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Improvement of ethanol production from sweet sorghum juice under high gravity and very high gravity conditions: Effects of nutrient supplementation and aeration

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ABSTRACT

Ethanol production by *Saccharomyces cerevisiae* NP 01 from sweet sorghum juice was conducted under nutrient supplementation and/or aeration conditions. Yeast extract (YE) and dried spent yeast (DSY) were used as nitrogen supplements. Under high gravity (HG, ~200 g l^{-1} of total sugar) conditions, the addition of either 6 g l^{-1} of YE or 9 g l^{-1} of DSY did not increase the ethanol concentration (*P*), but it promoted the rate of ethanol production or ethanol productivity (Q_p). The Q_p value of the control treatment (no supplement) was 1.96 g l^{-1} h^{-1}. The *P* (93.4–94.0 g l^{-1}) and Q_p (3.89–3.92 g l^{-1} h^{-1}) values under the YE and DSY supplementation were comparable, indicating that DSY could be used to replace YE in ethanol production. Under very high gravity (VHG, ~280 g l^{-1} of total sugar) conditions, the supplementation of nitrogen and trace elements (Zn^{2+}, Mg^{2+} and Mn^{2+}) coupled with the aeration supply were required to improve the ethanol production efficiency. Under the supplementation (in g l^{-1}) of DSY (13.5), Zn^{2+} (0.01), Mg^{2+} (0.05) and Mn^{2+} (0.04) in the presence of aeration supply at 0.05 vvm for 12 h, the *P* and Q_p values were 126.3 g l^{-1} and 2.11 g l^{-1} h^{-1}, respectively. The *P* and Q_p values under the same supplementation without aeration were 114.8 g l^{-1} and 1.91 g l^{-1} h^{-1}, respectively, and these values under no supplementation and no aeration were 108.0 g l^{-1} and 1.50 g l^{-1} h^{-1}, respectively. In addition, glycerol (the main by-product during ethanol fermentation) under aeration conditions was ~3 g l^{-1} lower than that without aeration.

1. Introduction

Bioethanol from agricultural raw materials as the feedstock has become interesting as an alternative energy source to petroleum-based fuels, because it is both renewable and environmentally friendly. In Thailand, the main raw materials for ethanol fermentation are sugarcane molasses and tapioca starch (Nguyen and Gheewala, 2008; Laopaiboon et al., 2009) However, these raw materials are becoming insufficient due to the need for many ethanol plants. Recently, sweet sorghum (*Sorghum bicolor* {L.} Moench) has been considered as an alternative promising source for ethanol production because the juice from its stalks contains high amounts of fermentable sugar and a lot of trace elements that are essential for yeast growth and ethanol fermentation (Laopaiboon et al., 2009). In addition, it can be cultivated at a wide range of ambient temperature, including tropical climate areas (Sree et al., 1999). Moreover, sweet sorghum has other advantages such as short time to maturity which is in between 90 and 120 days, lower requirement for fertiliser, high water utilisation efficiency (1/3 of sugarcane and 1/2 of corn), and higher tolerance to salinity and drought comparing to sugarcane and corn (Gnansounou et al., 2005; Ratnavathi et al., 2010; Wu et al., 2010).

Typically, initial sugar concentrations used in fuel ethanol industry are under normal gravity (NG, less than 180 g l^{-1} of total sugar) and high gravity (HG, 180–220 g l^{-1} of total sugar) conditions (Bai et al., 2008). To increase ethanol fermentation efficiency, very high gravity (VHG) technology has been introduced because it can improve ethanol productivity, resulting in increased cost effectiveness. This technology involves the preparation and fermentation of mashes containing over 250 g l^{-1} of total sugar (Bai et al., 2008). High levels of ethanol production under VHG conditions can reduce capital costs as well as energy costs per litre of ethanol. In addition, the risk of bacterial contamination is minimal. However, the fermentation under high sugar content or VHG

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conditions may cause adverse effects on yeast metabolism because of high osmotic pressure and high ethanol concentrations produced (Pratt-Marshall et al., 2003). In industrial ethanol production, *Saccharomyces cerevisiae* is the main ethanol-producing organism (Siqueira et al., 2008). It can ferment under high sugar concentrations when adequate amounts of essential nutrients are provided (Reddy and Reddy, 2006).

To obtain high efficiency of ethanol production by yeast, many factors such as nitrogen, trace elements, vitamins and/or aeration on ethanol production have been investigated. Nitrogen is one of the essential nutrients for ethanol fermentation, especially under VHG conditions (Bai et al., 2008). It promotes yeast growth, the rate of ethanol production as well as ethanol tolerance (Bafrncová et al., 1999). Yeast extract (YE) is widely used in laboratory-scale studies as a nitrogen source for yeast growth and a nutrient supplement in ethanol fermentation (Laopaiboon et al., 2009; Khongsay et al., 2012). However, YE is not appropriate for industrial ethanol production due to its high cost. Thus, it is important to exploit low-cost nitrogen sources to provide nutritional requirements for yeast growth and fermentation. Several authors have continuously investigated low-cost nitrogen sources for the improvement of yeast growth and ethanol production under VHG fermentation, such as horse gram (*Dolichos biflorus*) flour, finger millet (*Eleusine coracana* L.) flour (Reddy and Reddy, 2005, 2006), corn steep liquor (Pereira et al., 2010) and fresh yeast autolysate (Jones and Ingledew, 1994). In this study, we are interested in a by-product from the Brewery industry, dried spent yeast (DSY), which is composed of high nitrogen and many essential trace elements (Sridee et al., 2011). Therefore, it may be used as a low-cost nutrient supplement instead of YE for industrial ethanol fermentation. Apart from carbon and nitrogen sources, trace elements such as zinc (Zn), magnesium (Mg) and manganese (Mn) have been reported to promote the sugar conversion rate and are required for several metabolic pathways as cofactors, resulting in enhanced ethanol tolerance and ethanol production particularly, under VHG fermentation (Wang et al., 2007; Palukurty et al., 2008; Xue et al., 2008; Zhao et al., 2009; Pereira et al., 2010).

