รายงานวิจัยฉบับสมบูรณ์

โครงการ การผลิตเอทานอลจากน้ำคั้นลำต้นข้าวฟ่างหวานโดยใช้เทคโนโลยี VHG

Ethanol production from sweet sorghum stem juice using very high gravity (VHG) technology

โดย รศ. ดร. ลักขณา เหล่าไพบูลย์

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มิถุนายน 2551

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สนับสนุนโดยสำนักงานกองทุนสนับสนุนการวิจัย

กิตติกรรมประกาศ

งานวิจัยนี้สำเร็จลุล่วงลงได้ผู้วิจัยใคร่ขอขอบคุณสำนักงานกองทุนสนับสนุนการวิจัย ที่ให้การสนับสนุนงบประมาณในการทำวิจัย

ขอขอบพระคุณ รองศาสตราจารย์ ดร. เพ็ญจิตร ศรีนพคุณ ภาควิชาวิศวกรรมเคมี คณะวิศวกรรมศาสตร์ มหาวิทยาลัยเกษตรศาสตร์ ที่ให้ความกรุณาเป็นนักวิจัยที่ปรึกษาของโครงการวิจัย

ขอขอบพระคุณ ผู้ช่วยศาสตราจารย์ ดร. ไพบูลย์ ด่านวิรุทัย ที่ให้ความอนุเคราะห์ยีสต์ *Saccharomyces cerevisiae* NP 01

ขอขอบพระคุณ รองศาสตราจารย์ ดร. ประสิทธิ์ ใจศิล ที่ให้ความอนุเคราะห์ในการจัดหาวัตถุดิบที่ใช้ในการวิจัย

ขอขอบพระคุณ รองศาสตราจารย์ ดร. พัฒนา เหล่าไพบูลย์ ที่ให้คำปรึกษาเกี่ยวกับวิธีวิเคราะห์โดยเครื่อง Gas Chromatography และ High Performance Liquid Chromatography และเป็นกำลังใจให้ตลอด

ขอขอบคุณ นายสุนันท์ นวลเพ็ง นางสาวประภาพันธ์ ศิริขันธ์แสง นักศึกษาปริญญาโท ภาควิชาเทคโนโลยีชีวภาพ คณะเทคโนโลยี มหาวิทยาลัยขอนแก่น ที่ช่วยเหลือในการทำวิจัย

และขอขอบคุณภาควิชาเทคโนโลยีชีวภาพ คณะเทคโนโลยี มหาวิทยาลัยขอนแก่น และศูนย์วิจัยการหมักเพื่อเพิ่มมูลค่าผลิตภัณฑ์ทางการเกษตร มหาวิทยาลัยขอนแก่น ที่ได้เอื้อเฟื้อสถานที่และอุปกรณ์ เครื่องมือทั่วไป และเครื่องมือวิเคราะห์ต่าง ๆ ที่ใช้ในงานวิจัย

บทคัดย่อ

รหัสโครงการ : MRG4980199

ชื่อโครงการ : การผลิตเอทานอลจากน้ำคั้นลำต้นข้าวฟ่างหวานโดยใช้เทคโนโลยี VHG

ชื่อนักวิจัย : รศ. ดร. ลักขณา เหล่าไพบูลย์ มหาวิทยาลัยขอนแก่น

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ระยะเวลาโครงการ : 2 ปี (1 กรกฎาคม 2549 ถึง 30 มิถุนายน 2551)

การคัดเลือกและประเมินความสามารถของ *Saccharomyces cerevisiae* 3สายพันธุ์ ที่สามารถผลิตเอทานอลได้สูง) TISTR 5048, TISTR 5339 และ NP 01) ในการหมักเอทานอลจากอาหารสังเคราะห์ภายใต้สภาวะ VHG ที่อุณหภูมิ 30 องศาเซลเซียส พบว่า สายพันธุ์ NP 01 ที่เลี้ยงในน้ำตาลกลูโคส 280 กรัมต่อลิตร ให้ความเข้มข้นของเอทานอลสูงสุดคือ 0.11 ± 104.68 กรัมต่อลิตร เมื่อใช้ *S. cerevisiae* NP 01 หมักเอทานอลจากน้ำคั้นลำต้นข้าวฟ่างหวานที่แปรผันแหล่งไนโตรเจน)ยีสต์เอ็กซ์แทร็กท์ร่วมกับเปปโตน หรือแอมโมเนียมซัลเฟต (และแหล่งคาร์บอนเสริม (น้ำตาลทราย หรือกากน้ำตาล) เพื่อเติมลงในน้ำคั้นให้ได้ปริมาณของแข็งที่ละลายได้ถึงระดับ VHG โดยหมักในขวดรูปชมพู่ที่ป้องกันอากาศเข้าขนาด 500 มิลลิลิตร ที่สภาวะนิ่ง พบว่า การใช้น้ำตาลทรายเสริมในน้ำคั้นให้มีปริมาณของแข็งที่ละลายได้ 28 องศาบริกซ์ และเติมยีสต์เอ็กซ์แทร็กท์ 3 กรัมต่อลิตร และเปปโตน 5 กรัมต่อลิตร ได้ประสิทธิภาพการผลิตเอทานอลสูงสุด คือได้ความเข้มข้น อัตราผลผลิต และประสิทธิภาพการหมักเอทานอล เป็น 0.54 ± 120.68 กรัมต่อลิตร 0.01 ± 2.01 กรัมต่อลิตรต่อชั่วโมง และ 0.20 ± 93.76 เปอร์เซ็นต์ ตามลำดับ การแปรผันความเข้มข้นของยีสต์เอ็กซ์แทร็กท์ (6 ,3 ,0 และ 9 กรัมต่อลิตร) ในน้ำคั้นลำต้นข้าวฟ่างหวานที่มีปริมาณของแข็งที่ละลายได้ 28 องศาบริกซ์ เพื่อใช้ผลิตเอทานอลพบว่า น้ำคั้นที่เติมยีสต์เอ็กซ์แทร็กท์ 9 กรัมต่อลิตร ให้ประสิทธิภาพการผลิตเอทานอลสูงสุด คือได้ความเข้มข้น อัตราผลผลิต และประสิทธิภาพการหมักเอทานอล เท่ากับ ± 120.24 3.35กรัมต่อลิตร 0.08 ± 3.01 กรัมต่อลิตรต่อชั่วโมง และ 0.55 ± 91.37 เปอร์เซ็นต์ ตามลำดับ เมื่อเพิ่มขนาดการหมักเอทานอลจากน้ำคั้นลำต้นข้าวฟ่างหวานที่เติมยีสต์เอ็กซ์แทร็กท์ 9 กรัมต่อลิตร เป็น 5ลิตร และ 50 ลิตร และหมักภายใต้สภาวะที่มีการกวน 100 รอบต่อนาที พบว่า การหมักในถังปฏิกรณ์ชีวภาพขนาด 5 ลิตร ได้ความเข้มข้น อัตราผลผลิต และประสิทธิภาพการหมักเอทานอล เป็น 0.11 ± 139.51กรัมต่อลิตร 0.00 ± 3.49 กรัมต่อลิตรต่อชั่วโมง และ 0.69 ± 91.66 เปอร์เซ็นต์ ตามลำดับ ในขณะที่การหมักในถังปฏิกรณ์ชีวภาพขนาด 50 ลิตร ได้ความเข้มข้นของเอทานอล 0.20 ± 119.53)กรัมต่อลิตร (และ อัตราผลผลิตเอทานอล 0.01 ± 2.13) กรัมต่อลิตรต่อชั่วโมง (ต่ำกว่าเมื่อหมักในถังปฏิกรณ์ชีวภาพขนาด 5 ลิตร การหมักเอทานอลแบบกะซ้ำในถังปฏิกรณ์ชีวภาพขนาด 5 ลิตร โดยดึงน้ำหมักออก 50 เปอร์เซ็นต์ และเติมน้ำหมักใหม่เข้าไปแทนที่ทันทีในปริมาตรที่เท่ากัน พบว่าประสิทธิภาพการผลิตเอทานอลต่ำลงในกะที่ 2 ถึงกะที่ 8 คือได้ความเข้มข้นของเอทานอลในกะที่ 2 ถึงกะที่ 8 เป็น 103.37 ± 0.28 ถึง 109.53 ± 1.06 กรัมต่อลิตร

คำหลัก : น้ำคั้นลำต้นข้าวฟ่างหวาน การหมักภายใต้สภาวะ VHG เอทานอลเชื้อเพลิง *Saccharomyces cerevisiae*

Abstract

Project Code : MRG4980199

Project Title : Ethanol production from sweet sorghum stem juice using very high gravity (VHG) technology

Investigator : Assoc. Prof. Dr. Lakkana Laopaiboon, Khon Kaen University

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Project Period : 2 years (1 July 2006 - 30 June 2008)

The selection and evaluation of the performance of three high-ethanol-producing strains, *Saccharomyces cerevisiae* (TISTR 5048, TISTR 5339 and NP 01), for VHG ethanol fermentation in a synthetic medium under VHG conditions at 30°C found that NP 01 cultured in 280 g glucose l^{-1} gave the maximum ethanol concentration with the value of 104.68 ± 0.11 g l^{-1}. Ethanol production from sweet sorghum juice (extracted from its stalks) by *S. cerevisiae* NP 01 under VHG fermentation and various nitrogen sources (yeast extract and peptone or $(NH_4)_2SO_4$) and carbon supplements (sucrose or sugarcane molasses) was investigated. The fermentation was carried out in 500-ml air-locked Erlenmeyer flasks under static condition. The results showed that when the sweet sorghum juice was supplemented with sucrose (to obtain total soluble solids of 28°Bx), 3 g yeast extract l^{-1} and 5 g peptone l^{-1}, ethanol production efficiency was maximum. The concentration, productivity and yield efficiency of ethanol were 120.68 ± 0.54 g l^{-1}, 2.01 ± 0.01 g $l^{-1}h^{-1}$ and 93.76 ± 0.20 %, respectively. Yeast extract concentration (0, 3, 6 and 9 g l^{-1}) supplemented in the juice was varied for ethanol production. The maximum ethanol production efficiency was obtained when 9 g l^{-1} of yeast extract was supplemented to the juice. The ethanol concentration, productivity and yield efficiency were 120.24 ± 3.35 g l^{-1}, 3.01 ± 0.08 g l^{-1} h^{-1} and 91.37 ± 0.55%, respectively. Scale up batch ethanol fermentation from the sweet sorghum juice containing 9 g l^{-1} of yeast extract was further carried out in 5-litre and 50-litre bioreactors with the agitation rate of 100 rev min^{-1}. The ethanol concentration, productivity and yield efficiency in the 5-litre bioreactor were 139.51 ± 0.11 g l^{-1}, 3.49 ± 0.00 g l^{-1} h^{-1} and 91.66 ± 0.69%, respectively. Lower ethanol concentration (119.53 ± 0.20 g l^{-1}) and ethanol productivity (2.13 ± 0.01 g l^{-1} h^{-1}) were obtained in the 50-litre bioreactor. In the repeated-batch fermentation in the 5-litre bioreactor, the juice was withdrawn at 50% of the working volume and the same amount of the fresh juice was immediately replaced. Lower ethanol production efficiency was observed in the subsequent batches. Ethanol concentrations in batch 2 to 8 were in the range of 103.37 ± 0.28 to 109.53 ± 1.06 g l^{-1}.

Keywords : Sweet sorghum juice, VHG fermentation, fuel ethanol, *Saccharomyces cerevisiae*

สารบัญ

Manuscript I

SELECTION OF *SACCHAROMYCES CEREVISIAE* AND INVESTIGATION OF ITS PERFORMANCE FOR VHG FERMENTATION

ABSTRACT

This research aims to select and evaluate the performance of three high-ethanol-producing strains of *Saccharomyces cerevisiae* (TISTR 5048, TISTR 5339 and NP 01) in very high gravity (VHG) ethanol fermentation. The maximum specific growth rates (μ_{max}) of TISTR 5048 and NP 01 grown in yeast extract malt extract broth containing 150 g glucose l^{-1} were 0.49 and 0.46 h^{-1} respectively while μ_{max} of TISTR 5339 could not be determined due to cell flocculation. The ethanol production by TISTR 5048 and NP 01 was further carried out in batch mode at 30ºC under normal gravity fermentation (240 g glucose l^{-1}) and VHG fermentation (280 and 320 g glucose l^{-1}) and the initial cell concentration was 1×10^{8} cells ml^{-1}. The results showed that TISTR 5048 cultured in 240 and 280 g glucose l^{-1} gave the maximum ethanol concentration (P) with the value of 99.58 g l^{-1}, while NP 01 cultured in 280 and 320 g glucose l^{-1} gave the maximum P with the value of 104.68 g l^{-1}. Ethanol productivities (Q_p) of NP 01 were slightly higher than those of TISTR 5048 at all conditions tested.

Keyword: ethanol, fermentation, normal gravity, very high gravity, *Saccharomyces cerevisiae*

Introduction

Very high gravity (VHG) ethanol fermentation is one of process improvements for the fuel ethanol production. It aims at increasing both ethanol concentration and fermentation rate. It can reduce capital costs, energy costs per litre of alcohol as well as the risk of bacterial contamination (Thomas et al., 1996; Bvochora et al., 2000; Narendranath and Power, 2005). The VHG process involves preparation and fermentation of mashes containing at least 27 g of dissolved solids per 100 g mash (Bafrncová et al., 1999; Bayrock and Ingledew, 2001; Bai et al., 2004a; Bai et al., 2004b). Under normal gravity, dissolved solid concentrations of 20-24 g per 100 g mash (Narendranath and Power, 2005) and suitable environmental parameters, the higher initial sugar concentration is used, the higher ethanol concentration is produced. However, ethanol tolerance and the ability to accumulate high ethanol concentrations are strain-dependent characteristics (Kosaric and Vardar-Sukan, 2001) especially under VHG conditions. In addition, environmental parameters such as temperature, osmotic pressure and carbon dioxide levels may directly affect yeast growth and ethanol productivity (Nagashima, 1990).

Saccharomyces cerevisiae is one of the ethanol-producing organisms used in industrial processes. Under VHG conditions if appropriate environment and all required nutrients in adequate amounts were provided, *S. cerevisiae* could ferment increased amount of sugars in the medium (Reddy and Reddy, 2005; Reddy and Reddy, 2006). In addition, it could produce and tolerate high ethanol concentrations (Thomas et al., 1994; Thomas et al., 1996: Bafrncová et al., 1999). Successful VHG fermentation is therefore dependent not only on the optimal composition of a fermentation medium, but also on the yeast strain.

In Thailand, *S. cerevisiae* TISTR 5048 and TISTR 5339 are recommended as high-ethanol-producing strains under the normal gravity conditions (Arunpairojana et al., 2000) and *S. cerevisiae* NP01 was found to be a high ethanol producer in a Thai rice wine (Rittiplang, 2006). However, none has studied ethanol production using those strains under the VHG conditions. Thus, this research aims to select and evaluate the performance of the three high-ethanol-producing strains of *S. cerevisiae*

in VHG ethanol fermentation in a synthetic ethanol production medium, and to raise the final ethanol concentration in a batch system.

Materials and methods

Microorganisms and growth conditions

S. cerevisiae TISTR 5048 and TISTR 5339 were obtained from MIRCEN, Bangkok, Thailand, and *S. cerevisiae* NP01 was isolated from Loog-pang (Chinese yeast cake) for Sato (Thai rice wine) making (Rittiplang, 2006). The yeasts were grown in yeast extract malt extract (YM) broth containing 10, 150 and 240 g glucose l^{-1} on a rotating shaker at 100 rpm, 30ºC. Maximum specific growth rate (μ_{max}) of the yeasts was calculated by determining viable cells using methylene blue staining technique (Zoecklien et al., 1995). The yeast cells in log phase grown in the YM broth giving μ_{max} were harvested and used as inoculum for ethanol production.

Ethanol production medium

Ethanol production medium (EP) consisted of (g l^{-1}) yeast extract (HiMedia laboratory, India), 3; peptone (HiMedia laboratory, India), 5; $MgSO_4.7H_2O$ (Fluka, Switzerland), 0.025; KH_2PO_4 (Merck, Switzerland), 0.5; $CaCl_2.2H_2O$ (BDH, England), 1; $(NH_4)_2PO_4$ (BDH, England), 1; $MnSO_4.6H_2O$ (BDH, England), 0.5; $Zn(NO_3)_2$ (Ajax, New Zealand), 0.2 and glucose (Glucose-D Patar, Thailand), 240, 280 or 320. The EP medium was transferred into a 500-ml air-locked Erlenmeyer flask with a final working volume of 400 ml and autoclaved at 110°C for 15 min.

Ethanol fermentation

The fermentation was carried out in batch mode under static condition at 30ºC. The sterile EP medium at various initial glucose concentrations was inoculated with the *S. cerevisiae* strains to give the final cell concentrations of approximately 1×10^8 cells ml^{-1}. The samples were collected at time intervals for further analyses.

Analytical methods

The cell numbers of the fermentation broth were determined by direct counting method using haemacytometer (Zoecklien et al., 1995). The biomass yield (Y_{xs}) was calculated as the actual viable cells produced and expressed as cells per g sugar utilized (cells g glucose^{-1}). The fermentation broth was centrifuged at 13,000 rev min^{-1} for 10 min. The supernatant was then determined for total residual sugars by a phenol sulfuric acid method (Mecozzi, 2005). Percentage of glucose utilization was calculated as the ratio of the consumed mass of glucose to the initial mass of glucose. Ethanol concentration was analyzed by gas chromatography (Shimadzu GC-14B, Japan, Solid phase: polyethylene glycol (PEG-20M), carrier gas: nitrogen, 150^{o}C isothermal packed column, injection temperature 180 ^{o}C, flame ionization detector temperature 250 ^{o}C; C-R7 Ae plus Chromatopac Data Processor) and 2-propanol was used as an internal standard (Modified from Laopaiboon et al., 2007). The ethanol yield (Y_{ps}) was calculated as the actual ethanol produced and expressed as g ethanol per g glucose utilized (g g^{-1}). The volumetric ethanol productivity (Q_p) and the percentage of conversion efficiency or yield efficiency (E_y) were calculated by the following equations:

$$Q_p = \frac{P}{t}$$

and

$$E_y = \frac{Y_{ps} \times 100}{0.51}$$

where P is the actual ethanol concentration produced (g l^{-1}), t is the fermentation time (h) giving the highest ethanol concentration and 0.51 is the maximum theoretical ethanol yield of glucose consumption.

All the experiments were performed in duplicate and the results were expressed as mean ± SD of the duplicated experiments. The means were analyzed by Univariate using SPSS 15.0 for Windows program (SPSS Inc., 2006) with the general linear model procedure. DUNCAN test for multiple comparisons of the means was used for judging the significance of difference at the probability, $p \leq 0.05$.

Results and discussion

Effects of glucose on cell growth

Microbial growth patterns of the three high-ethanol-producing strains of *S. cerevisiae*; TISTR 5048, TISTR 5339 and NP 01, were investigated under various initial glucose concentrations. Figure 1 shows the growth curve of the yeasts in the YM broth containing glucose at concentrations of 10, 150 and 240 g l^{-1}. No lag phase was observed after the yeast cells were inoculated into the YM broth at all sugar concentrations, and stationary phase occurred at 12 to 15 h of the cultivation except for TISTR 5339. Cell concentrations of TISTR 5339 grown under glucose concentrations of 150 and 240 g l^{-1} did not increase after 3 h of the experiments due to cell flocculation. Consequently, the growth rate of this strain under both conditions could not be determined.

