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Dual substrate strategy to enhance butanol production using high cell inoculum and its efficient recovery by pervaporation

Sweta Yadav, Garima Rawat, Priyanka Tripathi, R.K. Saxena *

Department of Microbiology, University of Delhi South Campus, New Delhi 110021, India

HIGHLIGHTS

- An efficient process for ABE fermentation wherein no acetone is produced.
- A higher butanol yield of 17.75 g/L was noted when glucose was supplemented.
- Production was successfully scale up in 300 L bioreactor.
- Efficient recovery of biobutanol *via* pervaporation.

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ABSTRACT

The present study deals with the development of an efficient ABE fermentation process using mixed substrate strategy for butanol production wherein no acetone was produced. For this, glucose was supplemented in the medium containing glycerol as main substrate which leads to a higher butanol production of 17.75 g/L in 72 h by *Clostridium acetobutylicum* KF158795. Moreover, the high cell inoculum also resulted in an increased ABE productivity of 0.46 g/L/h. Further, industrial scalability of the process was also successfully validated in a 300 L fermenter. Furthermore, potential of the Polymeric (PolyRMem) and Zeolite (ZeoMem) membranes for separation of butanol from fermentation broth was also studied by testing the pervaporation performance through which the butanol was successfully recovered.

1. Introduction

Biofuel, defined as liquid or gas fuel is receiving increased attention in response to the depletion of petroleum fuels and environmental issues. Bio-butanol in particular is mainly produced through acetone–butanol–ethanol (ABE) fermentation with clostridia. Now-a-days researchers are exploiting a number of substrates for ABE fermentation, among which glycerol can be a potential candidate for butanol production. As the metabolic pathway responsible for glycerol fermentation naturally occurs in Clostridia and thus has been explored for butanol production. The clostridial biphasic fermentation model consisting of acidogenic and solventogenic phases has been well accepted.

However, butanol production by fermentation of glycerol has been limited to the relatively low butanol titre and productivity as compared to those achieved by other sugar-based ABE fermentation processes (Taconi et al., 2009). It was reported earlier that ABE fermentation often failed due to the exhaustion of nutrients, and adding nutrients lead to an increase in substrate consumption and product formation. Therefore, nutrients supplementation in the medium can maintain stable fermentation for an extended period (Ezeji et al., 2004a). Compared to batch and continuous fermentations, fed-batch fermentation offers a number of advantages, including the utilization of highly concentrated substrate, which can reduce hydraulic load and wastewater generated in the process (Ezeji et al., 2004a,b, 2005, 2007b; Qureshi and Blaschek, 2001). Previously it was also reported that if glucose–glycerol mixture is used as substrate, glucose catabolism is used by the cells to produce energy through the acetate–butyrate production and NADH, whereas glycerol is used chiefly in the utilization of the reducing power (Abbad-Andaloussi et al., 1998). Therefore, an idea of glucose fed in the glycerol medium may result in an increase in the butanol production, wherein the basal medium supports initial growth and production, and the feed medium prevents depletion of nutrients and increases the production phase.

The objective of this study was to investigate the potential of glycerol and glucose to produce acetone, ethanol and butanol by *Clostridium acetobutylicum* KF158795. These two substrates not only enhanced the bacterial cell biomass but also increased the

* Corresponding author. Tel.: +91 11 24116559.
E-mail address: rks.micro2012@gmail.com (R.K. Saxena).

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productivity of ABE 0.3 g/L/h. As a result, an increased butanol production of 17.75 g/L with no acetone was observed when glucose was fed in the medium containing glycerol. Moreover, an initial study of high cell inoculum on ABE fermentation resulted in an increased ABE productivity of 0.46 g/L/h. Further, the production was also successfully scaled up to 300 L fermentor, as no studies to date have described efficient butanol production at such pilot scale. However, for any industrially important molecule, it is of utmost important to extract it from the fermentation broth. Therefore, the separation performance *via* pervaporation from the fermentation broth was also investigated and the successful extraction of butanol proved the efficiency of the process.

2. Methods

2.1. Culture conditions

The culture was procured from the laboratory stock culture collection, Department of Microbiology, University of Delhi South Campus, New Delhi, India. Furthermore, the bacterium (*C. acetobutylicum* 1) was identified on the basis of 16S rRNA analysis and the genome sequence has been submitted to NCBI GenBank under the accession No. KF158795. The modified AnS medium consisting of Glycerol (4.5%), Yeast extract (5.0%), K_2HPO_4 (0.3%), Na_2CO_3 (0.6%), $(NH_4)_2SO_4$ (0.1%), $CaCl_2$ (0.02%), $MgCl_2{\cdot}6H_2O$ (0.02%), Na_2S (0.002%) was used to grow and maintain the bacterial culture in anaerobic bottles. In order to ensure the removal of dissolved oxygen in the medium, CO_2 was sparged to achieve strict anaerobic condition. Bottles were then capped with butyl rubber bungs and sealed with aluminium crimps followed by autoclaving at 121 °C for 15 min. These sterile anaerobic bottles containing AnS medium were inoculated with 2% (v/v) of inoculum raised in the same medium.

