after another 12 h. After 36 h of batch fermentation in the first stage (60 h for the third stage), the CSJ was continuously pumped into the first stage of bioreactor by peristaltic pump (Baoding Chuangrui Precision Pump Co., Ltd., Baoding, Hebei, China). At the same time, the fermentation broth in the first, second and the third stages were also continuously pumped into the second and third stage bioreactor and the storage tank, respectively, with the similar dilution rate of that in the first stage bioreactor. After 150 h of fermentation, when the steady state was achieved, the dilution rate of each stage was stepwise increased. Samples were taken periodically (for every ~12 h and ~24 h in batch and continuous fermentation process, respectively) for residual sugars and solvents analysis. And the batch fermentations were repeated for 3 times.

2.4. Calculations

Formulas for the parameters are listed as below.

According to the method of Van Hecke et al. (2013), the overall dilution rate of the three-stage continuous fermentation system can be calculated as:

$$\frac{1}{D_{ov}} = \frac{1}{D_1} + \frac{1}{D_2} + \frac{1}{D_3} \quad (1)$$

where D_{ov} refers to the overall dilution rate of the process, and D_1, D_2 and D_3 are the specific dilution rate of the first stage, the second stage and the third stage, respectively.

The productivity of ABE solvent and butanol can be calculated by:

$$P_{ov} = D_{ov} * C_i \quad (2)$$

where P_{ov} is the overall productivity of the process, C_i represents to the concentration of component i at the end of the third stage.

Where $P_{i,n}$ refers to the productivity of component i in number n stage of fermentation.

The overall fermentable sugar consumption rate can be described by: