

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



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ABSTRACT

Ethanol production by *Saccharomyces cerevisiae* NP 01 from sweet sorghum juice was conducted under nutrient supplementation and/or aeration conditions. Yeast extract (YE) and dried spent yeast (DSY) were used as nitrogen supplements. Under high gravity (HG, ~200 g l⁻¹ of total sugar) conditions, the addition of either 6 g l⁻¹ of YE or 9 g l⁻¹ of DSY did not increase the ethanol concentration (*P*), but it promoted the rate of ethanol production or ethanol productivity (*Q_p*). The *Q_p* value of the control treatment (no supplement) was 1.96 g l⁻¹ h⁻¹. The *P* (93.4–94.0 g l⁻¹) and *Q_p* (3.89–3.92 g l⁻¹ h⁻¹) values under the YE and DSY supplementation were comparable, indicating that DSY could be used to replace YE in ethanol production. Under very high gravity (VHG, ~280 g l⁻¹ of total sugar) conditions, the supplementation of nitrogen and trace elements (Zn²⁺, Mg²⁺ and Mn²⁺) coupled with the aeration supply were required to improve the ethanol production efficiency. Under the supplementation (in g l⁻¹) of DSY (13.5), Zn²⁺ (0.01), Mg²⁺ (0.05) and Mn²⁺ (0.04) in the presence of aeration supply at 0.05 vvm for 12 h, the *P* and *Q_p* values were 126.3 g l⁻¹ and 2.11 g l⁻¹ h⁻¹, respectively. The *P* and *Q_p* values under the same supplementation without aeration were 114.8 g l⁻¹ and 1.91 g l⁻¹ h⁻¹, respectively, and these values under no supplementation and no aeration were 108.0 g l⁻¹ and 1.50 g l⁻¹ h⁻¹, respectively. In addition, glycerol (the main by-product during ethanol fermentation) under aeration conditions was ~3 g l⁻¹ lower than that without aeration.

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1. Introduction

Bioethanol from agricultural raw materials as the feedstock has become interesting as an alternative energy source to petroleum-based fuels, because it is both renewable and environmentally friendly. In Thailand, the main raw materials for ethanol fermentation are sugarcane molasses and tapioca starch (Nguyen and Gheewala, 2008; Laopaiboon et al., 2009). However, these raw materials are becoming insufficient due to the need for many ethanol plants. Recently, sweet sorghum (*Sorghum bicolor* {L.} Moench) has been considered as an alternative promising source for ethanol production because the juice from its stalks contains high amounts of fermentable sugar and a lot of trace elements that are essential for yeast growth and ethanol fermentation (Laopaiboon et al., 2009). In addition, it can be cultivated at a wide

range of ambient temperature, including tropical climate areas (Sree et al., 1999). Moreover, sweet sorghum has other advantages such as short time to maturity which is in between 90 and 120 days, lower requirement for fertiliser, high water utilisation efficiency (1/3 of sugarcane and 1/2 of corn), and higher tolerance to salinity and drought comparing to sugarcane and corn (Gnansounou et al., 2005; Ratnavathi et al., 2010; Wu et al., 2010).

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

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

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

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

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

2. Materials and methods

2.1. Microorganism and inoculum preparation

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

2.2. Identification of the yeast isolate

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

2.3. Raw material and nutrient supplementation

Sweet sorghum (cv. KKK40) was obtained from the Department of Plant Science and Agricultural Resources, Faculty of Agriculture, Khon Kaen University, Thailand. The juice was extracted from its stalks by sugarcane juice extractor, and was kept at -18 °C until use.

The nutrient supplements used in this study were YE (Himedia laboratory, India), dried spent yeast (DSY, a by-product from brewery industry), ZnSO₄·7H₂O (analytical grade, BDH, England), MgSO₄·7H₂O (analytical grade, BDH, England) and MnSO₄·H₂O (analytical grade, BDH). The DSY was donated from Beerthip Brewery (1991) Co., Ltd., Phra Nakhon Sri Ayutthaya, Thailand.

