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Capability of sweet sorghum stalks as supporting materials for yeast immobilization to produce ethanol under various fermentation processes

Pongthep Ariyajaroenwong[a], Pattana Laopaiboon[b,c], Lakkana Laopaiboon[b,d,*]

[a] Graduate School, Khon Kaen University, Khon Kaen 40002, Thailand
[b] Department of Biotechnology, Faculty of Technology, Khon Kaen University, Khon Kaen 40002, Thailand
[c] Fermentation Research Center for Value-Added Agricultural Products, Khon Kaen University, Khon Kaen 40002, Thailand
[d] Center for Alternative Energy Research and Development, Khon Kaen University, Khon Kaen 40002, Thailand

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ABSTRACT

Unpeeled sweet sorghum stalk (SSS) pieces sizing 6- to 20-mm diameter and 6-mm thick were used as carriers for *Saccharomyces cerevisiae* NP 01 immobilization to produce ethanol. The diluted sweet sorghum juice containing 100 g/l of total sugar without nutrient supplementation was a suitable medium for yeast cell immobilization, and 18 h incubation time was sufficient for the immobilization process. In repeated-batch ethanol fermentation from sweet sorghum juice (240 g/l of total sugar) at 30 °C, the average ethanol concentration (P), productivity (Q_P) and yield ($Y_{P/S}$) by the immobilized yeast cells were 93.4 g/l, 1.30 g/l h and 0.47 g/g, respectively. When the continuous system was operated in a double-tubular packed-bed bioreactor with 50% bed height, the average P, Q_P and $Y_{P/S}$ were comparable to those of the repeated-batch fermentation. Supplementation the juice with 6 g/l of yeast extract resulted in significant increases in P, Q_P and $Y_{P/S}$ to 105.7 g/l, 2.43 g/l h and 0.48 g/g, respectively at the dilution rate of 0.023/h. These results demonstrated that the unpeeled SSS pieces were successfully used as low-cost carriers for yeast cell immobilization to produce ethanol under both repeated-batch and continuous systems.

1. Introduction

Bioethanol, an alternative to petroleum-based fuels, can be produced from biomass or sugar-yielding crops by fermentation of microorganisms. The use of fuel ethanol can reduce the toxic exhaust emissions and greenhouse gases from vehicles [1]. One of the prime sugar-yielding crops being investigated for ethanol production is sweet sorghum {*Sorghum bicolor* (L.) Moench}. Sweet sorghum is a high biomass crop that contains a large amount of fermentable sugars, particularly glucose and sucrose, in its stalk [2]. These sugars can be directly converted to ethanol by means of fermentation processes. The juice from its stalk also contains nutrients and trace elements that are essential for yeast growth and ethanol production [2,3].

Fermentation process development is very important for improvement of efficient ethanol production [4]. In many research works, batch, repeated-batch, fed-batch and continuous fermentation were used to produce ethanol from sugars by free yeast cells [5-7]. However, using free cells for ethanol fermentation often encounters difficulties such as substrate or product inhibition from direct contact between the cells and medium [8], and losing time for new inocula preparation and cleaning for each batch. To mitigate these problems, cell immobilization is introduced in ethanol fermentation processes. The use of cell immobilization system can reduce the cost of ethanol production as it offers several advantages over free cell system, *i.e.* higher yeast cell concentration resulting in higher fermentation rate, recycling utilization of the yeast and lower product inhibition [9]. However, carrageenan and alginate which are widely used as carriers for cell immobilization in laboratory scales are expensive. Therefore, low-cost carriers or supporting materials are required for cell immobilization in large scale applications.

In this study, unpeeled sweet sorghum stalk (SSS) pieces were chosen as the carriers for yeast cell immobilization by absorption method and the juice from its stalk was used as an ethanol production medium. The method of cell immobilization was simplified. Stability and durability of the carriers were investigated by ethanol fermentation using *Saccharomyces cerevisiae* NP 01 immobilized on the SSS pieces under repeated-batch and continuous processes.

* Corresponding author. Tel.: +66 4336 2121; fax: +66 4336 2121.
E-mail address: lakcha@kku.ac.th (L. Laopaiboon).

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2. Materials and methods

2.1. Microorganism and inoculum preparation

The yeast *S. cerevisiae* NP 01 [2] was inoculated into yeast extract malt extract (YM) medium containing 10 g/l of glucose and incubated on a rotating shaker at 200 rev/min, 30 °C for 18 h. The culture was sub-cultivated into YM medium containing 20 g/l of glucose using 10% (v/v) inoculum size and incubated for another 18 h before use as inoculum for cell immobilization [10].

2.2. Raw material and ethanol production medium

The sweet sorghum juice extracted from sweet sorghum stalks (cv. KKU 40) was obtained from Division of Agronomy, Faculty of Agriculture, Khon Kaen University, Thailand. The juice containing total soluble solids of 18 °Bx was concentrated to 75 °Bx and stored at 4 °C until use.

Ethanol production (EP) medium was prepared by dilution of the concentrated juice with distilled water to obtain the total sugar concentration of ~240 g/l without nutrient supplementation.

2.3. Cell immobilization on carriers

The active yeast cells were inoculated into three media to obtain the cell concentration of $\sim 1 \times 10^8$ cells/ml. The three media were (1) YM medium containing 20 g/l of glucose, designated as YM20; (2) sweet sorghum juice containing 100 g/l of total sugar, designated as SSJ100 and (3) YM medium containing 100 g/l of glucose, designated as YM100. After the desired cell concentration was attained, sterile unpeeled sweet sorghum stalk (SSS) pieces with diameter in the range of 6–20 mm and thickness of 6 mm were aseptically transferred into the cultures, and the cultures were incubated at 30 °C for 12, 18 or 24 h. Then, the SSS pieces were aseptically removed and washed with sterile EP medium and used as for the study of ethanol production.

