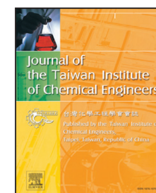
Journal of the Taiwan Institute of Chemical Engineers 000 (2016) 1–7

Contents lists available at ScienceDirect

Journal of the Taiwan Institute of Chemical Engineers

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



Bioethanol production from potato starch by a novel vertical mass-flow type bioreactor with a co-cultured-cell strategy

Yu-Kuo Liu*, Po-Min Lien

Graduate Institute of Biochemical and Biomedical Engineering, Chang Gung University, Kwei-Shan, Taoyuan City 33302, Taiwan, ROC

ARTICLE INFO

Article history:
Received 4 October 2015
Revised 21 January 2016
Accepted 27 January 2016
Available online xxx

Keywords:
Bioethanol
Co-immobilization culture
SSF
VMFB

ABSTRACT

The purpose of this study was to develop a vertical mass-flow type bioreactor (VMFB) consisting of both aerobic and anaerobic zones to produce ethanol by Simultaneous Saccharification and Fermentation (SSF). Potato starch and microorganisms of *Aspergillus awamori*, *Rhizopus japonicus*, and *Zymomonas mobilis* were used. The highest ethanol concentration with 6.0 % (w/v) initial starch were 2.99 g/L by traditional two-stage Separate Hydrolysis and Fermentation (SHF, co-suspension culture, flask, 48 h), 17.71 g/L (SHF, co-immobilization culture, flask, 84 h), and 21.68 g/L (SSF, co-cultured in VMFB, 27 h), respectively. In the repeated-batch process (3 cycles) using the VMFB, stable ethanol production was 95.97 g/L after 72 h. Increase of the starch concentration from 6.0% to 12.0% (w/v) resulted in higher ethanol production of 60.18 g/L after 51 h. Another type of starch was tested, and 27.91 g/L of ethanol was obtained from 6.0% (w/v) cassava starch. The results showed that the performance of VMFB cannot only enhance the ethanol yield, but also has great benefit for high fermentation efficiency and less cost.

© 2016 Taiwan Institute of Chemical Engineers. Published by Elsevier B.V. All rights reserved.

1. Introduction

According to the depletion of petroleum based economy sources and Kyoto Protocol for global warming, developments of new energy and alternative energy are urgent. The application of biomass energy is one of the potential alternative energy [1], as alcohol could be produced from natural starch and cellulose containing biomass resources. Biomass alcohol is now considered a renewable energy [2]. Starch was chosen as a biomass resource, because of its rich content in wide range of plants including potato, corn, cassava, rice and wheat [3,4]. Among the starch-rich raw materials, potato is a high biomass-producing and energy-efficient crop [5], and is considered as a potential bioethanol feedstock for Asian countries.

With respect to the technical aspect of saccharin fermentation, developing a technology for simultaneous saccharification and fermentation (SSF) becomes important. The new technology offers short fermentation time and also avoids the feedback inhibition of glucoamylase from the end products of saccharification [6]. Srichuwong et al. reported the SSF bioprocess for bioethanol production from potato, in which maximum ethanol concentration, average ethanol productivity rate, and ethanol yield are 131 g/L, 1.82 g/L h and 89.7% [7]. According to the recent emergent demands of alternative energy sources, strategies of efficient production, and immobilized microbial cell systems for conversion of biomass to fuels become more and more important [8]. Different immobilization processes for ethanol production have been compared with conventional processes without immobilization, in which the co-immobilization culture is the core technology to achieve SSF [9–14]. Co-immobilized three strains of microorganisms with different carriers (gel bead and loofa sponge) indicated that the co-immobilized cells could be batch-cultured repeatedly and continuously for long-time production of ethanol [15]. In addition, bioethanol obtained from SSF can be blended with petroleum for automotive powertrain [16,17].

