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Repeated-batch ethanol fermentation from sweet sorghum juice by free cells of *Saccharomyces cerevisiae* NP 01

Pongthep Ariyajarearnwong[1], Lakkana Laopaiboon[2,3]*, Prasit Jaisil[4] and Pattana Laopaiboon[2]

[1]Graduate School, Khon Kaen University, Khon Kaen 40002, Thailand.
[2]Department of Biotechnology, Faculty of Technology, Khon Kaen University, Khon Kaen 40002, Thailand.
[3]Fermentation Research Center for Value Added Agricultural Products, Khon Kaen University, Khon Kaen 40002, Thailand.
[4]Department of Plant Science and Agricultural Resources, Faculty of Agriculture, Khon Kaen University, Khon Kaen 40002, Thailand.

Low-cost inoculum preparation (IP) media for ethanol production were developed. It was found that sweet sorghum juice (SSJ) containing 100 g l^{-1} of total sugar without nutrient supplement could be used as the low-cost IP medium instead of the typical IP medium or yeast extract malt extract (YM) medium. Ethanol production from the SSJ (total soluble solids of 24 °Bx) by the inoculum of *Saccharomyces cerevisiae* NP 01 in batch and repeated-batch fermentations was then investigated. The fermentations were carried out under static condition in 500 ml air-locked Erlenmeyer flasks at 30°C and the initial yeast cell concentration was 1×10^8 cells ml^{-1}. In the batch fermentation, the concentration (*P*), productivity (*Q_p*) and yield (*Y_{p/s}*) of ethanol were 110.09 ± 0.81 g l^{-1}, 2.29 ± 0.01 g l^{-1} h^{-1} and 0.51 ± 0.02, respectively. In the repeated-batch fermentation, the yeasts could be used at least eight successive batches without a marked decrease in ethanol production. The repeated-batch fermentation with fill and drain volume at 75% of the working volume gave higher ethanol production efficiencies than those at 50% of the working volume in terms of total ethanol production rate (g h^{-1}). The average *P*, *Q_p* and *Y_{p/s}* of the eight successive batches at 75% fill and drain volume in a 2 L bioreactor at the agitation rate of 100 rev min^{-1} were 93.30 ± 9.44 g l^{-1}, 1.21 ± 0.43 g l^{-1} h^{-1} and 0.48 ± 0.03, respectively.

Key words: Repeated-batch, ethanol fermentation, low-cost nutrient, sweet sorghum juice, *Saccharomyces cerevisiae*.

INTRODUCTION

Nowadays, the ethanol industry utilizes raw materials rich in saccharides such as sugar cane or sugar beets and raw materials rich in starch such as corn and wheat (Rudolf et al., 2009). In Thailand, the main raw materials used for ethanol production are sugarcane molasses and cassava. Regarding the energy policy of the Thai government, ethanol production will be increased to 3,000,000 L day^{-1} in year 2012 and to 9,000,000 L day^{-1} in year 2022 (Department of Energy Business, 2010). Therefore, it is possible that Thailand may face a shortage of sugar cane molasses and cassava.

Sweet sorghum [*Sorghum bicolor* (L.) Moench] is a high biomass- and sugar-yielding C4 plant (Bryan, 1990) containing high fermentable sugars and insoluble

*Corresponding author. E-mail: lakcha@kku.ac.th. Tel: +66 43 362121, Fax: +66 43 362121.

Abbreviations:**IP**, Inoculum preparation; **YM**, yeast extract malt extract; ***Q_p***, ethanol productivity; ***P***, ethanol concentration; ***Y_{p/s}***, ethanol yield; **YE**, yeast extract; **ME**, malt yeast; **PE**, peptone; **DNS**, dinitrosalicylic acid; **GC**, gas chromatography; **DSY**, dried spent yeast; μ, specific growth rate; **SSJ**, sweet sorghum juice.

carbohydrates (cellulose and hemicellulose) (Bennett and Anex, 2009). Its grain and stem can be used as substrates for the production of sugar, alcohol syrup, fodder, fuel, bedding, roofing, fencing and paper (Rajvanshi and Nimbkar, 2005). Due to the energy crisis, renewable carbohydrate materials including sweet sorghum have become more of interest for biological transformation into ethanol for use as fuel or fuel additive (Göksungur and Zorlu, 2001). Sweet sorghum has been noted for its potential as an energy crop because it can be cultivated at almost all temperature and tropical climate areas (Bennett and Anex, 2009) with the growing period of 120 to 150 days (Wu et al., 2010). The juice from the stalks contains many essential trace elements for microbial growth and ethanol production (Dajui, 2010; Laopaiboon et al., 2009). In addition, the pH of the juice is in the range of the optimum pH for yeast growth and ethanol production (pH 4.0 to 5.5) (Narendranath and Power, 2005).