2.4. Ethanol production medium

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

2.5. Experiments

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

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

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

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

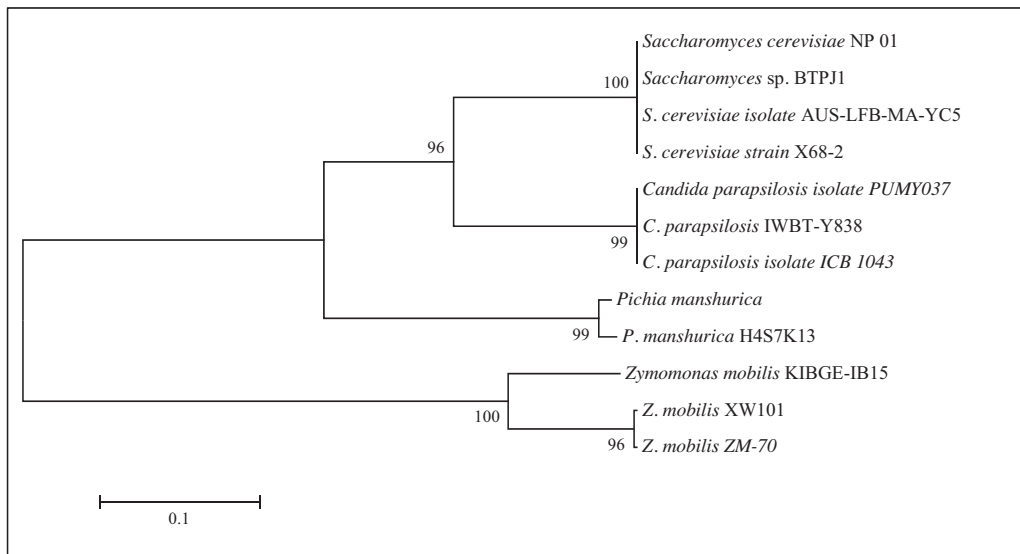


Fig. 1. Phylogenetic analysis of yeast NP 01. Clustering was performed by the neighbour-joining method, MEGA (molecular evolutionary genetics analysis) version 4. The percentages at the nodes indicate the levels of bootstrap support for the branch point based on 1000 bootstrap replicates.

medium at the rates of 0.05, 0.20 and 0.35 vvm (volume of air supplied per volume of fermentation medium per minute) for the first 12 h of the fermentation (Alfenore et al., 2004; Bai et al., 2004; Cot et al., 2006; Limtong et al., 2007; Breisha, 2010; Lin et al., 2011).

2.6. Fermentation conditions

The fermentation was carried out at 30°C in batch mode with an agitation rate of 100 rev min⁻¹ under the presence or absence of aeration. The yeast NP 01 was inoculated into the sterile HG and VH media to obtain the initial cell concentration of $\sim 5 \times 10^7$ cells ml⁻¹ (Sridee et al., 2011).

2.7. Analytical methods

The viable yeast cells and residual total sugar were determined using a haemocytometer with methylene blue staining (Zoecklein et al., 1995) and phenol sulphuric acid method (Mecozzi, 2005), respectively. The ethanol concentration (P) and glycerol were analysed by gas chromatography (Laopaiboon et al., 2009), and high

performance liquid chromatography (Sirisantimethakom et al., 2008), respectively. The ethanol yield ($Y_{p/s}$, g g⁻¹) and volumetric ethanol productivity (Q_p , g l⁻¹ h⁻¹) were calculated (Deesuth et al., 2012; Khongsay et al., 2012).

3. Results and discussion

3.1. Identification of the yeast strain

The yeast NP 01 isolate was previously identified as *S. cerevisiae* according to morphological and biochemical characteristics described by Rittiplang (2006). However, this strain has not yet been identified by molecular techniques, which is remarkable due to its high sensitivity and speed (Alcoba-Florez et al., 2005). Identification of the NP 01 strain was conducted using molecular analysis based on nucleotide sequences of the D1/D2 domain of 26S rDNA to confirm the previous identification. The results showed that the sequences of the D1/D2 domain of 26S rDNA from yeast NP 01 and the strain *S. cerevisiae* from the database were identical. Moreover,