Main growth kinetic parameters (μ_{max}, glucose utilization and biomass yield) of the three strains are shown in Tables 1 and 2. When μ_{max} of the three strains were compared, the strain giving the highest μ_{max} under glucose concentration of 10, 150 and 240 g l^{-1} was TISTR 5339, TISTR 5048 and NP 01, respectively (Table 1). Under 10 g l^{-1} of glucose, the sugar was almost utilized and the maximum biomass yields were obtained. Even though YM broth containing 10 g glucose l^{-1} is used as a standard medium for yeast inoculum preparation, in this experiment it is not suitable for inoculum preparation. The total biomass produced under 10 g glucose l^{-1} was significantly ($p \leq 0.05$) lower than those produced under the other two glucose concentrations (Table 2). If 10 g l^{-1} of glucose is used for inoculum preparation, massive volume of culture medium is needed to obtain high biomass. Another important reason is that the inoculum or cells prepared under a high initial glucose concentration will be acclimatized under high sugar concentrations, which is useful for ethanol fermentation under VHG condition. Therefore, TISTR 5339 was not selected for further studies because it was unable to grow under high glucose concentrations. Biomass yields of both TISTR 5048 and NP 01 under 150 g glucose l^{-1} were similar to those under 240 g glucose l^{-1}. However, glucose utilization under 240 g l^{-1} was relatively low at only 45-50 %, whereas glucose utilized under 150 g l^{-1} was quite high, at approximately 81-91 % depending on the yeast strains. In addition,

the biomass yields of the two strains grown under 150 g glucose l^{-1} were similar. Therefore, both TISTR 5048 and NP 01 were further investigated for VHG ethanol fermentation and the YM broth containing 150 g l^{-1} of glucose would be used for inoculum preparation for the subsequent experiments.

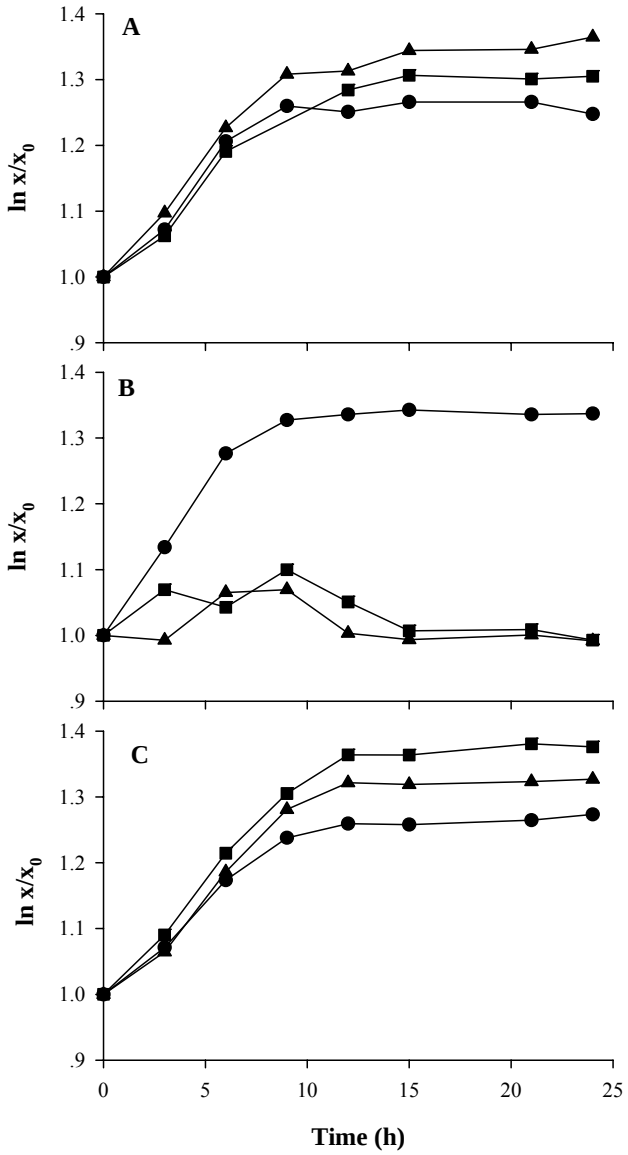

Figure 1 Growth curves of *S. cerevisiae* TISTR 5048 (A), *S. cerevisiae* TISTR 5339 (B) and *S. cerevisiae* NP 01 (C) at various glucose concentrations in YM medium at 30°C, 100 rpm. ●: glucose 10 g l^{-1}, ▲: glucose 150 g l^{-1} and ■: glucose 240 g l^{-1}.

Table 1 Maximum specific growth rate (μ_{max}) of the *S. cerevisiae* strains grown in YM medium containing various glucose concentrations at 30°C, 100 rpm.

Strains	μ_{max} (h^{-1}) (mean ± SD)		
	10 g glucose l^{-1}	150 g glucose l^{-1}	240 g glucose l^{-1}
TISTR 5048	0.43 ± 0.03	0.49 ± 0.03	0.45 ± 0.02
TISTR 5339	0.62 ± 0.02	-	-
NP 01	0.39 ± 0.04	0.46 ± 0.01	0.48 ± 0.01

Table 2 Glucose utilization and biomass yield of the *S. cerevisiae* strains in YM medium containing various glucose concentrations.

Strains	Initial glucose concentration (g $l^{-1)}$	Glucose utilized (g l^{-1})	Glucose Utilized (%)	Cell produced (cells ml^{-1})	Y_{xs} [a]
TISTR 5048	10	9.76 ± 0.02	94.68 ± 0.21	(5.37 ± 0.40)×10^7	(5.50 ± 0.42) ×10^6
	150	98.55 ± 1.41	91.13 ± 0.17	(1.30 ± 0.05) ×10^8	(1.32 ± 0.03) ×10^6
	240	82.91 ± 1.80	45.04 ± 1.29	(8.43 ± 1.91) ×10^7	(1.02 ± 0.21) ×10^6
TISTR 5339	10	11.05 ± 0.05	94.33 ± 0.14	(6.81 ± 0.07) ×10^7	(6.16 ± 0.03) ×10^6
	150	73.73 ± 12.60	61.44 ± 4.60	-	-
	240	35.86 ± 4.69	17.83 ± 2.02	-	-
NP 01	10	10.37 ± 0.22	96.52 ± 0.00	(7.22 ± 0.50) ×10^7	(6.96 ± 0.33) ×10^6
	150	98.82 ± 0.64	81.33 ± 0.12	(1.95 ± 0.00) ×10^8	(1.97 ± 0.01) ×10^6
	240	93.64 ± 0.51	49.52 ± 0.44	(1.79 ± 0.13) ×10^8	(1.91 ± 0.15) ×10^6

[a] Biomass yield (cells per g glucose utilized).

Normal gravity and VHG ethanol fermentations

The time profiles of total soluble solids, total residual sugar, reducing sugar, ethanol and cell numbers of the fermentation broth during normal gravity and VHG fermentations by TISTR 5048 and NP 01 are illustrated in Figures 2 to 4. At all experimental conditions, the cell concentrations were relatively constant throughout 40 h of the fermentations and they were less than one log reduction at the end of the experiments. The sugars were almost completely consumed under normal gravity fermentation (240 g glucose l^{-1}). Under VHG conditions at the initial sugar concentrations of 280 and 320 g l^{-1}, stuck fermentation was observed with approximately 42 to 60 and 96 to 107 g l^{-1} of reducing sugar remaining in the fermentation broth, respectively. In addition, the results showed that the sugars remaining in the fermentation broth using NP 01 were 3 to 7% lower than those using TISTR 5048 at all initial sugar concentrations. This indicated that the sugar utilization was strain-dependent and NP 01 had a better capability of glucose utilization than TISTR 5048. Nagashima (1990) reported that as the ethanol concentration increased, a decrease in growth rate was the first incident observed. However, the results obtained from this study revealed that both TISTR 5048 and NP 01 could tolerate high ethanol concentrations up to approximately 100 g l^{-1} before some yeast cells died.

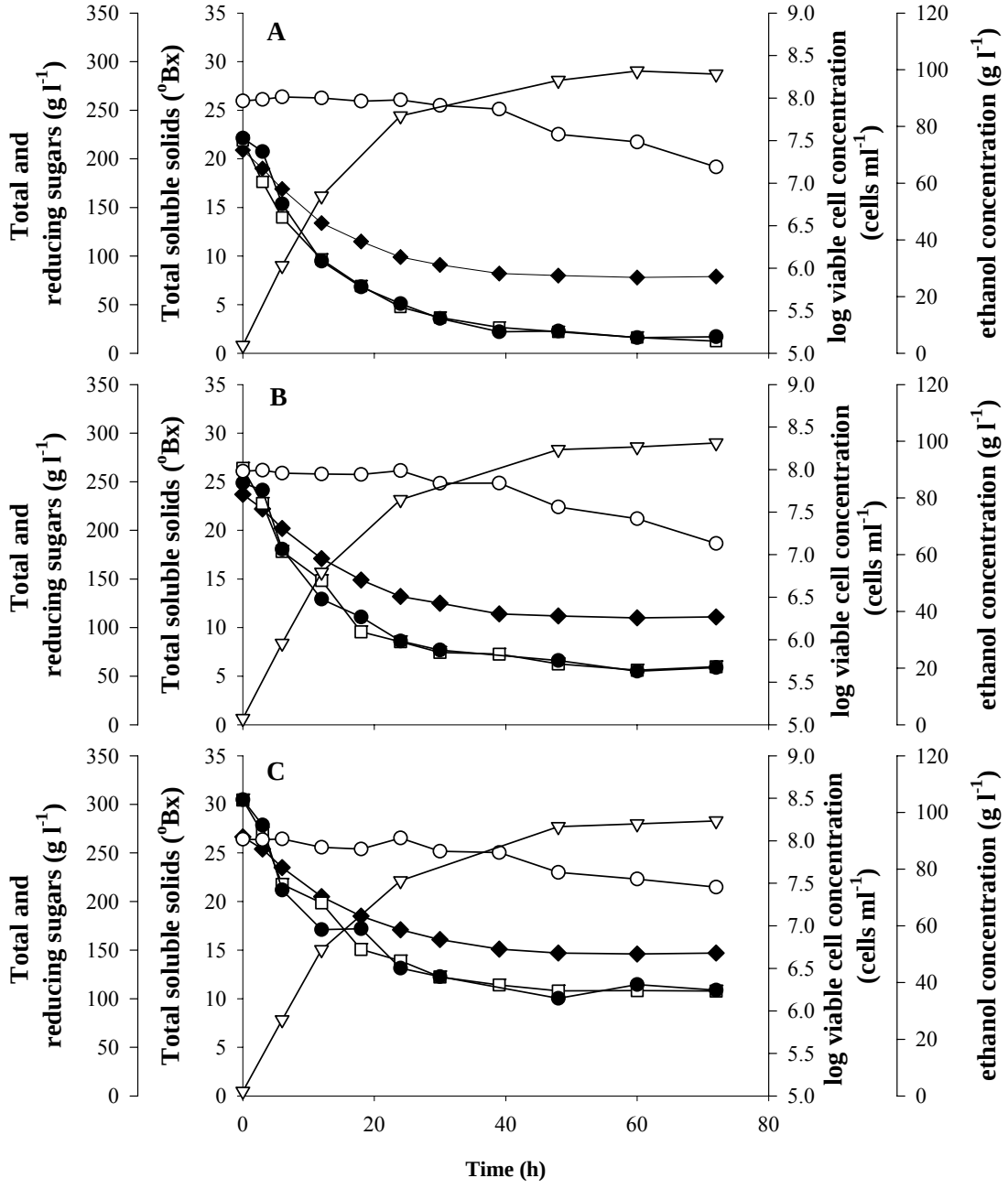

Figure 2 Fermentation kinetics during ethanol production by *S. cerevisiae* TISTR 5048 in medium containing glucose concentration of 240 (A), 280 (B) and 320 (C) g l^{-1}. ♦: total soluble solids (°Bx), ●: total sugar, □: reducing sugar, ○: log viable cell concentration and ∇: ethanol concentration.

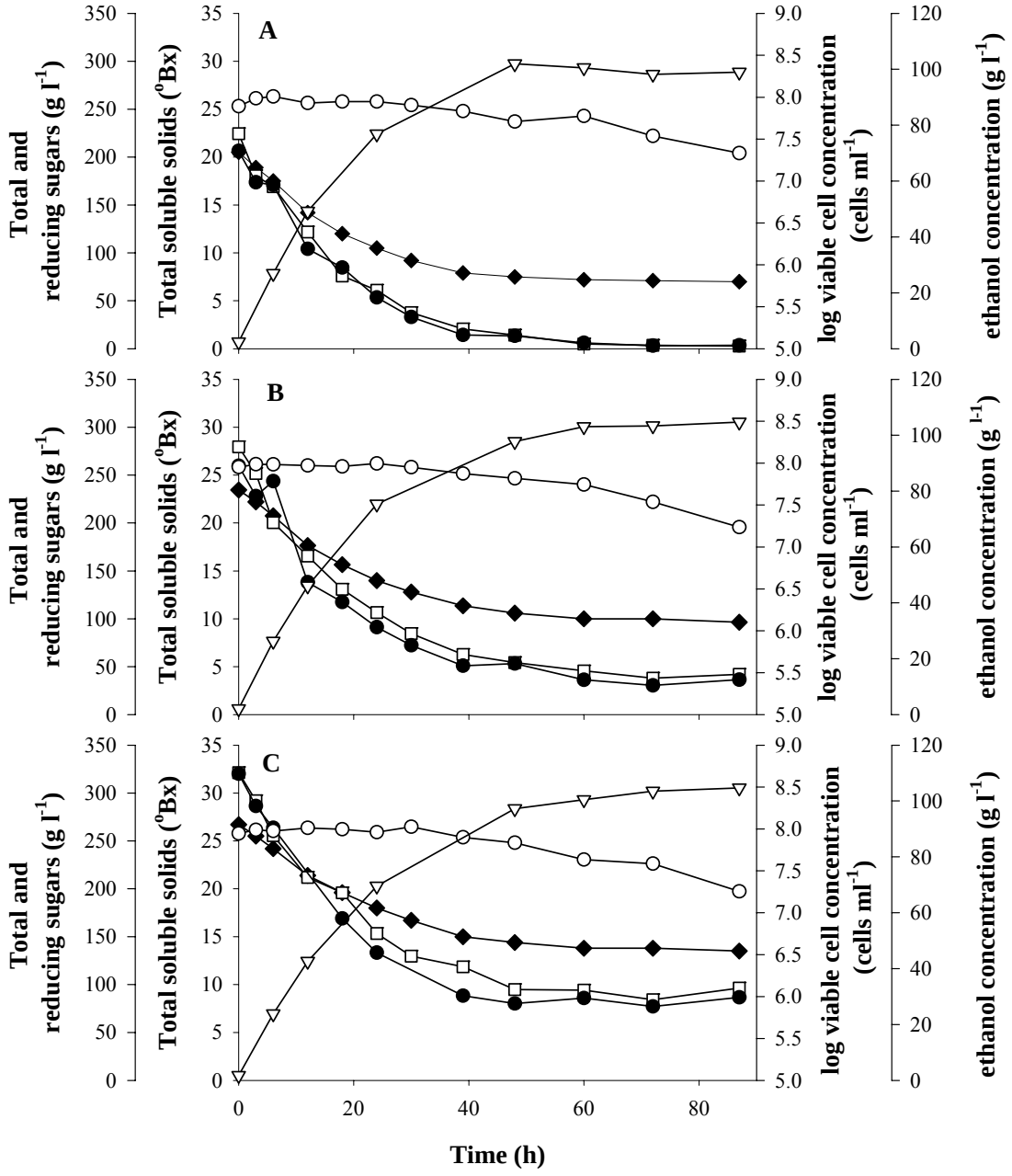

Figure 3 Fermentation kinetics during ethanol production by *S. cerevisiae* TISTR NP 01 in medium containing glucose concentration of 240 (A), 280 (B) and 320 (C) g l^{-1}. ♦: total soluble solids (°Bx), ●: total sugar, □: reducing sugar, ○: log viable cell concentration and ∇: ethanol concentration.

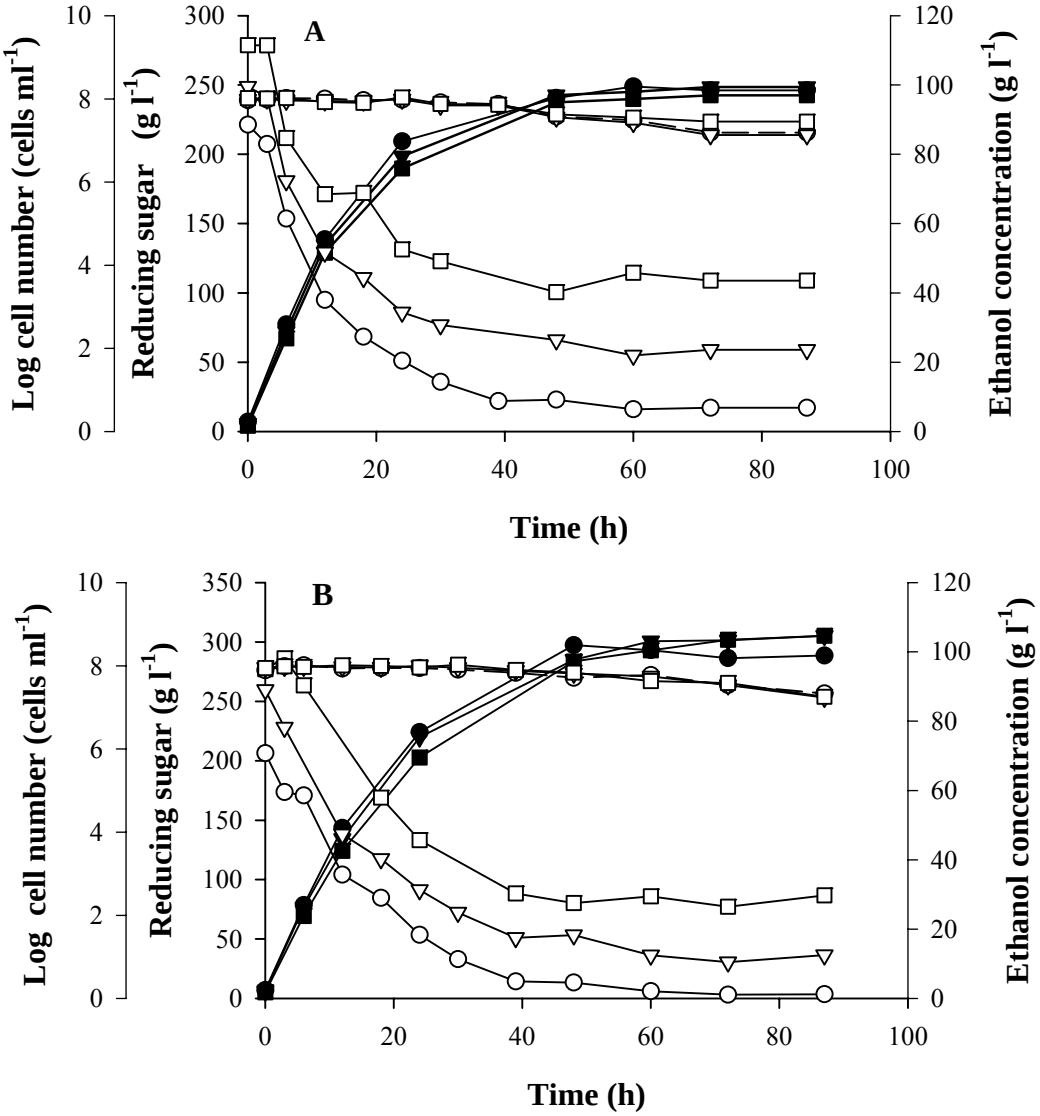

Figure 4 Effects of initial glucose concentration on cell growth, sugar utilization and ethanol production during batch ethanol fermentation by *S. cerevisiae* TISTR 5048 (A) and *S. cerevisiae* NP 01 (B) : glucose 240 g l^{-1} (●,○), glucose 280 g l^{-1} (■,□), glucose 320 g l^{-1} (▲,Δ); cell numbers (open symbol,_ _ _), reducing sugar (open symbol,___) and ethanol (close symbol).

Table 3 summarizes the important fermentation kinetic parameters at various initial glucose concentrations by TISTR 5048 and NP 01. When fermentation kinetic parameters were compared, the ethanol concentration produced, P, using TISTR

5048 under the normal gravity (99.58 g l^{-1}) was not significantly ($p \leq 0.05$) different from that under the VHG at 280 g glucose l^{-1} (99.42 g l^{-1}). The results suggested that this strain was suitable for ethanol fermentation under normal gravity more than under VGH condition. When NP 01 was used, the VHG fermentations gave higher ethanol concentration than under normal gravity fermentation. However, further increase in glucose concentration from 280 to 320 g glucose l^{-1} did not lead to an increase in ethanol concentration. The P at 280 and 320 g glucose l^{-1} were almost the same, implying that NP 01 was a suitable strain for VHG fermentation at the initial glucose concentration not exceeding 280 g l^{-1}. When the main fermentation kinetic parameters of the two strains were compared, the results showed that P and Q_p of NP 01 were higher than those of TISTR 5048 under both normal gravity and VHG fermentations, while Y_{ps} of both strains were similar under the normal gravity condition. Under VHG conditions, however, ethanol yield efficiencies (E_y) of TISTR 5048 was about 10% higher than those of NP 01. The results implied that additional by-products such as glycerol, succinate, alpha-ketogutarate, butanediol and diacetyl (Zoecklein et al. 1995) might be produced by NP 01 during the fermentations. TISTR 5048 gave lower P than NP 01 suggesting that TISTR 5048 might be less ethanol tolerant.