One of the key concepts to achieve high ethanol production efficiency is based on a high cell con-centration because the ethanol productivity is propor-tional to the biocatalyst concentration during ethanol fermentation (Ikegami et al., 1998). *Saccharomyces cerevisiae* is one of the main biocatalysts or ethanol-producing organisms used in industrial processes. In typical ethanol fermentation, the yeast cells are cultured in yeast extract-malt extract (YM) medium. However, the prices of the YM medium compo-nents such as glucose, yeast extract, malt extract and peptone are relatively expensive. To minimize the cost of inoculum preparation (IP) medium, low-cost substrates and/or nutrient supplements such as sweet sorghum juice (SSJ) (Dajui, 2010; Laopaiboon et al., 2009) and dried spent yeast (DSY) (Sridee et al., 2009; Ferreira et al., 2010) may be used instead of the expensive components.

Apart from the development of new sweet sorghum cultivars with high grain and sugar yield (Almodares et al., 2007), fermentation process development is also im-portant for efficient ethanol production from SSJ (Laopaiboon et al., 2007). In this study, we are interested in repeated-batch fermentation because this process has several advantages compared to conventional batch fer-mentation such as no new inoculum requirement for each batch (Bajpai and Bajpai,1988) and long-term productivi-ty (Anastassiadis and Rehm 2006). In addition, no time is wasted for cleaning and resterilization, and the opera-tional control is easier than that of a continuous mode.

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. The repeated-batch process has been used to improve several bio-product formation such as citric acid (Anastassiadis and Rehm, 2006), hydrogen (Poggi-Varaldo et al., 2009), L-lactic acid (Akao et al., 2007; Wu and Jiang, 2009) and ethanol (Choi et al., 2009; Ma et al., 2009; Staniszewski et al., 2009). The

main important factors affecting the production efficiency in the repeated-batch process are cell concentration, fermentation time and recycling volume (Chen et al., 2008; Choi et al., 2009; Staniszewski et al., 2009).

The aim of this study was to develop a low-cost IP medium for ethanol production and to improve ethanol production efficiency from SSJ using repeated-batch fermentation (fill and drain technique) by *S. cerevisiae* NP 01. The effects of fill and drain volume in the repeated-batch system on ethanol production were also investigated.

MATERIALS AND METHODS

Composition of nutrient supplements

The main composition of sweet sorghum, yeast extract (YE) and DSY was analyzed by Central Laboratory (Thailand) Co., Ltd., Khon Kaen, Thailand.

Microorganism, inoculum preparation, inoculum preparation (IP) medium

S. cerevisiae NP 01 isolated from Loog-pang (Chinese yeast cake) from Nakorn Panom province, Thailand, was inoculated into a 250 ml Erlenmeyer flask containing 150 ml of YM medium as the first medium. The medium contained (in g l^{-1}) YE 3, malt extract (ME) 3, peptone (PE) 5 and glucose 10. The flask was incubated on a rotating shaker at 150 rev min^{-1}, 30°C for 15 h.

To increase cell concentration, the yeasts (10% inoculum size) were transferred into 500 ml Erlenmeyer flasks with 350 ml of modified IP media as shown in Table 1. The flasks were further incubated under the conditions previously mentioned and the viable yeast cell concentration was measured every 3 h for 24 h. Specific growth rate (μ) of the yeasts in the IP media was calculated. The IP medium giving the highest μ was used for inoculum preparation for ethanol production.

Raw materials

SSJ cv. KKU40 modified from cv. Keller was obtained from the department of Agronomy, Faculty of Agriculture, Khon Kaen University, Thailand. After extraction, the juice containing total soluble solids of 18°Bx, was concentrated to 75°Bx and kept at -18°C until use. DSY obtained from Beerthip Brewery (1991) Co., Ltd., Bang Baan, Phra Nakhon Sri Ayutthaya, Thailand was kept at room temperature until use.

Ethanol production medium

The concentrated SSJ was diluted with distilled water to the total soluble solids of 24°Bx and used as an ethanol production medium. The ethanol production medium was transferred into a 500 ml air-locked Erlenmeyer flask (working volume of 400 ml) or a 2 L bioreactor (working volume of 1500 ml) and autoclaved at 110°C for 15 (for the 500-ml flask) or 28 min (for the 2 L bioreactor) (Laopaiboon et al., 2009).

Fermentation processes

Batch fermentation system

The yeasts grown in the optimum IP medium were inoculated into

Table 1. Composition of yeast inoculum preparation (IP) media.

Medium code*	Composition (g l^{-1})
YM 1 (control 1)	YE 3, ME 3, PE 5 and glucose 20
YM 2 (control 2)	YE 3, ME 3, PE 5 and glucose 100
SSJ 1	SSJ containing total sugar of 20 (SSJ 20)
SSJ 2	SSJ 20 and DSY 11
SSJ 3	SSJ 20, YE 1.5, ME 1.5 and PE 2.5
SSJ 4	SSJ containing total sugar of 100 (SSJ 100)
SSJ 5	SSJ 100 and DSY 11
SSJ 6	SSJ 100, YE 1.5, ME 1.5 and PE 2.5

* YM, Yeast extract malt extract medium; **SSJ**, sweet sorghum juice; YE, yeast extract; ME, malt extract; PE, peptone; DSY, dried spent yeast.

the sterile ethanol production medium in the 500 ml air-locked Erlenmeyer flask to give the initial cell concentration of 1×10^8 cells ml^{-1}. The fermentation was operated at 30°C under static condition. The samples were collected at time intervals for analysis.