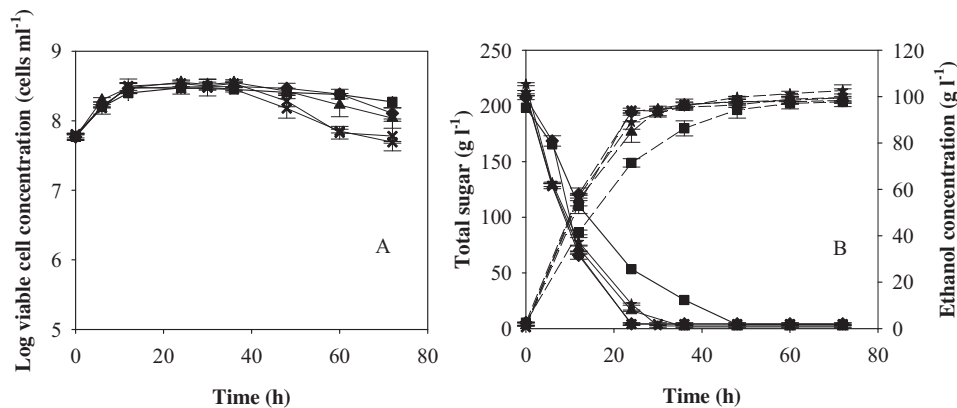


Fig. 2. Batch culture profiles of ethanol fermentation from sweet sorghum juice under HG conditions (~ 200 g l⁻¹ of total sugar) with and without nutrient supplementation; control or no supplement (■), 3 g l⁻¹ of YE (▲), 6 g l⁻¹ of YE (◆), 4.5 g l⁻¹ of DSY (★) and 9.0 g l⁻¹ of DSY (×). (A) Log viable cell concentration, and (B) total sugar (solid lines) and ethanol concentration (dash lines).

Table 1
Fermentation parameters and assimilable nitrogen utilization of ethanol production from sweet sorghum juice containing $\sim 200 \text{ g l}^{-1}$ of total sugar under various nutrient supplementations.

Nitrogen source ^a	P (g l^{-1})	Q_p ($\text{g l}^{-1} \text{ h}^{-1}$)	$Y_{p/s}$	t (h)	Assimilable nitrogen (mg l^{-1})	
					Initial (mg l^{-1})	Utilized (mg l^{-1})
None	94.3 ± 3.6^a	1.96 ± 0.07^a	0.48 ± 0.02^b	48	326 ± 15^a	$280 \pm 19^{b,c}$
YE3	94.4 ± 0.6^a	3.14 ± 0.02^c	0.45 ± 0.00^a	30	448 ± 0^d	294 ± 14^c
YE6	93.4 ± 0.6^a	3.89 ± 0.02^d	0.46 ± 0.01^a	24	568 ± 12^e	334 ± 15^d
DSY4.5	92.8 ± 1.5^a	3.11 ± 0.03^c	0.45 ± 0.00^a	30	336 ± 0^a	251 ± 3^a
DSY9	94.0 ± 1.0^a	3.92 ± 0.04^d	0.46 ± 0.00^a	24	363 ± 4^b	274 ± 1^b
DSY9 ^{**}	95.3 ± 0.6^a	2.65 ± 0.02^b	0.46 ± 0.0^a	36	383 ± 5^c	290 ± 11^c

P = ethanol concentration, Q_p = ethanol productivity ($Q_p = P/t$), $Y_{p/s}$ = ethanol yield and t = fermentation time.

^{a,b,c,d,e} Means followed by the same letter within the same column are not significantly different using Duncan's multiple range test at the level of 0.05; The results are mean \pm SD of independent triplicate.

^{*} YE3 = 3 g l^{-1} of YE; YE6 = 6 g l^{-1} of YE; DSY4.5 = 4.5 g l^{-1} of DSY and DSY9 = 9 g l^{-1} of DSY.

^{**} In 2-l fermenter.

phylogenetic analysis (Fig. 1) confirmed that yeast strain NP 01 was *S. cerevisiae*.

3.2. Effects of nitrogen supplementation on ethanol fermentation under HG condition

In this study, the raw juice containing total soluble solids of 17°Brix was extracted from sweet sorghum stalks in laboratory scale. Sucrose was then chosen as the carbon adjunct in the raw juice for the preparation HG (20°Brix containing 200 g l^{-1}) and VHG (28°Brix containing 280 g l^{-1}) media (Laopaiboon et al., 2009). In industrial scale, the juice extracted from many tons of the sweet sorghum stalks has to be kept in concentrated form (as molasses) to reduce storage area and the risk of bacterial contamination. To prepare HG or VHG medium for ethanol production, the concentrated juice (about $65\text{--}70^\circ \text{Brix}$ containing about $650\text{--}700 \text{ g l}^{-1}$ of total sugar) must be diluted to the desired concentrations. Therefore, no sucrose is required for the preparation of HG and VHG media in the large scale.