As approximately 15% of initial sugar concentration still remained at the end of the VHG fermentation at 280 g glucose l^{-1} by NP 01, complete sugar utilization may be achieved by optimization of aeration rate, agitation rate and nutrient supplementations (Bafrncová, 1999; Alfenore et al., 2004).

Table 3 Fermentation kinetic parameters of ethanol production at various initial glucose concentrations by *S. cerevisiae* TISTR 5048 and *S. cerevisiae* NP 01.

Strains	Glucose concentrations ($g\ l^{-1}$)	Parameters (mean ± SD) [a]				
		P ($g\ l^{-1}$)	Q_p ($g\ l^{-1}\ h^{-1}$)	Y_{ps} ($g\ g^{-1}$)	E_y (%)	t *(h)
TISTR 5048	240	99.58 ± 1.06	1.66 ± 0.02	0.50 ± 0.01	98	60
	280	99.42 ± 1.40	1.66 ± 0.03	0.49 ± 0.01	96	60
	320	97.01 ± 0.48	1.61 ± 0.01	0.50 ± 0.01	98	60
NP 01	240	101.95 ± 0.50	2.12 ± 0.01	0.48 ± 0.02	94	48
	280	104.68 ± 0.11	1.75 ± 0.00	0.44 ± 0.00	86	60
	320	104.68 ± 0.00	1.75 ± 0.00	0.44 ± 0.01	86	60

[a] P, ethanol concentration produced; Q_p, volumetric ethanol productivity; Y_{ps}, ethanol yield; E_y, yield efficiency and t, fermentation time.

* Fermentation time.

: The experiments were performed in duplicate.

Conclusions

The results obtained from this study have demonstrated that among the three *S. cerevisiae* strains, NP 01 was found to be the most suitable strain for ethanol production under VHG fermentation. At total sugar concentration of 280 $g\ l^{-1}$, P, Q_p and Y_{ps} were 104.68 $g\ l^{-1}$, 1.75 $g\ l^{-1}h^{-1}$ and 0.44 $g\ g^{-1}$, respectively. To achieve the goals of VHG fermentation, which are the improvement of ethanol production efficiency and complete sugar utilization, environmental parameters such as aeration rate and stirring speed during VHG fermentation as well as nutrient supplementation should be further studied.

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References

Alfenore, S., Cameleyre, X., Benbadis, L., Bideaux, C., Uribelarrea, J.L., Goma, G., Molina-Jouve, C., Guillouet, S.E. 2004. Aeration strategy: a need for very high ethanol performance in *Saccharomyces cerevisiae* fed-batch process. **Appl. Microbiol. Biotechnol**., 63, 537-542.

Arunpairojana, V., Sawannachart, C., Chatanon L., Budhaka, P. 2000. **TISTR Culture Collection:** List of cultures, 6th ed., Bangkok: Bangkok MIRCEN.

Bayrock, D.P., Ingledew, W.M. 2001. Application of multistage continuous fermentation for production of fuel alcohol by very-high-gravity fermentation technology. **J. Ind. Microbiol. Biotechnol**., 27, 87–93.

Bafrncová, P., Šmogrovičová, D., Sláviková, I., Pátková, J., Dömény, Z. 1999. Improvement of very high gravity ethanol fermentation by media supplementation using *Saccharomyces cerevisiae*. **Biotechnol. Lett**., 21,337-341.

Bai, F.W., Chen, L.J., Zhang, Z., Anderson, W.A., Moo-Young, M. 2004a. Parameter oscillation in very high gravity continuous ethanol fermentation and their attenuation on multi-stage packed column bioreactor system. **Biotechnol. Bioeng**., 88, 558-566.

Bai, F.W., Chen, L.J., Zhang, Z., Anderson, W.A., Moo-Young, M. 2004b. Continuous ethanol production and evaluation of yeast cell lysis and viability loss under very high gravity medium conditions. **J. Biotechnol**., 110, 287-293.

Bvochora, J.M., Read, J.S., Zvauya, R. 2000. Application of very high gravity technology to the cofermentation of sweet stem sorghum juice and sorghum grain. **Ind. Crop. Prod**., 11, 11-17.

Kosaric, N., Vardar-Sukan, F. 2001. Potential source of energy and chemical products. In Roehr, M. (Ed). **The Biotechnology of Ethanol: Classical and future applications**. Weinheim: Wiley-VCH Verlag GmbH.

Laopaiboon, L., Thanonkeo, P., Jaisil, P., Laopaiboon, P. 2007. Ethanol production from sweet sorghum juice in batch and fed-batch fermentations by *Saccharomyces cerevisiae*. **World J Microbiol Biotechnol**., 23, 1497-1501.

Mecozzi, M. 2005. Estimation of total carbohydrate amount in environmental samples by the phenol-sulphuric acid method assisted by multivariate calibration. **Chemom. Intell. Lab. Syst**., 79, 84-90.

Nagashima, M. 1990. Progress in ethanol production with yeasts. In Hubert,V. Rene, De M. (Eds.). **Yeast: Biotechnology and Biocatalysis.** New York: Marcel Dekker.

Narendranath, N.V., Power, R. 2005. Relationship between pH and medium dissolved solids in terms of growth and metabolism of Lactobacilli and *Saccharomyces cerevisiae* during ethanol production. **Appl. Environ. Microbiol**., 71, 2239-2243.

Reddy, L.V.A., Reddy, O.V.S. 2005. Improvement of ethanol production in very high gravity fermentation by horse gram (*Dolichos biflorus*) flour supplementation. **Lett. Appl. Microbiol**., 41, 440-444.

Reddy, L.V.A., Reddy, O.V.S. 2006. Rapid and enhanced production of ethanol in very high gravity (VHG) sugar fermentations by *Saccharomyces cerevisiae*: Role of finger millet (*Eleusine coracana L.*) flour**. Process Biochem**., 41,726-729.

Rittiplang, J. 2006. **Specific selection of microorganisms for high efficiency loog-pang preparation for quality rice wine production.** Master Thesis in Biotechnology, Graduate School, Khon Kaen University.

Thomas, K.C., Hynes, S.H., Ingledew, W.M. 1994. Effects of particulare materials and osmoprotectants on very-high-gravity ethanolic fermentation by *Saccharomyces cerevisiae*. **Appl. Environ. Microbiol**., 60, 1519–1524.

Thomas, K.C., Hynes, S.H., Ingledew, W.M. 1996. Practical and theoretical considerations in the production of high concentrations of alcohol by fermentation. **Process Biochem**., 31, 321–331.

Zoecklien, B.W., Fugelsang, K.C., Gump, B.H., Nury, F.S. 1995**. Wine Analysis and Production.** New York: Chapman & Hall.

Manuscript II

ETHANOL PRODUCTION FROM SWEET SORGHUM JUICE USING VHG TECHNOLOGY: EFFECTS OF CARBON AND NITROGEN SUPPLEMENTATIONS

ABSTRACT

Ethanol production from sweet sorghum juice by *Saccharomyces cerevisiae* NP 01 was investigated under very high gravity (VHG) fermentation and various nitrogen sources (yeast extract and peptone or $(NH_4)_2SO_4$). Sucrose or sugarcane molasses was added to the juice to obtain total soluble solids of 24, 28, 32 and 34°Bx. The experiments were carried out at 30°C in 500-ml air-locked Erlenmeyer flasks under static condition. When sucrose was used as an adjunct, the results showed that the sweet sorghum juice containing total soluble solids of 28°Bx, 3 g yeast extract l^{-1} and 5 g peptone l^{-1} gave the maximum ethanol production efficiency. The concentration, productivity and yield of ethanol were 120.68 ± 0.54 g l^{-1}, 2.01 ± 0.01 g $l^{-1}h^{-1}$ and 93.76 ± 0.20 %, respectively at the fermentation time of 60 h. When molasses was used as an adjunct, the juice containing total soluble solids of 32°Bx, 3 g yeast extract l^{-1} and 5 g peptone l^{-1} gave the maximum ethanol concentration, productivity and yield efficiency with the values of 109.34 ± 0.78 g l^{-1}, 1.52 ± 0.01 g $l^{-1}h^{-1}$ and 83.62 ± 1.42 %, respectively at the fermentation time of 72 h. These results imply that molasses may contain some inhibitors for yeast metabolism resulting in lower ethanol production efficiency compared to that of using sucrose as an adjunct.

Keywords: *S. cerevisiae*; ethanol production; VHG fermentation: sweet sorghum juice; sugarcane molasses

Introduction

Ethanol production as an alternative fossil fuel energy resource has been a subject of great interest since the oil crisis in the 1970s. To increase the productivity and cost effectiveness of ethanol production, many process improvements have been studied including very high gravity (VHG) technology. VHG fermentation technology is defined as the preparation and fermentation to completion of mashes containing 270 grams or more of dissolved solids per litre (Bayrock and Ingledew, 2001). It has several advantages for industrial applications such as the increase in both the ethanol concentration and the rate of fermentation by reducing capital costs, energy costs per litre of alcohol and the risk of bacterial contamination (Thomas et al., 1996).

Apart from sugarcane (in Brazil), corn grain (in USA), tapioca starch and sugarcane molasses (in Thailand), other agricultural raw materials rich in fermentable carbohydrates, including sweet sorghum, have been of particular interest for biological transformation into ethanol to use as fuel or fuel additive (Schaffert, 1995; Göksungur and Zorlu, 2001). Sweet sorghum has been promised as a large scale energy crop because its stalks contain high fermentable sugar and it can be cultivated at nearly all temperatures and tropical climate areas (Sree et al., 1999). It is also one of the most drought resistant agricultural crops because of its capacity to remain dormant during the driest periods (Woods, 2000).

It was reported that under appropriate environmental and nutritional conditions, *Saccharomyces cerevisiae* can produce and tolerate high ethanol concentrations (Thomas et al., 1996; Bafrncová et al., 1999). VHG fermentation process exploits the observation that the growth of *S. cerevisiae* is promoted and prolonged when very low but adequate levels of oxygen are present and assimilable nitrogen levels are not limiting (Casey and Ingledew, 1986). Several investigators have observed that yeast extract (Casey et al., 1984; Thomas and Ingledew, 1990; Jones et al., 1994; Bafrncová et al., 1999), ammonium (Jones et al., 1994), urea (Jones and Ingledew, 1994a), calcium and magnesium (Dombek and Ingram, 1986) have protective effects either on growth and fermentation or viability, which stimulate the fermentation rate and ethanol production. Our previous study showed that

Saccharomyces cerevisiae NP 01 isolated from Long-pang (Chinese yeast cake) for Sato (Thai rice wine) making was a high-ethanol-producing strain under VHG condition (Laopaiboon et al., in press). As total soluble solids in the sweet sorghum juice cv. KKU 40 has only 18°Bx (grams per 100 ml), ethanol fermentation under VHG supplemented with other carbon sources in order to raise the sugar content in the juice needs to be investigated. Characteristics of raw sweet sorghum juice cv. KKU 40 are shown in Table 1.

Table 1 Characteristics of raw sweet sorghum juice cv. KKU 40

Constituents	Contents
pH	4.9
Total soluble solid (°Bx)	18
Reducing sugar (g l^{-1})	37.65
Total sugar (g l^{-1})	173.02
Fructose (g l^{-1})	11.46
Glucose (g l^{-1})	14.22
Sucrose (g l^{-1})	124.05
NH_4^+-N (ppm)	21.4
NO_3^--N (ppm)	4.4
Total P (ppm)	20
Total K (ppm)	1790
Total Na (ppm)	170
Total S (ppm)	120
Total Ca (ppm)	166
Total Mg (ppm)	194
Total Fe (ppm)	2
Total Mn (ppm)	3
Total Cu (ppm)	0.3
Total Zn (ppm)	1.4

The aim of this study was to compare the efficiency of ethanol production from sweet sorghum juice supplemented with sucrose or sugarcane molasses under VHG fermentation using *S. cerevisiae* NP 01. The influences of yeast extract and peptone (YEP) or $(NH_4)_2SO_4$ as nitrogen sources for ethanol production were also studied.

Materials and methods

Microorganism and inoculum preparation

S. cerevisiae NP 01 isolated from Long-pang (Chinese yeast cake) from Nakhon Phanom province, Thailand was inoculated into a 250-ml Erlenmeyer flask containing 150 ml of yeast extract malt extract (YM) medium. The medium contained (in g l^{-1}) yeast extract 3, peptone 5, malt extract 3 and glucose 10. The flask was incubated on a rotating shaker at 100 rev min^{-1}, 30ºC for 15 h. To increase cell concentration, the yeast was transferred into a 500-ml Erlenmeyer flask with 360 ml of the YM medium containing 150 g l^{-1} of glucose to give the initial cell concentration of 1×10^6 cells ml^{-1}. The flasks were further incubated under the conditions previously mentioned. After 15 h, the cells were harvested and used as an inoculum for ethanol production.

Raw materials

Sweet sorghum juice (cv. KKU 40) extracted from its stalks was obtained from the Department of Agronomy, Faculty of Agriculture, Khon Kaen University, Thailand. After extraction, the juice was kept at -18ºC until use. Sugarcane molasses obtained from Mitr Phu Viang Sugar Mill, Nongrua, Khon Kaen, Thailand was kept at 4ºC until use.

Ethanol production medium

Sweet sorghum juice containing total soluble solids of 18°Bx was adjusted with sucrose or molasses to give total soluble solids of 24, 28, 32 and 34°Bx. Then the juices were supplemented with 8 g l^{-1} of YEP (3 g yeast extract and 5 g peptone) or 1.3 g l^{-1} of $(NH_4)_2SO_4$, and used as ethanol production (EP) medium. The EP

medium was transferred into a 500-ml air-locked Erlenmeyer flask with a final working volume of 400 ml and autoclaved at 110°C for 15 min. Molasses containing total soluble solids of 24, 28 and 32°Bx was also used as media for ethanol production.

Fermentation conditions

The sterile EP medium containing various sugar and nitrogen supplements was inoculated to give the initial yeast cell concentration of 1×10^8 cells ml^{-1}. The fermentation was carried out in batch mode at 30°C under static condition. The samples were withdrawn at time intervals for analysis.

Analytical methods

The viable yeast cell numbers and total soluble solids of the fermentation broth were determined by direct counting method using haemacytometer and hand-held refractometer, respectively. The fermentation broth was centrifuged at 13,000 rev min^{-1} for 10 min. The supernatant was then determined for total residual sugars and reducing sugar by phenol sulfuric acid method and dinitrosalicylic acid (DNS) method, respectively. Ethanol concentration (P, g l^{-1}) was analyzed by gas chromatography (Shimadzu GC-14B, Japan, Solid phase: polyethylene glycol (PEG-20M), carrier gas: nitrogen, 150^{o}C isothermal packed column, injection temperature 180^{o}C, flame ionization detector temperature 250^{o}C; C-R7 Ae plus Chromatopac Data Processor) and 2-propanol was used as an internal standard (Modified from Laopaiboon et al., 2007). The ethanol yield (Y_{ps},) was calculated as the actual ethanol produced and expressed as g ethanol per g glucose utilized (g g^{-1}). The volumetric ethanol productivity (Q_p, g l^{-1} h^{-1}) and the percentage of conversion efficiency or yield efficiency (E_y) were calculated by the following equations:

$$Q_p = \frac{P}{t}$$

and

$$E_y = \frac{Y_{ps} \times 100}{0.54}$$

where P is the ethanol concentration (g l^{-1}), t is the fermentation time (h) giving the highest ethanol concentration and 0.54 is the maximum theoretical ethanol yield of sucrose consumption. Fermentable nitrogen or formol nitrogen in the fermentation broth was analyzed by formol titration method (Zoecklein et al., 1995).

Results and discussion

VHG fermentation with sucrose as an adjunct and influences of various nitrogen sources to ethanol production

Standard ethanol production medium (Melzoch et al., 1994) contains 3 g l^{-1} of yeast extract and 5 g l^{-1} of peptone which total fermentable nitrogen equals 1,129 mg l^{-1}. Therefore in this study yeast extract and peptone at those concentrations were supplemented in the sweet sorghum juice as nitrogen sources. To compare the effects of nitrogen source on ethanol production, the same amount of fermentable nitrogen in $(NH_4)_2SO_4$ (1.3 g l^{-1}) was supplemented in the juice.

The time profiles of total soluble solids, residual total sugar, reducing sugar, yeast cell and ethanol during batch fermentation of *S. cerevisiae* NP 01 from the sweet sorghum juice supplemented with sucrose at the initial soluble solids of 24, 28 and 32°Bx and various nitrogen sources are shown in Figures 1, 2 and 3. Sugar consumption and ethanol production at the various conditions were compared in Figure 4. The sugars were almost completely consumed under high gravity (HG) fermentation (the initial soluble solids of 24°Bx). Under VHG conditions at the initial soluble solids of 28 and 32°Bx, stuck fermentation was observed with approximately 49 to 73 and 120 to 158 g l^{-1} of reducing sugar remaining in the fermentation broth, respectively. The amount of sugar remaining was also dependent on supplemented nitrogen sources. Not all reducing sugar in the media were completely utilized by the yeast. This might be due to thermal stress as described by Jones and Ingledew (1994b). They found that the amount of sugar that could be fermented decreased when fermentation temperature was above 25°C. However, lower temperature might cause lower ethanol productivity. This was supported by Bai et al. (2008) who reviewed that the negative impact of high temperature on the ethanol fermentation performance was much worse under the VHG conditions than the regular

fermentation. In addition, the fermentation in this study was carried out in the air-locked Erlenmeyer flask under static condition, where the conditions might not be optimal for complete fermentation under VHG levels.

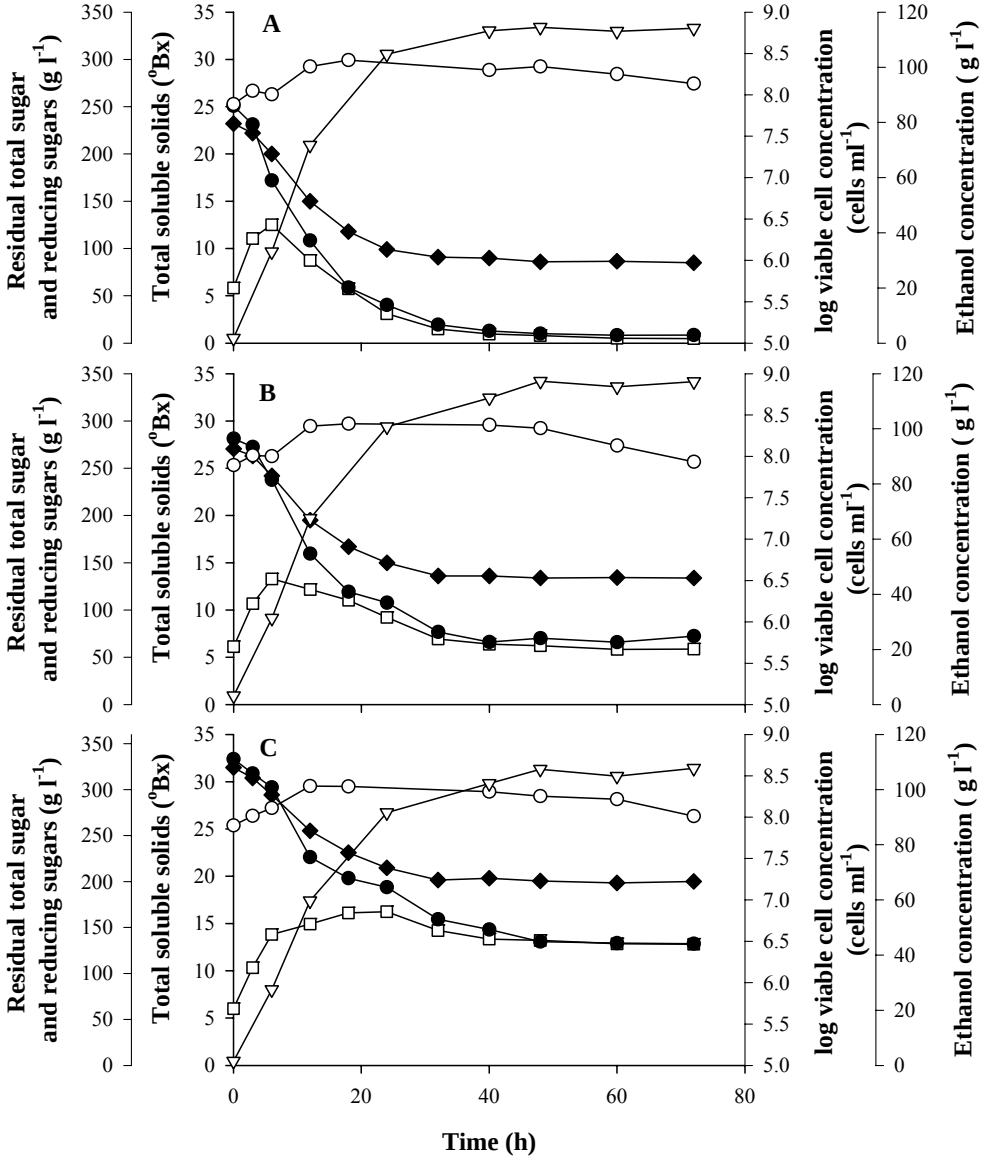

Figure 1 Fermentation kinetics during ethanol production by *S. cerevisiae* NP 01 from sweet sorghum juice supplemented with sucrose at various initial soluble solids without extra nitrogen source: 24°Bx (A), 28°Bx (B) and 32°Bx (C). ♦: total soluble solids (°Bx), ●: total sugar, □: reducing sugar, ○: log viable cell concentration and ∇: ethanol concentration.

