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Improvement of a continuous ethanol fermentation from sweet sorghum stem juice using a cell recycling system

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ABSTRACT

The process variables (aeration rate and recycle ratio) of a continuous ethanol fermentation with a cell recycling system (CRS) by *Saccharomyces cerevisiae* NP 01 from sweet sorghum stem juice were optimized using response surface methodology (RSM). The relationship between intracellular composition and fermentation efficiency was also investigated. RSM results revealed that the optimum aeration rate and recycle ratio were 0.25 vvm and 0.625, respectively. The validation experiment under the optimum conditions indicated high precision and reliability of the experiment, achieving an actual ethanol concentration (P_E) of 99.28 g/l, which was very close to the predicted value (98.01 g/l), and a very high ethanol productivity (Q_P) of 7.94 g/l h. The intracellular composition of the yeast cells (*i.e.*, unsaturated fatty acids (UFAs), total fatty acids (TFAs), ergosterol and trehalose) was positively related to the fermentation efficiency and yeast adaptive response under ethanol stress. A higher ratio of UFAs/TFAs and ergosterol strongly promoted yeast viability and ethanol fermentation. Additionally, high trehalose content was observed when the yeast was subjected to stress conditions.

1. Introduction

Bio-ethanol is an alternative energy for substitute for fossil fuels. It can be blended with gasoline as a transportation fuel, which can reduce the emission of hydrocarbons and carbon monoxide into the environment. Ethanol production can be achieved via microbial fermentation processes from many agricultural residues that are locally produced, cheap and abundant. Currently, sugarcane molasses and cassava are the primary raw materials for industrial ethanol production in Thailand (Khongsay et al., 2014). From 2008 to 2020, the ethanol production goals of the Thai government are expanded from 3,000,000 to 9,000,000 l/day (Silalertruksa and Gheewala, 2010). Therefore, it is possible that Thailand may face a shortage of sugarcane molasses and cassava for ethanol production. Sweet sorghum (*Sorghum bicolor* (L.) Moench) is an attractive raw material for ethanol production due to its rapid growth (90–120 days), high content of fermentable sugars in its stalks, high adaptation in dry and warmer areas, and high tolerance to drought and salinity (Wu et al., 2010).

Several fermentation processes including batch (Breisha, 2010; Nuanpeng et al., 2010; Deesuth et al., 2015), fed batch (Alfenore et al., 2004; Cheng et al., 2009) and continuous fermentation (Bai et al., 2004; Purwadi and Taherzadeh, 2008; Govindaswamy and Vane, 2010) have been applied for ethanol production. However, continuous systems for ethanol fermentation have several distinct advantages over other systems in terms of ethanol concentration and productivity (Brandberg et al., 2007).

Ethanol production by *Saccharomyces cerevisiae* has been employed and developed for achieving high fermentation efficiency. However, often low productivities and incomplete sugar utilization were observed under high gravity fermentations (Liu et al., 2011; Deesuth et al., 2015). To achieve high ethanol production rates, as well as high ethanol concentrations, the fermentation system requires high cell concentrations under optimum environmental conditions (Laopaiboon et al., 2007; Sridee et al., 2011). Typically, aerobic conditions are required for yeast growth. Thus, the cell number under aerobic conditions is much higher than those under anaerobic conditions (Alfenore et al., 2004). However, excess oxygen may lead to high cell density, resulting in low ethanol concentration. In many studies, the positive effects of aeration on ethanol fermentation were reported in terms of the biomass, sugar consumption, ethanol and productivity (Alfenore et al., 2004; Lin et al., 2011). Additionally, aeration also affects the stability of continuous fermentations (Kida et al., 1989). Control of the circulation and retention of cells in the system is one of the techniques to improve ethanol fermentation efficiency. Continuous

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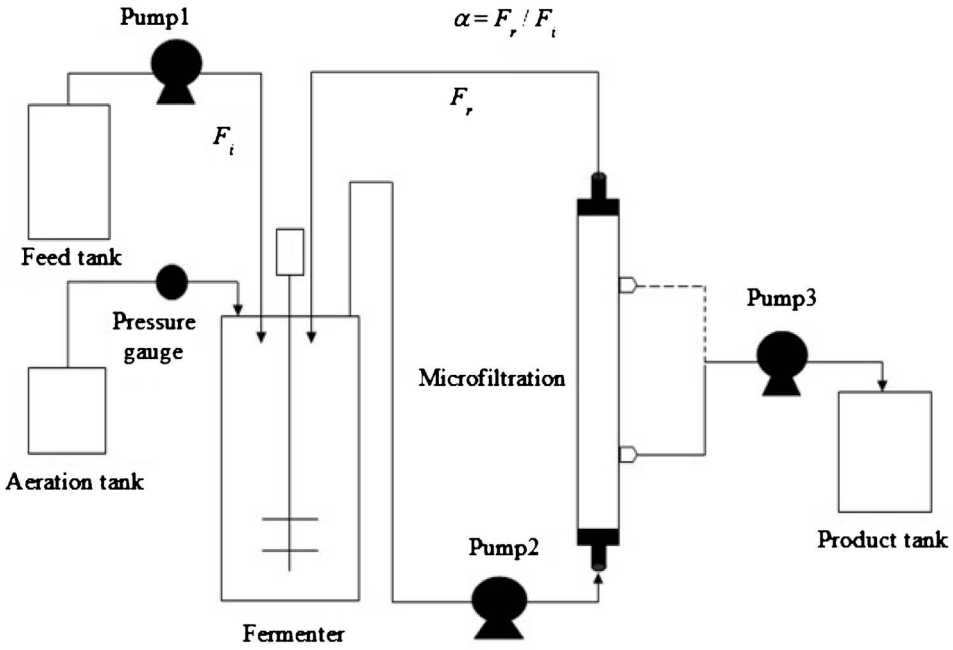