In ethanol production, the deficiency of nitrogen can cause the reduction of yeast growth and ethanol yield (Thomas et al., 1996; Vvochora et al., 2000). In batch fermentation from the sweet sorghum juice under the HG conditions ($\sim 200 \text{ g l}^{-1}$ of total sugar), with and without nutrient supplementation, the yeast cell numbers increased within 12 h (Fig. 2A). In the control treatment (no supplement), 3 and 6 g l^{-1} of YE supplementation (YE3 and YE6), the cell concentrations slightly decreased after 60 h, while under 4.5 and 9 g l^{-1} DSY supplementation (DSY4.5 and DSY9), the cell concentrations obviously decreased after 48 h (Fig. 2A). The total sugar in the control treatment was almost completely consumed at 48 h, with only 3.4 g l^{-1} of total sugar remaining in the broth (Fig. 2B). The P and $Y_{p/s}$ values were relatively high at 94.3 g l^{-1} , corresponding to the Q_p and $Y_{p/s}$ values of $1.96 \text{ g l}^{-1} \text{ h}^{-1}$ and 0.48 g g^{-1} (Table 1). To improve the Q_p value, nitrogen sources were supplemented. In YE3 and YE6, the P , $Y_{p/s}$ and sugar consumption were not significantly different from those of the control treatment, but the Q_p values increased to 3.14 and $3.89 \text{ g l}^{-1} \text{ h}^{-1}$, respectively. In Thailand, the cost of yeast extract (HiMedia Laboratory, India), and DSY (Beerthip Brewery (1991), Co., Ltd.) are approximately US\$ 100 and 1 per kg, respectively. Due to the high cost of YE, DSY4.5 and DSY9, containing the same amount of total nitrogen as found in YE3 and YE6, respectively, were employed. The results showed that the P , Q_p and $Y_{p/s}$ values of DSY4.5 and DSY9 were similar to those of YE3 and YE6, respectively. The Q_p values of DSY4.5 and DSY9 were 3.11 and $3.92 \text{ g l}^{-1} \text{ h}^{-1}$, respectively. These results clearly indicated that both nitrogen supplements did not have a positive effect on yeast growth but they promoted the rate of ethanol production or the ethanol productivity under the HG fermentation. Although the total nitrogen content of YE3 and YE6 were equal to those of

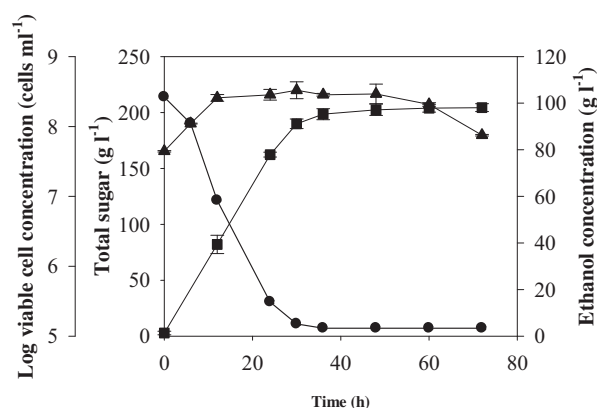


Fig. 3. Batch culture profiles of ethanol fermentation from sweet sorghum juice under HG conditions ($\sim 200 \text{ g l}^{-1}$ of total sugar and 9 g l^{-1} of DSY) in the 2-l fermenter. Log viable cell (▲), total sugar (●) and ethanol concentration (■).

DSY4.5 and DSY9, respectively, the initial assimilable nitrogen contents were different (Table 1). Assimilable nitrogen utilised during the ethanol production increased with increasing YE concentrations, but these values were similar under DSY supplementation and control (Table 1). These findings implied that not only was the nitrogen source in YE and DSY utilised, but other trace elements containing the supplements might also play an important role in promoting the rate of ethanol production.