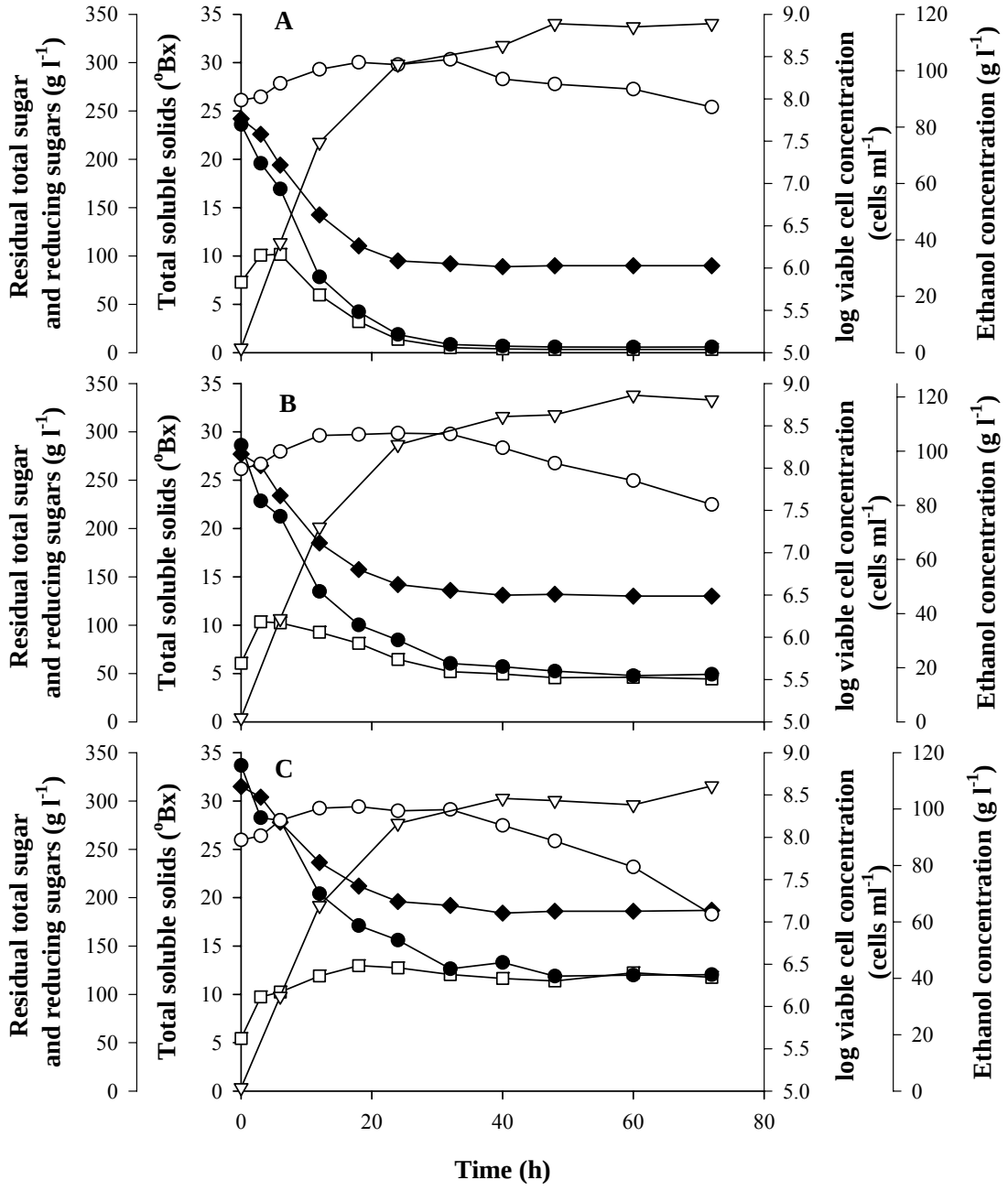

Figure 2 Fermentation kinetics during ethanol production by *S. cerevisiae* NP 01 from sweet sorghum juice supplemented with sucrose at various initial soluble solids and YEP: 24°Bx (A), 28°Bx (B) and 32°Bx (C). ♦: total soluble solids (°Bx), ●: total sugar, □: reducing sugar, ○: log viable cell concentration and ∇: ethanol concentration.

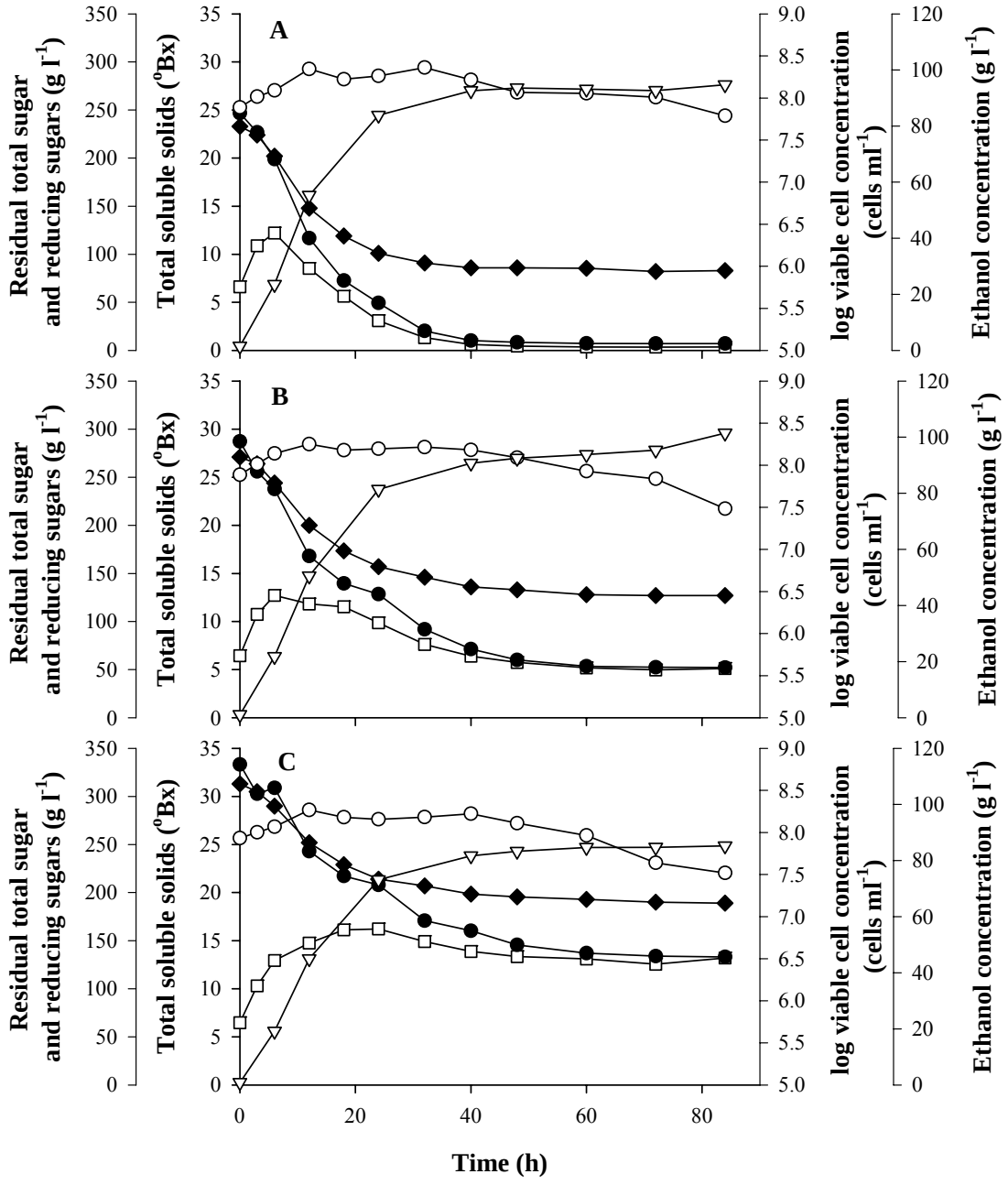

Figure 3 Fermentation kinetics during ethanol production by *S. cerevisiae* NP 01 from sweet sorghum juice supplemented with sucrose at various initial soluble solids and $(NH_4)_2SO_4$: 24°Bx (A), 28°Bx (B) and 32°Bx (C). ♦: total soluble solids (°Bx), ●: total sugar, □: reducing sugar, ○: log viable cell concentration and ∇: ethanol concentration.

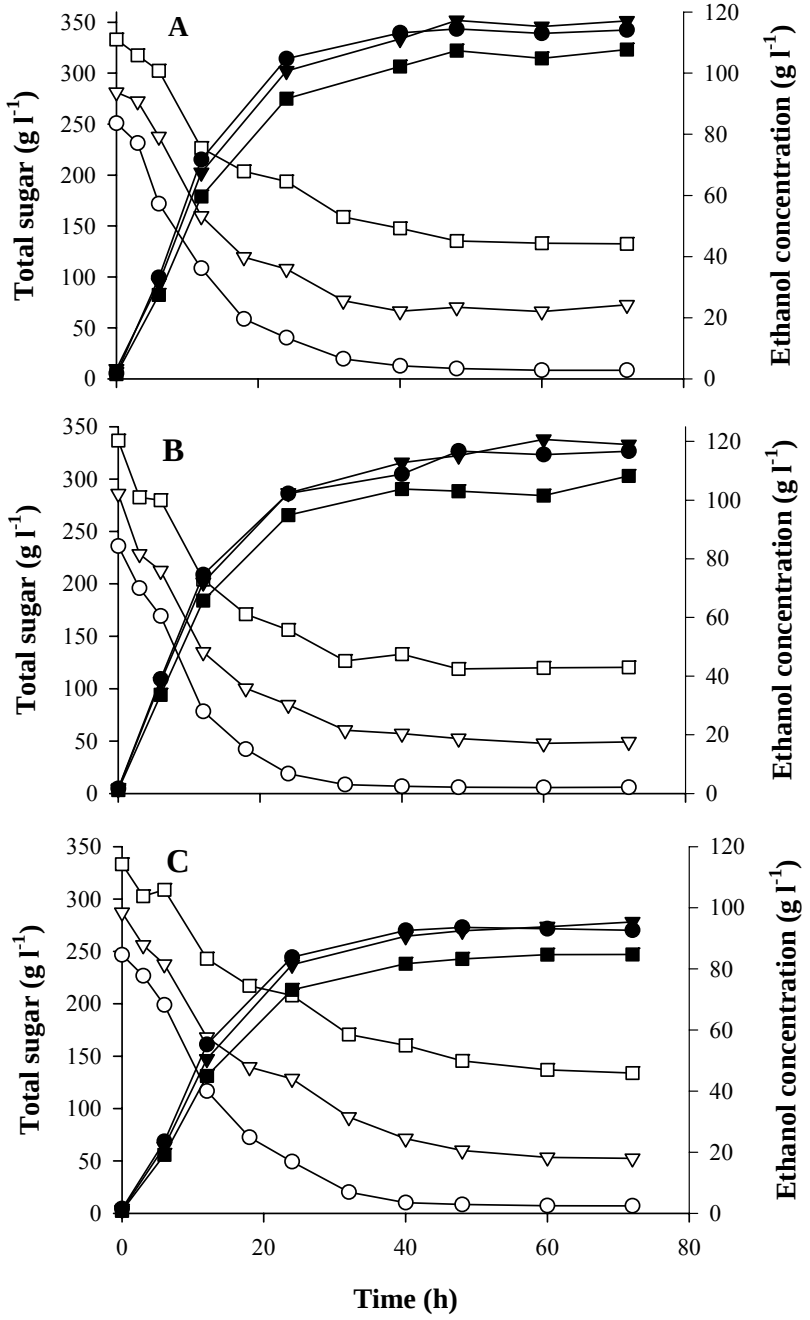

Figure 4 Sugar consumption and ethanol production during batch ethanol fermentation by *S. cerevisiae* NP 01 from sweet sorghum juice supplemented with sucrose at various initial soluble solids and nitrogen sources: 24ºBx (○, ●), 28ºBx (▽, ▼) and 32ºBx (□, ■), total sugar (open symbol) and ethanol (close symbol). (A) no extra nitrogen source, (B) supplemented with YEP and (C) supplemented with $(NH_4)_2 SO_4$.

Table 2 summarizes the important kinetic parameters (P, Q_p and Y_{ps}) of the ethanol fermentation under various conditions. The results showed that initial total soluble solids or initial sugar concentration had significant effects on the main kinetic parameters. The juice containing initial total soluble solids of 28°Bx and YEP gave the maximum ethanol concentration with the value of 120.68 ± 0.54 g l^{-1}. At the initial total soluble solids of 24 and 28°Bx, YEP enhanced the final ethanol concentrations approximately 3% compared to those of the control (no extra nitrogen supplement). However, the fermentation time was prolonged than that of the control resulting in lower ethanol productivity (Table 2). When $(NH_4)_2SO_4$ at the same amount of fermentable nitrogen detected in YEP was used as a nitrogen supplement, P, Q_p and Y_{ps} obtained were significantly lower than those of the control and the juice supplemented with YEP, respectively. The explanation for this result is still under investigation. However, $(NH_4)_2SO_4$ seems not to be suitable for use as a nitrogen supplement in sweet sorghum juice for ethanol production, and hence it would not be used for subsequent experiments.

Table 2 Kinetic parameters of ethanol production from sweet sorghum juice supplemented with sucrose at various initial total soluble solids and nitrogen sources by *S. cerevisiae* NP 01.

Extra nitrogen sources	Initial total soluble solids (°Bx)	Parameters (mean ± SD)[a]				
		P (g l^{-1})	Q_p (g $l^{-1}h^{-1}$)	Y_{ps} (g g^{-1})	E_y (%)	t* (h)
None	24	113.20 ± 0.81	2.83 ± 0.02	0.48 ± 0.03	89.07 ± 6.12	40
	28	117.28 ± 0.14	2.44 ± 0.00	0.53 ± 0.01	98.12 ± 3.06	48
	32	107.39 ± 1.07	2.24 ± 0.02	0.53 ± 0.00	99.07 ± 0.00	48
YEP	24	116.71 ± 0.85	2.43 ± 0.02	0.51 ± 0.01	93.93 ± 1.89	48
	28	120.68 ± 0.54	2.01 ± 0.01	0.51 ± 0.00	93.76 ± 0.20	60
	32	108.23 ± 0.16	1.50 ± 0.00	0.50 ± 0.01	92.53 ± 2.12	72
$(NH_4)_2SO_4$	24	93.58 ± 0.73	1.95 ± 0.02	0.43 ± 0.00	79.40 ± 0.77	48
	28	101.48 ± 0.06	2.11± 0.00	0.52 ± 0.02	95.84 ± 2.79	48
	32	83.31 ± 0.06	1.74 ± 0.00	0.53 ± 0.02	98.12 ± 3.50	48

[a] The results were expressed as mean ± SD.

*Fermentation time.

: The experiments were performed in duplicate.

Bai et al. (2008) reported that assimilation nitrogen is the most important component in the fermentation medium. In this study, utilization of fermentable nitrogen in ethanol fermentation under various media is shown in Table 3. In the juice without nitrogen supplementation, the amount of fermentable nitrogen utilized decreased when sugar concentration in the juice increased. The results suggested that yeast nitrogen assimilation might be repressed under high sugar concentrations. Under HG conditions (24°Bx), the amount of nitrogen utilized in all media was similar. However, under VHG conditions at the same initial soluble solids, the yeast utilized fermentable nitrogen in the juice containing extra nitrogen sources more than the juice without nitrogen supplementation.

In this study, fermentable nitrogen remaining in the control medium (without nitrogen supplementation) was 40-60% depending on the initial total soluble in the juice. This clearly suggested that nitrogen was not limited in the control medium, but

the capability of nitrogen utilization by yeast might depend on other factors, especially, under VHG conditions. However, the amount of nitrogen consumption was not always related to ethanol production efficiency. Nitrogen utilized in the control medium was lower than that in the juice supplemented with $(NH_4)_2SO_4$, but ethanol production from the control medium was higher.

Table 3 Fermentable nitrogen utilized during ethanol production from sweet sorghum juice supplemented with sucrose at various initial total soluble solids and nitrogen sources by *S. cerevisiae* NP 01.

Extra nitrogen sources	Initial total soluble solids (°Bx)	Fermentable nitrogen (mg l^{-1}) [a]	
		Initial	Utilized
None	24	657.28 ± 7.21	391.12 ± 1.80
	28	626.91 ± 0.00	294.29 ± 16.22
	32	594.96 ± 1.80	215.31 ± 19.82
YEP	24	1138.96 ± 10.81	420.40 ± 21.62
	28	1149.15 ± 18.02	402.60 ± 50.45
	32	1098.19 ± 18.02	287.90 ± 10.81
$(NH_4)_2SO_4$	24	1114.40 ± 7.92	439.60 ± 3.96
	28	1094.80 ± 3.96	380.80 ± 7.92
	32	1061.20 ± 11.88	313.60 ± 7.92

[a] The results were expressed as mean ± SD.

: The experiments were performed in duplicate.

The results also showed that *S. cerevisiae* NP01 was a suitable microorganism for ethanol fermentation under VHG levels. It could survive and retain its metabolism under very high ethanol concentrations up to 120 g l^{-1} (15 % by volume) (Table 2) with high viable cells remaining in the fermentation broth (Figure 5).