Repeated-batch fermentation system

The repeated-batch system in the 500 ml flask was first carried out in batch mode as described above. When the total residual sugars in the broth had dropped slowly as found in the batch fermentation system, the fermented broth was withdrawn at 75 and 50% of the working volume and the same amount of the fresh juice was immediately replaced to initiate the next batch. This method was called "fill and drain technique" (Anastassiadis and Rehm, 2006). Eight successive batches were performed. The fill and drain volume giving the maximum ethanol production was selected for the fermentation in the 2 L bioreactor.

In the 2 L bioreactor, the fermentation was operated as in the flask, but the medium was agitated at 100 rev min^{-1}. Eight successive batches were performed.

Analytical methods

The cell numbers and the total soluble solids in the fermentation broth were determined by direct counting method using haemacytometer with methylene blue staining technique and hand-held refractometer (Zoecklien et al., 1995), respectively. The μ was calculated from the slope of natural log viable cell concentration in log phase against time. The fermentation broth was centrifuged at 13,000 rev min^{-1} for 10 min. Then, the supernatant was determined for residual total sugar and reducing sugar by phenol-sulfuric acid method (Mecozzi, 2005) and dinitrosalicylic acid (DNS) method (Bailey, 1988), respectively. Ethanol concentration (*P*) was analyzed by gas chromatography (Shimadzu GC-14B, Japan, solid phase: polyethylene glycol (PEG-20M), carrier gas (200 KPa): nitrogen, 150°C isothermal packed column, injection temperature 180°C, flame ionization detector temperature 250°C; gas chromatography (GC) solution analysis Version 2.30) and 2-propanol was used as an internal standard (modified from Laopaiboon et al., 2007). The ethanol yield ($Y_{p/s}$) was calculated and expressed as g ethanol produced per g sugar utilized (g g^{-1}). The volumetric ethanol productivity (Q_p, g l^{-1} h^{-1}) was calculated by the following equation:

$$Q_p = P / t$$

Where, *P* is the actual ethanol concentration produced (g l^{-1}) and *t*

is the fermentation time (h) giving the highest *P* for batch and repeated-batch fermentations.

RESULTS AND DISCUSSION

Composition and cost of nutrient supplements

Some nutrient components of the concentrated SSJ, YE and DSY are shown in Table 2. The DSY, a by-product from brewery's industry, was evaluated as a complex nitrogen source because it contained high levels of nitrogen and minerals. Total nitrogen content in DSY was about 60% of that in YE. Potassium, sodium, zinc and chloride contents were much lower than those of YE whereas calcium, magnesium and iron contents were higher. Other components detected in DSY were comparable with those of yeast extract. In Thailand, the cost of yeast extract (HiMedia laboratory, India) and DSY (Beerthip Brewery (1991), Co., Ltd.) was approximately US$ 67 and 0.7 per kg, respectively.

Several researches used spent yeast cells, crude yeast autolysate or spent yeast hydrolysate as a nutrient supplement for the production of ethanol (York and Ingram, 1996), lactic acid (Rivas et al., 2004; Marica et al., 2007), succinic acid (Jiang et al., 2009), biomass of *Bacillus thuringiensis* (Saksinchai et al., 2001), disodium guanosine-5'-monophosphate (5'-GMP) (Sombutyanuchit et al., 2001), S-adenosyl-L-methionine (AdoMet) and glutathione (GSH) (Liu et al., 2004), microbial medium (Zvidzai et al., 2007) and a commercial YE (Tanguler and Erten, 2008).

Growth of *S. cerevisiae* NP 01 in inoculum preparation media

We previously studied the effects of glucose concentration on *S. cerevisiae* NP 01 grown in YM medium (Laopaiboon et al., 2008). It was found that the yeasts grown in YM medium containing 150 g l^{-1} of glucose gave significantly higher biomass yield than those containing

Table 2. Some nutrient components of concentrated SSJ cv. KKU40, YE (HiMedia laboratory, India) and DSY from Beerthip Brewery (1991) Co., Ltd., Bang Baan, Phra Nakhon Sri Ayutthaya, Thailand.

Component	Concentrated SSJ	Yeast extract	DSY
Total soluble solid (°Bx)	75	-	-
Total N (%)	0.2760	10.6500	6.4400
Total P (%)	0.1640	1.1500	1.2500
Total K (%)	5.0550	4.9600	1.5900
Total Na (%)	0.3940	0.8200	0.3400
Total Ca (%)	0.0640	0.0240	0.0670
Total Mg (%)	0.0638	0.0540	0.1740
Total Fe (%)	0.0004	0.0052	0.0081
Total Mn (%)	0.0003	ND*	ND*
Total Cu (%)	0.0002	ND*	ND*
Total Zn (%)	0.0023	0.0087	0.0044
Total Cl (%)	-	0.2500	0.1100
Total S (%)	0.0230	0.3500	0.3500

*ND, Not detected; N, nitrogen; P, phosphorus; K, potassium; Na, sodium; Ca, calcium; Mg, magnesium; Fe, iron; Mn, manganese; Cu, cupper; Zn, zinc; Cl, chlorine; S, sulphur.

