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Acetone–butanol–ethanol fermentation in a continuous and closed-circulating fermentation system with PDMS membrane bioreactor

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HIGHLIGHTS

- Continuous and closed-circulating ABE fermentation.
- Fermentation intermittent coupling with pervaporation.
- Fermentation continuous coupling with pervaporation.
- Overall kinetic parameters.
- Time course profiles of fermentation kinetics.

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ABSTRACT

Acetone–butanol–ethanol (ABE) fermentation by combining a PDMS membrane bioreactor and *Clostridium acetobutylicum* was studied, and a long continuous and closed-circulating fermentation (CCCF) system has been achieved. Two cycles of experiment were conducted, lasting for 274 h and 300 h, respectively. The operation mode of the first cycle was of fermentation intermittent coupling with pervaporation, and the second cycle was of continuous coupling. The average cell weight, glucose consumption rate, butanol productivity and butanol production of the first cycle were 1.59 g L^{-1}, 0.63 g L^{-1} h^{-1}, 0.105 g L^{-1} h^{-1} and 28.03 g L^{-1}, respectively. Correspondingly, the four parameters of the second cycle were 1.68 g L^{-1}, 1.12 g L^{-1} h^{-1}, 0.205 g L^{-1} h^{-1} and 61.43 g L^{-1}, respectively. The results indicate the fermentation behaviors under continuous coupling mode were superior to that under intermittent coupling mode. Besides, two peak values were observed in the time course profiles, which means the microorganism could adapt the long CCCF membrane bioreactor system.

1. Introduction

The production of *n*-butanol with solvent-producing strains of *clostridium*, commonly known as ABE (acetone–butanol–ethanol) fermentation is one of the largest biotechnological processes ever developed (Van Hecke et al., 2012). Butanol and acetone are excellent organic solvents and important industrial chemicals, which are widely used in chemical industries, such as pharmaceuticals, agrochemicals and organic syntheses. In recent years, butanol is regarded as genuine biofuel due to its considerably higher combustion value than ethanol and blending in fuels more readily than ethanol (Kumar and Gayen, 2011; Thirmal and Dahman, 2012).

Many researches on ABE fermentation have been indexed. Qureshi et al. investigated butanol production from wheat straw by simultaneous saccharification and fermentation in five different batch fermentation processes (Qureshi et al., 2008). Cheng et al. studied bio-butanol production by solvent-producing bacterial microflora and the maximum butanol concentration of 12.4 g L^{-1} was obtained in batch fermentation (Cheng et al., 2012). However, as known, the economics of the ABE bio-fermentation are hampered by the high cost of substrates and butanol toxicity leading to cell inhibition, low product accumulation, low space-time-yields and high purification costs (Bankar et al., 2012; Garcia et al., 2011).

To relieve butanol inhibition and improve the fermentation efficiency, several separation technologies have been investigated for extracting products and controlling their concentrations in fermentation broth, such as distillation (Wang et al., 2012), gas stripping (Ezeji et al., 2003; Qureshi and Blaschek, 2001), extraction (Bankar et al., 2012; Kraemer et al., 2011), adsorption (Qureshi et al., 2005), perstraction (Qureshi and Maddox, 2005) and pervaporation (Liu et al., 2011; Yen et al., 2012). For example, Lu et al. investigated fed-batch fermentation for butanol production from

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cassava bagasse hydrolysate in a fibrous bed bioreactor with continuous gas stripping, and a total of 76.4 g L^{-1} butanol was produced over the duration of 263 h (Lu et al., 2012). In another report, continuous two stage ABE fermentation by immobilized cells with integrated liquid–liquid extraction was explored and the improved fermentation performance was observed (Bankar et al., 2012). Furthermore, a long-term continuous butanol fermentation in combination with pervaporation was conducted and the system was evaluated (Van Hecke et al., 2012).

In the earlier work, a coupled system named continuous and closed-circulating fermentation (CCCF), which integrated fermentation with in situ pervaporation, was developed for aerobic ethanol production (Chen et al., 2012; Ding et al., 2011; Tang et al., 2007a). The results showed the feasibility and prospect of the long term CCCF system in ethanol production with improved fermentation performance. However, it was another challenging and interesting subject whether the CCCF system is suitable for the anaerobic butanol fermentation. In this work, two cycles of CCCF experiment with different coupling modes were conducted, both the overall kinetic parameters and time course profiles of fermentation were explored, intending to find the process principles of ABE fermentation in this specific system.