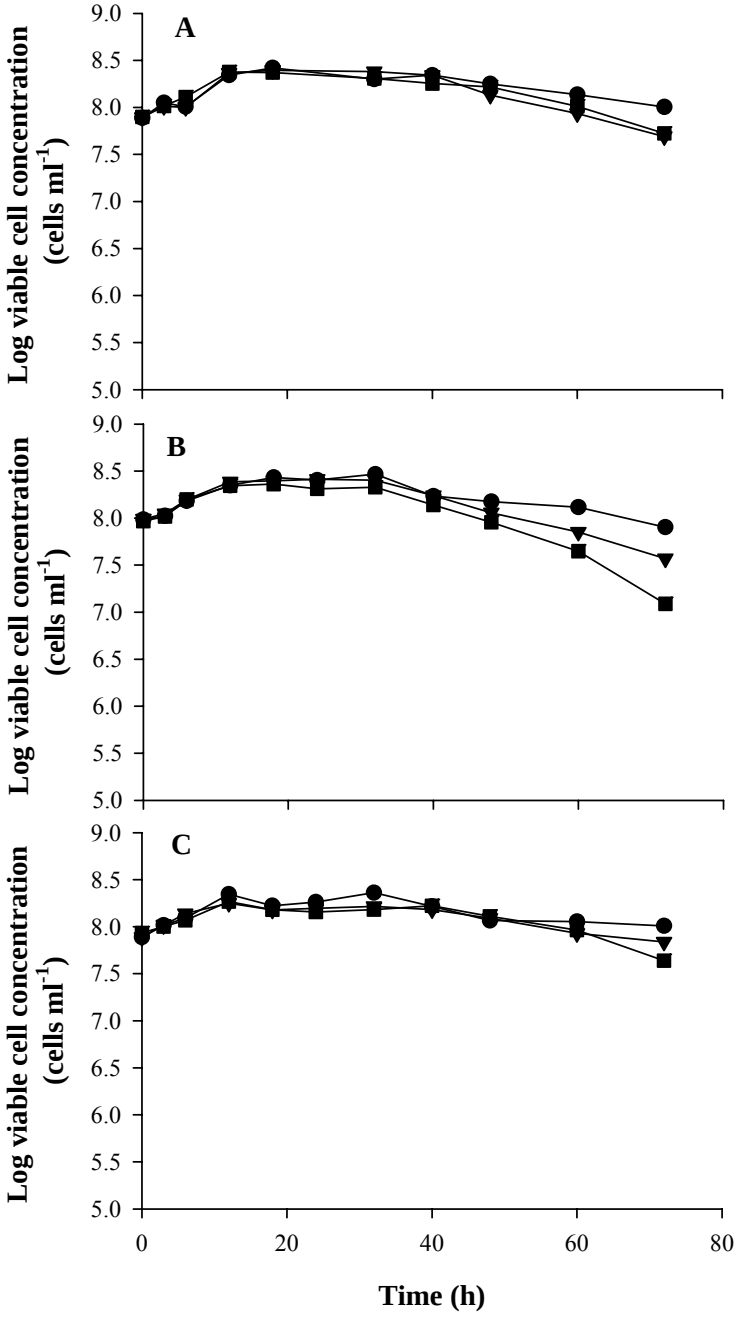

Figure 5 Viability of *S. cerevisiae* NP 01 during batch ethanol fermentation from sweet sorghum juice supplemented with sucrose at various initial total soluble solids and nitrogen sources: 24ºBx (●), 28ºBx (▼) and 32ºBx (■). (A) no extra nitrogen source, (B) supplemented with YEP and (C) supplemented with $(NH_4)_2\ SO_4$.

VHG fermentation using molasses as an adjunct and influences of YEP to ethanol production

The time profiles of total soluble solids, residual total sugar, yeast cell and ethanol concentration during batch fermentation from the sweet sorghum juice supplemented with molasses under the presence and absence of YEP are shown in Figures 6 and 7. Sugar consumption and ethanol production at various conditions were compared in Figure 8 and Table 4 summarizes the important kinetic parameters of the ethanol fermentation. Even though the cell numbers at specific fermentation time throughout the experiments under various conditions were similar (Figure 9), the cells could utilize more sugar in the fermentation broth containing YEP under the VHG levels resulting in higher ethanol concentration. However, the rate of ethanol fermentation (ethanol productivity) in the presence of YEP was significantly lower compared to that under no YEP due to longer fermentation time (Table 4). These findings imply that YEP may have some effects on yeast metabolism, which stimulates the fermentation rate and ethanol production under VHG conditions. The juice supplemented with molasses and YEP at the initial total soluble solids of 32°Bx gave the maximum ethanol concentration with the value of 109.34 ± 0.78 g l^{-1}. Under these conditions the productivity and yield of ethanol were 1.52 ± 0.01 g l^{-1} h^{-1} and 83.62 ± 1.42%, respectively.

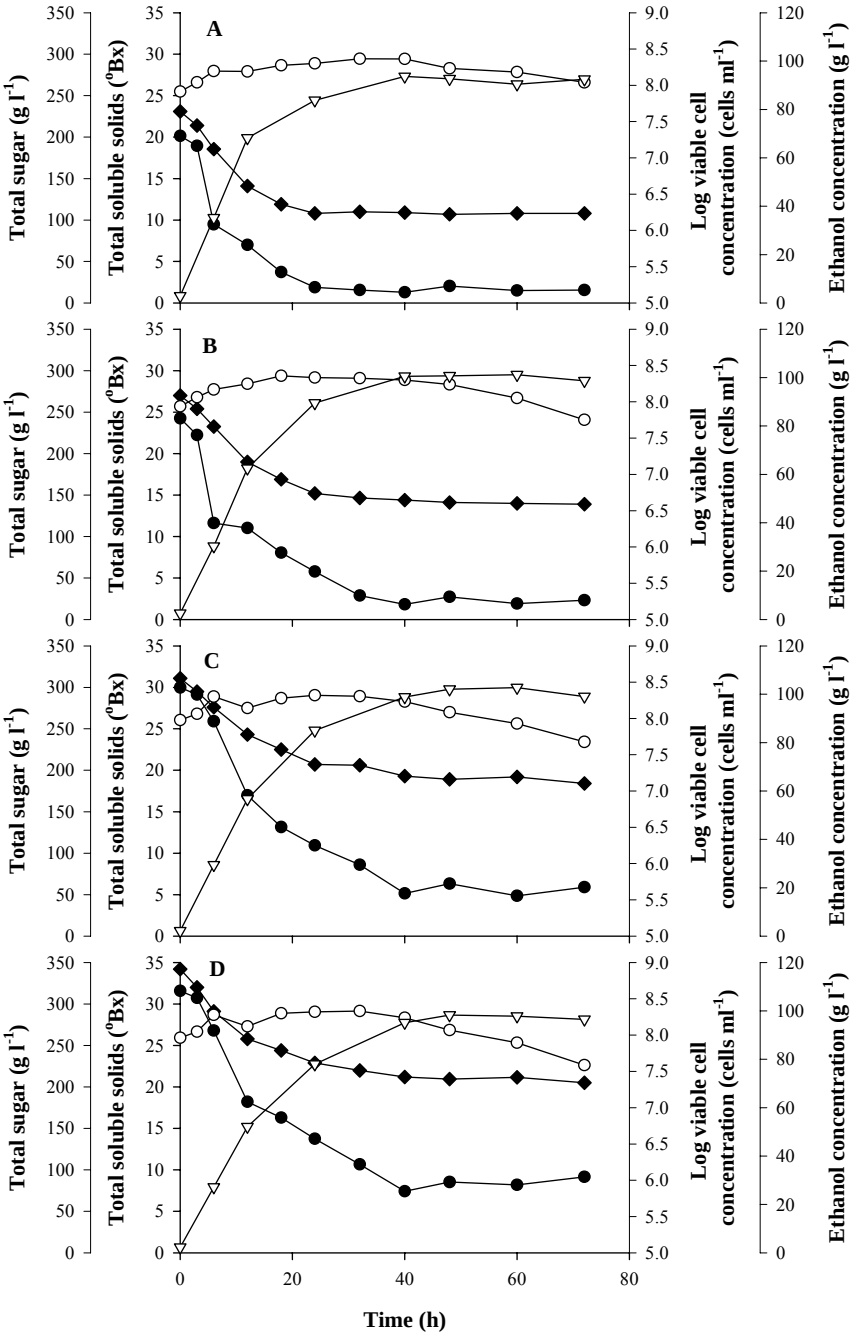

Figure 6 Fermentation kinetics during ethanol production by *S. cerevisiae* NP 01 from sweet sorghum juice supplemented with molasses at various initial total soluble solids without extra nutrient: 24°Bx (A), 28°Bx (B) 32°Bx (C) and 34°Bx (D). ♦: total soluble solids (°Bx), ●: total sugar, ○: log viable cell concentration and ∇: ethanol concentration.

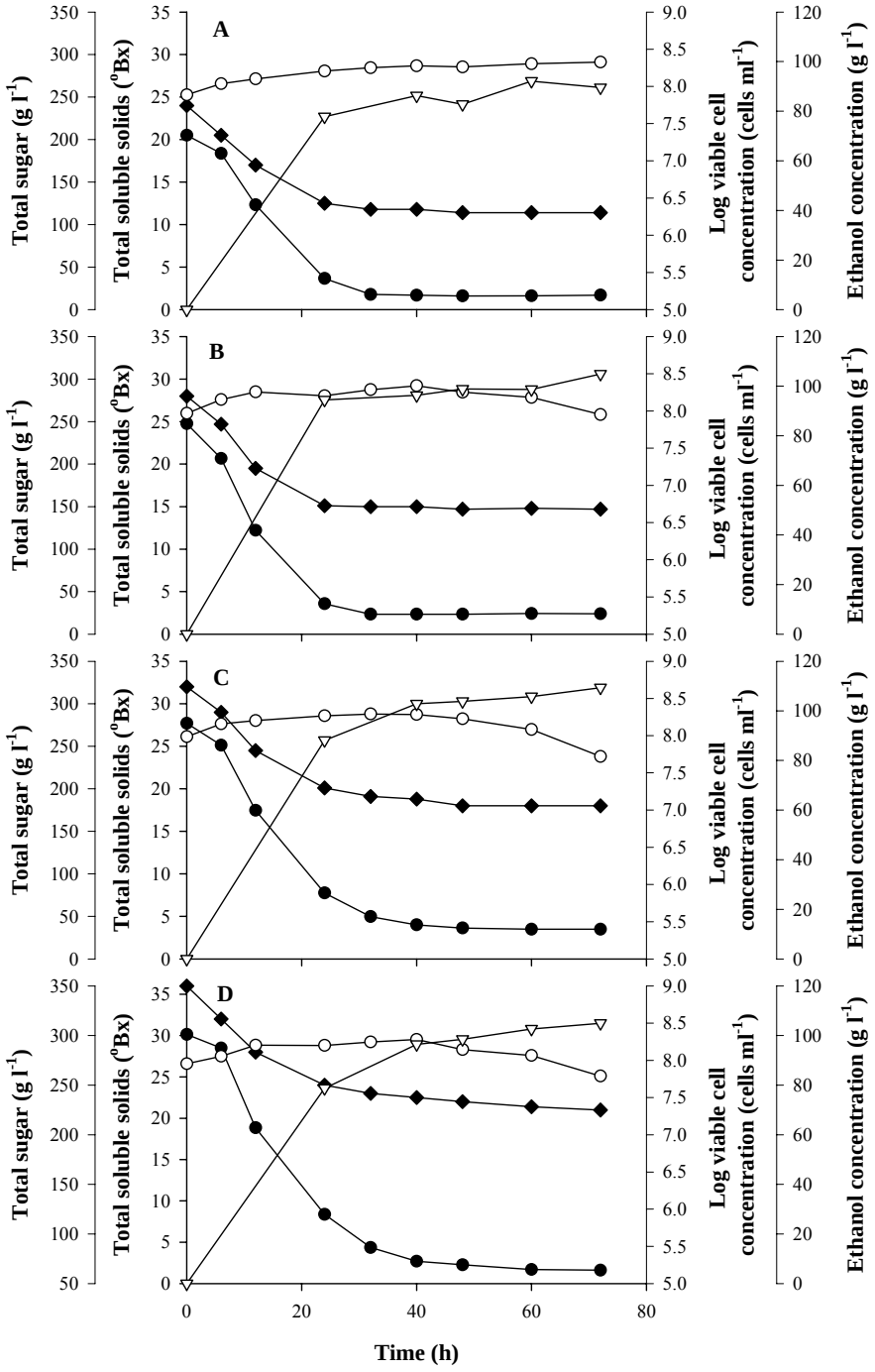

Figure 7 Fermentation kinetics during ethanol production by *S. cerevisiae* NP 01 from sweet sorghum juice supplemented with molasses at various initial total soluble solids and YEP: 24°Bx (A), 28°Bx (B) 32°Bx (C) and 34°Bx (D). ♦: total soluble solids (°Bx), ●: total sugar, ○: log viable cell concentration and ∇: ethanol concentration.

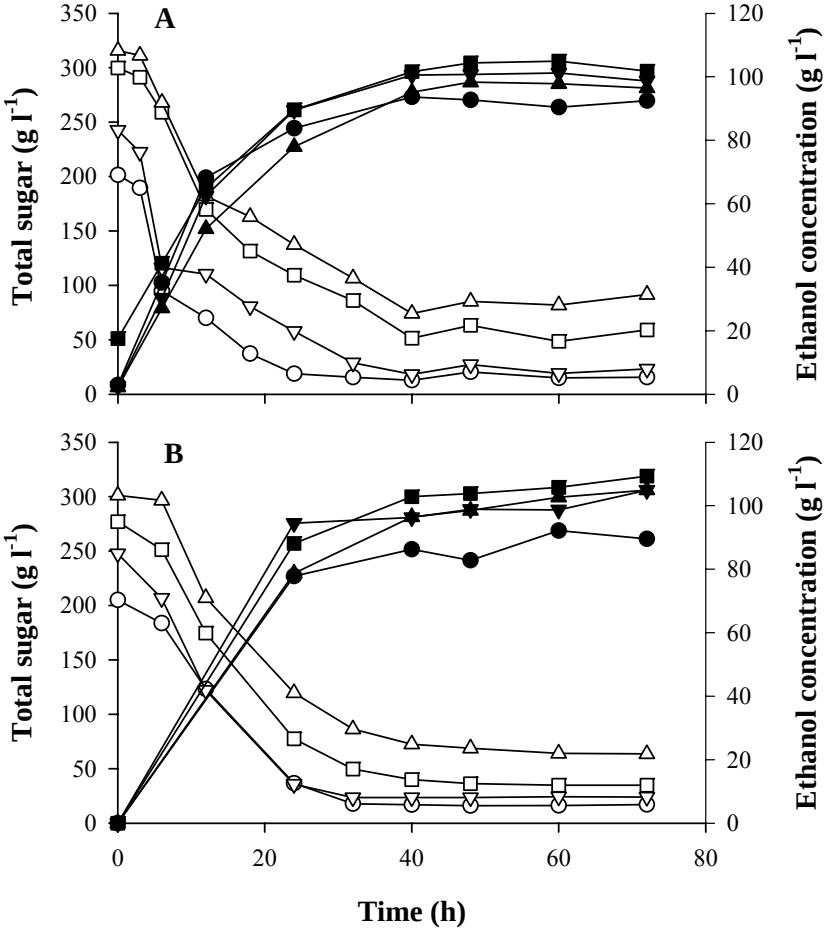

Figure 8 Sugar consumption and ethanol production during batch ethanol fermentation by *S. cerevisiae* NP 01 from sweet sorghum juice supplemented with molasses at various initial total soluble solids: 24ºBx (○, ●), 28ºBx (▽, ▼), 32ºBx (□, ■) and 34ºBx (△,▲), total sugar (open symbol) and ethanol (close symbol). (A) no extra nutrient, (B) supplemented with YEP.

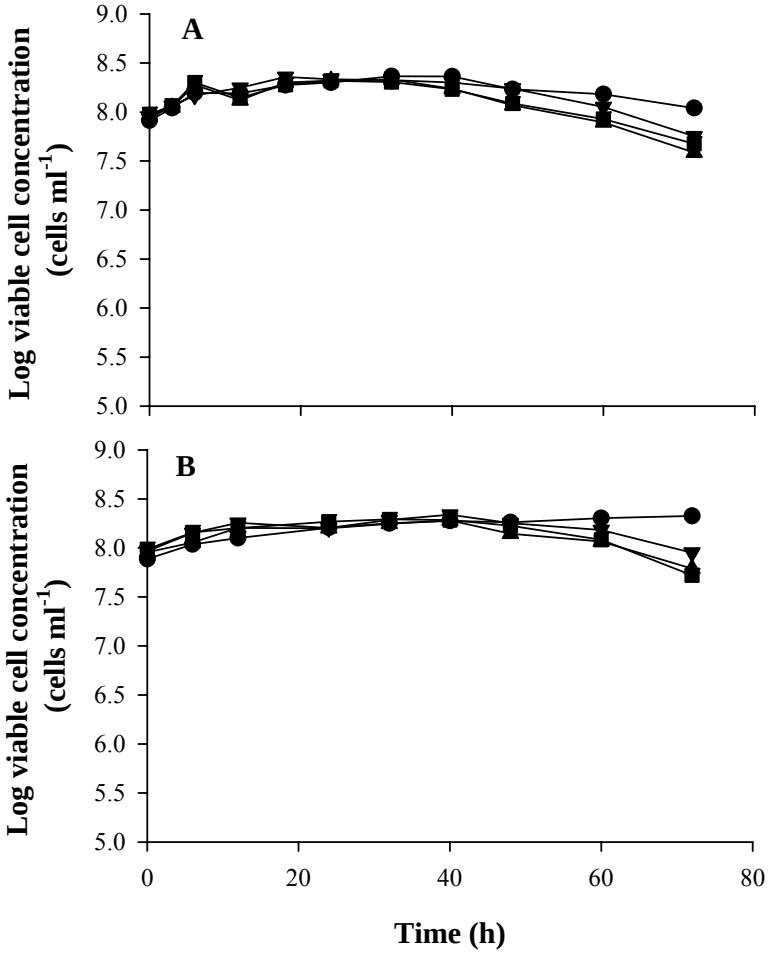

Figure 9 Viability of *S. cerevisiae* NP 01 during batch ethanol fermentation from sweet sorghum juice supplemented with molasses at various initial total soluble solids and nutrient: 24°Bx (●), 28°Bx (▼) 32°Bx (■) and 34°Bx (▲). (A) no extra nutrient, (B) supplemented with YEP.

Table 4 Kinetic parameters of ethanol production from sweet sorghum juice supplemented with molasses at various initial total soluble solids by *S. cerevisiae* NP 01.

Extra nitrogen sources	Initial total soluble solids (°Bx)	Parameters (mean ± SD)[a]				
		P (g l^{-1})	Q_p (g l^{-1} h^{-1})	Y_{ps} (g g^{-1})	E_y (%)	t* (h)
None	24	93.67 ± 0.88	2.34 ± 0.02	0.50 ± 0.00	91.95 ± 0.18	40
	28	100.54 ± 0.70	2.51 ± 0.02	0.45 ± 0.01	83.00 ± 1.75	40
	32	102.08 ± 2.63	2.13 ± 0.05	0.43 ± 0.00	79.93 ± 0.89	48
	34	98.29 ± 6.16	2.05 ± 0.13	0.43 ± 0.04	78.93 ± 6.58	48
YEP	24	92.15 ± 0.28	1.54 ± 0.00	0.49 ± 0.05	90.29 ± 8.90	60
	28	104.99± 1.51	1.46 ± 0.02	0.47 ± 0.04	86.86 ± 8.08	72
	32	109.34 ± 0.78	1.52 ± 0.01	0.45 ± 0.01	83.62 ± 1.42	72
	34	104.95 ± 2.18	1.46 ± 0.03	0.44 ± 0.02	81.80 ± 3.25	72

[a] The results were expressed as mean ± SD.

*Fermentation time.

: The experiments were performed in duplicate.

When the results of using sucrose and molasses were compared, all the important kinetic parameters (P, Q_p and Y_{ps}) of the ethanol fermentation including the amount of sugar utilized using molasses as an adjunct was significantly lower than those of using sucrose as an adjunct (Tables 2 and 4). These findings imply that molasses may contain some inhibitors for yeast metabolism. This conclusion was supported by the results of ethanol fermentation from only molasses at various initial total soluble solids (Table 5). When molasses at 32°Bx was used as EP medium, ethanol concentration and the amount of assimilation nitrogen were only 80 g l^{-1} and 82 mg l^{-1}, respectively.

Table 5 Kinetic parameters of ethanol production from only molasses at various initial total soluble solids by *S. cerevisiae* NP 01 and fermentable nitrogen utilized in the fermentation.

Initial total soluble solids (°Bx)	Parameters (mean ± SD)[a]					
	P (g l^{-1})	Q_p (g l^{-1} h^{-1})	Y_{ps} (g g^{-1})	E_y (%)	t* (h)	Fermentable nitrogen utilized (mg l^{-1})
24	60.59 ± 0.83	2.52 ± 0.03	0.50 ± 0.03	92.24 ± 6.12	24	250.38 ± 7.79
28	69.98 ± 0.25	1.75 ± 0.01	0.49 ± 0.01	90.95 ± 2.64	40	151.15 ± 4.58
32	79.90 ± 0.13	1.66 ± 0.00	0.49 ± 0.03	91.46 ± 6.23	48	82.42 ± 8.06

[a] The results were expressed as mean ± SD.