Operation cost and safety are two important issues for bioethanol production industry, and ways to reach the goals depend not only on microorganisms and fermentation protocols, but also on reactor design. A reactor that can be easily controlled for optimizing the fermentation environments and conditions for high yields of biomass and product is highly demanded. Continuous propionic acid fermentations from lactate by *Propionibacterium acidipropionici* were studied in spiral wound fibrous bed bioreactors with a high cell density of 37 g/L and reactor productivity of almost 4 times higher than that from conventional batch fermentation [18]. Another circulating loop bioreactor (CLB) was developed for ethanol production from raw cassava starch by *Aspergillus awamori* IAM 2389 and *Saccharomyces cerevisiae* IR2 immobilized on loofa sponge [19]. The CLB consists of an aerated riser and a non-aerated downcomer column connected at the top and bottom by cylindrical pipes. Under the optimal condition to

* Corresponding author. Tel.: +886 3 4227151×5328, 886 3 2118800×5328; fax: +886 3 4227151×65067, 886 3 2118668.
E-mail address: ykliu@mail.cgu.edu.tw, ykliu0919@yahoo.com.tw (Y.-K. Liu).

http://dx.doi.org/10.1016/j.jtice.2016.01.027
1876-1070/© 2016 Taiwan Institute of Chemical Engineers. Published by Elsevier B.V. All rights reserved.

Please cite this article as: Y.-K. Liu, P.-M. Lien, Bioethanol production from potato starch by a novel vertical mass-flow type bioreactor with a co-cultured-cell strategy, Journal of the Taiwan Institute of Chemical Engineers (2016), http://dx.doi.org/10.1016/j.jtice.2016.01.027

maintain a very high DO concentration in the riser column and a low DO concentration in the downcomer column, ethanol productivity increased by more than 100–1.17 g/L h. Although a well-designed bioreactor could offer better bioethanol production, there are only few studies about SSF bioreactor design in recent years [20]. In the SSF ethanol production by co-cultural microbial system, development of a bioreactor with characters of simple structure, energy-saving, and easy operation is imperative.

The aim of this study was to develop a novel bioreactor consisting of both aerobic and anaerobic reaction zones. Then the feasibility of co-immobilizing saccharification strains (*A. awamori* and *R. japonicas*) and fermentation strains (*Z. mobilis*) for bioethanol production through SSF of potato starch was tested, where polyurethane foam (aerobic reaction zone) and calcium-alginate beads (anaerobic reaction zone) were employed as carriers for the immobilization.

2. Materials and methods

2.1. Microorganism and media

The *Rhizopus japonicus* BCRC 33070, *Aspergillus awamori* BCRC 31509 and *Zymomonas mobilis* BCRC 10809 were obtained from the Bioresource Collection and Research Centre (BCRC) of Taiwan. The stock culture (pH 6.8) of both *R. japonicus* and *A. awamori* were aseptically grown on potato dextrose agar (PDA) petri plates (Difco Laboratories, USA) incubated at 30 °C for 5 days until sporulation was sufficient and then stored at 4 °C. The stock culture (pH 6.8) of *Z. mobilis* was cultured in the plate consisted of yeast extract (5.0 g/L), reducing sugars (20 g/L) and agar (20 g/L) incubated at 30 °C for 2 days. The precultural media consisted of yeast extract (10.0 g/L), KH_2PO_4 (1.0 g/L), $MgSO_4{\cdot}7H_2O$ (0.5 g/L), $(NH_4)_2SO_4$ (1.0 g/L), glucose (100.0 g/L), potato starch (5.0 g/L, Sigma) and distilled water were applied. The fermentation media consisted of yeast extract (2.0 g/L), polypeptone (5.0 g/L), KH_2PO_4 (1.0 g/L), $MgSO_4{\cdot}7H_2O$ (1.0 g/L), $FeSO_4{\cdot}7H_2O$ (0.01 g/L), $CaCl_2{\cdot}2H_2O$ (2.0 g/L), potato starch (60.0 g/L, Sigma), and distilled water were used.

2.2. Cell immobilization and cultivation procedure

The *Z. mobilis* cells (1.0×10^9 cells/ml) were mixed with sodium alginate to yield a 4.0% (w/v) Na-alginate solution. The alginate beads were prepared by thoroughly mixing the 3% sodium alginate solution and injecting into a 3% $CaCl_2$ solution with a no. 16 needle. Finally, the alginate beads containing bacteria were harvested by washing with phosphate buffer solution. After gelling, the micro-beads were placed in double distilled water to remove unreacted material. Micro-beads with cells were stored in physiological solution at 8 °C. Concentrated aqueous *R. japonicus* and *A. awamori* spores (2%) were used to inoculate in 500-ml Erlenmeyer flasks containing 200 ml of precultural media and agitated at 200 rpm on a rotary shaker at 30 °C. The initial pH value was approximately 4.8 and was not controlled during cultures. The *R. japonicus* (1.0×10^6 spores/ml) and *A. awamori* spores (1.0×10^6 spores/ml) were mixed and then cultivated in an polyurethane (PU) carrier in the upper aerobic reactor of a co-immobilized system.