Normally, ethanol can be produced via the glycolysis pathway under anaerobic conditions. However, several authors have observed that a small amount of aeration during ethanol fermentation is required to improve sugar utilisation and ethanol production efficiency under VHG conditions (Alfenore et al., 2004; Patrascu et al., 2009; Breisha, 2010). The amount of aeration required is dependent on various factors, e.g. yeast strains, nutrient availability and fermentation processes.

Therefore, the aim of this study was to compare and improve the efficiency of ethanol production using an ethanol-producing isolate or yeast NP 01 under HG and VHG fermentations from sweet sorghum juice by nitrogen supplementations (YE and DSY) coupling with the metals (Zn, Mg and Mn). The influence of aeration on ethanol production was also investigated.

2. Materials and methods

2.1. Microorganism and inoculum preparation

The yeast NP 01 isolate was isolated from dried starter used for Sato (Thai rice wine) from Nakhon Phanom province, Thailand. Preculture was carried out in yeast extract malt extract (YM) medium (Khongsay et al., 2012). The culture was incubated at 150 rev min^{-1}, 30 °C for 20 h. Subsequently, the yeast (10% inoculum size) was transferred into sweet sorghum juice containing 150 g l^{-1} of total sugar and incubated under the same conditions for 15 h.

2.2. Identification of the yeast isolate

Identification of the yeast NP 01 isolate was conducted using D1/D2 domain of 26S rDNA gene sequencing analysis (O'Donnell, 1993). Genomic DNA was isolated from yeast cells using the method modified from Harju et al. (2004). The gene was amplified by the PCR using DreamTaq polymerase (Fermentas, USA) and the primer pairs NL1 (5′GCATATCAATAAGCGGAGGAAAAG-3′) and NL4 (5′-GGTCCGTGTTTCAAGAC GG-3′) (O'Donnell, 1993), and genomic DNA isolated from yeast cells as the template. The PCR products were analysed by agarose gel electrophoresis on a 1% agarose gel and purified using the GF-1 AmbiClean Kit (Vivantis, USA). All procedures for DNA amplification and purification were carried out according to the manufacturers' instructions. DNA sequencing was performed by the First BASE Laboratories Sdn Bhd (Seri Kembangan, Selangor Darul Ehsan, Malaysia). The D1/D2 sequence was compared to the sequences of related species retrieved from the NCBI database using BLASTN. Phylogenetic analysis was performed using the neighbour-joining method with the program MEGA4 (Tamura et al., 2007) and the bootstrap analysis based on 1000 replicates.

2.3. Raw material and nutrient supplementation

Sweet sorghum (cv. KKU40) was obtained from the Department of Plant Science and Agricultural Resources, Faculty of Agriculture, Khon Kaen University, Thailand. The juice was extracted from its stalks by sugarcane juice extractor, and was kept at −18 °C until use.

The nutrient supplements used in this study were YE (Himedia laboratory, India), dried spent yeast (DSY, a by-product from brewery industry), $ZnSO_4 \cdot 7H_2O$ (analytical grade, BDH, England), $MgSO_4 \cdot 7H_2O$ (analytical grade, BDH, England) and $MnSO_4 \cdot H_2O$ (analytical grade, BDH). The DSY was donated from Beerthip Brewery (1991) Co., Ltd., Phra Nakhon Sri Ayutthaya, Thailand.

2.4. Ethanol production medium

Total soluble solids of raw sweet sorghum juice was adjusted from of 17° Bx to 20° Bx (HG conditions) and 28° Bx (VHG conditions) by the addition of sucrose (Laopaiboon et al., 2009), corresponding to total sugar concentrations of ~200 and 280 g l^{-1}, respectively. The juices were used as the ethanol production (EP) media, namely HG and VHG media, respectively.

2.5. Experiments

2.5.1. Effects of nitrogen supplementation on ethanol fermentation under HG conditions

The HG medium was added with 3 and 6 g l^{-1} of YE, and designated as YE3 and YE6, respectively; or supplemented with 4.5 and 9 g l^{-1} of DSY, and designated as DSY4.5 and DSY9, respectively. According to nitrogen content in YE and DSY, the total nitrogen of 1.5 g of DSY was equivalent to that of 1 g of YE (Chan-u-tit et al., 2013). The EP medium (400 ml) was transferred into a 500-ml air-locked Erlenmeyer flask and autoclaved at 110 °C for 28 min (Laopaiboon et al., 2009).

2.5.2. Effects of combination of nutrient supplementation and aeration on ethanol fermentation under VHG conditions

The VHG medium was added with 13.5 g l^{-1} of DSY (the same total nitrogen content as found in 9 g l^{-1} of YE), 0.01 g l^{-1} of Zn, 0.05 g l^{-1} of Mg and 0.04 g l^{-1} of Mn (Deesuth et al., 2012) before sterilisation. The fermentations were carried out in the 500-ml air-locked flask and a 2-l fermenter (Biostat®B, B. Braun Biotech, Melsungen, Germany). Aeration was supplied into the VHG