To confirm the results before applying these conditions in large scales, the ethanol production from the juice supplemented with 9 g l^{-1} of DSY or DSY9 was carried out in the 2-l fermenter (Fig. 3). It was found that the P value was similar to that when performed in the flask, but the Q_p value was lower due to the longer fermentation time ($\sim 12 \text{ h}$) in the 2-l fermenter (Table 1).

In this study, total sugar in the HG medium was almost consumed within 24–48 h depending on the nutrient supplements corresponding to the maximum P values of $93.4\text{--}95.3 \text{ g l}^{-1}$. Further improvement of the ethanol fermentation in terms of the P values was investigated under VHG conditions. However, high sugar concentrations in the medium can cause sluggish fermentation and eventually decrease the ethanol production efficiency (Pátková et al., 2000). Thus, more nutrient supplements and aeration would be applied in ethanol fermentation under the VHG conditions.

3.3. Effects of the combination of nutrient supplementation and aeration on VHG fermentation

In our previous study, the optimum YE, Zn, Mg and Mn concentrations for ethanol fermentation from sweet sorghum juice containing $\sim 280 \text{ g l}^{-1}$ of total sugar were 9, 0.01, 0.05 and 0.04 g l^{-1} ,

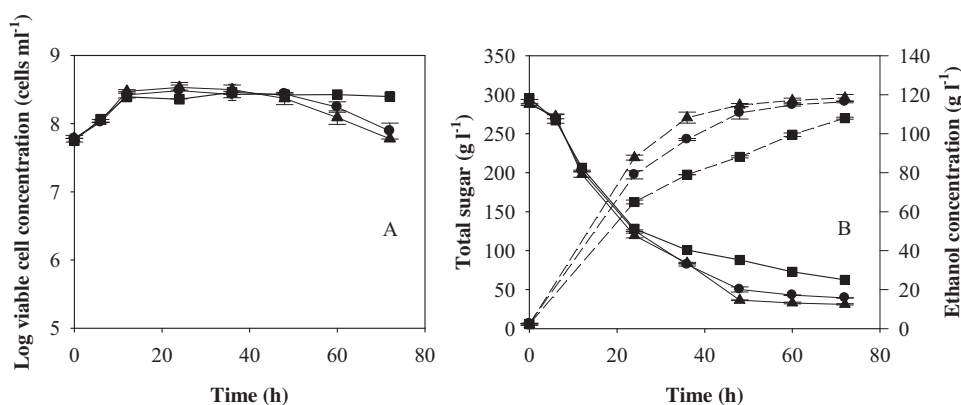


Fig. 4. Batch culture profiles of ethanol fermentation from sweet sorghum juice under VHG conditions ($\sim 280 \text{ g l}^{-1}$ of total sugar) with and without nutrient supplementation; control (■), 9 g l^{-1} of YE coupling with the metals (0.01 g l^{-1} of Zn, 0.05 g l^{-1} of Mg and 0.04 g l^{-1} of Mn) (▲) and 13.5 g l^{-1} of DSY coupling with the metals (●). (A) Log viable cell concentration, and (B) total sugar (solid lines) and ethanol concentration (dash lines).

respectively (Deesuth et al., 2012). In this study, 13.5 g l^{-1} of DSY (containing total nitrogen equal to that of 9 g l^{-1} of YE) was used instead of 9 g l^{-1} of YE. Under the conditions both with and without nutrient supplementation, the cell concentrations sharply increased within 12 h (Fig. 4A). The cell concentration in the control treatment remained constant throughout the experiment, whereas the values under the nutrient supplementation conditions decreased after 48 h (Fig. 4A). The lowest sugar consumption was observed in the control experiment, whereas those of other two treatments were comparable (Fig. 4B). The residual total sugar in the control treatment was $\sim 62 \text{ g l}^{-1}$, while these values in the other treatments ranged from 31 to 39 g l^{-1} . The P values in the EP medium supplemented with YE and the metals were slightly higher than those with DSY and the metals within 48 h (Fig. 4B). After that, the P values of the two VHG media were similar, while the P value in the control treatment was the lowest throughout the experiment.

