Journal of the Taiwan Institute of Chemical Engineers 60 (2016) 119–123

Contents lists available at ScienceDirect

Journal of the Taiwan Institute of Chemical Engineers

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

Performance of fed-batch acetone–butanol–ethanol (ABE) fermentation coupled with the integrated *in situ* extraction-gas stripping process and the fractional condensation

Kuan-Ming Lu[a], Yu-Sheng Chiang[a], Yin-Rong Wang[a], Rei-Yu Chein[b], Si-Yu Li[a,*]

[a] Department of Chemical Engineering, National Chung Hsing University, Taichung 402, Taiwan
[b] Department of Mechanical Engineering, National Chung Hsing University, Taichung 402, Taiwan

ARTICLE INFO

Article history:
Received 28 June 2015
Revised 19 October 2015
Accepted 26 October 2015
Available online 28 November 2015

Keywords:
Clostridium acetobutylicum
Fed-batch acetone–butanol–ethanol (ABE) fermentation
Integrated extraction-gas stripping
Fractional condensation

ABSTRACT

In this study, fed-batch acetone–butanol–ethanol (ABE) fermentation was coupled with the integrated *in situ* extraction-gas stripping process. The total glucose consumptions were 255 and 310 g/L at the gas flow rates of 0.5 and 1.0 liter per minute (lpm), respectively. The ABE productivity and yield were 0.65 g/L/h and 0.43 g-solvent/g-glucose at the gas flow rates of 0.5 lpm. When the gas flow rates increased from 0.5 to 1.0 lpm, the ABE productivity and yield became 0.69 g/L/h and 0.48 g-solvent/g-glucose, respectively. The fractional condensation was operated by using two different cold traps where temperatures for the first and second one were 2 and −196°C, respectively. It was found that 71–81 and 64–73% of total stripped water was condensed in the 2 °C cold trap at gas flow rates of 0.5 and 1.0 lpm, respectively. Therefore, high ABE concentrations of 360–460 g/L were found in the second cold trap. The overall separation factor for butanol was calculated to be up to 34 where the raffinate solution is the fermentation broth and the extract solution is the condensate in the −196 °C cold trap.

1. Introduction

Butanol is a carbon neutral, low polluting, and sustainable fuel alternatives [1–3]. It can be fermentatively produced by acetone–butanol–ethanol (ABE) fermentation where *Clostridium* species are microbial catalysts that are responsible for it [4–6]. Batch, fed-batch, and chemo-stat reactors are three common type processes for running ABE fermentation [7–11]. Among these methods, fed-batch fermentation has attracted attentions because the continuous feeding of fresh medium dilutes the butanol toxicity while provides more glucose for fermentation [8,12]. Performance of fed-batch ABE fermentation can be further enhanced by incorporating *in situ* product removal (ISPR) technique, such as adsorption [13], pervaporation [14], gas stripping [15], and liquid–liquid extraction [16,17].

A novel integrated extraction-gas stripping ISPR technique has been developed in our previous study [18]. One advantage of the integrated *in situ* extraction-gas stripping process is that since butanol in the oleyl alcohol (O.A.) phase is continuously removed by nitrogen stripping, only small amount of oleyl alcohol is needed without the concern of butanol saturation during ABE fermentation. The other advantage is that since butanol is stripped from the non-volatile O.A. phase instead of aqueous fermentation broth, the selectivity toward butanol during gas stripping is significantly elevated and has been shown to be comparable to pervaporation [18]. This method can effectively improve performance of batch ABE fermentation where the glucose consumption of 121 ± 2 g/L was achieved in a single batch [18]. Accompanied with the high glucose consumption were the significant increase in butanol productivity and yield by 112 and 56%, respectively. In this study, fed-batch fermentation combined with the integrated *in situ* extraction-gas stripping technique is developed in this study and performance of this novel fed-batch fermentation process is presented. Meanwhile, the fractional condensation is coupled aiming to further elevate the purity of recovered butanol. During which, separation factor and solvent removal rates were discussed in this study.

2. Materials and methods

2.1. Bacterial strain and medium

C. acetobutylicum ATCC824 was used as the bacterial strain for fed-batch ABE fermentation. The spore stock preparation, spore germination, and pre-culture preparation were conducted as previously described [11].

* Corresponding author. Tel.: +886 42284 0510x509.
E-mail address: syli@dragon.nchu.edu.tw (S.-Y. Li).

