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REVIEW PAPER

BIOETHANOL PRODUCTION FROM SWEET SORGHUM STALK JUICE BY ETHANOL-TOLERANT *SACCHAROMYCES CEREVISIAE* STRAINS: AN OVERVIEW

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Sweet sorghum (Sorghum bicolor (L.) Moench) represent an emerging alternative feedstock with great potential for bioethanol production. Sweet sorghum stalks can be crushed to extract the sugar-rich juice (containing mainly sucrose, glucose and fructose) which can be directly fermented into ethanol with high efficiency by *Saccharomyces cerevisiae* yeast. This fast-acting microorganism with high ethanol-tolerance displays high ethanol yields and maintains high cell viability during the fermentation under very high gravity conditions (with high total sugar concentrations). The objective of this study is to review the current state of knowledge on the bioethanol production from stalk juice of different sweet sorghum varieties by *Saccharomyces cerevisiae* (industrial and isolated yeast strains), assessing also the effects of process variables, nutrient supplementation and feeding systems (batch and fed-batch fermentations) on the final ethanol concentration.

Keywords: bioethanol, sweet sorghum, stalk juice, *Saccharomyces cerevisiae*

Fermentable Sugars Profile of Sweet Sorghum Juice

Sweet sorghum (Sorghum bicolor (L.) Moench), a C4 plant with high photosynthetic efficiency and high productivity (Almodares and Darany, 2006), is the fifth-most important cereal crop worldwide after rice, wheat, maize and barley (Ramatoulaye *et al.*, 2016), cultivated for food (as grain), forage, fiber and sugar (Yuan *et al.*, 2008). In terms of environmental impact, sweet sorghum cultivation is more water efficient, requires less energy input, fertilization and agrochemical application than sugarcane (Nasidi *et al.*, 2010). Sweet sorghum has the following advantages: (i) fast lifecycle (between 90 and 120 days); (ii) drought tolerance and ability to grow in infertile conditions; (iii) stems rich in directly fermentable sugars (40–60 ton/ha); (iv) second-generation bioethanol production; and (v) favorable energy balance (Fernandes *et al.*, 2014).

Degrees Brix (°Bx), defined as grams of sucrose per 100 grams of solution, is used as a rapid field estimate for sugar concentration of sweet sorghum juice and is based on the refractive index of pure sucrose solution measured with a refractometer. Uchimiya *et al.* (2017) analyzed stalk juices from 23 sweet sorghum cultivars and noticed that the maximum Brix value was 20.1 g of sucrose per 100 g of solution. Nasidi *et al*. (2010) reported that freshly harvested sweet sorghum stalk juice had a minimum of 12% sucrose content and about 18.7% Brix extracted juice sugar content. Umakanth *et al.* (2012) demonstrated that sweet sorghum hybrids can also be exploited to address ethanol production from juice, obtaining a Brix content in the range of 12.9° to 16.2° in the hybrids. Rao *et al.* (2013) compared the performance of sweet sorghum hybrids and open pollinated varieties and reported no differences in stalk juice degrees Brix between the two test groups (juice degrees Brix recorded at physiological maturity varied between 15.9 and 19.6%), but observed that the test hybrids as a group have recorded 10 and 18% higher sugar and bioethanol yields, respectively, than experimental open pollinated varieties.

The quality of juice is influenced by the genotype and growing conditions (the stage of growth and the environment) (Olweny *et al.*, 2013). Harvesting sweet sorghum at the pre-flowering stage is recommended because the content of soluble sugars in the juice reaches the maximum level (16–23°Bx), in contrast to maturity stage where the sugar content decreases by 20–25% (Dar *et al.*, 2018). Sweet sorghum stalk juice usually contain approximately 16–18% fermentable sugars (mainly in the form of sucrose, glucose and fructose) (Table 1), which can be directly fermented into ethanol by yeast (Jia *et al.*, 2013).

Table 1. Juice sugar composition profile of different sweet sorghum varieties

Sweet sorghum variety	Concentration (g/L)			Reference
	Sucrose	Glucose	Fructose	
Dale	52.73	41.47	24.59	Ebrahimiaqda and Ogden, 2017
Sugar T	66.02	31.59	12.12	Ebrahimiaqda and Ogden, 2017
350 FS	46.49	33.71	15.46	Ebrahimiaqda and Ogden, 2017
M81E	42.22	34.65	20.93	Ebrahimiaqda and Ogden, 2017
M81E	42.00	35.00	21.00	Ebrahimiaqda and Ogden, 2018
M81E	83.00	44.00	21.00	Imam and Capareda, 2011
Umbrella	89.00	31.00	20.00	Imam and Capareda, 2011
Sugar Drip	42.38	23.17	28.95	Luo *et al.*, 2014
Theis	139.60	16.71	14.49	Bunphan *et al.*, 2015
BJ248	136.27	16.46	14.22	Bunphan *et al.*, 2015
SPV1411	115.26	20.19	16.39	Bunphan *et al.*, 2015
KKU40	131.66	18.28	15.43	Bunphan *et al.*, 2015
KKU40	124.05	20.85	16.80	Laopaiboon *et al.*, 2009
SSV2	102.71	27.58	13.69	Nasidi *et al.*, 2013
SSV2	113.93	32.07	15.50	Nasidi *et al.*, 2013
KSV8	36.41	19.73	9.67	Nasidi *et al.*, 2013
KSV8	55.67	21.76	10.52	Nasidi *et al.*, 2013
SIL05	61.0	38.0	28.4	Sasaki *et al.*, 2014
SIL05	62.3	35.9	26.8	Sasaki *et al.*, 2015

