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Characterization of gas stripping and its integration with acetone–butanol–ethanol fermentation for high-efficient butanol production and recovery

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ABSTRACT

Two-stage gas stripping coupled with acetone–butanol–ethanol fermentation in a fibrous bed bioreactor was established for energy-efficient butanol recovery. The impacts of process parameters including butanol concentration, temperature and cell density with feed, gas flow rate, and cooling temperature on the efficiency of the gas stripping system were studied. High butanol concentration, low cell density and cooling temperature increased butanol titer in the condensate. The butanol titer in the condensate increased when stripping temperature increased from 25 to 55 °C, and decreased when temperature was above 55 °C. The optimal gas flow rate was 1.6 L/min, above which more water was stripped off and the condensate was diluted. After process optimization, 48.5 g/L butanol (73.3 g/L ABE) was produced in the fed-batch fermentation with *in situ* gas stripping due to the reduced butanol inhibition on cells. The condensate containing 147.2 g/L butanol (199.0 g/L ABE) was produced by the first-stage gas stripping, while a highly concentrated condensate containing 515.3 g/L butanol (671.1 g/L ABE) was obtained from the second-stage gas stripping. This process can significantly reduce energy consumption in the final product recovery.

1. Introduction

Butanol is now receiving increasing scientific and public attentions since it has been considered as an advanced biofuel, which could be produced through acetone–butanol–ethanol (ABE) fermentation by various *Clostridium* spp. [1]. Due to the severe butanol toxicity to cells, even though up to 2% (w/v) butanol could be produced through metabolic engineering and mutagenesis of Clostridia for the improvement of butanol tolerance and productivity [2,3], the fermentation broth is still dilute for butanol to be recovered and the recovery requires intensive energy consumption [4–6].

Adsorption [7,8], liquid-liquid extraction [9,10], pervaporation [11,12] and gas stripping [1,13] have been evaluated as promising techniques for *in situ* butanol recovery process, which could improve productivity by mitigating butanol inhibition. Even these techniques are more economically competitive comparing to conventional distillation for butanol recovery from the dilute fermentation broth, the deficiency in recovering a high titer of butanol is still the challenge.

The main advantages of gas stripping, comparing to other recovery methods, are simple operation, no harm to culture, and low capital investment for facilities [13,14]. However, more energy for further purification may be required after gas stripping in previous studies, which produced the condensate with comparatively low butanol (70 g/L) or ABE titer (120 g/L) [15,16]. Until recently, gas stripping could produce a highly concentrated product in our studies and thus has been considered as an energy-saving butanol recovery method [17,18], and yet its potential in butanol recovery and energy saving for biobutanol production has not been fully explored nor optimized.

The optimization of the process parameters in the recovery process is critical for the performance of gas stripping coupled with ABE fermentation, and thus the effects of process parameters including (1) butanol titer in the feed solution, (2) stripping temperature in feed solution, (3) cell density in the feed solution, (4) gas flow rate, and (5) cooling temperature were investigated systematically, indicating that all these parameters had significant effect on gas

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stripping. Based on these process characterization, two-stage gas stripping, using the fermentation off-gas (H_2 and CO_2) produced by the microorganism, was employed to couple with fed-batch ABE fermentation in a fibrous bed bioreactor fermentation system. An extremely high butanol titer of 515.3 g/L (or ABE titer of 671.1 g/L) was obtained in the final product condensate, which was much higher than those obtained in our recent studies [17,18], and significantly higher than those in other studies [1,19–21]. Furthermore, the energy consumption for butanol recovery could be reduced significantly with this two-stage gas stripping process, providing a highly energy-efficient technique for biobutanol production.

2. Materials and methods

2.1. Characterization: gas stripping for butanol separation in model system

The separation of butanol by gas stripping was studied with the spinner flask containing 500 ml of the butanol/water binary model solution or fermentation broth with the butanol, acetone and ethanol ratio of 6:3:1. The solution containing ~10 g/L butanol at 37 °C was sparged with air at a flow rate of 1.6 L/min, unless otherwise noted. The stripping gas was passed through the condenser, and the condensate was collected in a flask immersed in a cold water bath with cooling temperature of 0 °C. The impacts of process parameters on butanol titer in condensate, butanol stripping rate, and butanol selectivity were investigated. These process parameters were: temperature in feed solution (25–60 °C), butanol titer in feed solution (3.9–82.6 g/L), cell density in feed solution (0–15 g dry cell weight/L), gas flow rate (0.8–6.4 L/min), and cooling temperature (−10 °C to 15 °C). To evaluate the effect of cell density on gas stripping, the fermentation broths with different cell dry weights were employed in this study. Liquid samples were taken from the spinner flask and condensate storage tank periodically, and then were assayed to calculate the butanol titer in condensate, butanol stripping rate, and butanol selectivity. The butanol stripping rate (g/L/h) was given as W/Lh, where L was the liquid volume in the spinner flask, and h was the time during which butanol in condensate W was collected. The butanol selectivity was calculated as $\alpha = [y/(1-y)]/[x/(1-x)]$, where x and y were weight fractions of butanol in the feed solution/fermentation broth and condensate, respectively.