10 g l^{-1} of glucose. However, the sugar consumption at 150 g l^{-1} of glucose was only 80% of the initial value. Therefore, in this study, 100 g l^{-1} of initial sugar concentration in the IP media was used to complete sugar utilization. The compositions of the IP media in Table 1 were then designed according to the typical YM medium (YM 1). In SSJ 2 and SSJ 5, DSY at 11 g l^{-1} was used as a nutrient supplement to replace YE, ME and PE (at total amount of 11 g l^{-1}) in the YM1, whereas only half the amounts of the three nutrients were formulated in SSJ 3 and SSJ 6 in order to reduce the cost of IP media.

The time profiles of viable cell concentration during batch cultures of *S. cerevisiae* NP 01 in the IP media containing total sugar of 20 (YM 1, SSJ 1, SSJ 2 and SSJ 3) and 100 g l^{-1} (YM 2, SSJ 4, SSJ 5 and SSJ 6) are shown in Figure 1. No lag phase was observed after the yeast cells were incubated into all media. This might be due to good adaptation of yeast cells to the new environment. The average cell numbers at the stationary phase of SSJ 1, 2 and 3 (the IP media containing 20 g l^{-1} of total sugar) were not significantly different (Table 3). However, they were significantly different from that of the control (YM 1) ($p \le 0.05$) (Table 3). In the IP media containing the total sugar of 100 g l^{-1}, the SSJ 6 gave the highest cell numbers at the stationary phase (log 8.55 ± 0.03 cells ml^{-1}) and the viable cell concentrations of SSJ 4, 5 and 6 were significantly higher than that of the control (YM 2) (log 8.13 ± 0.04 cells ml^{-1}).

Bai et al. (2008) reported that nitrogen was the most important component in the fermentation medium. Therefore, we used DSY as a low-cost nitrogen supplement. Phunjumpa et al. (2006) also reported that the yeast cell concentration increased 2.5 times of the control medium (without supplementation) when they were cultured in Melzoch media (Melzoch et al., 1991)

and tamarind juice supplemented with 1 g l^{-1} of DSY. However, in our experiment, under the same initial sugar concentration, the maximum viable cell concentrations of SSJ 2 and 5 (with DSY supplementation) were not different from those of SSJ 1 and 4 (without DSY supplementation) (Table 3). This finding implied that the SSJ contained sufficient essential nutrients for yeast growth.

When μ during log phase were calculated (Table 3), the lowest μ was observed in the YM 2, while μ in the other IP media were similar with a range of 0.36 to 0.41 h^{-1}. The sugar utilization of *S. cerevisiae* NP 01 under various IP media was relatively high with at least 82% of the initial value (Table 3). The IP media containing 100 g l^{-1} of total sugar gave higher maximum yeast cell concentration than those containing only 20 g l^{-1} and the sugar was almost completely consumed in the former. These results indicate that the increase in total sugar in the IP media up to 100 g l^{-1} led to an increase in the maximum cell concentration (Table 3).

The IP media containing higher sugar concentration gave higher maximum cell concentration and the aim of this experiment was to develop a low-cost IP medium; therefore, the SSJ containing 100 g l^{-1} of total sugar without nutrient supplement (SSJ 4) was selected to be the IP medium for batch and repeated-batch ethanol fermentations.

Batch ethanol fermentation

To increase cell concentration, the yeast cells were cultured in the SSJ 4 medium at 30°C with the agitation rate of 150 rev min^{-1} for 15 h. The cells were then harvested and used as inoculum for ethanol production under the batch fermentation. Figure 2 shows the time
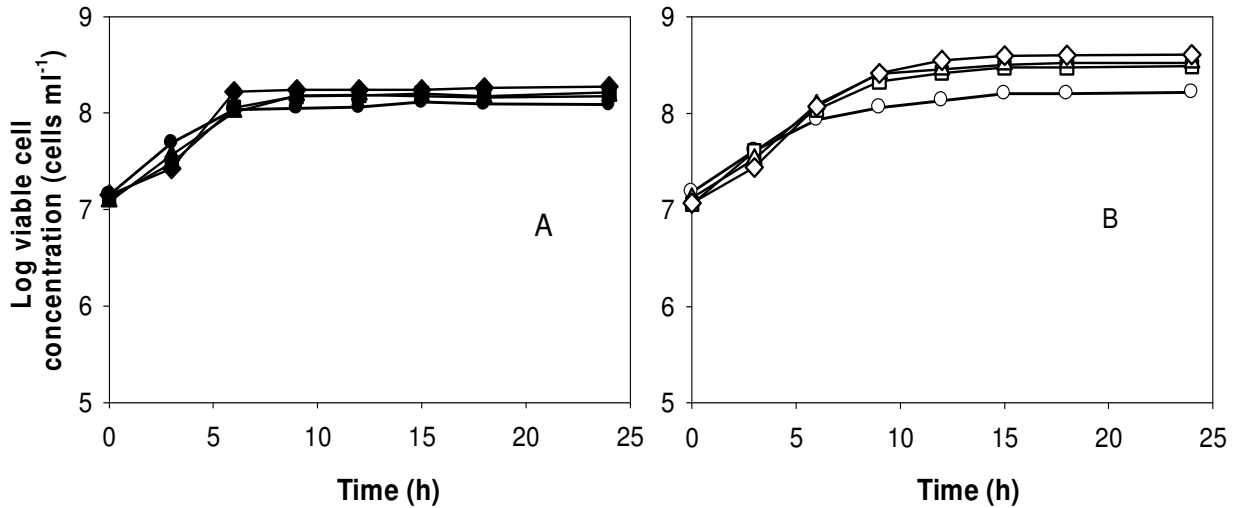
Figure 1. Growth curve of *S. cerevisiae* NP 01 in various IP media containing the total sugar concentration of 20 (A) and 100 (B) g l^{-1}. ●: YM 1, ■: SSJ 1, ▲: SSJ 2, ◆: SSJ 3, ○: YM 2, □: SSJ 4, Δ: SSJ 5 and ◊: SSJ 6.