Using the juice without nutrient supplement, the P , Q_p and $Y_{p/s}$ were 108.0 g l^{-1} , $1.50 \text{ g l}^{-1} \text{ h}^{-1}$ and 0.46, respectively, at 72 h (Table 2). When 9 g l^{-1} of YE or 13.5 g l^{-1} of DSY (the same total nitrogen content) was used as the nitrogen supplement under the optimum metal (Zn, Mg and Mn) concentrations, the P value of both conditions increased to $\sim 115 \text{ g l}^{-1}$, but the Q_p value under the YE supplementation was slightly higher than that under DSY supplementation due to a shorter fermentation time by 12 h. However, the $Y_{p/s}$ values at all conditions were the same, indicating that the metabolic pathway during the ethanol fermentation under the different nutrient conditions was unchanged. The results obtained confirmed that DSY could be used instead of the expensive nitrogen supplement or YE under the VHG fermentation.

The utilisation of assimilable nitrogen and the main by-product or glycerol production from the three VHG media (with and without the nutrient supplements) were compared (Table 3). According to nitrogen content, the total nitrogen content of 9 g l^{-1} of YE and 13.5 g l^{-1} of DSY was equal (Chan-u-tit et al., 2013). However, the assimilable nitrogen in DSY was only $\sim 57\%$ of that in YE (Table 3), implying that DSY contained more non-assimilable nitrogen than YE. The nitrogen utilisation in the medium supplemented with YE coupled with the metals was significantly higher than that of other conditions. However, the P and $Y_{p/s}$ values of the two VHG media were not different (Table 2). This again implied that some essential elements containing in YE might promote the rate of ethanol production. In addition, glycerol formation ($11.3\text{--}12.4 \text{ g l}^{-1}$) during fermentation in the three media was similar (Table 3), indicating that the metabolic pathway during the ethanol fermentation under no aeration was unchanged.

Many researchers have studied the effects of aeration during ethanol production under VHG conditions (Alfenore et al., 2004; Yan et al., 2009; Breisha, 2010; Lin et al., 2011). It was found that *S. cerevisiae* required a certain supply of elemental oxygen to synthesise ergosterol and the unsaturated fatty acids, which are essential for plasma membrane integrity (You et al., 2003). Lin et al. (2011) reported that a small amount of aeration during the initial stage of yeast growth resulted in increased ethanol production efficiency. Therefore, in this study, the combination effect of nutrient supplementation and aeration on the VHG fermentation was investigated under different aeration rates (0.05, 0.2 and 0.35 vvm) during log phase (12 h) (Alfenore et al., 2004; Bai et al., 2004; Cot et al., 2006; Limtong et al., 2007; Breisha, 2010; Lin et al., 2011).

Table 2

Fermentation parameters of ethanol production from sweet sorghum juice containing $\sim 280 \text{ g l}^{-1}$ of total sugar under various nutrient supplementations with and without aeration supply.

Aeration	Nutrient supplements	P (g l^{-1})	Q_p ($\text{g l}^{-1} \text{ h}^{-1}$)	$Y_{p/s}$	t (h)
No	No supplement	108.0 ± 0.5^a	1.50 ± 0.01^a	0.46 ± 0.00^a	72
	YE and metals*	114.7 ± 0.8^b	2.39 ± 0.02^e	0.46 ± 0.00^a	48
	DSY and metals**	114.8 ± 0.5^b	1.91 ± 0.01^c	0.46 ± 0.01^c	60
0.05 vvm, 12 h	DSY and metals*	117.2 ± 1.3^c	2.44 ± 0.03^f	0.49 ± 0.00^c	48
	DSY and metals**	126.3 ± 1.5^e	2.11 ± 0.02^d	0.50 ± 0.01^c	60
	DSY and metals*	131.6 ± 0.4^f	1.83 ± 0.01^b	0.50 ± 0.01^c	72
	DSY and metals**	121.7 ± 0.3^d	2.54 ± 0.01^g	$0.47 \pm 0.01^{a,b}$	48
0.35 vvm, 12 h	DSY and metals**	116.0 ± 0.0^c	2.42 ± 0.00^f	0.48 ± 0.00^b	48

P = ethanol concentration, Q_p = ethanol productivity ($Q_p = P/t$), $Y_{p/s}$ = ethanol yield and t = fermentation time.

a,b,c,d,e,f,g Means followed by the same letter within the same column are not significantly different using Duncan's multiple range test at the level of 0.05; The results are mean \pm SD of independent triplicate.

* 9 g l^{-1} of YE, 0.01 g l^{-1} of Zn, 0.05 g l^{-1} of Mg and 0.01 g l^{-1} of Mn.