2.4. Fermentation processes

2.4.1. Batch process

The SSS pieces containing immobilized yeast cells at 25% of working volume were inoculated into the sterile EP medium (350 ml) in a 500-ml air-locked Erlenmeyer flask. The fermentation was operated at 30 °C under static condition.

2.4.2. Repeated-batch process

The repeated-batch system was first carried out as in batch mode until the residual total sugar in the broth had dropped slowly. Then, all fermented broth was withdrawn leaving only the carriers with immobilized cells in the flask. After that, the same amount of the sterile EP medium was added to start the next cycle [10].

2.4.3. Continuous process

Before studying continuous ethanol fermentation by the immobilized yeast cells, the optimum bed height under batch fermentation in a single-tubular packed-bed bioreactor were determined (Fig. 1a). The bioreactor was a double jacketed glass tube with 6-cm inner diameter and 35-cm height, and its working volume was 0.78 l. The SSS pieces with immobilized yeast cells were put into the single-tubular packed-bed bioreactor at 25, 50 and 75% of the bed height before feeding the sterile EP medium. The fermentation was operated at 30 °C under static condition.

In continuous process, two single-tubular packed-bed bioreactors were connected in series, namely a double-tubular packed-bed bioreactor with total working volume of 1.56 l (Fig. 1b). The double-tubular bioreactor was packed with the SSS pieces with immobilized yeast cells at the optimum bed height obtained from the batch study. The sterile EP medium was fed into the bioreactor at the dilution rates of 0.013, 0.023 and 0.045/h.

2.5. Analytical methods

During the fermentation processes, the fermented broth was collected at time intervals for analyses. Ten grams of the SSS pieces

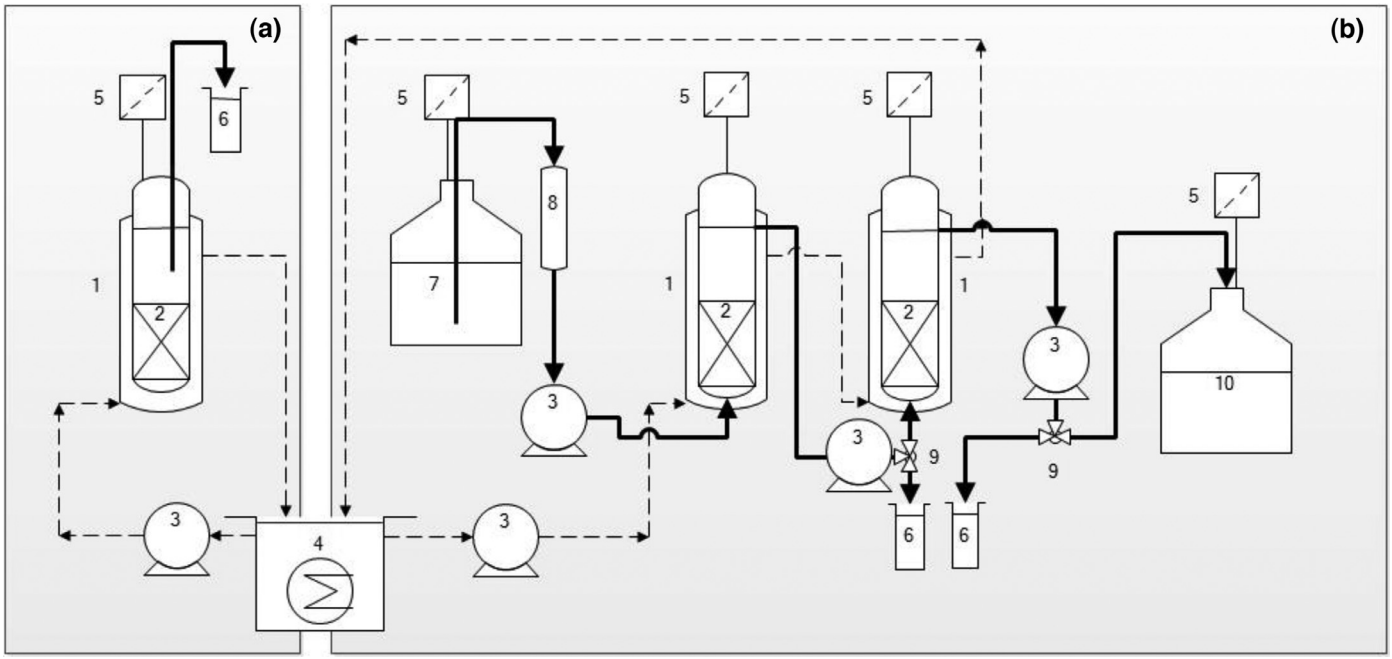

Fig. 1. (a) A single-tubular packed-bed bioreactor for batch fermentation and (b) a double-tubular packed-bed bioreactor for continuous fermentation: (1) double jacketed tubular bioreactor, (2) bed or carriers, (3) peristaltic pump, (4) cooling bath, (5) air filter, (6) sample tube, (7) influent tank, (8) flow meter (9) valve and (10) effluent tank; medium line (solid line) and cooling water line (dash line).

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