Journal of the Taiwan Institute of Chemical Engineers 63 (2016) 46–51

Contents lists available at ScienceDirect

Journal of the Taiwan Institute of Chemical Engineers

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

A novel static cultivation of bacterial cellulose production by intermittent feeding strategy

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ARTICLE INFO

Article history:
Received 1 December 2015
Revised 6 March 2016
Accepted 11 March 2016
Available online 7 April 2016

Keywords:
Bacterial cellulose
Static culture
Intermittent feeding strategy
Layer-by-layer production

ABSTRACT

Bacterial cellulose (BC) of insoluble extracellular polysaccharide can be produced by *Gluconacetobacter* strains. Because of its unique nano-structure, BC has found its applications in various fields. In this study, an intermittent feeding strategy was proposed for the static culture to produce BC film of arbitrary thickness in layer-by-layer form with high productivity. That is, simply by supplementing the nutrients directly to the top of BC film already formed, microorganisms would be able to utilize the oxygen and nutrients at the same time without any obstruction, thereby the production rate could be maintained at steady state. For example, the BC thickness was roughly 30 mm in this modified static process after 30 days cultivation compared to 2 mm in conventional static culture. Further, excessive amount of fresh medium addition would create a distance between the previous BC pellicle and the new air–liquid interface, thus resulting in a separated layer BC. For, example, once the distance is larger than some critical value (1 mm in this study with ATCC 11142), there would be layer-by-layer BC pellicles formed. With concept of this intermittent feeding strategy, BC products can be produced with arbitrary shape and thickness continuously and with high productivity.

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1. Introduction

Unlike the cellulose from wood pulp and cotton, bacterial cellulose (BC) was found to be free of the other polysaccharides such as hemicellulose and lignin; thereby its isolation and purification were relatively simple. Also, BC displayed other unique characteristics such as high Young's modulus, high water holding capacity, high degree of polymerization and ultra-fine network structure [1]. Thus, BC becomes a unique source of pure cellulose and gradually gains its attention. Commercial applications of BC has been initiated from its above-mentioned unique properties and developments and could have been found in various fields, *i.e.* food industry as Nata de coco, biomedical industry as artificial skins or wound care dressings, materials industry as acoustic of diaphragms, and paper industry as additives to enhance the strength of paper [2–5].

Gluconacetobacter xylinus (formerly called *Acetobacter xylinum*) has been known to produce highly pure cellulose for more than 100 years. Several techniques such as static culture and agitated culture for BC production were reported [2]. Traditionally, BC was produced by static culture in which cellulose was produced in the form of gelatinous pellicle at the air–liquid interface. It usually requires long time cultivation and extensive space to produce BC in static culture, thus resulting in low productivity. This drawback sometimes could be relaxed by developing a cost-effective medium to lower production cost or enhancing production rate by partial medium replacement [6,7]. Also, it is experimentally confirmed that the cellulose production rate depended proportionally on the surface area of the culture medium and was relatively unaffected by the depth and volume of the medium [8]. It is generally accepted that the reason why *G. xylinus* produce BC is to help them float and reach the oxygen-rich surface [7]. Once BC pellicles form at air–liquid interface to a certain thickness, the growth of cell as well as BC production stops because the aerobic zone (upper part of BC pellicles) lacks nutrient while the bottom portion of BC pellicles are short of oxygen. Prolonged fermentation would have very little contribution to BC production.

As to the agitated culture, the oxygen and nutrients transfer to microorganisms in agitated culture are sufficient, the growth of BC would not be limited at the air–liquid interface. Though the chemical structure of cellulose is similar to that of static culture and better productivity of BC may be expected, the morphology is very different. They are mainly in the form of pellet or conglomerate which depends on the culture method [9]. Furthermore, however,

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http://dx.doi.org/10.1016/j.jtice.2016.03.020
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it is also reported that, because of the genetic instability, some cells might convert into Cel$^-$ mutants in agitated culture, and gradually lose the cellulose productivity of BC [10,11]. Discussion of various operations such static/batch, continuous or semi-continuous could found in reference [5,7].

The objective in this study, using *Gluconacetobacter xylinus* as a model strain, was to provide a novel strategy for cellulose production by intermittent feeding under static culture. It was shown that the proposed strategy would produce BC products not only of any desired thickness in a layer-by-layer fashion continuously but also with high productivity.

2. Materials and methods

2.1. Medium composition and culture conditions

All the chemicals used were of analytical grade and commercially available. For cell-preculture, the pre-cultured medium used in this study contains the following constituents per liter: 25.0 g mannitol, 5.0 g yeast extract, 3.0 g peptone (pH 6.8). On the other hand, for BC production, Buffered Schramm & Hestrin's Medium (BSH medium) with a slight modification was used in this study, which contains the following constituents per liter: 20.0 g glucose, 5.0 g yeast extract, 5.0 g peptone, 2.7 g Na_2HPO_4 $12H_2O$ and 1.15 g citric acid (pH 5.5) [12].

