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Variation of fermentation redox potential during cell-recycling continuous ethanol operation

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ABSTRACT

Fermentation redox potential was monitored during cell-recycling continuous ethanol operation. The cell-recycling system (CRS) was operated using two hollow fibre (HF) membranes (pore sizes 0.20 and 0.65 μm) at three dilution rates (0.02, 0.04 and 0.08 h^{-1}). *Saccharomyces cerevisiae* NP 01 were recycled in the fermenter at a recycle ratio of 0.625. Aeration was provided at 2.5 vvm for the first 4 h and then further supplied continuously at 0.25 vvm. As steady state was established, results showed that the fermentation redox potential was lower for processes employing CRS than those without. At the same dilution rates, the sugar utilization and ethanol production with CRS were higher than those without CRS. The highest fermentation efficiency (87.94 g/l of ethanol, ~90% of theoretical yield) was achieved using a 0.2-μm HF membrane CRS at a dilution rate of 0.02 h^{-1}. It was found that 7.53–10.07% of the carbon derived from glucose was incorporated into the yeast. Further, at the same dilution rates, yeast in the processes with CRS incorporated less carbon into ethanol than in those grown without CRS. This result suggests that processes involving CRS utilize more carbon for metabolite synthesis than biomass formation. This indicated that the processes with CRS could utilize more carbon for metabolite synthesis than biomass formation.

1. Introduction

The continuous ethanol fermentation process has more advantages in terms of ethanol concentration and productivity over other fermentation systems. The fermentation efficiency, and specifically productivity, can be improved by increasing the dilution rate. Moreover, continuous fermentation can control product over-formation by adjusting feed sugar concentration and the accumulated ethanol can be removed to avoid its toxicity (Brandberg et al., 2007).

The continuous ethanol production by *Saccharomyces cerevisiae* has been developed for maximizing fermentation efficiency. However, the results obtained from many research works demonstrated low volumetric productivity and incomplete sugar utilization. This may be due to the fact that some of cell population in an effluent is withdrawn from the cultivation system during the continuous fermentation process. It was also reported that high cell density in the fermentation broth during the fermentation process significantly affected product concentration, productivity, as well as sugar consumption (Laopaiboon et al., 2007; Sridee et al., 2011). Therefore, to achieve high ethanol production efficiency, the fermentation system having a high cell concentration should be operated under optimum conditions. One technique to retain the cell concentration during the continuous fermentation process is through use of a cell recycling system (CRS). Generally, an increase in cell concentration can be achieved using a settler that has a high ability to flocculate yeasts or a membrane based cell recycling via a hollow fibre membrane module (Kwon et al., 2001; Tang et al., 2010; Wang et al., 2013). Despite the high operation costs of performing fermentation with a hollow fibre membrane, the high potential to retaining the cell concentration in the reactor makes this cell recycling equipment much more superior than the settler. Continuous fermentation coupled with CRS provides a more convenient way to maintain high cell numbers in the reactor of the fermentation system (Wang et al., 2013).

Typically, yeast growth under aerobic conditions is higher than that under anaerobic conditions, resulting in higher cell concentrations. It was reported that a small amount of aeration during ethanol fermentation improved sugar utilization and ethanol production efficiency (Patrascu et al., 2009; Thani et al., 2014). Also

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reported are the effects of aeration rate during ethanol fermentation, whereby it has been found to be related to biomass, sugar consumption, ethanol, and productivity (Lin et al., 2011).

Redox potential is an important parameter which can be applied to a fermentation process under micro or anaerobic conditions, especially when monitoring the dissolved oxygen level becomes impracticable. Redox potential involves the net balance between oxidizing and reducing powers of an oxidation and reduction reaction (Lin et al., 2010). During the fermentation process, NADH (serving as the electron donor) and dissolved oxygen (serving as an electron acceptor) are the main contributors which influence the changes in redox potential. Further, intracellular activity and dissolved oxygen correlate to the changes in redox potential during fermentation. The effect of controlling redox potential in ethanol fermentation was investigated and results demonstrated an influence on ethanol production, as well as formation of undesired byproducts during fermentation. (Yu et al., 2008; Lin et al., 2010).

Although several researchers have attempted to control the redox potential to ethanol fermentation (Lin et al., 2010; Jeon and Park, 2010; Liu et al., 2011) and to other metabolites (Sridhar and Eiteman, 1999; Kastner et al., 2003; Du et al., 2006; Liu et al., 2010, 2013; Chen et al., 2012), the changes and suitable levels of redox potential are strain and environmental dependent. During microaerobic fermentation, such as in ethanol fermentation, the amount of trace dissolved oxygen in the fermentation broth is a critical determinant for whether the microorganism proceeds with the desired fermentation route to produce the intended fermentation products or biomass. In the previous researches, the levels of redox potential at the end of fermentation period or steady state condition were varied depending on the fermentation condition and microorganism (Lin et al., 2010; Jeon and Park, 2010). However, the relationship between redox potential profile and ethanol fermentation in a continuous process with CRS has not been reported.

