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Research article

Improvement of ethanol production from sweet sorghum juice under batch and fed-batch fermentations: Effects of sugar levels, nitrogen supplementation, and feeding regimes

Niphaphat Phukoetphim [a], Apilak Salakkam [b,c], Pattana Laopaiboon [b,c], Lakkana Laopaiboon [b,c,*]

[a] Graduate School, Khon Kaen University, Khon Kaen 40002, Thailand
[b] Department of Biotechnology, Faculty of Technology, Khon Kaen University, Khon Kaen 40002, Thailand
[c] Fermentation Research Center for Value Added Agricultural Products, Khon Kaen University, Khon Kaen 40002, Thailand

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ABSTRACT

Background: Fermentation process development has been very important for efficient ethanol production. Improvement of ethanol production efficiency from sweet sorghum juice (SSJ) under normal gravity (NG, 160 g/L of sugar), high gravity (HG, 200 and 240 g/L of sugar) and very high gravity (VHG, 280 and 320 g/L of sugar) conditions by nutrient supplementation and alternative feeding regimes (batch and fed-batch systems) was investigated using a highly ethanol-tolerant strain, *Saccharomyces cerevisiae* NP01.
Results: In the batch fermentations without yeast extract, HG fermentation at 200 g/L of sugar showed the highest ethanol concentration (P_E, 90.0 g/L) and ethanol productivity (Q_E, 1.25 g/L·h). With yeast extract supplementation (9 g/L), the ethanol production efficiency increased at all sugar concentrations. The highest P_E (112.5 g/L) and Q_E (1.56 g/L·h) were observed with the VHG fermentation at 280 g/L of sugar. In the fed-batch fermentations, two feeding regimes, *i.e.*, stepwise and continuous feedings, were studied at sugar concentrations of 280 g/L. Continuous feeding gave better results with the highest P_E and Q_E of 112.9 g/L and 2.35 g/L·h, respectively, at a feeding time of 9 h and feeding rate of 40 g sugar/h.
Conclusions: In the batch fermentation, nitrogen supplementation resulted in 4 to 32 g/L increases in ethanol production, depending on the initial sugar level in the SSJ. Under the VHG condition, with sufficient nitrogen, the fed-batch fermentation with continuous feeding resulted in a similar P_E and increased Q_P by 51% compared to those in the batch fermentation.

1. Introduction

Bioethanol is an alternative energy source that is both renewable and environmentally friendly. It can be produced from agricultural raw materials such as corn grain, cassava, sugar cane, sugar cane molasses, and sweet sorghum, among others. Sweet sorghum, *Sorghum bicolor* (L.) Moench, is a potential alternative feedstock for bioethanol production because the juice from its stalks contains high levels of fermentable sugars, mainly sucrose, fructose, and glucose, and it has short life cycle of only 100–120 d. Moreover, it can be cultivated at almost all temperatures in tropical areas [1,2].

Saccharomyces cerevisiae is widely used in industrial ethanol production [3]. In addition to yeast strains, nutrients, and environmental conditions, the ability of yeast to produce ethanol also depends on the initial sugar concentration of the fermentation medium. In ethanol fermentation, 1 mol of glucose can be converted to 2 mol of ethanol and 2 mol of carbon dioxide. Therefore, a medium containing a higher sugar concentration will give a higher ethanol concentration. Typically, sugar concentrations for ethanol fermentation are divided into normal gravity (NG) (<180 g/L of sugar), high gravity (HG) (180–240 g/L of sugar), and very high gravity (VHG) conditions (≥250 g/L of sugar) [4,5]. However, high sugar concentrations or VHG conditions cause an increased osmotic pressure, which has negative effects on yeast cells. Bafrncovà et al. [6] reported that under appropriate environmental and nutritional conditions, *S. cerevisiae* could produce and tolerate high ethanol concentrations.

Fermentation process development has been very important for efficient ethanol production [7,8]. Ethanol fermentation can be performed in batch, fed-batch, and continuous modes. The batch fermentation is a closed culture system. Biomass and substrate are added into fermenter without removal of media during fermentation, and products are harvested at the end of the fermentation. The batch mode has disadvantages, particularly when microorganisms are either slow growing or strongly affected by substrate inhibition [9]. The

* Corresponding author.
E-mail address: lakcha@kku.ac.th (L. Laopaiboon).
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fed-batch mode is started as a batch mode with a small amount of biomass and substrate in the fermenter. Then, a feeding medium is fed, stepwise or continuously, to the fermenter when most of the initially added substrate has been consumed. This process can increase the total substrate content in the fermenter while maintaining a low substrate concentration during fermentation to reduce the negative effects of osmotic pressure on yeast. The advantages of this process include reduction of substrate inhibition, higher productivity, shortened fermentation time, and reduction of toxic effects of the medium components, which are present at high concentrations [10]. Stepwise feeding of fed-batch fermentation was previously demonstrated to be effective in enhancing ethanol production and yield from sweet sorghum juice (SSJ) under HG conditions [8]. In the current study, stepwise and continuous feedings were examined under VHG conditions to determine if these regimes could enhance fermentation efficiency at very high initial sugar concentrations.

Ethanol produced by yeast is toxic to the yeast itself. To achieve high-level ethanol production, yeast strains that can produce and tolerate high ethanol concentration should be used. *S. cerevisiae* NP01 and *S. cerevisiae* ATCC 4132 are considered robust ethanol-producing strains because of their ability to produce high ethanol titers under HG and VHG conditions [2,11]. However, their ethanol tolerance has not been examined. In the current study, the ability of these yeast strains to tolerate ethanol at various concentrations was tested. Improvement of ethanol production efficiency from SSJ under NG, HG, and VHG conditions by nutrient supplementation and alternative feeding regimes (batch and fed-batch systems) was subsequently investigated.