Table 3. Specific growth rate (μ) and sugar utilization of *S. cerevisiae* NP 01 in various IP media.

Medium code*	Initial total sugar concentration (g l^{-1})	μ (h^{-1})	Sugar Utilization (%)	Log cell concentration (cell ml^{-1})	
				Initial	Stationary phase
YM 1 (control 1)		0.34 ± 0.01	93.63 ± 0.35	7.11 ± 0.06	8.05 ± 0.03 (9 h)**
SSJ 1	20	0.36 ± 0.02	92.62 ± 0.57	7.16 ± 0.08	8.17 ± 0.03 (9 h)
SSJ 2		0.36 ± 0.02	82.14 ± 0.40	7.09 ± 0.06	8.17 ± 0.08 (9 h)
SSJ 3		0.41 ± 0.02	89.19 ± 0.14	7.17 ± 0.06	8.24 ± 0.03 (9 h)
YM2 (control 2)		0.28 ± 0.00	95.71 ± 0.13	7.32 ± 0.06	8.13 ± 0.04 (12 h)
SSJ 4	100	0.37 ± 0.02	92.22 ± 0.11	7.09 ± 0.10	8.42 ± 0.04 (12 h)
SSJ 5		0.37 ± 0.02	90.15 ± 0.29	7.12 ± 0.04	8.45 ± 0.03 (12 h)
SSJ 6		0.39 ± 0.02	91.53 ± 0.00	7.04 ± 0.16	8.55 ± 0.03 (12 h)

**, Fermentation time. The experiments were performed in duplicate and the results were expressed as mean ± SD.

profiles of some main compositions during batch fermentation of *S. cerevisiae* NP 01 from the SSJ without nutrient supplementation. At the beginning of the fermentation, the initial total sugar, reducing sugar and total soluble solids in the SSJ were 228.80 ± 3.07 g l^{-1}, 158.41 ± 4.82 g l^{-1} and 24.4 °Bx, respectively. The pH of the broth was slightly decreased from 4.60 to 4.22 at 12 h and then constantly throughout the experiment (data not shown). This might be due to carbondioxide produced by *S. cerevisiae* NP 01 during fermentation that was formed to carbonic acid in the fermentation broth and then changed to carbonate ion and proton (Shen et al., 2004). The viable cell concentration was increased about 3 times from 1.06×10^{8} cells ml^{-1} to 2.94×10^{8} cells ml^{-1} at 48 h. The total sugar and reducing sugar levels were markedly decreased and almost depleted at 48 h. The total sugar and reducing sugar remaining in the fermentation broth were 13.15 ± 0.31 and 3.95 ± 0.06 g l^{-1}, respectively. In addition, *P* was increased and reached

the maximum value of 110.09 ± 0.81 g l^{-1} at 48 h. The Q_p and $Y_{p/s}$ were 2.29 ± 0.01 g l^{-1} h^{-1} and 0.51 ± 0.02, respectively.

Effects of fill and drain volume on ethanol production in repeated-batch fermentation