** 13.5 g l^{-1} of DSY, 0.01 g l^{-1} of Zn, 0.05 g l^{-1} of Mg and 0.01 g l^{-1} of Mn.

Table 3
Utilisation of assimilable nitrogen and glycerol production of VHG ethanol fermentation from sweet sorghum juice under various nutrient supplementations with and without aeration supply.

Aeration	Nutrient supplements	Assimilable nitrogen (mg l^{-1})*		Glycerol concentration (g l^{-1})
		Initial	Utilized	
No	No supplement	319 ± 10^a	268 ± 17^b	11.3 ± 0.1^b
	YE and metals**	671 ± 8^c	381 ± 4^c	11.9 ± 0.4^c
	DSY and metals**	373 ± 14^b	235 ± 4^a	12.4 ± 0.1^d
0.05 vvm, 12 h	DSY and metals**	381 ± 4^b	272 ± 2^b	9.8 ± 0.1^a
0.2 vvm, 12 h	DSY and metals**	384 ± 8^b	287 ± 14^b	9.9 ± 0.1^a
0.35 vvm, 12 h	DSY and metals**	373 ± 8^b	283 ± 14^b	9.8 ± 0.6^a

a,b,c,d Means followed by the same letter within the same column are not significantly different using Duncan's multiple range test at the level of 0.05; The results are mean \pm SD of independent triplicate.

* At the fermentation time (see Table 2).

** See Table 2.

The viable yeast cells in the VHG medium increased in 12 h (Fig. 5A). These values under the aeration at 0.05 vvm were relatively constant throughout the fermentation, while they decreased after 48 h under the aeration at 0.2 and 0.35 vvm. Changes of the sugar consumption in the fermentation broth under all aeration conditions were similar. The total sugar concentrations under the aeration rates at 0.2 and 0.35 vvm remained constant at 48 h, whereas it was constant at 60 h under the aeration of 0.05 vvm. The total sugar remaining under the aeration rates at 0.05 and 0.2 vvm were similar at about $22\text{--}23 \text{ g l}^{-1}$, whereas this value was higher at the aeration of 0.35 vvm with the sugar remaining of approximately 43 g l^{-1} . Changes of the ethanol concentration under all conditions were similar in the first 48 h. After that, the ethanol values at the aeration of 0.05 vvm were continuously increased until 72 h with the highest P value of 131.6 g l^{-1} . However, the Q_p value under the aeration at 0.05 vvm was slightly lower than those under the aeration at 0.2 and 0.35 vvm due to a longer fermentation time. Yan et al. (2009) found that initial aeration and agitation improved the ethanol production up to 143.8 g l^{-1} . Interestingly, the nutrient supplementation with 13.5 g l^{-1} of DSY, low-cost nitrogen source, on VHG ethanol production under the lowest aeration rate (0.05 vvm), led to an increase in the final P value (131.6 g l^{-1}) at 72 h (Table 2). These results strongly indicated that aeration at appropriate levels could improve the ethanol production efficiency under the VHG conditions.

In the present study, glycerol productions under the DSY supplementation coupled with different aeration rates were similar ($9.8\text{--}9.9 \text{ g l}^{-1}$), but they were lower than that (12.4 g l^{-1}) under no aeration (Table 3). Similar glycerol productions under all aeration conditions might be due to the fact that the aeration was supplied only during log phase or the first 12 h of the fermentation.

The significant change of glycerol production was also reported by Alfenore et al. (2004). They found that oxygen had an impact on the formation of by-products, such as glycerol; under full aeration conditions the glycerol was decreased from 12.2 to 4.0 g l^{-1} . The glycerol content indicates the degree of microbial stress, as the higher the level of glycerol, the higher the microbial stress was (Sunder et al., 1996; Caridi, 2002). Similar results were observed by Reddy and Reddy (2006), who found that the supplementation of finger millet induced a marked reduction of glycerol production. Therefore, the decrease in glycerol production under the aeration conditions in our study indicated that the degree of yeast stress was less than that under no aeration, resulting in higher ethanol production efficiency.

