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Membrane-assisted extractive butanol fermentation by *Clostridium saccharoperbutylacetonicum* N1-4 with 1-dodecanol as the extractant

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ABSTRACT

A polytetrafluoroethylene (PTFE) membrane was used in membrane-assisted extractive (MAE) fermentation of acetone–butanol–ethanol (ABE) by *Clostridium saccharoperbutylacetonicum* N1-4. The growth inhibition effects of 1-dodecanol, which has a high partition coefficient for butanol, can be prevented by employing 1-dodecanol as an extractant when using a PTFE membrane. Compared to conventional fermentation, MAE–ABE fermentation with 1-dodecanol decreased butanol inhibition and increased glucose consumption from 59.4 to 86.0 g/L, and total butanol production increased from 16.0 to 20.1 g/L. The maximum butanol production rate increased from 0.817 to 0.979 g/L/h. The butanol productivity per membrane area was remarkably high with this system, i.e., 78.6 g/L/h/m^2. Therefore, it is expected that this MAE fermentation system can achieve footprint downsizing.

1. Introduction

A major problem during fermentation processes is severe end-product inhibition, which decreases cell growth and product concentration in the fermentation broth. *In situ* recovery of end products during fermentation is a key point in the overall process, which aims to solve this problem. Liquid–liquid extraction can be applied to recover a product using extractants, such as organic solvents, which results in improved cell growth and productivity by decreasing end-product inhibition (Ishii et al., 1985; Taya et al., 1985; Roffler et al., 1987; Ishizaki et al., 1999). The extraction efficiency depends on the partition coefficient of the extractant used and the targeted product. However, a major limitation is that an extractant with a high partition coefficient often leads to microbial toxicity because of direct contact between the fermentation broth and the extractant (Evans and Wang, 1987).

Membrane-assisted extractive (MAE) technique is an approach that can overcome this major limitation. In MAE fermentation, the two phases of extractant and fermentation broth are separated by a porous membrane. The membrane can be either hydrophilic or hydrophobic and the interface is immobilized by the impregnation of its pores with one of the two phases depending on the membrane affinity (Kiani et al., 1984; Eksangsri et al., 2005). This has advantages of no dispersion or emulsion formation between the two phases (Yeh and Huang, 1995; Juang et al., 2000; Sciubba et al., 2009). Furthermore, it prevents the cells from making direct contact with the extractant, and thus can reduce the microbial toxicity of the extractant. MAE technique allows the selection of a wide range extractants, including microbial toxic extractants, with high partition coefficient for *in situ* recovery of end products during fermentation.

Acetone–butanol–ethanol (ABE) fermentation by *Clostridium* species shows end-product inhibition, particularly butanol, which severely inhibits cell growth and substrate consumption, and butanol production at a concentration of 12–16 g/L (Jones and Woods, 1986). The low butanol production is the bottleneck to progress ABE fermentation as an industry although biobutanol (bio-based butanol) continues to receive attention as a source of fuel because of its superior properties compared to bioethanol in terms of higher energy density and lower volatility (Lee et al., 2008). Several MAE–ABE fermentation systems have already been investigated (Jeon and Lee, 1987; Grobben et al., 1993). Their studies used combinations of relatively hydrophobic polymeric membranes and non-toxic extractants, such as a silicone membrane and oleyl alcohol (Jeon and Lee, 1987), or a polypropylene membrane and a

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mixture of oleyl alcohol and decanol (Grobben et al., 1993). MAE fermentation decreases butanol inhibition and exhibits higher substrate consumption rate and butanol productivity compared with methods without extraction. However, the extraction rate was limited by the diffusion rate of butanol through the membrane, which resulted in a lower butanol productivity per membrane area due to the low affinity between butanol and the membrane used (Jeon and Lee, 1987; Grobben et al., 1993). There have been no reports of MAE–ABE fermentations with high butanol productivity per membrane area.

In this study, in order to achieve efficient butanol production, a combination of a polytetrafluoroethylene (PTFE) membrane and 1-dodecanol in MAE–ABE fermentation by *Clostridium saccharoperbutylacetonicum* N1-4 was investigated because 1-dodecanol has a high partition coefficient of 5.14 for butanol (Kim et al., 1999) and PTFE is more hydrophobic than membranes used in previously. Although 1-dodecanol is toxic to the strain N1-4 in the conventional extractive fermentation, the microbial toxicity was avoided in MAE technique using a PTFE membrane. Consequently, a drastic increase was achieved in butanol productivity per membrane area which was corresponding to 25.6-fold compared with a previous study using a different combination of extractant and membrane (Jeon and Lee, 1987).

