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Improvement of very-high-gravity ethanol fermentation from sweet sorghum juice by controlling fermentation redox potential

Naulchan Khongsay [a], Yen-Han Lin [b,*], Pattana Laopaiboon [c,d], Lakkana Laopaiboon [c,d,**]

[a] Graduate School, Khon Kaen University, Khon Kaen 40002, Thailand
[b] Department of Chemical and Biological Engineering, University of Saskatchewan, Saskatoon, SK S7N5A9, Canada
[c] Department of Biotechnology, Faculty of Technology, Khon Kaen University, Khon Kaen 40002, Thailand
[d] Fermentation Research Center for Value Added Agricultural Products, Khon Kaen University, Khon Kaen 40002, Thailand

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ABSTRACT

Effects of fermentation redox potential control (no control, −100 mV and −150 mV) on the improvement of ethanol productivity during very-high-gravity (VHG) fermentation from sweet sorghum juice (300 g/l of total sugar) were investigated. Results showed that redox potential controlled at −150 mV gave the highest ethanol production efficiencies (ethanol concentration or *P* value, 134.35 ± 1.67 g ethanol 1/l and ethanol productivity or Q_p value, 2.80 ± 0.03 g ethanol 1/l h) at the fermentation time of 48 h. Under the same condition without the redox potential control, the *P* and Q_p values were 115.34 ± 2.01 g/l and 2.14 ± 0.05 g/l h, respectively at the fermentation time of 54 h. To complete sugar utilization under redox potential controlled at −150 mV, 16 mM urea was supplemented into the VHG medium. Results showed that the *P* and Q_p values were 140.23 ± 1.88 g/l and 2.92 ± 0.01 g/l h, respectively corresponding to sugar utilization of 96%. When the initial total sugar in the juice was reduced to 250 g/l, no urea addition was required in order to complete sugar utilization, and the *P* and Q_p values were 121.54 ± 2.23 g/l and 3.38 ± 0.04 g/l h, respectively at the fermentation time of 36 h. These results clearly indicated that redox potential control coupling with supplementation of assimilable nitrogen levels significantly improved the ethanol production efficiency from the sweet sorghum juice under the VHG fermentation by *Saccharomyces cerevisiae* NP 01.

1. Introduction

Bioethanol is a clean and efficient energy and extensively accepted as a potential substitute for fossil fuels [1,2]. One of the main purposes of using bioethanol as an alternative fuel is to reduce CO_2 emission. It was reported that the use of bioethanol as a replacement for gasoline could reduce CO_2 emission from vehicles by 90% [3]. Nowadays, bioethanol is produced from agricultural biomass by fermentation of microorganisms. In Thailand, there are only two raw materials used for industrial ethanol production, *i.e.*, sugarcane molasses and cassava. These two materials are also used as substrates for many industries. According to the 15 year plan and target of biofuel development (year 2008–2022) of the Thai Government, the ethanol production is aimed at 3, 6.2 and 9.0 M l/day by year 2011, 2016 and 2022, respectively [4]. Therefore, Thailand may encounter a deficiency of the two raw materials for ethanol production.

Sweet sorghum [*Sorghum bicolor* (L.) Moench] is an attractive alternative energy crop for bioethanol production because its stalks contain a large amount of fermentable sugars (sucrose, fructose and glucose), and it can be cultivated at almost all temperatures including tropical climate areas [5]. Additionally, the juice squeezed from its stalks contains many essential trace elements for yeast growth and ethanol production [6]. In Thailand, sweet sorghum is mostly used as feed and the plantation area is still limited. However, Thai farmers have been encouraged to plant the sweet sorghum after harvesting sugarcane and cassava (growing season of sugarcane and cassava plantation is November–April). The highest yield of sweet sorghum is during May–October and the average yield of sweet sorghum cultivar KKU40 is about 15–25 dry ton/ha [7], which is comparable to that (20–30 dry ton/ha) reported by Wu et al. [8]. In addition, most areas for sugarcane and cassava plantation (in the northeastern and central parts) are located near ethanol plants. Currently, there are not enough raw materials for industrial ethanol production in out of growing season (May–October). Hence, if the farmers grow sweet

* Corresponding author. Tel.: +1 306 966 4764; fax: +1 306 966 4777.
** Corresponding author at: Department of Biotechnology, Faculty of Technology, Khon Kaen University, Khon Kaen 40002, Thailand. Tel.: +66 4336 2121; fax: +66 4336 2121.
E-mail addresses: naul_khong@hotmail.com (N. Khongsay), yenhan.lin@usask.ca (Y.-H. Lin), patlao@kku.ac.th (P. Laopaiboon), lakcha@kku.ac.th (L. Laopaiboon).

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sorghum during this period, the problem of raw material shortage can be alleviated.

The use of very-high-gravity (VHG) fermentation technology for bioethanol production can markedly increase ethanol concentrations and reduce energy consumption of distillation process [9,10]. The raw sweet sorghum juice cultivar KKU40 contains 170–180 g/l of total sugar. Therefore, it has to be concentrated to use for the VHG fermentation. In addition, the concentration process can reduce storage problem in terms of area for raw material storage and prevention bacterial contamination. During VHG fermentation, a decrease in yeast population is common, resulting in incompleteness of sugar utilization and decreasing fermentation rate [11]. However, it was reported that *Saccharomyces cerevisiae* could produce and tolerate high levels of ethanol under appropriate environmental and nutritional conditions [12–15].