2.3. Modified bioreactor configuration and use for bioethanol production

A modified vertical mass-flow type bioreactor, VMFB, with an upper aerobic zone and a lower anaerobic zone was designed to enhance bioethanol production (Fig. 1). It has a total working volume of 1.5 L and the fermentation media were circulated between aerobic upper- and anaerobic lower-zone by gravity-flow (upper to lower) and pump-flow (lower to upper) in the VMFB. The modified bioreactor has only one pump without air-compressor to allow energy-saving in a simple structure, and an easy operation for ethanol production by simultaneous saccharification and fermentation of potato starch using a co-cultural microbial system. Reducing sugar from the upper aerobic reactor was used as the substrate for bioethanol production in the lower anaerobic zone. Briefly, bioethanol was produced in a modified bioreactor by co-cultivating three strains, *R. japonicus*, *A. awamori* and *Z. mobilis*. First, in the precultivation step, both *R. japonicus* and *A. awamori* were co-cultured in the upper aerobic zone with fresh precultural media with full of fungi on a PU carrier for 3 days. The PU carrier served not only as a fungal carrier, but also as an enzyme filter during bioethanol production. Meanwhile, *Z. mobilis* was entrapped into the alginate beads. After precultivation, the second step was to remove the precultural media and then put the microbial-laden alginate beads in the lower anaerobic zone. Fresh fermentation medium for bioethanol production was then fed into the modified bioreactor, and the starch saccharification and ethanol fermentation were simultaneously performed.

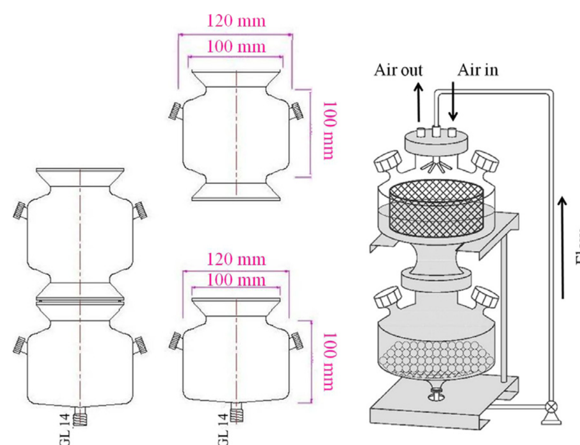


Fig. 1. Schematic diagram of VMFB with divided aerobic and anaerobic reaction zones in the modified bioreactor.

2.4. Analytical methods

For comparison purposes, total reducing sugar present in the hydrolyzate was estimated by dinitrosalicylic acid (DNS) method [21]. Briefly, 20 ml of concentrated HCl solution was added into 1 ml of the hydrolyzate solution at 90 °C for 5 min. Then, 0.05 ml of 5 N KOH was added into the solution to neutralize the acid. Finally, the DNS reagent was added into the solution. Samples were centrifuged and filtered through 0.2 mm filters for HPLC analysis. Concentrations of reducing sugars and bioethanol were determined using an Aminex HPX-87H column (Bio-Rad, USA) at 65 °C. All compounds were detected three times with a refractive index detector. The supernatant collected after centrifugation of the 3-day culture broth at 1500 × g for 10 min was used as the crude enzymes. The activity of α-amylase, β-amylase, and glucoamylase were measured according to the methods described by Jolanta Bryjak [22]. An enzyme activity unit (U) was defined as the amount of enzyme liberating 1 mg of maltose (α-amylase and β-amylase) or glucose (glucoamylase) per minute under the assay conditions. The starch conversion rate was calculated using the following equation [23]:

$$\text{Percentage conversion (on 100 g starch basis)} = \frac{\text{Reducing augar/glucose yield (\%dwb)} \times 100\%}{\text{starch(\%dwb)}}$$

Please cite this article as: Y.-K. Liu, P.-M. Lien, Bioethanol production from potato starch by a novel vertical mass-flow type bioreactor with a co-cultured-cell strategy, Journal of the Taiwan Institute of Chemical Engineers (2016), http://dx.doi.org/10.1016/j.jtice.2016.01.027

Download English Version:

https://daneshyari.com/en/article/690317

Download Persian Version:

https://daneshyari.com/article/690317

Daneshyari.com