*Fermentation time.

: The experiments were performed in duplicate

Conclusions

This research achieves the goal of VHG fermentation technology with at least 15% (v/v) of ethanol has been produced in the fermentation broth (Bai et al., 2008). The results obtained from this research have demonstrated that sucrose is a good adjunct to raise the total soluble solids of sweet sorghum juice to VHG levels for ethanol fermentations while molasses causes the lower ethanol production efficiency. However, other methods for raising total soluble solids in sweet sorghum juice such as concentrating the juice by evaporation should be considered to reduce the cost of the adjunct. Yeast extract and peptone promote ethanol fermentation under VHG levels. Particulate yeast cell wall products may replace yeast extract and peptone as they are far less expensive. In addition, the optimum conditions in terms of processing parameters and/or fermentation processes to achieve complete sugar utilization under VHG levels need to be further studied.

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References

Bai, F.W., Anderson, W.A., Moo-Young, M. 2008. Ethanol fermentation technologies from sugar and starch feedstocks. **Biotechnol. Adv**., 26, 89-105.

Bafrncová, P., Šmogrovičová, D., Sláviková, I., Pátková, J., Dőmény, Z. 1999. Improvement of very high gravity ethanol fermentation by media supplementation using *Saccharomyces cerevisiae*. **Biotechnol. Lett**., 21, 337–341.

Bayrock, D.P., Ingledew, W.M. 2001. Application of multistage continuous fermentation for production of fuel alcohol by very-high-gravity fermentation technology. **J. Ind. Microbiol. Biotechnol**., 27, 87-93.

Casey, G.P., Ingledew, W.M. 1986. Ethanol tolerance in yeasts. **CRC. Crit. Rev. Biotechnol**., 13, 219–280.

Casey, G.P., Magnus, C.A., Ingledew, W.M. 1984. High gravity brewing: effects of nutrition on yeast composition, fermentative ability, and alcohol production. **Appl. Environ. Microbiol**., 48, 639-646.

Dombek, K.M., Ingram, L.O. 1986. Magnesium limitation and its role in apparent toxicity of ethanol during yeast fermentation. **Appl. Environ. Microbiol**., 52, 975–81.

Göksungur, Y., Zorlu, N. 2001. Production of ethanol from beet molasses by Ca-alginate immobilized yeast cells in a packed-bed bioreactor. **Turk. J. Biol**., 25, 265–275.

Jones, A.M., Ingledew, W.M. 1994a. Fuel ethanol production: appraisal of nitrogenous yeast foods for very high gravity wheat mash fermentation. **Process Biochem**., 29, 483–488.

Jones, A.M., Ingledew, W.M. 1994b. Fuel alcohol fermentation: Optimization of temperature for efficiency very-high-gravity fermentation. **Appl. Environ. Microbial**., 60, 1048-1051.

Jones, A.M., Thomas, K.C., Ingledew, W.M. 1994. Ethanolic fermentation of blackstrap molasses and sugarcane juice using very high gravity technology. **J. Agric. Food. Chem**., 42, 1242-1246.

Laopaiboon, L., Nuanpeng, S., Srinophakun, P., Klanrit, P., Laopaiboon, P. in press. Selection of *Saccharomyces cerevisiae* and investigation of its performance for very high gravity ethanol fermentation. **Biotechnol.**

Laopaiboon, L., Thanonkeo, P., Jaisil, P., Laopaiboon, P. 2007. Ethanol production from sweet sorghum juice in batch and fed-batch fermentations by *Saccharomyces cerevisiae*. **World J. Microbiol. Biotechnol**., 23, 1497-1501.

Melzoch, K., Rychtera, M., Hábová, V. 1994. Effect of immobilization upon the properties and behaviour of *Saccharomyces cerevisiae* cells. **J. Biotechnol**., 32, 59-65.

Schaffert, R.E. 1995. Sweet sorghum substrate for industrial alcohol. In Dendy, D.A.V., (Ed.). **Sorghum and Millets: Chemistry and Technology.** Saint Paul: American Association of Cereal Chemists.

Sree, N.K., Sridhar, M., Rao, L.V., Pandey, A. 1999. Ethanol production in solid substrate fermentation using thermotolerant yeast. **Process Biochem**., 34, 115-119.

Thomas, K.C., Ingledew, W.M. 1990. Fuel alcohol production: effects of free amino nitrogen on fermentation of very-high-gravity wheat mash. **Appl. Environ. Microbiol**., 56, 2046–2050.

Thomas, K.C., Hynes, S.H., Ingledew, W.M. 1996 Practical and theoretical considerations in the production of high concentration of alcohol by fermentation. **Process Biochem**., 31, 321-331.

Woods, J. 2000. **Integrating Sweet sorghum and sugarcane for bioenergy: Modeling the potential for electricity and ethanol production in SE Zimbabwe,** Ph.D. Thesis, King's College, London.

Zoecklein, B.U., Fugelsang, K.C., Gump, B.H., Nury, F.S. 1995. **Wine Analysis and Production**. New York: Chapman & Hall.

Manuscript III

BATCH AND REPEATED-BATCH ETHANOL FERMENTATION FROM SWEET SORGHUM JUICE UNDER VERY HIGH GRAVITY CONDITIONS BY *SACCHAROMYCES CEREVISIAE*

ABSTRACT

The effects of yeast extract on ethanol fermentation from sweet sorghum juice by *Saccharomyces cerevisiae* NP01 under very high gravity (VHG) conditions were carried out in a 500-ml air-locked Erlenmeyer flask under static condition. The juice containing total soluble solids of 28°Bx was supplemented with yeast extract at concentrations of 0, 3, 6 and 9 g l^{-1}. The maximum ethanol production efficiency was obtained when 9 g l^{-1} of yeast extract was supplemented to the juice. The ethanol concentration, productivity, yield and yield efficiency were 120.24 ± 3.35 g l^{-1}, 3.01 ± 0.08 g l^{-1} h^{-1}, 0.49 ± 0.00 and 91.37 ± 0.55% at the fermentation time of 40 h. Scale up batch ethanol fermentation from sweet sorghum juice containing 9 g l^{-1} of yeast extract was further carried out in 5-litre and 50-litre bioreactors with the working volume of 3.5 litres and 40 litres, respectively. The agitation rate of the bioreactors was 100 rev min^{-1}. The ethanol concentration, productivity and yield in the 5-litre bioreactor were 139.51 ± 0.11 g l^{-1}, 3.49 ± 0.00 g l^{-1} h^{-1} and 0.49 ± 0.00, respectively at the fermentation time of 40 h. Lower ethanol concentration (119.53 ± 0.20 g l^{-1}) and ethanol productivity (2.13 ± 0.01 g l^{-1} h^{-1}) were obtained in the 50-litre bioreactor. In the repeated-batch fermentation in the 5-litre bioreactor, the juice was withdrawn at 50% of the working volume and the same amount of the fresh juice was immediately replaced. In the first batch, the concentration, productivity and yield of ethanol were 129.48 ± 2.68 g l^{-1}, 4.01 ± 0.09 g l^{-1} h^{-1} and 0.48 ± 0.02, respectively at the fermentation time of 32 h. Lower ethanol production efficiencies in terms of ethanol concentration and productivity were observed in the subsequent batches. Ethanol concentrations in batch 2 to 8 were in the range of 103.37 ± 0.28 to 109.53 ± 1.06 at the fermentation time of 64 to 72 h.

Keywords: batch fermentation, ethanol production, repeated-batch fermentation, *Saccharomyces cerevisiae*, sweet sorghum juice, VHG fermentation, yeast extract

Introduction

In recent years, research on improving ethanol production has been accelerating for both ecological and economic reasons, primarily for its use as an alternative to petroleum-based fuels. Therefore, the development of a fermentation process using economical raw materials is important for the biofuel ethanol production on a commercial scale (Tanaka et al., 1999 and Tao et al., 2005). One technology that is significantly changing ethanol fermentation is very high gravity (VHG) fermentation. The process involves preparation and fermentation of mashes containing at least 270 grams or more of dissolved solids per litre (Bayrock and Ingledew, 2001). The advantages exist through the use of VHG technology include the increase in both ethanol concentration and the rate of fermentation (Bafrncová et al., 1999) and the reduction in the level of bacterial contamination resulting in decrease in the cost of ethanol production.

The fermentation of VHG medium may have a negative effect upon the yeast performance due to the elevated osmotic pressure and the production of high levels of ethanol (Pratt-Marshall et al., 2003). An important consideration in VHG fermentation is that the yeast is subjected to considerable osmotic stress which reduces the growth and increases the loss of cell viability (Casey et al., 1984, Odumeru et al., 1992 and Thomas and Ingledew 1992). Successful VHG fermentation is therefore dependent on the yeast's ability to withstand increased osmotic stress and to tolerate high concentrations of ethanol. Our previous studies showed that *Saccharomyces cerevisiae* NP 01 was one of the optimum yeast strains for ethanol production under VHG levels. It could produce and resist ethanol at concentrations up to 120 g l^{-1} (15% v/v) (Nuanpeng et al., 2007 and Laopaiboon et al., in press).

Thomas et al. (1994) reported the effects of particulate materials (wheat bran, soy flour, sea sand, mash solids and alumina) and osmoprotectants (tryptone, yeast

extract, mixing of purine and pyrimidine bases and ergosterol-tween 80) on VHG fermentation. The particulate materials maintained the cell viability at high levels for a longer period of time, while the osmoprotectants stimulated sugar utilization and ethanol production.

Several authors observed that yeast extract (Casey et al. 1984; Thomas and Ingledew 1992; Thomas et al. 1993; Jones and Ingledew 1994; Jones et al., 1994; Bafrncová et al. 1999), ammonium (Leao and Van Uden 1983; Jones and Ingledew 1994; Niessen et al. 2000), magnesium (Dombek and Ingram 1986; Ciesarova et al. 1996; Birch and Walker 2000) or calcium (Nabais et al. 1988) had a protective effect on growth, fermentation, or cell viability, which overall stimulated the rate of ethanol production.

Apart from sugarcane (in Brazil), corn grain (in USA), tapioca starch and sugarcane molasses (in Thailand), other agricultural raw materials rich in fermentable carbohydrates, including sweet sorghum, have been of a particular interest for biological transformation into ethanol to use as fuel or fuel additive (Schaffert, 1995; Göksungur and Zorlu, 2001). Sweet sorghum has been promised as a large scale energy crop because its stalks contain high fermentable sugar and it can be cultivated at nearly all temperatures and tropical climate areas (Sree et al., 1999). It is also one of the most drought resistant agricultural crops because of its capacity to remain dormant during the driest periods (Woods, 2000).

The ethanol fermentation can be carried out in batch, fed-batch, repeated-batch or continuous modes (Vitolo, 1996). Fermentation processes are often conducted in batch mode where microbial cells are suspended. However, the batch process has important disadvantages, particularly when the microorganisms are either slow growing or strongly affected by product inhibition (Najafpour et al., 2004).

Repeated-batch fermentation is the fermentation that the portion of the fermentation broth is withdrawn at time intervals and the residual part of the broth is used as an inoculum for the next batch. This process aims to increase the productivity and it is interesting because it has several advantages compared to a conventional batch fermentation such as no new inocula requirement for each batch (Bajpai and Bajpai, 1988).

Nuanpeng et al. (2007) showed that 5 g l^{-1} of peptone and 3 g l^{-1} of yeast extract (based on standard ethanol production medium, Melzoch et al., 1994) could improve ethanol production efficiency from sweet sorghum juice. Due to high cost of peptone (about 2.3 times higher than yeast extract), in this study sweet sorghum juice supplemented with yeast extract at various concentrations was used for ethanol production under batch fermentation. Ethanol fermentation from the sweet sorghum juice in batch and repeated-batch modes were compared. Scale up ethanol production to a 50-litre bioreactor was also studied.

Materials and methods

Microorganism and inoculum preparation

S. cerevisiae NP 01 isolated from Long-pang (Chinese yeast cake) from Nakhon Phanom province, Thailand was inoculated into a 250-ml Erlenmeyer flask containing 150 ml of yeast extract malt extract (YM) medium. The medium contained (in g l^{-1}) yeast extract 3, peptone 5, malt extract 3 and glucose 10. The flask was incubated on a rotating shaker at 100 rev min^{-1}, 30ºC for 15 h. To increase cell concentration, the yeast was transferred into a 500-ml Erlenmeyer flask containing 360 ml of the YM medium containing 150 g l^{-1} of glucose to give the initial cell concentration of 1×10^{6} cells ml^{-1}. The flasks were further incubated under the previously mentioned conditions. After 15 h, the cells were harvested and used as an inoculum for ethanol production.

Raw materials

Sweet sorghum juice (cv. KKU 40) extracted from its stalks was obtained from the Department of Agronomy, Faculty of Agriculture, Khon Kaen University, Thailand. After extraction, the juice was kept at -18ºC until use.

Ethanol production medium

Sweet sorghum juice containing total soluble solids of 18°Bx was adjusted with sucrose to give total soluble solids of 28ºBx. Then the juices were supplemented with yeast extract and used as ethanol production (EP) medium.

Experiments

Ethanol production from the EP medium containing various yeast extract concentrations

The EP medium supplemented with 0, 3, 6 and 9 g l^{-1} of yeast extract was transferred into a 500-ml air-locked Erlenmeyer flask with a final working volume of 400 ml and autoclaved at 110°C for 15 min. After cooling, *S. cerevisiae* NP 01 was inoculated into the EP medium to give the final cell concentration of 1×10^8 cells ml^{-1}. The fermentation was conducted in batch mode at 30°C under static condition. The samples were withdrawn at time intervals for analysis. The optimum yeast extract concentration was selected for subsequent ethanol production experiments.

Batch ethanol production from the EP medium in 5-litre and 50-litre bioreactors

The EP medium containing the optimum yeast extract concentration was transferred into a 5-liter bioreactor with the working volume of 3.5 litres and autoclaved at 110°C for 60 min. *S. cerevisiae* NP 01 was inoculated to give the final cell concentration of 1×10^8 cells ml^{-1}. The fermentation was conducted at 30°C and the agitation rate of 100 rev min^{-1}. The samples were withdrawn at time intervals for analysis. The control EP medium (without yeast extract addition) was also conducted to compare ethanol production. In a 50-liter bioreactor, the working volume was 40 liters and the EP medium was sterilized at 90°C for 30 min and then processed as previous mentioned.

Ethanol production from the EP medium under repeated-batch fermentation

The repeated-batch fermentation was carried out in the 5-liter bioreactor with the working volume of 3.5 litres and the agitation rate of 100 rev min^{-1}. The fermentation was first carried out in batch mode until the level of total sugars in the broth had dropped and remained approximately 20% of the initial value. The fermentation medium was then withdrawn at 50% of the working volume and the same amount of the fresh EP medium was immediately replaced to initiate the next batch. Eight successive batches were performed.

Analyses

The viable yeast cell numbers and total soluble solids of the fermentation broth were determined by direct counting method using haemacytometer and hand-held refractometer, respectively. The fermentation broth was centrifuged at 13,000 rev min^{-1} for 10 min. The supernatant was then determined for residual total sugars and reducing sugar by phenol sulfuric acid method and dinitrosalicylic acid (DNS) method, respectively. Ethanol concentration (P, g l^{-1}) was analyzed by gas chromatography (Shimadzu GC-14B, Japan, Solid phase: polyethylene glycol (PEG-20M), carrier gas: nitrogen, 150^{o}C isothermal packed column, injection temperature 180^{o}C, flame ionization detector temperature 250^{o}C; C-R7 Ae plus Chromatopac Data Processor) and 2-propanol was used as an internal standard (Modified from Laopaiboon et al., 2007). The ethanol yield (Y_{ps}, g ethanol per g sugar utilized) was calculated as the actual ethanol produced and expressed as g ethanol per g glucose utilized (g g^{-1}). The volumetric ethanol productivity (Q_p) and the percentage of conversion efficiency or yield efficiency (E_y) were calculated by the following equations:

$$Q_p = \frac{P}{t}$$

and

$$E_y = \frac{Y_{ps} \times 100}{0.54}$$

where P is the actual ethanol concentration produced (g l^{-1}), t is the fermentation time (h) giving the highest ethanol concentration and 0.54 is the maximum theoretical ethanol yield of sucrose consumption.

Results and discussion

Effects of yeast extract on batch ethanol fermentation under VHG conditions

Figure 1 shows the time profiles of total soluble solids, total sugar, reducing sugar, yeast cell and ethanol during batch fermentation of *S. cerevisiae* NP 01 from sweet sorghum juice supplemented with various yeast extract concentrations. Sugar consumption, ethanol production and yeast cell viability under various conditions were compared in Figure 2. The results showed that the amount of residual sugar in the EP medium containing yeast extract was less than that of the medium without yeast extract. The lowest residual sugar concentration (42 g l^{-1}) was found in the medium containing 9 g l^{-1} of yeast extract. This value corresponded to 85% of sugar utilization. The results obtained was agreed with Thomas et al. (1994) who found that yeast extract stimulated sugar utilization and ethanol production. In addition, Stewart et al. (1988) reported that when the concentrations of peptone and yeast extract in the medium were increased, yeast could more tolerate to osmotic pressure and high temperature.

Table 1 summarizes the important kinetic parameters (P, Q_p and Y_{ps}) of the ethanol fermentation from sweet sorghum juice under various yeast extract concentrations. Yeast extract at 3 g l^{-1} seemed not to have any significant effect on ethanol production efficiency. P, Q_p and Y_{ps} in the absence (control) and the presence of 3 g l^{-1} of yeast extract were not different. Higher yeast extract concentration slightly increased in P and Q_p suggesting that yeast extract could stimulate ethanol production in terms of both ethanol concentration and the rate of ethanol production. However, yeast extract did not improve the efficiency of glucose conversion to ethanol. Yield efficiency or E_y at all conditions were similar ranging from 91 to 94% of the theoretical yield.

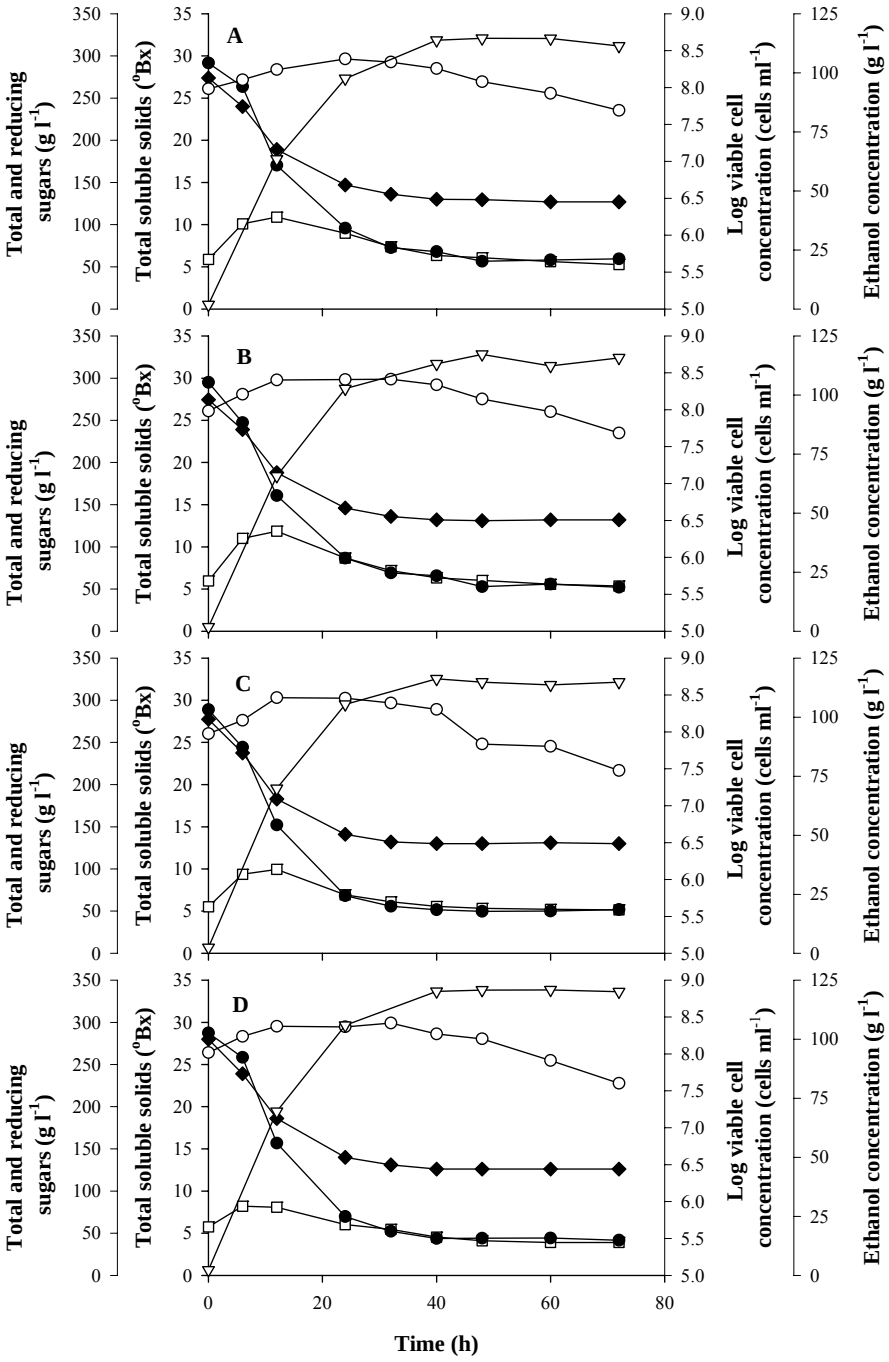

Figure 1 Fermentation kinetics during ethanol production by *S. cerevisiae* NP 01 from sweet sorghum juice containing various yeast extract concentrations (g l^{-1}): 0 (A), 3 (B), 6 (C) and 9 (D), ♦: total soluble solids (°Bx), ●: total sugar, □: reducing sugar, ○: log viable cell concentration and ∇: ethanol concentration.