http://dx.doi.org/10.1016/j.jtice.2015.10.044
1876-1070/

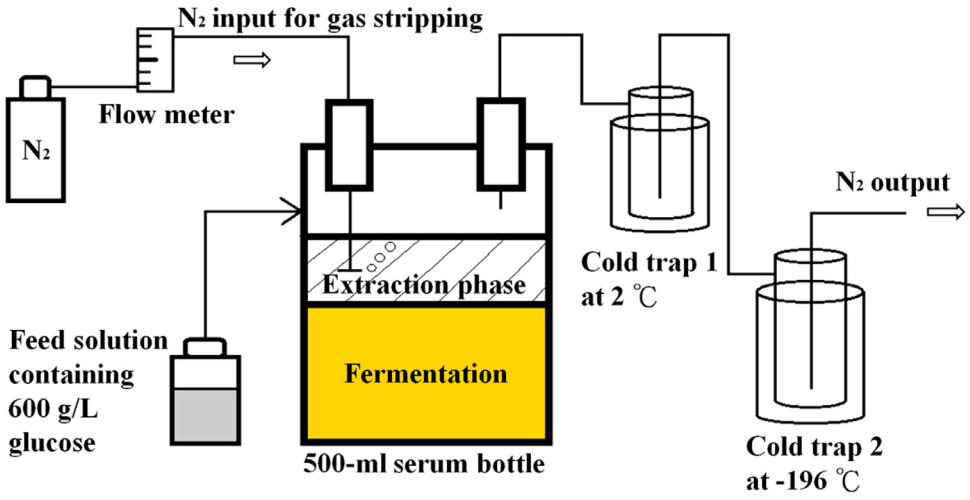

Fig. 1. Schematic of integrated *in situ* extraction-gas stripping process for fed-batch ABE fermentation. The implementation of the fractional condensation is presented.

2.2. Fed-batch ABE fermentation with the integrated in situ extraction-gas stripping process and the fractional condensation

Fed-batch ABE fermentation was achieved by firstly aseptically transferring 9 mL of *C. acetobutylicum* pre-culture solution to 300 mL of fresh Luria Broth (LB) medium containing 80 g/L glucose, 0.008 g/L $CaCl_2$, 0.1 g/L $FeSO_4{\cdot}7H_2O$, and 0.6 g/L $MgSO_4{\cdot}7H_2O$. A 500 mL serum bottle was used as a reactor for ABE fermentation. 40 mL fresh Luria Broth containing 600 g/L glucose, 0.008 g/L $CaCl_2$, 0.1 g/L $FeSO_4{\cdot}7H_2O$, and 0.6 g/L $MgSO_4{\cdot}7H_2O$ were fed into the reactor when the glucose concentration was below 10 g/L. Fermentation was conducted in a rotary shaker at 37 °C and 150 rpm.

The schematic of fed-batch ABE fermentation coupled with the integrated *in situ* extraction-gas stripping process was shown in Fig. 1. The 500 mL serum bottle held 300 mL of fresh medium and 100 mL of oleyl alcohol (80 –85%, Alfa Aesar®). Gas stripping of butanol was initiated at the fermentation time of 48 h. The nitrogen gas from the cylinder was delivered at a flow rate of 0.5 or 1.0 liter per minute (lpm) into the O.A. layer for butanol gas stripping where the nitrogen gas was sterilized using a 0.2 μm syringe filter (Millipore). The bubbling of nitrogen gas was achieved through a single 18-G syringe needle. Upon the initiation of gas stripping, the rotary speed decreased from 150 to 100 rpm.

As shown in Fig. 1, the fractional condensation was achieved by using two cold traps in series. The first cold trap was maintained at 2 °C (50% water and 50% ethylene glycol) and the second cold trap was maintained at −196 °C with liquid nitrogen. The sampling of the condensate was on the 24 h basis.

2.3. Analytical methods

The biomass concentration and the glucose concentration were respectively determined by the spectrophotometry and the dinitrosalicylic acid (DNS) method as described earlier [19]. The concentrations of acetone, butanol, ethanol, acetate, and butyrate were quantified by gas chromatography (GC) (Hewlett Packard HP 5890 Series II) as described earlier [19].

3. Results and discussion

3.1. Performance of fed-batch ABE fermentation coupled with the integrated in situ *extraction-gas stripping process*

Performance of fed-batch ABE fermentation coupled with the integrated *in situ* extraction-gas stripping process has been conducted where flow rates of 0.5 and 1.0 lpm were tested. It can be seen in Fig. 2a that when the gas flow rate of 0.5 lpm was applied in the extraction phase, the biomass concentration reached above 25 g/L and whole fed-batch fermentation time reached 168 h with the total glucose consumption of 255 g/L. The profile of the biomass and glucose concentrations reflects a fact that the prolonged fermentation results from the relief of butanol toxicity. Furthermore, since each supplement of fresh medium was achieved with 13% dilution rate, the fed-batch mode itself has little contribution to the dilution of butanol. It is therefore concluded that the relief of butanol toxicity is mainly achieved by the effective function of the integrated *in situ* extraction-gas stripping process. Fig. 2b showed that when the gas flow rate was

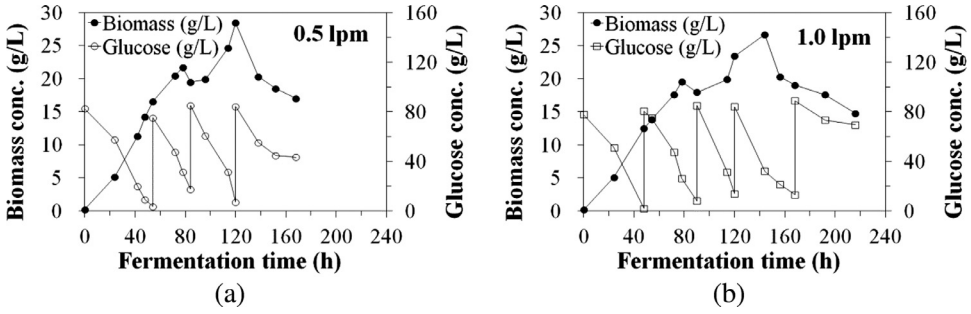

Fig. 2. Profile of the biomass and glucose concentration of fed-batch ABE fermentation with the gas stripping rate of (a) 0.5 lpm, (b)1.0 lpm.