2.2. Butanol production using glycerol as substrate

In the previous report, butanol production was carried out using glycerol as a sole carbon source to check the potential of the organism to utilize this substrate wherein 13.40 g/L of butanol with a total ABE of 18.27 g/L was produced after 72 h of incubation (Yadav et al., 2013). In the present study fed of glucose was provided in the basal medium containing glycerol as initial substrate.

2.3. Effect of glucose fed on ABE production

To begin with, butanol production was carried out in 500 mL bottles containing 200 mL of the optimized conditions. Here, the initial glycerol concentration was 45 g/L. The fermentation run was carried out at 37 °C for 96 h with constant agitation rate of 200 rpm. The initial pH of the medium was set at 6.5. An inoculum of 2.0% (v/v) was transferred into the medium. After inoculation, removal of dissolved oxygen was performed with filtered CO_2 gas to maintain anaerobic conditions. Fed-batch cultures were performed without pH control using a feed solution of glucose which was added after 24 h of cultivation.

2.4. Effect of high-cell-density Inoculum on ABE fermentation

High-cell-density fermentations offer many advantages over traditional fermentation, therefore, a simple, efficient, and reproducible high-cell-density fermentation protocol was attempted to examine its effect on growth and butanol production. For this, *C. acetobutylicum* KF158795 cultures were grown for 24 h. After that 500 ml of the broth was centrifuged and the pellet (6 g wet wt.) obtained was re-suspended in 10 ml of the fermentation medium under sterilized conditions. The suspension obtained was used as inoculum to inoculate the production medium (200 mL in 500 mL bottles) under the optimized conditions at 37 °C, 200 rpm. Samples (2 ml) were withdrawn periodically up to 72 h to examine ABE production and growth.

2.5. ABE fermentation in 30 L bioreactor

The production of butanol using the optimized fed batch conditions was identically translated in a 30 L fermenter with a working volume of 17 L. The fermentation run was initiated with glycerol as substrate using 2.0% inoculum, and the production was carried out at pH 6.5, 37 °C. Foaming was controlled by the addition of sterilized silicon antifoam (10%). Further, glucose was fed to the production medium at 24 h, complete anaerobiosis by CO_2 flushing and efficient mixing of nutrients by mild agitation ensured a favourable environment for production of ABE. Samples were withdrawn at regular time intervals and analysed for ABE production. The fermentation parameters were continuously monitored using microprocessor control probes.

2.6. Butanol production in pilot size (300 L) bioreactor

After scale up in 30 L bioreactor, the process was further scaled up to an industrial level fermenter (300 L) containing 225 L production medium. Carbon dioxide gas was swept over the headspace of the fermenter to remove oxygen and other gases to achieve strict anaerobic condition. Initially, glycerol (45 g/L) was taken as substrate and glucose was fed at the selected time to increase the production. Inoculum was raised in the 30 L bioreactor containing 9.0 L medium (4.0% inoculum). After 24 h of incubation the inoculum was transferred to the 300 L bioreactor. Samples were withdrawn after every 12 h till 132 h and evaluated for growth and ABE production. The fermentation parameters, such as temperature and pH were continuously monitored using microprocessor controlled probes.

2.7. Downstream processing of butanol by pervaporation

A schematic diagram of the pervaporation apparatus is presented in Fig. 1a, 1b and 1c. The experiments were carried out in a static pervaporation cell**.** The upper part of the cell, a cylindrical chamber acted as the feed compartment. The membrane was supported by a porous sintered stainless steel plate embedded in the lower part of the cell. The lower and the upper parts of the permeation cell were set in proper alignment, and a pressure tight seal between the membrane and the permeation cell was formed using two rubber O-rings. The feed solution was stirred continuously. Vacuum was applied to the permeate side of the membrane, and the permeate vapor was condensed and collected in a cold trap dipped in liquid nitrogen.

Permeate was sampled periodically (every 3 h) to determine the permeation rate and permeate composition under controlled temperature. In all experiments, the feed was kept at atmospheric pressure, whereas the permeate pressure was maintained below 5 mm Hg using a vacuum pump. The permeation rate was deter-

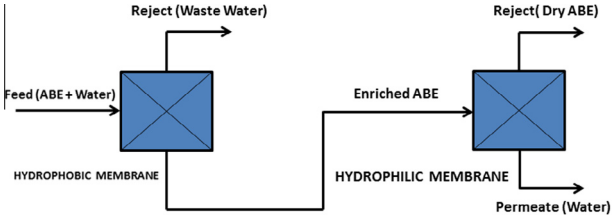

Fig. 1a. Picture of pervaporation (PV05) unit A and B: membrane–membrane hybrid.