Sridee et al. (2011) found that the constituent of DSY was composed of higher Mg^{2+} than that in YE. The effect of Mg^{2+} on ethanol production played a more important role in the cellular protection by increasing in the plasma membrane integrity (Petrov and Okorokov, 1990), resulting in membrane stability. Dombek and Ingram (1986) reported that supplementation of 0.5 mM Mg^{2+} prolonged exponential growth, leading to increased yeast cell mass and ethanol production rate. Thanonkeo et al. (2007) found that the cell viability increased when Mg^{2+} concentration was increased under heat and ethanol stress of *Zymomonas mobilis*, suggesting that Mg^{2+} exerted a protective role for *Z. mobilis* toward heat or ethanol stress, enabling cells to remain viable at high levels for relatively long periods. In our study, the viable yeast cells were almost constant from 12 h under DSY supplementation at the appropriate aeration rate (0.05 vvm). This might be due to the positive effect of Mg^{2+} in DSY, resulting in a higher concentration of ethanol being achieved at 72 h.

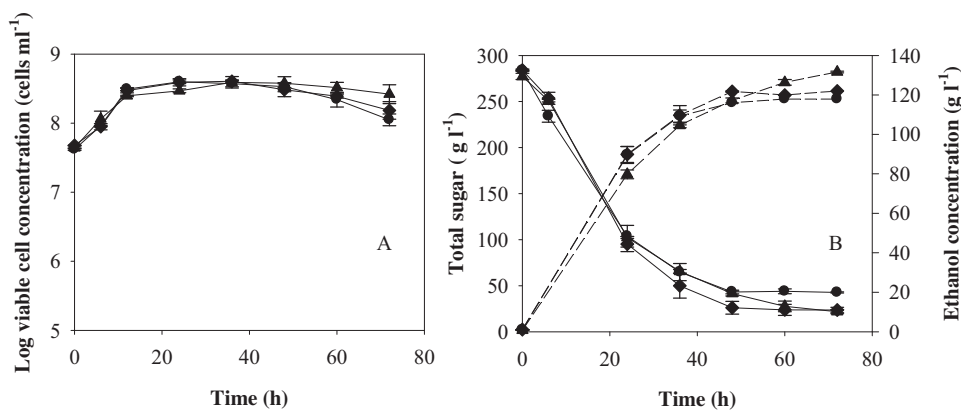


Fig. 5. Batch culture profiles of ethanol fermentation from sweet sorghum juice containing $\sim 280 \text{ g l}^{-1}$ of total sugar, 13.5 g l^{-1} of DSY and the metals (0.01 g l^{-1} of Zn, 0.05 g l^{-1} of Mg and 0.04 g l^{-1} of Mn) under different aeration rates for 12 h: 0.05 vvm (▲), 0.2 vvm (◆) and 0.35 vvm (●). (A) Log viable cell concentration, and (B) total sugar (solid lines) and ethanol concentration (dash lines).

Regarding the assimilable nitrogen utilised during ethanol production under various aeration supply (Table 3), it was found that under the aeration condition, the nitrogen utilised in the EP medium supplemented with DSY and the metals was increased $\sim 37\text{--}52\text{ mg l}^{-1}$, compared to that without aeration. More nitrogen utilised might be related to an increase in the final ethanol concentrations (Tables 2 and 3). Similar results were reported by Jones and Ingledew (1994), who found that supplementation of yeast autolysate into wheat mash under VHG fermentation significantly improved the rate of sugar utilisation and ethanol production.

4. Conclusions

Nutrient supplementation and aeration are the essential factors for promoting ethanol production efficiency from sweet sorghum juice. The by-product from the brewery industry, DSY, was successfully used as a low-cost nitrogen supplement. Under the HG conditions, only nitrogen (DSY) supplementation (9 g l^{-1}) was required for ~ 2 -fold improvement of the rate of ethanol production (Q_p). Under the VHG condition, the supplementation of nitrogen (13.5 g l^{-1} of DSY) and trace elements (0.01 g l^{-1} of Zn, 0.05 g l^{-1} of Mg and 0.04 g l^{-1} of Mn) coupled with small amount of aeration supply improved the ethanol production efficiency (P , Q_p and $Y_{p/s}$). In addition, the formation of by-products was reduced by 25% under the aeration supply. High ethanol fermentation efficiency can be achieved under both HG and VHG conditions with different requirements. Therefore, economic analysis of the HG and VHG fermentations including distillation cost should be considered before applying in industrial ethanol production.

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