2.2. Microorganism

The *Gluconacetobacter xylinus* subsp. *xylinus* (ATCC 11142) used in this study was bought from Bioresource Collection and Research Center (BCRC, Taiwan). The stock culture was maintained on an agar plate at 4 °C. Thereafter, one loop of inoculum from the agar plate was pre-cultured for 1 day under orbital shaking at 26 °C in a 250 ml conical flask containing 50 ml pre-cultured medium. Then the pre-cultured broth was used as inoculum and seeded into the modified BSH medium at level of 5%.

2.3. Fermentation containers

In this study, several types of containers were used such as 250 ml conical flask, the box with several individual space (each space with L: 3.6 cm×W: 3.6 cm×H: 2.7 cm), test tube (1.8 cm diameter), a box (L: 8.0 cm×W: 11.0 cm×H: 17.0 cm), and glasses petri dish (15.0 cm diameter, H: 2.5 cm). All of containers are sterilized by autoclave or by 95% ethanol solution.

2.4. Analytical methods

To purify cellulose, first the BC products were separated with the culture broth by a simple sieve (60 mesh). All of the BC products were cut into pieces (smaller than1.0 cm × 1.0 cm) and treated with 0.5 N NaOH to release trapped medium and bacterial cells at 90 °C for 30 min. The BC products were rinsed extensively with distilled water until the pH of the water became neutral. The purified cellulose products were dried in an oven at 70 °C to constant weight and then weighed.

2.5. Quality consistence of BC

A diffusional cell of two well-stirred and water-jacked chambers was used to measure the BC products permeability of various thicknesses. The donor chamber ($V_d = 30$ ml) was filled with 20 g/L glucose while the receptor chamber ($V_r = 30$ ml) was deionized water and the opening area for BC was 1.31 cm^2 (A). The samples periodically taken from two chambers were analyzed for their glucose concentration by DNS method. Thus the permeability (P) for the solute (glucose) through the BC could be calculated by the following equation.

$$P = \frac{1}{\beta t} \ln \frac{[C_{0,d} - C_{0,r}]}{[C_{1,d} - C_{1,r}]} \quad (1)$$

In the equation above, C stands for glucose concentration while its subscripts (d, r and 0, l) denote donor, receptor chambers as well as initial and various sampling times (t). The parameter $\beta (= \frac{A}{L}(\frac{1}{V_d} + \frac{1}{V_r}))$ is a geometric constant while L is thickness of BC.

3. Results

In the static culture of *Gluconacetobacter* or many similar strains, BC pellicle is mainly formed at the air–liquid interface where oxygen is sufficient. The BC product starts with the formation of island-like fragments on the air–liquid interface and gradually covers the whole interface. Fig. 1 showed a typical result of static culture in a 250 ml conical flask with 50 ml medium. It showed that the BC yield increased rapidly at the beginning and reached 2.0 g/L on the 7th day cultivation. However, the production

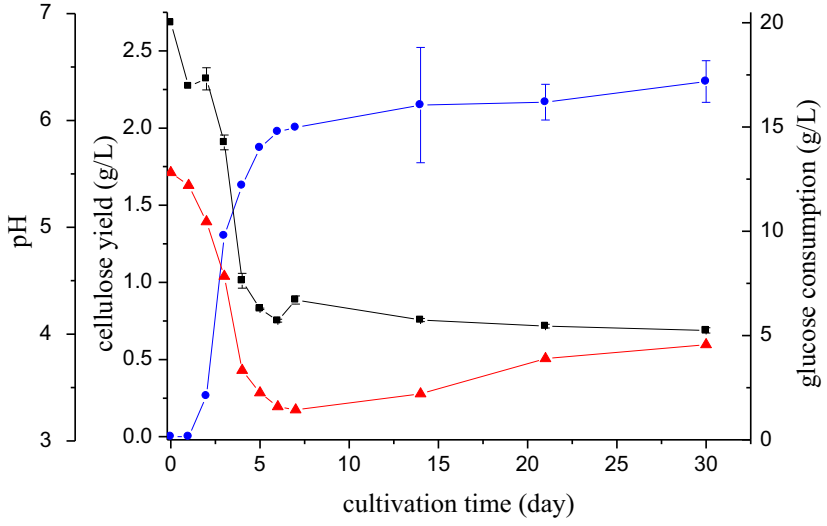

Fig. 1. BC yield (●), the glucose concentration (■), and pH(▲) profiles during the fermentation in a 250 ml conical flask with 50 ml medium.

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