The maximum ethanol concentration was obtained when 9 g l^{-1} of yeast extract was supplemented into the juice with the value of 120.24 ± 3.35 g l^{-1} (Table 1). This value was similar to that using sweet sorghum juice supplemented with 3 g l^{-1} of yeast extract and 5 g l^{-1} of peptone (YEP) (Table 2). In addition, ethanol productivity of the juice supplemented with 9 g l^{-1} of yeast extract was also higher than that of the juice supplemented with YEP. Therefore, in the subsequent experiments, YEP recommended in Melzoch medium (1994) was replaced by 9 g l^{-1} of yeast extract for ethanol production from sweet sorghum juice.

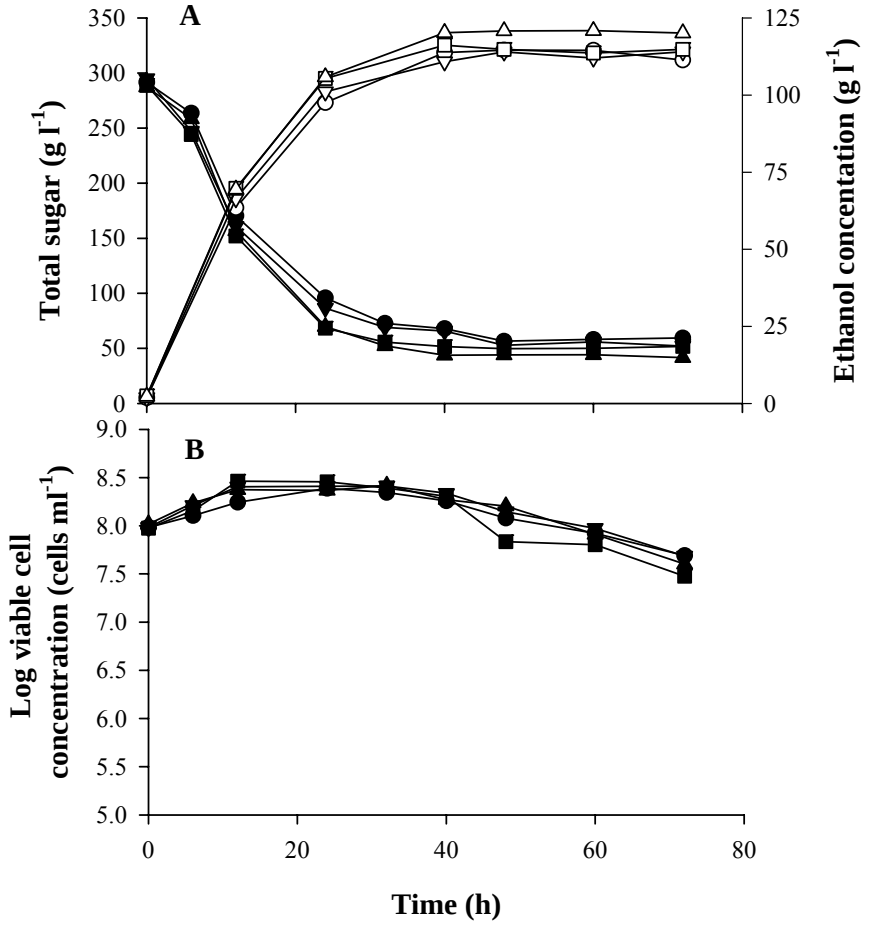

Figure 2 Sugar consumption, ethanol production and yeast viability during batch ethanol fermentation by *S. cerevisiae* NP 01 from sweet sorghum juice containing various yeast extract concentrations (g l^{-1}): 0 (●,○), 3 (▼,∇), 6 (■,□) and 9 (▲,Δ). (A) total sugar (close symbol) and ethanol concentration (open symbol) and (B) yeast cell concentration.

Table 1 Kinetic parameters of ethanol production in 500-ml Erlenmeyer flasks from sweet sorghum juice containing various yeast extract concentrations by *S. cerevisiae* NP 01.

Yeast extract concentration (g l^{-1})	Parameters (mean ± SD) [a]				
	P (g l^{-1})	Q_p (g $l^{-1}h^{-1}$)	Y_{ps} (g g^{-1})	E_y (%)	t * (h)
0 (control)	113.76±0.22	2.84±0.01	0.51±0.00	94.20±0.82	40
3	113.21±0.10	2.83±0.01	0.50±0.00	93.51±0.50	40
6	116.21±4.02	2.91±0.10	0.49±0.01	90.71±1.92	40
9	120.24±3.35	3.01±0.08	0.49±0.00	91.37±0.55	40

[a] P: ethanol concentration, Q_p: ethanol productivity, Y_{ps}: ethanol yield, E_y: yield efficiency.

* Fermentation time. The experiments were performed in duplicate.

Table 2 Comparison kinetic parameters of ethanol production in 500-ml Erlenmeyer flasks from sweet sorghum juice containing 9 g l^{-1} of yeast extract or YEP by *S. cerevisiae* NP 01.

Extra nitrogen sources	Parameters (mean ± SD) [a]				
	P (g l^{-1})	Q_p (g $l^{-1}h^{-1}$)	Y_{ps} (g g^{-1})	E_y (%)	t * (h)
9 g l^{-1} of yeast extract	120.24±3.35	3.01±0.08	0.49±0.00	91.37±0.55	40
YEP[b]	120.68 ± 0.54	2.01 ± 0.01	0.51 ± 0.00	93.76 ± 0.20	60

[a] P: ethanol concentration, Q_p: ethanol productivity, Y_{ps}: ethanol yield, E_y: yield efficiency.

* Fermentation time. The experiments were performed in duplicate.

[b] YEP: 3 g l^{-1} of yeast extract and 5 g l^{-1} of peptone

Scale up ethanol production from sweet sorghum juice

The effects of yeast extract on ethanol production from sweet sorghum juice under VHG fermentation were studied in small scale (the 500-ml air-locked Erlenmeyer flasks) under static condition. Scale up ethanol production should be investigated to confirm the results before launching to a commercial scale.

Figures 3 and 4 show the time profiles of total soluble solids, total sugar, reducing sugar, yeast cell and ethanol during batch fermentation of *S. cerevisiae* NP 01 from sweet sorghum juice with and without 9 g l^{-1} of yeast extract under VHG conditions in the 5-litre and 50-litre bioreactors, respectively. Sugar consumption, ethanol production and cell viability during ethanol fermentation from sweet sorghum juice with and without yeast extract supplementation in the different bioreactors were compared in Figure 5. The results clearly showed that yeast extract significantly stimulated the rate of sugar utilization and ethanol production in all bioreactors especially in the 50-litre bioreactor. The results were agreed with Thomas and Ingledew (1990) who reported that the fermentation of wheat mash containing 350 g l^{-1} dissolved solids were completed in 8 days at 20°C. When the mash was supplemented with 0.9% yeast extract, the fermentation time was reduced to 3 days with ethanol concentration of 17.1% (v/v).

In the 5-litre bioreactor, the sugar was almost utilized regardless of the presence of yeast extract. This might be due to the effect of agitation. The results also indicated that the agitation rate at 100 rev min^{-1} was suitable for mixing the fermentation broth for ethanol production. However, in the 50-litre bioreactor with the agitation rate of 100 rev min^{-1}, complete sugar utilization did not occur. The residual sugar concentration in the broth with and without yeast extract was 13 and 45 g l^{-1}, respectively. This may be due to no baffle in the 50-litre bioreactor resulting in vortex and swirl phenomena in the bioreactor.

Table 3 summarizes the important kinetic parameters and cell viability during ethanol production at the different bioreactors. The maximum ethanol concentration and its productivity were obtained in the 5-litre bioreactor containing the juice supplemented with yeast extract with the values of 139.51 ± 0.11 g l^{-1} and 3.49 ± 0.00 g l^{-1} h^{-1}, respectively.

Figure 5C shows the yeast viability during ethanol fermentation from sweet sorghum juice with and without yeast extract supplementation. The results indicated that yeast extract did not promote yeast viability in the 500-ml and 5-litre bioreactors. However, at 12 hours of the fermentation in the 50-litre bioreactor, viable cell concentration in the broth containing yeast extract was approximately 1.5 times higher than that in the broth without yeast extract. The cell concentrations in the fermentation broths were relatively constant throughout the experiments.

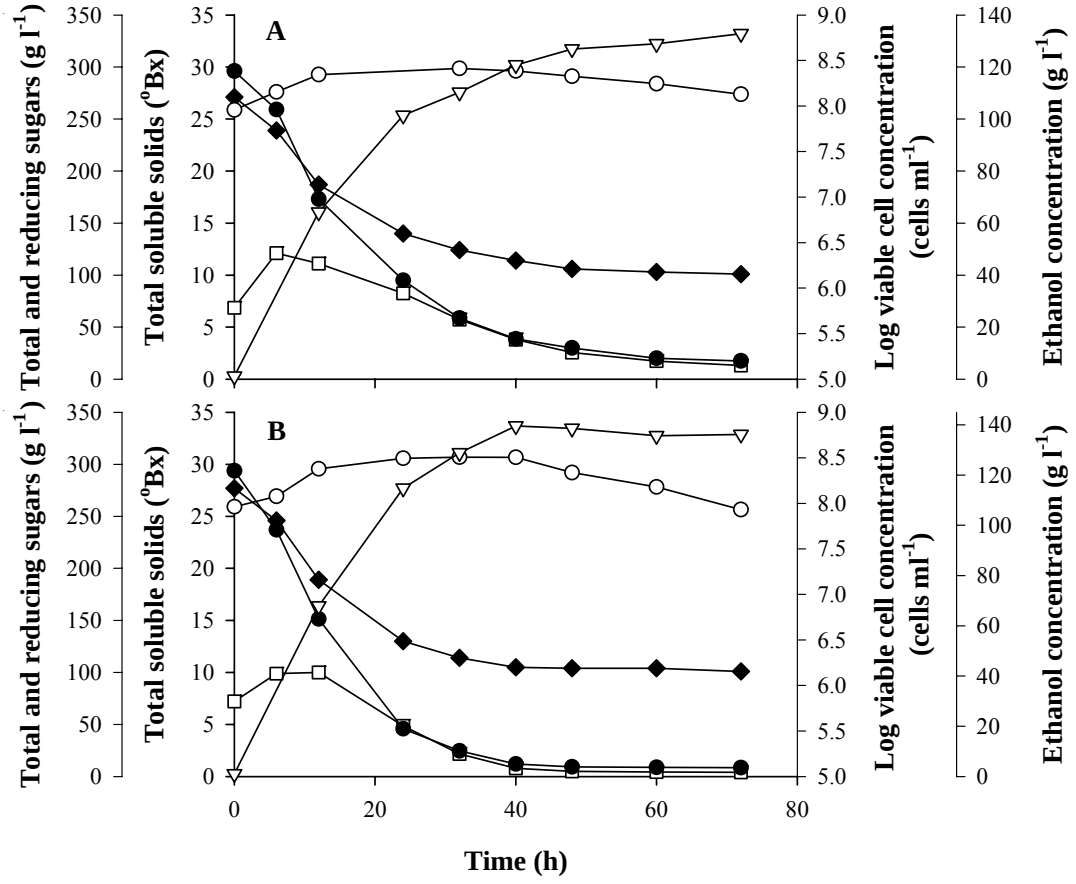

Figure 3 Fermentation kinetics during ethanol production by *S. cerevisiae* NP 01 from sweet sorghum juice with and without 9 g l^{-1} of yeast extract under VHG conditions in a 5-litre bioreactor: without (A) and with yeast extract (B) ♦: total soluble solids (°Bx), ●: total sugar, □: reducing sugar, ○: log viable cell concentration and ∇: ethanol concentration.

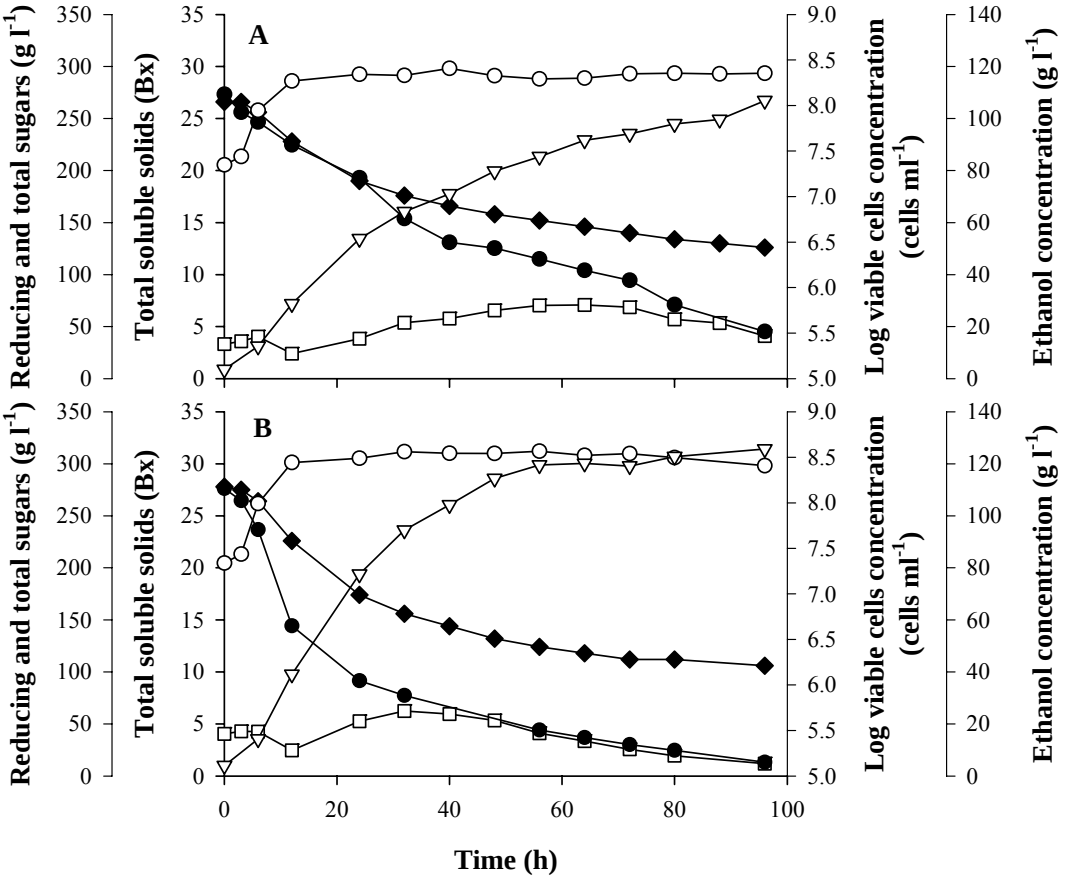

Figure 4 Fermentation kinetics during ethanol production by *S. cerevisiae* NP 01 from sweet sorghum juice with and without 9 g l^{-1} of yeast extract under VHG conditions in a 50-litre bioreactor: without (A) and with yeast extract (B) ♦: total soluble solids (°Bx), ●: total sugar, □: reducing sugar, ○: log viable cell concentration and ∇: ethanol concentration.

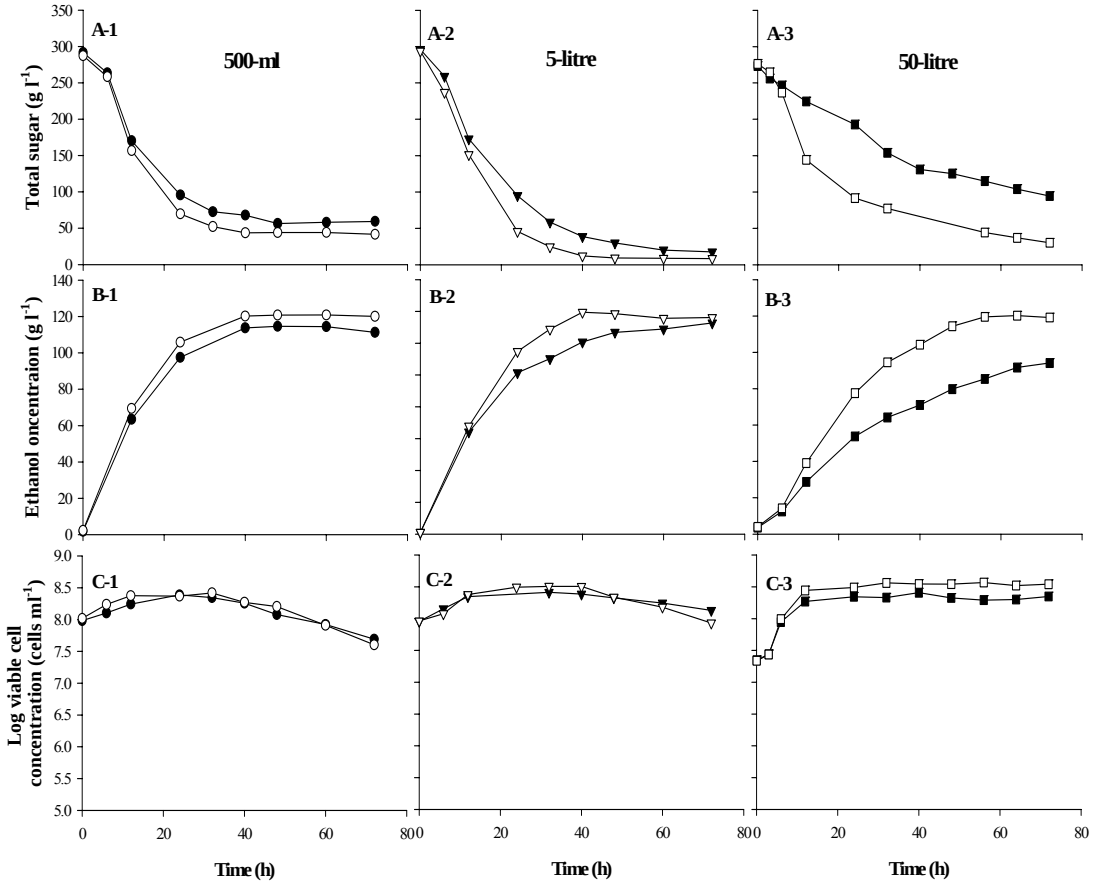

Figure 5 Fermentation kinetics during ethanol production by *S. cerevisiae* NP 01 from sweet sorghum juice with and without 9 g l^{-1} of yeast extract under VHG conditions in different bioreactors: (A) total sugar, (B) ethanol concentration and (C) yeast viability; (1) 500-ml Erlenmeyer flask, (2) 5-litre and (3) 50-litre; close symbol: without yeast extract and open symbol: with 9 g l^{-1} of yeast extract.