It was observed that under VHG fermentation process the growth of *S. cerevisiae* was promoted and prolonged when low levels of oxygen was present and the assimilable nitrogen levels were not limited [6,16,17]. The aeration supply at low levels to improve ethanol tolerance of yeast cells under VHG fermentation has been discussed [18,19]. Yeast normally requires oxygen for lipid synthesis (sterols and unsaturated fatty acids), which are essential for plasma membrane integrity [20–22]. Additionally, the supplementation of free amino nitrogen (FAN) leads to higher final ethanol concentration [23] as these assimilable nitrogens promotes yeast growth and multiplication [1].

Ethanol fermentation by *S. cerevisiae* is facultative to nearly anaerobic fermentation process wherein dissolved oxygen (DO) level is so low that a DO electrode becomes insensitive to changes in DO concentration in the fermenter. In contrast, redox (*i.e.*, reduction–oxidation) potential is the net outcome between reducing and oxidizing powers within a system. Biologically, it reflects the momentary metabolic status of oxidization or reduction of yeast cells in a fermentation process. Both NADH (electron donor) and DO (electron acceptor) resulting from agitation or sparging, are the major contributors to the change of redox potential [24].

The aim of this study is to investigate the feasibility of applying redox potential control and locally screened yeast strain (*S. cerevisiae* NP 01) to the locally grown sweet sorghum for enhancing bioethanol production in terms of sugar utilization and ethanol concentration in VHG fermentation. The use of the raw material readily available near the ethanol plant sites not only lowers the transportation cost (raw material), but also minimizes CO_2 emission.

2. Materials and methods

2.1. Microorganism and inoculum preparation

S. cerevisiae NP 01 isolated from Loog-pang (Chinese yeast cake) from Nakhon Phanom province, Thailand was inoculated into 100 ml of the sweet sorghum juice (100 g/l of total sugar) supplemented with 1.0% yeast extract and 16 mM urea. Then, it was incubated on a rotating shaker at 150 rpm, 32 °C for 12 h. The active cells were harvested and used as an inoculum for ethanol production.

2.2. Raw materials

Sweet sorghum juice extracted from its stalks (cv. KKU 40) was obtained from Department of Plant Science and Agricultural Resources, Faculty of Agriculture, Khon Kaen University, Thailand. The juice (18 °Bx of total soluble solids) was concentrated to 74 °Bx and stored at 4 °C prior to use.

2.3. Ethanol production medium

The concentrated sweet sorghum juice was diluted to the desired total sugar concentrations (300 and 250 g/l). Then the juice was supplemented with 9 g/l of yeast extract, and it was used as an ethanol production (EP) medium. The EP medium for testing excess assimilable nitrogen was added with 16 mM urea (as much as 40% nitrogen composition containing in the yeast cell). The EP medium was transferred into a 2-l fermenter with a final working volume of 1 l and autoclaved at 121 °C for 15 min.

2.4. Fermentation conditions

The batch ethanol fermentation was controlled at 32 °C and the agitation rate of 150 rpm. The initial yeast cell concentration in the sterile EP medium was $\sim 2 \times 10^7$ cells/ml for all experiments. The fermentation was operated in batch mode. The fermenter was equipped with an autoclavable ORP electrode that was custom-made and ordered through Cole-Palmer Inc. (12 mm × 250 mm Vernon Hills, IL, USA). Samples were withdrawn at time intervals (6–12 h) for analysis. Redox potentials were acquired by using Labview (Version 8.5, national instrument, Austin, TX, USA), and the proportional integrate derivate (PID) control algorithm was implemented to maintain redox potential at a desired level by adjusting aeration rate, where air was pre-filtered through 0.2 μm membrane. The planned redox potential levels were −100, −150 mV, and without redox potential control. When the measured redox potential was lower than the planned level, the sterile air (0.82 vvm) was supplied to the fermenter [16], and the required amount of air was determined by the PID algorithm. The ethanol fermentation without redox potential control was also carried out as the control treatment. All experiments were performed in triplicate.

2.5. Sample and data analyses

The viable yeast cell numbers in the fermentation broth was determined by direct counting method using haemacytometer with methylene blue staining [25]. The residual total sugars in the broth were determined in terms of total carbohydrate by phenol sulfuric acid method [26]. Ethanol concentration (P, g/l) was analyzed by gas chromatography using 23-DB column (J&W Scientific, Canada, 60 m × 0.25 mm × 0.25 μm) with helium as carrier gas and flame ionization detection (Agilent, 7890A) at the following conditions: injection temperature, 200 °C; initial oven temperature, 70 °C; flame ionization detector temperature, 225 °C. The ethanol yield ($Y_{p/s}$) was calculated and expressed as g ethanol produced per g total sugar utilized (g/g), and the ethanol productivity (Q_p, g/l h) was calculated by ethanol concentration produced (P, g/l) divided by fermentation time. Percentage of sugar utilization was calculated as the actual total sugar utilized divided by the initial total sugar concentration and multiplied by 100. The calculated results were expressed as mean ± SD, and statistical analysis was carried out using SPSS 17.0 for Windows.

3. Results and discussion

3.1. VHG ethanol fermentation under different redox potential levels

The time profiles of pH, viable cell, redox potential, total sugar and ethanol concentrations during batch fermentation under redox potential control at −100 mV and −150 mV are shown in Fig. 1. The pH of the medium was relatively constant in the range of 4.20–4.46 (Fig. 1A) which was in the optimum range for yeast growth and ethanol production [27]. The pH changes during the course of fermentation in all conditions were similar indicating