Fig. 1. Schematic diagram of the continuous ethanol fermentation with the CRS (modified from Doran, 1995). Feed flow rate, (F_i); recycle stream flow rate, (F_r); recycle ratio, (α).

ethanol fermentation with a cell recycling system (CRS) can be used to maintain high cell numbers in a bioreactor, resulting in an increase in the fermentation efficiency (Tang et al., 2010; Wang et al., 2013). However, the combined effects of aeration and a CRS on ethanol production have not been previously reported. To achieve high fermentation efficiency, the optimal conditions of aeration and a CRS in terms of aeration rate and recycle ratio were investigated.

Additionally, it was reported that cell membrane composition, *i.e.*, fatty acids (saturated and unsaturated fatty acids), sterols (ergosterol) and trehalose (a storage carbohydrate) are directly related to yeast growth and the progression of an ethanol fermentation (Mannazzu et al., 2008; Pereira et al., 2011). Furthermore, aeration during fermentation affected the concentration of these compounds. During ethanol fermentation, dissolved oxygen is required for yeast to facilitate the synthesis of unsaturated fatty acids (UFAs) and ergosterol for their structural cell membrane (Bardi et al., 1999; Rosenfeld and Beauvoit, 2003; Lin et al., 2011; Pereira et al., 2011). At high ethanol concentrations and osmotic pressures (high sugar concentration), fatty acids and ergosterol are strongly related to ethanol tolerance, fluidity and membrane integrity (Piper, 1995; Mannazzu et al., 2008). Trehalose in yeast is a strong protectant to maintain structural integrity of plasma membranes under stress conditions (Mahmud et al., 2009). Additionally, the concentration of intracellular trehalose can be used as an indicator of environmental stress in yeast cells (Conlin and Nelson, 2007; Tang et al., 2010). Trehalose content in yeast decreased when the cells were cultivated with no aeration, resulting in a decrease in cell activity (Tang et al., 2010). However, there is no report on the intracellular composition (lipids, sterols and trehalose) of yeast cells derived from continuous ethanol fermentation with a CRS.

The aims of this study were to optimize the aeration rate and recycle ratio of a CRS for high fermentation efficiency from sweet sorghum stem juice by *S. cerevisiae* NP 01. The changes in intracellular composition of the yeast membrane (intracellular fatty acids, ergosterol and trehalose) under continuous ethanol fermentation with and without a CRS were studied.

2. Materials and methods

2.1. Microorganism and inoculum preparation

S. cerevisiae NP 01 (Deesuth et al., 2015) was cultivated in 150 ml of yeast extract malt extract (YM) broth (yeast extract, 3 g/l; peptone, 5 g/l; malt extract, 3 g/l and glucose 10 g/l) on a rotary shaker at 200 rpm and 30 °C for 18 h. This pre-culture was transferred into sterile sweet sorghum stem juice containing 100 g/l of total sugar to obtain an initial cell concentration of ~5 × 10^6 cells/ml. The culture was grown under the same conditions for 18 h before use as the inoculum for ethanol production.

2.2. Raw material and ethanol production (EP) medium

Sweet sorghum stem juice *cv.* KKU40 was obtained from Faculty of Agriculture, Khon Kaen University, Thailand. The raw juice containing total soluble solids of 17°Bx was concentrated to 68°Bx and stored at 4 °C until use. To prepare the ethanol production (EP) medium, the concentrated juice was diluted with distilled water to obtain a total sugar concentration of 230 g/l before supplementation with yeast extract (6 g/l) (Thani et al., 2014). The EP medium was transferred into a 2-l fermenter with a final working volume of 1.0 l and autoclaved at 110 °C for 28 min (Laopaiboon et al., 2009).

2.3. CRS using a microfiltration bioreactor

The microfiltration module (UJP–0047R, Pall Corporation Co. Ltd, Japan) for cell recycling was the hollow fiber with a pore size of 0.65 μm, 0.31 m in length and surface area of 0.02 m^2. The hollow fibre was connected to the 2-l fermenter and other equipment as shown in Fig. 1 (modified from Doran, 1995).

2.4. Fermentation condition of the CRS

The ethanol fermentation was first carried out in a batch system using *S. cerevisiae* NP 01 at an initial cell concentration of ~2.0 × 10^7 cells/ml, at 30 °C and an agitation speed of 200 rpm. Aeration was provided at 2.5 vvm for the first 4 h of the fermentation