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Short Communication

Repeated ethanol production from sweet sorghum juice concentrated by membrane separation

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HIGHLIGHTS

- Sweet sorghum juice was concentrated by membrane separation.
- Sequential batch fermentation was done with concentrated juice.
- Ethanol was repeatedly produced with high levels by *Saccharomyces cerevisiae*.
- Exogenous nutrient sources were not necessary.
- Growth of *S. cerevisiae* was observed in concentrated juice.

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ABSTRACT

Sequential batch fermentation from sweet sorghum juice concentrated by membrane separation (ultrafiltration permeation and nanofiltration concentration) to increase sugar contents, was investigated. Ethanol production at 5th batch fermentation by *Saccharomyces cerevisiae* BY4741 attained 113.7 ± 3.1 g L^{-1} (89.1 ± 2.2% of the theoretical ethanol yield) from 270.0 ± 22.6 g L^{-1} sugars, corresponding to 98.7% of ethanol titer attained at the 1st batch fermentation. This titer was comparable to ethanol production of 115.8 ± 0.6 g L^{-1} (87.1 ± 2.7% of the theoretical ethanol yield) obtained at 5th batch fermentation with 3 g L^{-1} yeast extract and 6 g L^{-1} polypeptone. Increase of cell density in the concentrated sweet sorghum juice was observed during sequential batch fermentation, as indicated by increased OD_{600}. Utilization of sweet sorghum juice as the sole source, membrane separation, and *S. cerevisiae* was a cost-effective process for high ethanol production.

1. Introduction

The use of ethanol as a fuel or gasoline enhancer is appearing; this compound is renewable and environmentally friendly, and is believed to be one of best alternatives to petroleum-derived fuels (Sánchez and Cardona, 2008; Gray et al., 2006). Global ethanol production is currently derived from sugar and starch feedstocks (Bai et al., 2008). One such feedstock is sweet sorghum [*Sorghum bicolor* (L.) Moench], a plant that has sugar-rich stalks. Sorghum exhibits the great potential as an energy crops because the plant tolerates drought environments and cold temperatures, permitting growth across a wide geography, including temperate and tropical climate areas (Zabed et al., 2014; Ratnavathi et al., 2011). Sorghum has several additional advantages, including a short growing period of 4–5 months, as well as reduced requirements for fertilizer and water compared to other sugar crops (Sipos et al., 2009). Recently, sweet sorghum juice has been employed for ethanol fermentation using *Saccharomyces cerevisiae* (Sasaki et al., 2014; Ratnavathi et al., 2011; Laopaiboon et al., 2009). To increase ethanol production and decrease the energy requirement for distillation, starting sugar concentrations have been increased by adding exogenous sugar (Laopaiboon et al., 2009).

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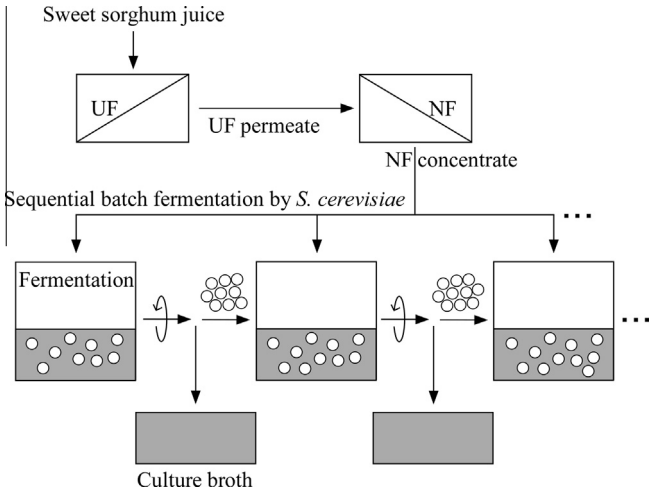

Fig. 1. Schematic flow of sequential batch fermentation. Sweet sorghum juice was permeated through ultrafiltration (UF) to remove residues. Nanofiltration (NF) increased sugar concentrations. NF concentrate was applied to 48 h of ethanol fermentation with *S. cerevisiae* BY4741. Then, culture broth was recovered after centrifugation to precipitate *S. cerevisiae* cells and new NF concentrate was added. Ethanol fermentation was restarted after mixing.

Another strategy for increasing starting sugar concentrations is membrane separation. Membrane separation can fractionate complex mixtures and is a cost-effective technology (He et al., 2012). Membrane separation for sweet sorghum juice involved passage through multiple membranes (Sasaki et al., 2014). The first, the ultrafiltration (UF) membrane, has a molecular-weight cut-off of 150,000 g mol^{-1}, permitting the passage of sugars in the sweet sorghum juice while retaining polymeric compounds and large residues. Subsequently, a nanofiltration (NF) membrane, with molecular-weight cut-off of 150 g mol^{-1}, permits concentration of the sugars contained in the UF membrane permeate. Sweet sorghum juice subjected to the combination of UF permeation and NF concentration yielded production of ethanol at high levels (131.4–133.5 g L^{-1}) upon fermentation with *S. cerevisiae* (Sasaki et al., 2014). The UF/NF membrane separation system also increases the starting concentration of amino acids (nitrogen source) in the substrate (Sasaki et al., 2014). In sequential fed-batch fermentation from the sweet sorghum juice, exogenous nutrient, i.e., nitrogen source is also contained together with sugar (Laopaiboon et al., 2007). These observations have led to interest in the use of sequential batch fermentation of concentrated sweet sorghum juice in the bio-production of ethanol.