In the repeated-batch fermentation in the flask, the juice was withdrawn at 75 and 50% of the working volume, and the same amount of the fresh juice was immediately replaced. At the 75% fill and drain volume (Figure 3: 1A-1E) in the first batch, the changes of total soluble solids, total sugar, reducing sugar, cell concentration and pH were similar to those found in the batch system. In batch 2 to 8, the initial total soluble solids, total sugar and reducing sugar concentrations were lower than those in batch 1 because the fresh ethanol production medium added was diluted with the remaining medium in the
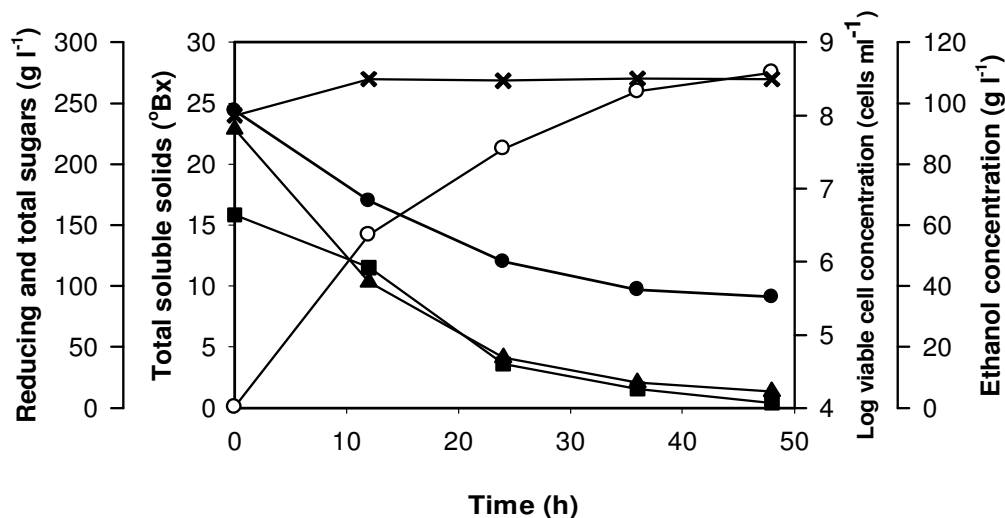
Figure 2. Batch culture profile of ethanol production from sweet sorghum juice containing the total soluble solids of 24 °Bx (●, total soluble solids; ▲, total sugar; ■, reducing sugar; ×, log viable cell concentration; ○, ethanol concentration).

flask (25% of total working volume) and the fermentation time was increased to 60 h. At the end of batch 2 to 8, the viable cell concentrations were relatively constant with approximately 0.5 log scale lower than that of batch 1, while the ethanol concentrations ranged from 88.52 ± 4.04 to 105.32 ± 0.81 g l^{-1}. Lower P in batch 2 to 8 might be due to lower cell concentration in the broth when compared with those of the first batch. The total amount of ethanol was 237.13 g in 2.5 L of the fermentation broth, and the total rate of ethanol production was 0.51 g h^{-1}. The total fermentation time of the eight successive batches at 75% fill and drain was 468 h. At the 50% fill and drain volume, the changes of parameters measured were similar to those of 75% fill and drain (Figure 3: 2A-2E). The total amount of ethanol was 161.70g in 1.8 L of the fermentation broth, and the total rate of ethanol production was 0.39 g h^{-1}. The total fermentation time of the eight successive batches was 420 h.

The average ethanol production efficiencies of the eight successive batches are summarized in Table 4. P, Q_p and $Y_{p/s}$ of the fermentation with 75 and 50% fill and drain were not significantly different (p < 0.05). However, the total rates of ethanol production at 75% fill and drain (0.51 g h^{-1}) was higher than that of 50% fill and drain (0.39 g h^{-1}). This might be because the amount of fermented broth being removed at 75% fill and drain was more than that of 50% fill and drain. This caused the ethanol in the broth at the beginning of each batch to be more diluted, which might prevent or reduce the product inhibition effect that might occur. Therefore, 75% fill and drain was selected for repeated-batch fermentation in the next experiment.

The results obtained from this study were similar to those of Choi et al. (2009) who reported that higher fill

and drain volume (20%) gave higher P and cell concentration when compared with the lower fill and drain volumes (5 and 10%). However, they found that the decrease in the fill and drain volume gave higher Q_p. Chen et al. (2008) indicated that the initial cell concentration affected P and Q_p in repeated-batch fermentation using free cells. The contradictory result was observed by immobilized cells. Staniszewski et al. (2009) reported that the decrease in the fill and drain volume in repeated-batch fermentation by immobilized cells gave a slight increase in P.