2. Materials and methods

2.1. Microorganisms

S. cerevisiae NP01 (accession number KP866701) was isolated from Loog-pang (Chinese yeast cake) for Sato (Thai rice wine) making and was identified by gene sequencing analysis using the D1/D2 domain of 26S rDNA [5], and *S. cerevisiae* ATCC 4132 was isolated from molasses distillery yeast. The yeasts were inoculated into 100 mL of yeast extract and malt extract (YM) medium (containing yeast extract, 3 g/L; malt extract, 3 g/L; peptone, 5 g/L; and glucose, 10 g/L) and incubated at 200 rpm and 30°C for 18 h. Then, the cultures (10% inoculum size) were transferred into 350 mL of SSJ containing 100 g/L of sugar [12] and incubated under the same conditions. After 15 h, the cells were harvested and used as inocula for ethanol fermentations.

2.2. Raw materials and ethanol production medium

Sweet sorghum *cv*. KKU40 was obtained from the Division of Agronomy, Faculty of Agriculture, Khon Kaen University, Thailand. To prevent bacterial contamination and improve storage stability after extraction, the juice (17 °Bx) was heated to approximately 90°C to concentrate to 65 °Bx, cooled, and stored at 4°C until use. It was diluted with distilled water to 160, 200, 240, 280, and 320 g/L of sugar and optionally supplemented with 9 g/L of yeast extract [13] before use as an ethanol production (EP) medium.

2.3. Ethanol tolerance

S. cerevisiae NP01 or *S. cerevisiae* ATCC 4132 was inoculated into 50 mL of SSJ containing 100 g/L of sugar to attain an initial cell concentration of ~5×10^7 cells/mL. Then ethanol was added to the cultures at 0, 6, 9, 12, 15, and 18% (v/v). The setup was incubated at 30°C and 100 rpm for 24 h. The yeast viability was measured at regular time intervals. The yeast strain that showed higher ethanol tolerance was used in subsequent experiments. Q4

2.4. Batch ethanol fermentation

EP media with and without 9 g/L of yeast extract were transferred into 500-mL air-locked Erlenmeyer flasks with a working volume of 400 mL and autoclaved at 110°C for 28 min [2]. The active cells of the more ethanol-tolerant strain were inoculated into sterile EP media to obtain an initial cell concentration of ~5×10^7 cells/mL. The fermentation was performed at 30°C with an agitation rate of 100 rpm. The samples were withdrawn at regular time intervals for analyses.

2.5. Fed-batch ethanol fermentation

Two feeding regimes for the fed-batch fermentation were used under VHG conditions. The first regime was stepwise feeding. Here, the fermentation was first performed in batch mode with sterile EP medium using 50% of the total working volume [8,14]. After 12 or 24 h, an equal volume of fresh sterile EP medium was carefully added into the flasks. The second regime was continuous feeding. Here, the other half of fresh EP medium was fed continuously at flow rates of 1X (10 g sugar/h), 2X (20 g sugar/h), and 4X (40 g sugar/h) to achieve final total sugar concentrations in the range of a VHG condition.

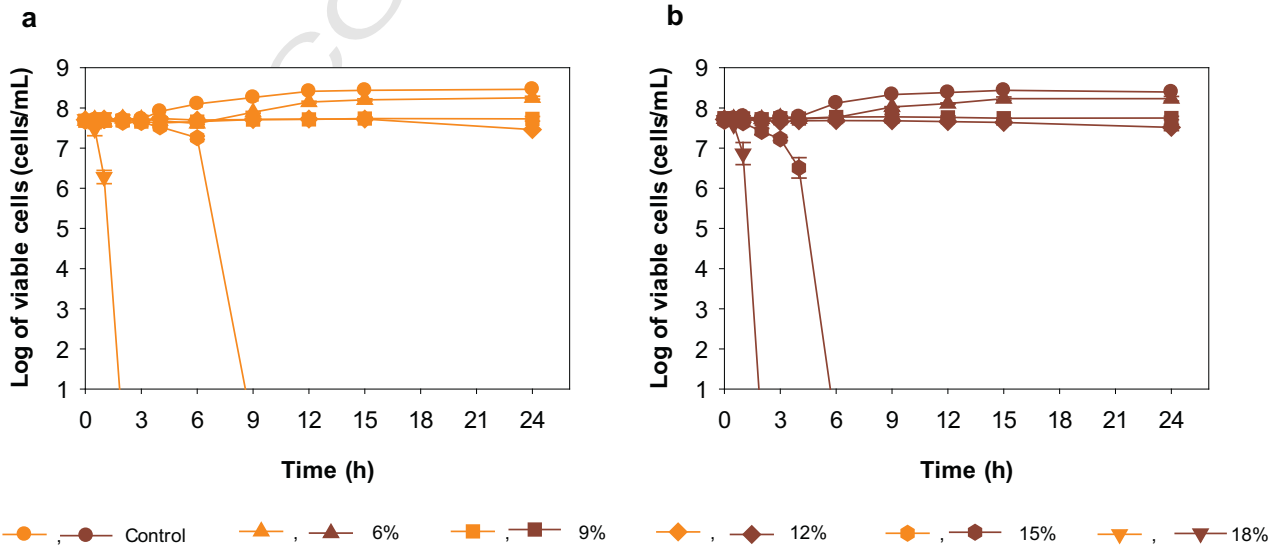

Fig. 1. Time profiles of cell survival of *S. cerevisiae* NP01 (a) and *S. cerevisiae* ATCC 4132 (b) in the presence of ethanol at different concentrations.