The aim of the present work was to investigate the sequential batch production of ethanol by *S. cerevisiae* BY4741 grown on sweet sorghum juice concentrated by UF permeation and NF concentration (Fig. 1). Sequential batch processes with and without nitrogen sources (yeast extract and polypeptone) were compared to investigate the need for addition of exogenous nutrients and test the utility of membrane separation on sequential batch ethanol fermentation. These insights into membrane separation of sweet sorghum juice would help repeated high ethanol production and growth of *S. cerevisiae* BY4741 without exogenous nutrients.

2. Methods

2.1. Raw material

Japanese sweet sorghum juice cv. SIL-05 was obtained from the Togo Field Science and Education Center of Nagoya University (Aichi, Japan) in 2013. The juice was extracted using a juice extractor (Okuhara Tekko, Okinawa, Japan). The juice (pH 5.2) contained 62.3 g L^{-1} sucrose, 35.9 g L^{-1} glucose, and 26.8 g L^{-1} fructose. The juice was stored at −20 °C until use.

2.2. Membrane separation process

Polyvinylidene fluoride UF membrane RS50 (molecular weight cut-off 150,000 g mol^{-1}) and polyamide NF membrane ESNA3 (molecular weight cut-off 150 g mol^{-1}) were obtained from Nitto Denko Corporation (Osaka, Japan). The membranes were cut into circles (diameter: 7.5 cm; effective area: 32 cm^2). RS50 membranes were soaked in 50% (v v^{-1}) ethanol solution for 15 min, then in deionized water for 15 min, and finally soaked overnight in deionized water before use.

Membrane separation experiments were carried out at 25 °C using a flat membrane test cell (diameter: 104 mm, height: 147 mm, working volume: 380 mL; model C40-B; Nitto Denko Corporation) as described previously (Sasaki et al., 2014). A pressure of 0.5 and 2.8 MPa was applied for UF permeation and NF concentration, respectively, using nitrogen gas and a pressure control valve. The process steps were: UF permeation → NF concentration → sequential ethanol fermentation (Fig. 1). The sweet sorghum juice was passed through a UF membrane, then the permeate was concentrated using a NF membrane. This concentrate was fermented by *S. cerevisiae* BY4741.

2.3. S. cerevisiae preparation

S. cerevisiae strain BY4741 (Genotype: *MATa his3Δ1 lue2Δ0 met15Δ0 ura3Δ0*) (Brachmann et al., 1998) was purchased from the American Type Culture Collection (ATCC) as ATCC No. 404002. The cells were aerobically propagated for 24 h at 30 °C and 150 r min^{-1} in 5 mL YPD medium [10 g L^{-1} yeast extract (Becton, Dickinson and Company, Tokyo, Japan), 20 g L^{-1} polypeptone (Wako Pure Chemical Industries, Ltd, Osaka, Japan), 20 g L^{-1} glucose] and then cultivated for 24 h in 500 mL of YPD medium. The cells were collected by centrifugation at 3000×*g* for 10 min at 4 °C and washed twice with distilled water.

2.4. Sequential batch fermentation

S. cerevisiae BY4741 was inoculated into sweet sorghum juice concentrated by the membrane separation; fermentation was performed in triplicate. The initial concentration of *S. cerevisiae* was 50 g L^{-1} of wet cells, corresponding to 10 g L^{-1} of dry cells and 5×10^8 cells mL^{-1}. Ethanol fermentation was performed under limited-oxygen and atmospheric pressure conditions at 30 °C with mild agitation (35 r min^{-1}) in 50-mL polypropylene bottles (Corning Inc., NY, USA) equipped with an outlet for CO_2 and set in a Thermo Block Rotator SN-06BN heat block (Nissin, Tokyo, Japan) as described previously (Matano et al., 2012). Each bottle had a working volume of 10 mL. The pH was not controlled and remained at approximately 5.2. After 48 h of fermentation, culture was centrifuged at 3000×*g* for 10 min at 4 °C. Then, 9.5 mL supernatant was removed and same amount of sweet sorghum juice concentrated by membrane separation was added in clean bench. Batch fermentation was started after vortex to mix *S. cerevisiae* with sweet sorghum juice concentrated by membrane separation. This batch fermentation was repeated 5 times for 240 h. 0.5 mL supernatants were withdrawn at 0, 48, 96, 144, and 192 h after centrifugation and 0.5 mL fermentation cultures were sampled at 195, 198, 201, 216, and 240 h in clean bench. For comparison, 1-time batch fermentation was conducted under the same conditions for 48 h by inoculating 50 g L^{-1} of wet cells of *S. cerevisiae* and 0.5 mL samples were withdrawn at 0, 3, 6, 9, 24, and 48 h. The ethanol yield was expressed as grams of ethanol produced per grams of combined sucrose, glucose, and fructose consumed. A

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