A continuous fermentation process with CRS is illustrated in Fig. 1. In general, the process requires cell circulation during the fermentation process. Yeast cells in the fermenter are removed from the fermenter and then recycled through the HF membrane back into the fermenter via a peristaltic pump. The objectives of this experiment were to evaluate and compare the performance of CRS with two hollow fibre (HF) membranes (at two pore sizes, 0.20 and 0.65 μm) during continuous ethanol fermentation by *S. cerevisiae* NP 01 to continuous ethanol fermentation without CRS (the control). A small amount of aeration was also supplied throughout the fermentation to improve fermentation efficiency. The relationships between the redox potential profile, yeast growth, ethanol production, and dilution rate under continuous fermentation at a constant initial glucose concentration of ~230 g/l were investigated. The effects of CRS in a continuous fermentation process on fermentation efficiency and amount of carbon incorporation by yeast were also discussed.

2. Materials and methods

2.1. Microorganism and ethanol production medium

S. cerevisiae NP 01 (Deesuth et al., 2015) was cultivated in a 250 ml Erlenmeyer flask containing 150 ml of pre-culture medium (yeast extract, 10 g/l; sodium glutamate, 1 g/l; and urea, 16 mM) (Lin et al., 2010). The flask was incubated at 30 °C on a rotary shaker set to 150 rpm for 18 h. The ethanol production medium consisted of glucose (230 g/l), yeast extract (10 g/l), sodium glutamate (1 g/l), $MgSO_4 \cdot 7H_2O$ (2 mM), KH_2PO_4 (3.67 mM), urea (16 mM), $(NH_4)_2SO_4$ (1 mM), and various mineral salts (in μM unit) including H_3BO_3, 24; Na_2MoO_4, 1.5; $MnSO_4 \cdot H2O$, 20; $CuSO_4$, 10; KI, 1.8; $FeCl_3 \cdot 6H_2O$, 100; $CaCl_2 \cdot 2H_2O$, 82; $ZnSO_4 \cdot 7H_2O$, 1000 (Lin et al., 2010). The medium was sterilized at 121 °C for 15 min.

2.2. Microfiltration bioreactor and fermentation conditions

The two microfiltration modules (CFP–2-D–4, A/G Technology Corporation, USA and UJP-0047, Pall Corporation, Japan) used for the cell recycling system were composed of HF membranes with pore sizes of 0.20 and 0.65 μm. These modules were then connected to the continuous fermentation system and are termed, 0.20-μm CRS and 0.65-μm CRS. The overall design for continuous ethanol fermentation with CRS is shown in Fig. 1. The fermentation process was done in a jar fermenter (Model: Omni Culture, New York, NY, USA) with a final working volume of 1 l, and the agitation rate was kept at 200 rpm. The fermenter was equipped with an autoclavable oxidation-reduction potential (ORP) electrode (12 mm × 250 mm, Cole-Palmer Inc, Vernon Hills, IL, USA). The redox potential was automatically measured using LabView (Version 8.5, National Instrument, Austin, TX, USA) and recorded periodically throughout the course of fermentation. The system was first carried out in batch mode where the aeration rate of 2.5 vvm was supplied for first 4 h of the fermentation to stimulate yeast growth, and the aeration rate of 0.25 vvm was further provided for the remainder of the fermentation process (Khongsay et al., 2014). Continuous ethanol fermentation was initiated at 18 h by feeding the sterile ethanol production medium into the system at various dilution rates (0.02, 0.04 and 0.08 h^{-1}). During the continuous ethanol fermentation process, the NP 01 strain was recycled to the fermenter with the recycle ratio (α) of 0.625 (equivalent to 50 ml/h) (Thani, 2016). The recycle ratio was calculated from the flow rate of the return stream (F_r) divided by the feed flow rate (F_i) to the fermenter. The samples were collected aseptically at specific time intervals for analysis. Continuous fermentations without CRS at the three dilution rates were used as the control runs.

2.3. Analytical methods

The fermentation broth was sampled every 6–8 h. One portion of the broth was used for the enumeration of the yeast cell number under a microscope. The viable and the dead cells were differentiated using the methylene violet staining procedure (Smart et al., 1999). The pH was measured with a pH meter. The remaining broth was centrifuged at 10,000 rpm for 10 min. The supernatant was analyzed using HPLC (Model: HP 1100 series, Agilent Technologies, Mississauga, ON, Canada) that was equipped with a refractive index (RI) detector (Model: 1200 series from Agilent Technologies) in order to determine the following metabolites: glucose, ethanol, glycerol, trehalose, and acetic acid. An ion exclusion column (Model: ORH–801, Transgenomic, Inc., Omaha, NE, USA) was used to separate the metabolites. The mobile phase was 8.5 mM H_2SO_4, and the flow rate was set at 0.4 ml/min. The temperature of column and RI detector were kept at 65 and 35 °C, respectively.

2.4. Calculation methods

The percentage of cell viability was determined by calculating the number of viable cells divided by total cell number of both viable and dead cells and multiplied by 100. The ethanol productivity (Q_P, g/l-h) was calculated by the ethanol produced (g/l) under steady state condition multiplied by the dilution rate (h^{-1}). The ethanol yield ($Y_{P/S}$, g/g) was calculated as the actual ethanol produced (g/l) divided by the sugar utilized (g/l) under steady state condition. The fermentation efficiency (%) was defined as the actual ethanol yield divided by the theoretical ethanol yield (0.51 g ethanol/g glucose) and multiplied by 100. To calculate the effects of CRS on continuous ethanol production (%), the difference