Repeated-batch ethanol fermentation in the bioreactor

The profiles of the main compositions during the repeated-batch fermentation in the 2 L bioreactor at 75% fill and drain were the same as those in the flask (data not shown). The ethanol production efficiencies of each batch in the bioreactor are summarized in Table 5. The average P, Q_p and $Y_{p/s}$ of the eight successive batches in the bioreactor were similar to those in the flask with the values of 93.30 ± 9.44 g l^{-1}, 1.21 ± 0.43 g l^{-1} h^{-1} and 0.48±0.03, respectively. Total ethanol production of the eight successive batches was 851.98 g ethanol in 10.25 L of the fermentation broth, and the rate of ethanol production was 1.82 g h^{-1} with the total fermentation time of 468 h. Because of our limitation in the raw materials, only eight repeated-batches were performed in this study. More successive batches (16 cycles) of repeated-batch ethanol fermentation from sweet sorghum was reported by Chohnan et al. (2011). However, the working volume used in each cycle was 10 ml only. Ozmihci and Kargi
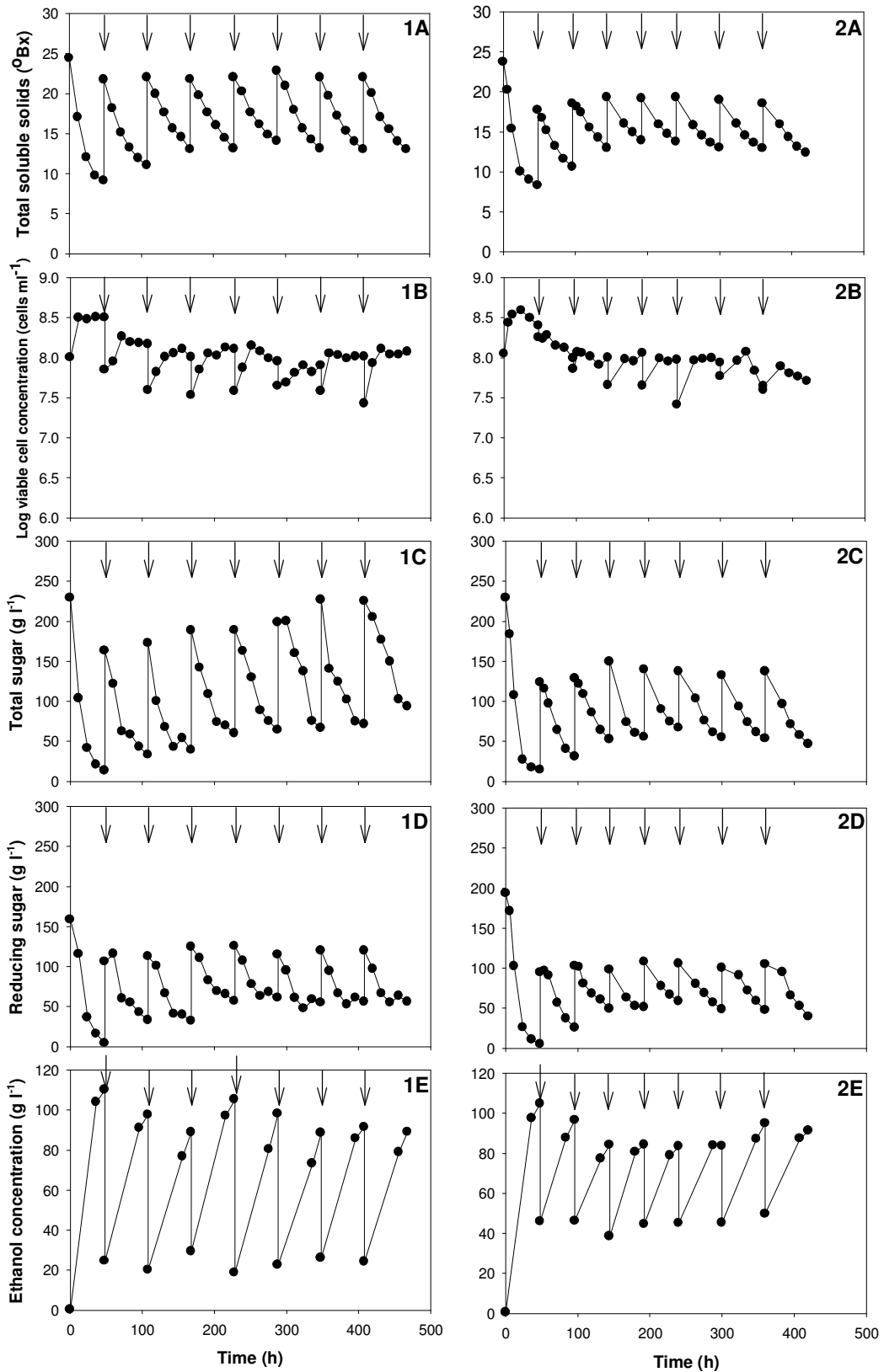
Figure 3. Repeated-batch ethanol fermentation at 75% fill and drain from sweet sorghum juice containing the total soluble solids of 24 °Bx in the 500 ml air-locked flask (A, total soluble solids; B, log viable cell concentration; C, total sugar; D, reducing sugar; E, ethanol concentration). The arrows indicate the start time of each batch.

Table 4. Comparison kinetic parameters of ethanol production from sweet sorghum juice by *S. cerevisiae* NP 01 using repeated-batch fermentation at different fill and drain volumes in the 500 ml flask.