Table 3 Kinetic parameters of ethanol production in different bioreactors from sweet sorghum juice with and without 9 g l^{-1} of yeast extract by *S. cerevisiae* NP 01.

Bioreactor volume	Yeast extract concentration (g l^{-1})	Parameters (mean ± SD)[a]					Cell concentration (cells ml^{-1}) at	
		P (g l^{-1})	Q_p (g $l^{-1}h^{-1}$)	Y_{ps} (g g^{-1})	E_y (%)	t* (h)	0 h	t* (h)
500-ml	0	113.76 ±0.22	2.84 ±0.01	0.51 ±0.00	94.20 ±0.82	40	9.75×10^7	2.59×10^8
	9	120.24 ± 3.35	3.01 ± 0.08	0.49 ± 0.00	91.37 ±0.55	40	1.06×10^8	2.85×10^8
5-litre	0	128.93 ± 1.01	2.15 ± 0.02	0.52 ± 0.02	95.38 ±3.06	60	9.38×10^7	3.06×10^8
	9	139.51 ± 0.11	3.49 ± 0.00	0.49 ± 0.00	91.66 ± 0.69	40	9.63×10^7	4.09×10^8
50-litre	0	106.85 ±0.25	1.11 ±0.02	0.47 ±0.01	86.85 ±0.50	96	2.25×10^7	2.26×10^8
	9	119.53 ±0.20	2.13 ±0.01	0.51 ±0.01	95.36 ±0.42	56	2.20×10^7	3.68×10^8

[a] P: ethanol concentration, Q_p: ethanol productivity, Y_{ps}: ethanol yield, E_y: yield efficiency.

* Fermentation time.

In a large scale bioreactor, it is difficult to prepare high initial cell concentrations up to 1×10^8 cell ml^{-1}. Therefore, the initial cell concentration in the 50-litre bioreactor was only 2.2×10^7 cell ml^{-1}. The cell concentration reached to 8.8×10^7 cells ml^{-1} in 6 hours and subsequently grew up to 12 hours with the values of approximately 2.8×10^8 and 1.9×10^8 cells ml^{-1} in the broth with and without yeast extract supplementation, respectively. Then the cell number was relatively constant throughout the experiments. As the yeast cells rapidly grew in the first 6 hours (growth phase), the fermentation time in the 50-litre bioreactor was extended from 40 to 56 hours compared to that in the 5-litre bioreactor.

Ethanol concentration from the juice containing yeast extract in the 50-litre bioreactor (120 g l^{-1}) was lower than that in the 5-litre bioreactor (140 g l^{-1}). This may be due to some carbon sources in the 50-litre bioreactor were utilized for microbial growth in the first 12 hours.

Ethanol production from sweet sorghum juice under repeated-batch fermentation

In the repeated-batch fermentation in the 5-litre bioreactor, the juice was withdrawn at 50% of the working volume and the same amount of the fresh juice was immediately replaced. Fermentation broth at 50% of the working volume was withdrawn in this study because higher percentage of the withdrawal may cause lower initial cell concentration in the next batch. This will directly affect on ethanol productivity as mentioned by Laopaiboon et al. (2007). The time profiles of total sugar, ethanol concentration and cell viability of the ethanol fermentation from sweet sorghum juice supplemented with yeast extract are shown in Figure 6. In the first batch, the sugar was almost utilized and the P, Q_p and Y_{ps} were 129.48 ± 2.68 g l^{-1}, 4.01 ± 0.09 g l^{-1} h^{-1} and 0.48 ± 0.02, respectively at 32 h (Table 5.4). In batch 2 to 8, the residual sugar concentrations in the broth were approximately 54 to 82 g l^{-1}, while the ethanol concentrations were similar ranging from 101.44 ± 6.09 to 109.53 ± 3.58 g l^{-1} at the fermentation time of 64 to 72 h. Initial sugar concentrations of fermentation broth in batch 2 to 8 were approximately 139 to 190 g l^{-1} resulting in lower final ethanol concentration compared to that in batch 1. Lower ethanol productivity in batch 2 to 8 may be due to lower cell concentration in the broth (Figure 6 and Table 4). Cell recycling should be performed to increase initial cell concentration in the subsequent batches.

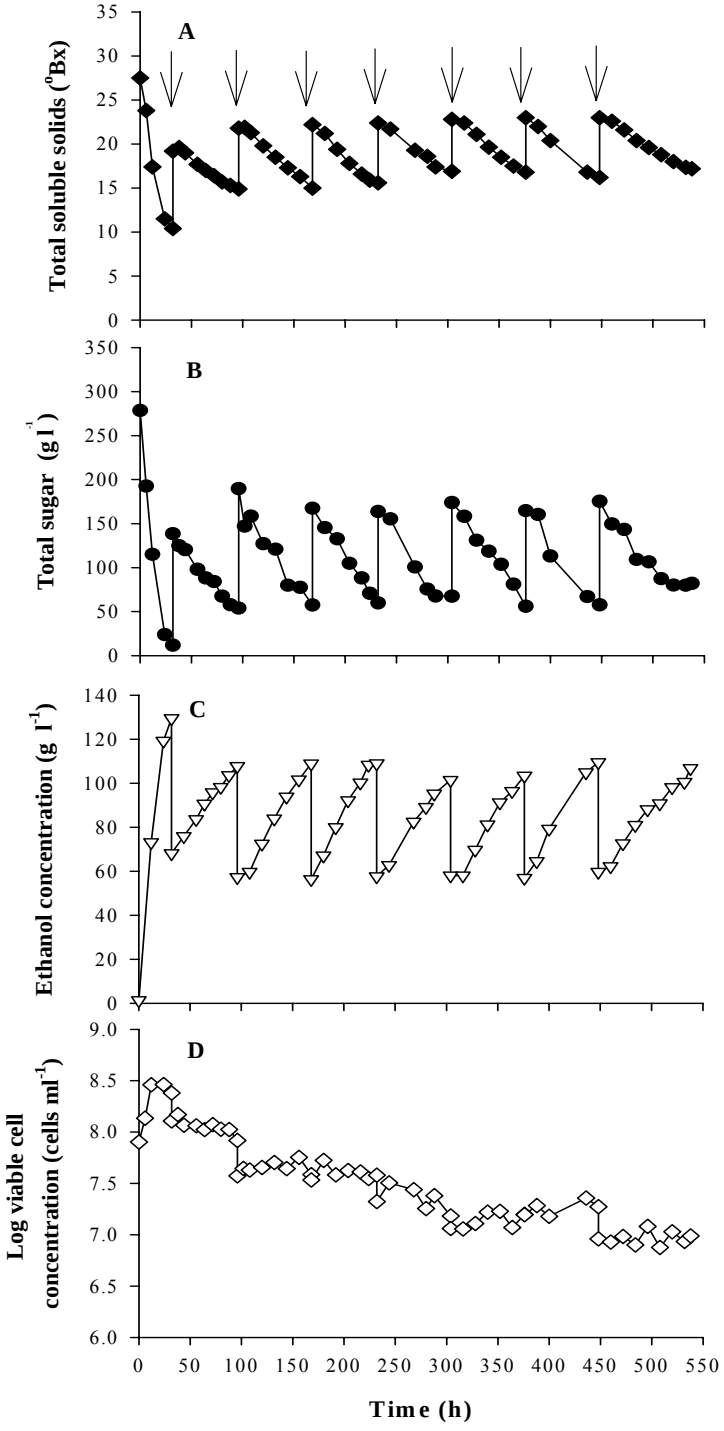

Figure 6 Fermentation kinetics during ethanol production in repeated-batch mode by *S. cerevisiae* NP 01 from sweet sorghum juice containing 9 g l^{-1} of yeast extract: total soluble solids (A), total sugar (B), ethanol concentration (C) and cell viability (D). The arrows indicate the start time of each batch.

Table 4 Kinetic parameters of ethanol production from the sweet sorghum juice in repeated-batch fermentation.

	Parameters (mean ± SD)[a]						Cell concentration (cells ml^{-1})	
Batch number	P [b] ($g\ l^{-1}$)	P [c] ($g\ l^{-1}$)	Q_p ($g\ l^{-1}h^{-1}$)	Y_{ps} ($g\ g^{-1}$)	E_y (%)	t [d] (h)	Initial	t [d] (h)
1	129.48 ± 2.96	129.48 ± 2.96	4.01 ± 0.09	0.48 ± 0.02	89.05 ± 3.03	32	8.24×10^7	3.28×10^8
2	107.80 ± 0.39	39.70 ± 0.39	0.62 ± 0.01	0.47 ± 0.01	86.81 ± 2.37	64	1.87×10^8	1.72×10^8
3	108.90 ± 7.26	51.67 ± 7.26	0.72 ± 0.10	0.46 ± 0.01	84.93 ± 1.75	72	8.60×10^7	8.80×10^7
4	109.02 ± 0.39	52.65 ± 0.39	0.82 ± 0.01	0.49 ± 0.02	90.72 ± 3.12	64	7.85×10^7	9.10×10^7
5	101.44 ± 6.09	43.81 ± 6.09	0.61 ± 0.08	0.48 ± 0.14	84.34 ± 2.39	72	5.04×10^7	4.50×10^7
6	103.37 ± 3.35	45.35 ± 3.35	0.63 ± 0.05	0.42 ± 0.03	77.01 ± 4.70	72	2.79×10^7	2.92×10^7
7	109.53 ± 3.58	52.61 ± 3.58	0.73 ± 0.05	0.49 ± 0.02	91.10 ± 4.18	72	2.91×10^7	3.60×10^7
8	102.46 ± 2.68	44.76 ± 2.68	0.53 ± 0.03	0.45 ± 0.01	82.92 ± 2.09	72	1.78×10^7	2.02×10^7

[a] P: ethanol concentration, Q_p: ethanol productivity, Y_{ps}: ethanol yield, E_y: yield efficiency.

[b]: Final ethanol concentration

[c]: Ethanol concentration produced

[d]: Fermentation time

Conclusions

Yeast extract, agitation and initial yeast cell concentration are important parameters to promote the rate of sugar consumption and ethanol production from sweet sorghum juice under VHG fermentations. Particulate yeast cell wall products may replace yeast extract as it is far less expensive, and will be suitable for industrial scale uses. Ethanol production from sweet sorghum juice at VHG levels under repeated-batch fermentation could be carried out for at least eight successive batches. To improve ethanol production efficiency in the repeated-batch fermentation, cell

recycling or cell immobilization should be performed to increase the initial cell concentration in the subsequent batches.

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References

Bafrncová, P., Šmogrovičová, D., Sláviková, I., Pátková, J., Dőmény, Z. 1999. Improvement of very high gravity ethanol fermentation by media supplementation using *Saccharomyces cerevisiae*. **Biotechnol. Lett**., 21, 337–341.

Bajpai, P, Bajpai P.K. 1988. Repeated fed batch fermentation for single cell protein production from prehydrolysate of a pulp mill. **Enzyme Microb. Technol**., 10, 280-283.

Bayrock, D.P., Ingledew, W.M. 2001. Application of multistage continuous fermentation for production of fuel alcohol by very-high-gravity fermentation technology. **J. Ind. Microbiol. Biotechnol**., 27, 87-93.

Birch, R.M., Walker, G.M. 2000. Influence of magnesium ions on heat shock and ethanol stress responses of *Saccharomyces cerevisiae*. **Enzyme Microb. Technol**., 26, 678–687.

Casey, G. P., Magnus, C. A., Ingledew, W. M. 1984. High gravity brewing: effects of nutrition on yeast composition, fermentative ability, and alcohol production. **Appl. Environ. Microbiol**., 48, 639-646.

Ciesarova, Z., Smogrovicova, D., Domeny, Z. 1996. Enhancement of yeast ethanol tolerance by calcium and magnesium. **Folia. Microbiol**., 41, 485–488.

Dombek, K.M., Ingram, L.O. 1986. Magnesium limitation and its role in apparent toxicity of ethanol during yeast fermentation. **Appl. Environ. Microbiol**., 52, 975–981.

Göksungur, Y., Zorlu, N., 2001. Production of ethanol from beet molasses by Ca-alginate immobilized yeast cells in a packed-bed bioreactor. **Turk. J. Biol**., 25, 265–275.

Jones, A.M., Ingledew, W.M. 1994. Fuel ethanol production: appraisal of nitrogenous yeast foods for very high gravity wheat mash fermentation. **Process Biochem**., 29, 483–488.

Jones, A.M., Thomas, K.C., Ingledew, W.M., 1994. Ethanolic fermentation of blackstrap molasses and sugarcane juice using very high gravity technology. **J. Agric. Food Chem**., 42, 1242-1246.

Laopaiboon, L., Nuanpeng, S., Srinophakun, P., Klanrit, P., Laopaiboon, P., in press. Selection of *Saccharomyces cerevisiae* and Investigation of Its Performance for Very High Gravity Ethanol Fermentation. **Biotechnol.**

Laopaiboon, L., Thanonkeo, P., Jaisil, P., Laopaiboon, P. 2007. Ethanol production from sweet sorghum juice in batch and fed-batch fermentations by *Saccharomyces cerevisiae*. **World J. Microbiol. Biotechnol**., 23, 1497-1501.

Leao, C., Van Uden, N. 1983. Effects of ethanol and other alcohols on the general amino acid permease of *Saccharomyces cerevisiae*. **Biotechnol. Bioeng**., 26, 403–405.

Melzoch, K., Rychtera, M., Hábová, V. 1994. Effect of immobilization upon the properties and behaviour of *Saccharomyces cerevisiae* cells. **J. Biotechnol**., 32, 59-65.

Nabais, R.C., Sa Correia, I., Viegas, C.A., Novais, J.M. 1988. Influence of calcium ion on ethanol tolerance of *Saccharomyces bayanus* and alcoholic fermentation by yeasts. **Appl. Environ. Microbiol**., 54, 2439–2446.

Najafpour, G., Younesi, H., Ismail, K.S.K. 2004. Ethanol fermentation in an immobilized cell reactor using *Saccharomyces cerevisiae*. **Bioresour. Technol**., 92, 251-260.

Niessen, T.L., Kielland-Brandt, M.C., Nielsen, J., Villadsen, J. 2000. Optimization of ethanol production in *Saccharomyces cerevisiae* by metabolic engineering of the ammonium assimilation. **Metab. Eng**., 2, 69–77.

Nuanpeng, S., Laopaiboon, P., Srinophakun, P., Klanrit, P., Laopaiboon, L. 2007. Ethanol production from sweet sorghum juice by using VHG technology: Effects of carbon and nitrogen supplementations. **Proceeding of 2nd International Conference on Fermentation Technology for Value Added Agricultural Product.** Khon Kaen.

Odumeru, J. A., D'Amore, T., Russell, I., Stewart, G. G. 1992. Effects of heat shock and ethanol stress on viability of a *Saccharomyces uvarum* (*carlsbergensis*) brewing yeast strain during fermentation brewing yeast strain during fermentation of high gravity wort. **J. Ind. Microbiol**., 10, 111-116.

Pratt-Marshall, P.L., Bryce, J.H., Stewart, G.G. 2003. The effects of osmotic pressure and ethanol on yeast viability and morphology. **J. Inst. Brew**., 109, 218–228.

Schaffert, R.E. 1995. Sweet sorghum substrate for industrial alcohol. In Dendy D.A.V., (Ed.). **Sorghum and Millets: Chemistry and Technology.** Saint Paul: American Association of Cereal Chemists.

Sree, N.K., Sridhar, M., Rao, L.V., Pandey, A. 1999. Ethanol production in solid substrate fermentation using thermotolerant yeast. **Process Biochem**., 34, 115-119.

Stewart, G. G., T. D'Amore, C. J. Panchal, and I. Russell. 1988. Factors that influence the ethanol tolerance of brewer's yeast strains during high gravity wort fermentations. **Master Brew. Assoc. Am**., 25:47-53.

Tanaka, K., Hilary, Z.D., Ishizaki, A. 1999. Investigation of the utility of pineapple juice and pineapple waste material as low-cost substrate for ethanol fermentation by Zymomonas mobilis. **J. Biosci. Bioeng**., 87, 642–646.

Tao, F., Miao, J.Y., Shi, G.Y., Zhang, K.C. 2005. Ethanol fermentation by an acid-tolerant *Zymomonas mobilis* under non-sterilized condition. **Process Biochem**., 40, 183–187.

Thomas, K.C., Ingledew, W.M. 1990. Fuel alcohol production: effects of free amino nitrogen on fermentation of very-high-gravity wheat mash. **Appl. Environ. Microbio**l., 56, 2046–2050.

Thomas, K.C., Ingledew, W.M. 1990. Production of 21% (v/v) ethanol by fermentation of very high gravity (VHG) wheat mashes. **J. Ind. Microbiol**., 10, 61–68.

Thomas, K.C., Hynes, S.H., Ingledew, W.M. 1994. Effects of particulate materials and osmoprotectants on very-high-gravity ethanolic fermentation by *Saccharomyces cerevisiae*. **Appl. Environ. Microbiol**., 60**,** 1519–1524.

Thomas, K.C., Hynes, S.H., Jones, A.M., Ingledew, W.M. 1993. Production of fuel alcohol from wheat by VHG technology. **Appl. Biochem. Biotechnol**., 43, 211–226.

Vitolo, M. 1996. Production of ethanol and invertase by *S. cerevi*siae grown in blackstrap molasses. In Chartier, P., Ferrero, G.L., Henius, U.M., Hultberg, S., Sachau, J., Wiinblad, M. (Eds). **Proceedings of the 7th Biomass for Energy and the Environment.** Copenhagen (Denmark). Oxford: Pergamon Press.

Woods, J., 2000. **Integrating Sweet sorghum and sugarcane for bioenergy: Modeling the potential for electricity and ethanol production in SE Zimbabwe**, Ph.D. Thesis, King's College, London.

Output จากโครงการวิจัย

1. ผลงานที่ตีพิมพ์ในวารสารนานาชาติ

1.1 Laopaiboon, L., Nuanpeng, S., Srinophakun, P., Klanrit, P. and Laopaiboon, P. (2008) Selection of *Saccharomyces cerevisiae* and investigation of its performance for very high gravity ethanol fermentation. **Biotechnology.** 7(3): 493-498.

2. ผลงานที่เสนอเพื่อขอตีพิมพ์ในวารสารนานาชาติ

2.1 Laopaiboon, L., Nuanpeng, S., Srinophakun, P., Klanrit, P. and Laopaiboon, P. Ethanol production from sweet sorghum juice by using VHG technology: Effects of carbon and nitrogen supplementations, **World Journal of Microbiology and Biotechnology (submitted on April, 18, 2008)**.

3. ผลงานที่เสนอในที่ประชุมวิชาการ

3.1 Nuanpeng, S., Laopaiboon, P., Srinophakun, P., Klanrit, P. and Laopaiboon, L. (2006) Selection of Saccharomyces cerevisiae and investigation of its performance for very high gravity ethanol fermentation. In : *18th Annual Meeting of the Thai Society for Biotechnology, Biotechnology: Benefits & Bioethics*, November 2-3, 2006, Bangkok, Thailand.

3.2 Nuanpeng, S., Laopaiboon, P., Srinophakun, P., Klanrit, P. and Laopaiboon, L. (2007) Ethanol production from sweet sorghum juice by using VHG technology: Effects of carbon and nitrogen supplementations In : *2nd International Conference on Fermentation Technology for Value Added Agricultural Products*, May 23-25, 2007, Khon Kaen, Thailand.

3.3 Nuanpeng, S., Laopaiboon, P., Srinophakun, P., Klanrit, P. and Laopaiboon, L. (2007) Batch and repeated batch ethanol fermentations from sweet sorghum juice under very high gravity conditions by *Saccharomyces cerevisiae*. In : *The 5th International Symposium on Biocontrol and Biotechnology*, November 1-3, 2007, Nong Khai, Thailand.

ภาคผนวก

ผลงานที่ตีพิมพ์ในวารสารนานาชาติ

1. Laopaiboon, L., Nuanpeng, S., Srinophakun, P., Klanrit, P. and Laopaiboon, P. (2008) Selection of *Saccharomyces cerevisiae* and investigation of its performance for very high gravity ethanol fermentation. **Biotechnology.** 7(3): 493-498.

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