2.2. Culture and media

The strain used in this study is *Clostridium acetobutylicum* JB200 derived from ATCC 55025. The seed medium, Clostridial growth medium (CGM), contains 30 g/L glucose, 2 g/L yeast extract, 1 g/L tryptone, minerals, and vitamins in a phosphate buffer, as described in Xue's study [17]. After inoculation, the seed culture was incubated at 37 °C for about 16 h until active growth was observed. P2 medium was used for ABE study, containing: 100 g/L glucose 1 g/L yeast extract, 0.5 g/L KH_2PO_4, 0.5 g/L K_2HPO_4, 2.2 g/L ammonium acetate, vitamins (1 mg/L para-amino-benzoic acid, 1 mg/L thiamin and 0.01 mg/L biotin), and mineral salts (0.2 g/L $MgSO_4{\cdot}7H_2O$, 0.01 g/L $MnSO_4{\cdot}H_2O$, 0.01 g/L $FeSO_4{\cdot}7H_2O$, 0.01 g/L NaCl). P2 medium was prepared according to the procedures described previously [12]. The media were sterilized by autoclaving at 121 °C and 15 psig for 30 min. The media were purged with nitrogen for 1 h through a sterile 0.2 μm filter before inoculation, to ensure the anaerobic environment.

2.3. Batch fermentation

Batch fermentation was carried out without gas stripping and the fibrous bed bioreactor (FBB) system. The P2 medium of ~100 g/L initial glucose was used in batch fermentation, which was conducted at 37 °C and pH 5.0. OD at 600 nm was used as an indicator for cell density, and glucose and fermentation products were analyzed using the withdrawn samples from the fermentation broth.

2.4. Fed-batch fermentation with cell immobilization

100 ml of actively growing cells (~16 h) was inoculated into 1 L of the P2 medium in the spinner flask and then the culture was maintained at 37 °C and pH 5.0 by the addition of 2 N NH_4OH, and agitated at 150 rpm. Fermentation was maintained for ~30 h until optical density (OD_{600}) reached 12.0. Then, the fermentation broth was recirculated by a peristaltic pump through the fibrous bed bioreactor (FBB) for cells to be immobilized effectively onto the fibrous matrix until at the end of the fermentation, as illustrated in Fig. 1. The FBB was made of a glass column (50 mm × 400 mm, 250 mL working volume) packed with spiral wound cotton towel and stainless steel wire cloth as described previously [17]. When residual glucose decreased to ~10 g/L, 400 g/L of concentrated glucose was continuously pumped into the spinner flask to supply carbon source for ABE fermentation.

2.5. Fermentation system coupled with two-stage gas stripping

Gas stripping system including two condensers (Pyrex Graham coil, 30 mm × 300 mm, Fisher) for vapor condensation, pumps, cooling system, thermostat and storage tanks was connected to the FBB fermentation system illustrated in Fig. 1. The spinner flask, FBB and condensers were sterilized by autoclaving separately for 45 min and aspetic connection afterwards. To ensure an oxygen-free environment, pure nitrogen was used to purge the whole system before inoculation. For the first-stage gas stripping, the off-gas (CO_2 and H_2) was circulated at 1.6 L/min through the fermentation broth and the condenser using a peristaltic pump, which was turned on when butanol concentration in the fermentation broth reached 8 g/L. The ratio of CO_2 and H_2 may vary during the ABE fermentation, which had no effect on the performance of gas stripping. The condenser was maintained at ~0 °C which is the optimal condition that can be operated; the condensate was collected in a flask immersed in a cold water bath, which was pumped into the storage tank for phase separation.

In the first-stage condensate storage tank mentioned above, the organic phase consisting of ~80% (w/w) butanol was transferred to the second-stage condensate storage tank, and the aqueous phase with ~8% (w/w) butanol was heated to 55 °C for gas stripping again. The air existed in the system was circulated and employed for the second-stage gas stripping. The second-stage condensate storage tank collected both the final condensate from the second-stage gas stripping and the organic phase from the first-stage stripping. Analysis was done for samples from fermentation broth in the spinner flask, the first- and second-stage condensate storage tanks.

2.6. Analytical methods

Broth samples were withdrawn periodically and the cells in the broth were removed by centrifugation at 13,200 × g for 5 min. The supernatant was collected for glucose and fermentation products analysis. Optical density at 600 nm was measured with a spectrophotometer (UV-16-1, Shimadzu, Columbia, MD), as an indicator for the biomass. Cell dry weight was measured by the method described previously [22]. The glucose concentration was determined with YSI 2700 Select Biochemistry Analyzer (Yellow Springs, Ohio). Butanol, acetone, ethanol, acetic acid and butyric acid were determined using a gas chromatograph (GC-2014 Shimadzu, Columbia, MD) equipped with a flame ionization detector (FID) and a fused silica column (Stabilwax-DA, 30 m long, 0.25 μm