Batch number	Parameter (mean ± SD)*					
	75% fill and drain			50% fill and drain		
	P (g l^{-1})	Q_p (g l^{-1} h^{-1})	$Y_{p/s}$ (g g^{-1})	P (g l^{-1})	Q_p (g l^{-1} h^{-1})	$Y_{p/s}$ (g g^{-1})
1	109.08 ± 0.71	2.27 ± 0.02	0.51 ± 0.02	104.69 ± 0.13	2.18 ± 0.01	0.51 ± 0.00
2	97.55 ± 0.24	1.22 ± 0.01	0.53 ± 0.00	96.47 ± 0.79	1.05 ± 0.02	0.53 ± 0.00
3	88.87 ± 3.99	1.15 ± 0.06	0.51 ± 0.02	84.19 ± 0.77	0.79 ± 0.05	0.50 ± 0.00
4	105.32 ± 0.81	1.27 ± 0.00	0.53 ± 0.00	84.25 ± 1.70	0.95 ± 0.04	0.49 ± 0.01
5	98.08 ± 3.74	1.32 ± 0.07	0.53 ± 0.00	83.42 ± 0.41	0.81 ± 0.01	0.53 ± 0.03
6	88.52 ± 4.04	1.04 ± 0.11	0.45 ± 0.03	83.58 ± 0.07	0.64 ± 0.04	0.47 ± 0.03
7	91.38 ± 5.07	1.09 ± 0.09	0.45 ± 0.02	94.90 ± 0.75	0.83 ± 0.03	0.53 ± 0.00
8	88.97 ± 1.93	1.08 ± 0.06	0.45 ± 0.01	91.20 ± 1.61	0.69 ± 0.05	0.46 ± 0.03
average	95.97 ± 7.95	1.30 ± 0.41	0.50 ± 0.04	90.34 ± 7.87	0.99 ± 0.49	0.50 ± 0.03

*P, Q_p and $Y_{p/s}$, Ethanol concentration, ethanol productivity and ethanol yield of the eight successive batches, respectively.

Table 5. Kinetic parameters of ethanol production from sweet sorghum juice by *S. cerevisiae* NP 01 using repeated-batch fermentation at 75% fill and drain in the 2 L bioreactor.

Batch number	Parameter (mean ± SD)*		
	P (g l^{-1})	Q_p (g l^{-1} h^{-1})	$Y_{p/s}$ (g g^{-1})
1	108.95 ± 0.57	2.25 ± 0.01	0.53 ± 0.01
2	105.22 ± 2.02	1.25 ± 0.03	0.53 ± 0.00
3	96.86 ± 1.26	1.13 ± 0.04	0.47 ± 0.01
4	91.25 ± 0.00	1.05 ± 0.00	0.47 ± 0.04
5	88.56 ± 0.75	1.05 ± 0.00	0.48 ± 0.01
6	85.08 ± 0.20	0.94 ± 0.06	0.47 ± 0.02
7	85.53 ± 1.40	1.01 ± 0.05	0.47 ± 0.03
8	84.94 ± 0.10	1.03 ± 0.01	0.46 ± 0.01
average	93.30 ± 9.44	1.21 ± 0.43	0.48 ± 0.03

* P, Q_p and $Y_{p/s}$, Ethanol concentration, ethanol productivity and ethanol yield of the eight successive batches, respectively.

(2007) studied ethanol production from cheese whey powder solution at sugar concentration of 125 g l^{-1} using repeated fed-batch fermentation. The total working volume was 5 L. They found that high ethanol production rate in five cycles (5.3 g h^{-1}) was obtained at the total fermentation time of 336 h. The higher ethanol production rate as compared with that of our study might be due to the differences in microorganism, working volume and initial sugar concentration. The initial sugar at higher concen-trations significantly retarded the ethanol production rate (Laopaiboon et al., 2008). In our study, the initial sugar concentration was about 2 times higher than that reported by Ozmihci and Kargi (2007).

Table 6 compares the ethanol fermentation by repeated-batch process from various low-cost raw materials. It is clearly shown that the SSJ is one of the candidate raw materials for scale-up ethanol production. In this study, the P, Q_p values in batch 2 to 8 were lower

than those in batch 1. To improve the ethanol production efficiencies, cell recycling (Choi et al., 2009) or cell immobilization (Yu et al., 2007) should be considered to increase the initial cell concentration in the subsequent batches.

Conclusion

The SSJ containing 100 g l^{-1} of total sugar without any nutrient supplement (SSJ 4 medium) could be used directly as a low-cost IP medium instead of the typical or YM medium. The use of this IP medium can significantly reduce both the cost and preparation time. Ethanol production from SSJ by *S. cerevisiae* NP 01 using repeated-batch fermentation could be carried out at least eight successive batches. The fill and drain volume in the repeated-batch system did not affect the ethanol

Table 6. Ethanol production from various low-cost raw materials using repeated-batch fermentation.

Raw material	Working volume of each cycle (L)	Fill and drain volume (%)	Number of cycle	P * (g l^{-1})	Reference
Molasses medium (25% w/v)	3.0	75	6	106	Kida et al. (1991)
Molasses medium (22% w/v)	3.0	75	6	92	Morimura et al. (1997)
Cheese whey powder (125 g l^{-1})	5.0	60	5	63	Ozmihci and Kargi (2007)
Casava medium (185 g l^{-1} of sugar)	4.0	20	10	85	Choi et al. (2009)
Kitchen refuse (130 g l^{-1} of sugar)	< 1	60	>10	80	Ma et al. (2009)
Sweet sorghum juice (100 g l^{-1} of sugar)	0.01	100	16	44-48	Chohnan et al. (2011)
Sweet sorghum juice (230 g l^{-1} of sugar)	1.5	75	8	93	This study

* P, Ethanol concentration.

production efficiencies in terms of P, Q_p and $Y_{p/s}$ However, higher fill and drain volume gave higher total ethanol production rate (g ethanol h^{-1}) than the lower volume.

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