2. Methods

2.1. Culture and inoculum preparation

Clostridium acetobutylicum strain CICC 8012 was obtained from China Center of Industrial Culture Collection (Beijing, China). Initially, sporulated cells were activated by heat shock at 100 °C for 1 min. The activated spore culture of 5 mL was inoculated to 100 mL sterile medium in a glass bottle and cultivated anaerobically for 24 h at 37 °C. The medium used for seed culture and fermentation was identical and contained (for 1 L) glucose 60 g, yeast extraction 3 g, phosphate buffer (KH_2PO_4 0.5 g, K_2HPO_4 0.5 g, ammonium acetate 2.2 g), vitamins (para-amino-benzoic acid 1 mg, thiamin 1 mg, biotin 0.01 mg), mineral salts ($MgSO_4{\cdot}7H_2O$ 0.2 g, $MnSO_4{\cdot}H_2O$ 0.01 g, $FeSO_4{\cdot}7H_2O$ 0.01 g, NaCl 0.01 g). Glucose and yeast extraction were sterilized by autoclaving at 121 °C for 20 min. Phosphate buffer, vitamins and mineral salts were filter-sterilized through 0.45 μm sterile membrane.

2.2. CCCF integrated with pervaporation

The CCCF set-up was adopted from the previous study with some modifications (Chen et al., 2012). Experiments were carried out in a 10 L jacketed glass bioreactor with the fermentation volume of 8 L. After sparging with nitrogen, the bioreactor was inoculated with 10% inoculums of highly motile cells of *C. acetobutylicum* CICC 8012. Two cycles of fermentation with different operation modes were conducted in the experiments. For the first cycle, the operation mode was of fermentation intermittent coupling with pervaporation, which means fermentation was running for 24 h every day, while pervaporation was only operated from 8:30 to 20:30 (12 h) each day. For the second cycle, the operation mode for the first 192 h was continuous coupling, which means both fermentation and pervaporation were running for 24 h every day. Then, the operation mode was shifted to intermittent coupling which was the same as the first cycle. During the two cycles of fermentation, the temperature of pervaporation and fermentation was the same 37 °C controlled by a thermostat, and the pH value was adjusted to no lower than 4 with ammonia water of 25 wt.%. Glucose and distilled water were added every 2 h in order to maintain the glucose concentration of about 30 g L^{-1} and broth volume of 8 L. The permeation vapor released at the membrane downstream (with an absolute pressure of 2000 Pa) was collected in cold traps immersed in a −30 °C refrigerator.

2.3. Pervaporation membrane and module

The pervaporation membrane used here was in-house developed composite polydimethylsiloxane (PDMS) membrane, and two kinds of modules were applied in the two cycles of CCCF respectively. A circular flat-plate membrane module providing an effective membrane area of 0.024 m^2 was used in the first cycle of CCCF, and a quadrate flat-plate membrane module providing an effective membrane area of 0.08 m^2 was used in the second cycle. The preparation procedure of the membrane and the structure of the module had been fully described in the previous work (Li et al., 2004; Shi et al., 2007; Tang et al., 2007b).

2.4. Analysis

Biomass concentration was determined in three terms of dry cell weight, cell number and Optical Density (OD), respectively. For dry cell weight, the broth sample was first centrifuged at 12,000 rpm for 5 min, then cells at the bottom was washed with distilled water and finally dried in an oven at 80 °C. For cell number, broth sample was moderately diluted and then counted by a microscope in a counting chamber after staining with lodine solution. For OD, the broth sample was directly determined by a spectrophotometer at 540 nm. Solvents (acetone, butanol, ethanol) were quantified by gas chromatography (GC112A, shanghai, China) equipped with a FFAP capillary column (30 m × 0.32 mm × 0.5 μm) with flame ionization. Injector temperature 180 °C, detector temperature 200 °C, column temperature linearly ramping from 60 to 180 °C at 20 °C min^{-1}. Nitrogen was used as the carrier gas. Isobutanol was used as an internal standard, and the injection volume was 1 μL. Butyric acid and acetic acid were quantified with an Agilent series 1200 high performance liquid chromatography (HPLC) system equipped with a Agilent Hi-Plex H (300 × 7.7 mm,8 μm). The mobile phase was 5 mM degassed H_2SO_4 at a flow rate of 0.8 mL min^{-1} and a column temperature of 35 °C. A UV detector was used and the wavelength was 210 nm. The concentrations of glucose were determined using the 3,5-dinitrosalicyclic acid (DNSA) method (Bergmeyer and Grassel, 1983). Production in g L^{-1} was calculated by the summation of solvent amounts in the broth and the amounts removed by the PDMS membrane in g dividing by the volume of the broth in L. Productivity in g L^{-1} h^{-1} was calculated by dividing production in g L^{-1} by the used fermentation time in h. Yield in g g^{-1} was calculated by the solvents produced in g dividing the total consumed glucose in g.

3. Results and discussion

3.1. Pervaporation performance of the PDMS membrane

Table 1 shows the pervaporation performance of the PDMS membrane in the two cycles of CCCF. As mentioned in Section 2.3, different membrane modules providing different membrane areas were used in the two cycles of experiment. The average fluxes of the two cycles were 783.91 and 566.45 g m^{-2} h^{-1}, respectively, with the corresponding separation factor of 10.2 and 7.03. The results indicated that the circular flat-plate membrane module has better flow channel structure than the quadrate flat-plate membrane module. However, neither blocking nor breaking was observed in the two cycles of pervaporation. And both membrane modules showed stable pervaporation behaviors during the long CCCF process.