2. Methods

2.1. Microorganism and medium

The *C. saccharoperbutylacetonicum* strain N1-4 (ATCC 13564) was used in this study. For spore preparation of the strain N1-4, the refresh culture broth was stored at 4 °C in fresh potato glucose medium (PG medium) after 24 h of cultivation at 30 °C. PG medium contained the following components per liter of distilled water: 150 g of grated fresh potato, 10 g of glucose, 0.5 g of $(NH_4)_2SO_4$, and 3 g of $CaCO_3$ (Lee et al., 1995). The prepared medium was placed in boiling water bath for 1 h with mixing every 10 min, and then was filtered through gauze. The medium was sterilized at 121 °C for 1 h in an autoclave. Tryptone–yeast extract–acetate medium (TYA medium) was used for the pre-culture and main culture (Tashiro et al., 2004). TYA medium comprised 20 or 90 g of glucose, 2 g of yeast extract (Difco Laboratories, Detroit, MI, USA), 6 g of tryptone (Difco Laboratories), 3 g of CH_3COONH_4, 0.3 g of $MgSO_4{\cdot}7H_2O$, 0.5 g of KH_2PO_4, and 10 mg of $FeSO_4{\cdot}7H_2O$ per liter of distilled water. The medium was adjusted to pH 6.5 and sterilized at 115 °C for 15 min.

2.2. Fermentation

To prepare the seed culture, 1 mL of spore suspension was aseptically transferred into 9 mL of PG medium, and then the mixture was incubated in an AnaeroBox (Hirasawa Co. Ltd., Tokyo, Japan) chamber at 30 °C for 24 h after applying a heat shock for 1 min in boiling water. Ten milliliters of the seed culture was transferred into 40 mL of TYA medium at a glucose concentration of 20 g/L and incubated at 30 °C for 16 h for use as a pre-culture.

The main fermentation was initially performed in a 1-L flask with a working volume of 0.5 L. The batch fermentation was initiated by inoculating the pre-culture broth at a rate of 10% (v/v). For the first 8 h, the fermentation was performed in an AnaeroBox chamber at 30 °C. Next, 450 mL of the fermentation broth was transferred into the apparatus used for MAE fermentation. The fermenter was custom made to provide an effective membrane area of 50.2 cm^2. As a control for comparison with MAE fermentation, a fermenter without an extractive section was used. A PTFE membrane (pore size: 1.0 μm, diameter: 90 mm and thickness: 75 μm) (Advantec, Tokyo, Japan) was used for the separation of the broth and extractant. Four hundred milliliters of 1-dodecanol (Wako Pure Chemical Industries, Osaka, Japan) and oleyl alcohol (Nacalai Tesque, Kyoto, Japan) were used as extractants. During butanol extraction, the broth and extractant were separately agitated at 200 rpm and maintained at 30 °C, and samples were periodically collected for monitoring ABE and glucose concentrations. After collection, the cell densities were measured at 600 nm using a spectrophotometer (Bio-Rad SmartSpec™ Plus; Bio-Rad, California, USA), and broth samples were immediately centrifuged at 20,000×*g* for 3 min. The supernatants were further filtered through cellulose acetate filter membrane (pore size: 0.45 μm) (Advantec). The solutions obtained and organic samples were stored at −20 °C for further analysis.

2.3. Solvent toxicity

The inhibitory effect of 1-dodecanol and oleyl alcohol on the growth of the strain N1-4 was investigated by injecting 5 mL of solvent into a test tube containing 5 mL of active cells grown for 8 h in an AnaeroBox chamber at 30 °C. The solvent toxicity was determined by comparing the cell density and glucose consumption between cultures with and without extractants after 48 h of cultivation. The cell density of cultures was measured directly at 660 nm using a spectrophotometer (TAITEC MiniPhoto 518R; TAITEC Co. Ltd, Tokyo, Japan).

2.4. Chromatographic analyses

The ABE concentrations in the aqueous and organic phase samples were measured by gas chromatography. The analysis was performed using a 7890A GC System (Agilent Technologies, USA) equipped with a flame ionization detector. Components were separated on a 15 m capillary column (Innowax; i.d.: 0.53 mm; 19095 N-121; Agilent Technologies). Helium was used as the carrier gas at a flow rate of 3.7 mL/min. The oven temperature was programmed to increase from 50 to 170 °C at a rate of 10 °C/min. The injector and detector temperatures were set at 250 °C.

The glucose concentration in the fermentation broth was measured by high performance liquid chromatography (HPLC) (LaChrom Elite, Hitachi High Technologies, Tokyo, Japan). The components were separated on a SUGAR SH 1011 column (Shodex, Tokyo, Japan) at 50 °C using 3 mM $HClO_4$ as the mobile phase at a flow rate of 1.0 mL/min.

3. Results and discussion

3.1. Conventional batch ABE fermentation without extraction

Conventional batch fermentation of the strain N1-4 with 90 g/L glucose was performed as a control for comparison with MAE fermentation. Fig. 1 shows the concentrations of ABE and glucose and the cell density. The product concentrations at 42 h of fermentation were 4.80 g/L acetone, 16.0 g/L butanol, and 0.559 g/L ethanol. The cell growth began to cease with the accumulation of more than 13.5 g/L butanol in the broth and the maximum optical density (OD) was 15.6. The glucose consumption also ceased at a butanol concentration of approximately 16 g/L, which resulted in a consumed glucose level and remaining glucose level of 59.4 g/L and 29.6 g/L, respectively, after fermentation. From these data, it was suggested that approximately 16 g/L butanol inhibited cell growth, glucose consumption and thus further butanol production by the strain N1-4.