The sucrose content in the sweet sorghum stalk juice is predominant and stable throughout the growth stages compared to glucose and fructose contents which varies depending on the harvest period (Sakellariou-Makrantonaki *et al.*, 2007).

The total sugar content in the juice has an important influence on the fermentation efficiency. Wu *et al.* (2010) reported that in order to achieve high efficiency in batch fermentation (at 30°C for 72 h, using dry yeast Ethanol Red), the sugar content in the sweet sorghum concentrated juices (M81E variety) should not exceed 20% (93–94% efficiency), compared to 25% sugars (86–89% efficiency) and 30% sugars (72–77% efficiency), otherwise, both the high sugar content and the resulting high ethanol concentration will exert inhibitory effects on the yeast (resulting in an incomplete fermentation of fructose and higher glycerol contents). The results showed that a significant amount of residual sugars (approximately 4–17% of the original sugars) remained in the concentrated juices with 25% and 30% sugars. According to a study conducted by Imam and Capareda (2011), the fermentation efficiency of sweet sorghum juice from two varieties (Umbrella and M81E, with total sugar concentration of 140 and 148 g/L, respectively), inoculated with dry yeast Ethanol Red for a period of 72 h at 32°C, was greater than 90% (for 25% of sugar content in the juice) but lower (79% efficiency) for 30% of sugar content in the juice. Also, residual sugars of 3% and 10% remained in the highly concentrated juices of 25% and 30%, respectively.

Industrial and Isolated Yeast Strains for Bioethanol Fermentation from Sweet Sorghum Juice

Bioethanol production from free sugar containing juices is more attractive than ethanol from starch or lignocellulosic biomass due to the elimination of expensive stages such as pretreatment or hydrolysis to get fermentable sugars (Zabed *et al.*, 2014). *Saccharomyces cerevisiae* is the most commonly employed yeast in industrial ethanol production due to its high ethanol-productivity, high ethanol-tolerance and the ability to ferment a wide range of sugars (Azhar *et al.*, 2014). While soluble sugars from sweet sorghum juice (mainly in the form of sucrose) can be converted to ethanol through a simple process of direct fermentation (using common industrial biocatalyst such as *Saccharomyces cerevisiae*), the bagasse produced after juice extraction (mainly in the form of glucose and xylose) needs to be submitted through a complex process (pretreatment and enzymatic hydrolysis) in order to produce a mixture of pentose and hexose sugars which can then be fermented to produce ethanol (Castro *et al.*, 2017).

Some of the industrial *Saccharomyces cerevisiae* yeast strains used in bioethanol fermentation from sweet sorghum juice are as follows: CAT-1; ATCC 24858; ATCC 7754; CICC 1308; Y940 and TISTR 5048.

Saccharomyces cerevisiae CAT-1 is a suitable yeast for bioethanol production from cellulosic biomass on a large scale, being the most widely used strain in the Brazilian ethanol plants due to its outstanding capacity of competing with native yeasts, surviving and dominating during industrial fermentation process (Basso *et al.*, 2008; Costa *et al.*, 2014). This yeast is able to ferment sugars such as glucose, fructose, sucrose, mannose, maltose, raffinose and galactose, while other sugars such as xylose, cellobiose, mannitol and lactose cannot be fermented by this strain (Camargo *et al.*, 2018). The main advantages of using this yeast strain in the ethanol fuel industry are as follows: (i) reducing ethanol production costs by increasing ethanol yield or simplifying fermentation operations, and also by reducing antifoam consumption; and (ii) high resistance to pH shocks during breaks in the fermentation and recycling processes (Basso *et al.*, 2008; da Silva *et al.*, 2018). *Saccharomyces cerevisiae* ATCC 24858 is a very viable yeast for the fermentation of enzymatically generated sugars into bioethanol. At moderate reaction temperature of 30°C, this microorganism has the ability to ferment hexose sugars (such as glucose), but is not able to metabolize pentose sugars (such as xylose) (Boakye-Boaten *et al.*, 2015).

Saccharomyces cerevisiae strain ATCC 7754 can achieve better values of bioethanol concentration (37.5 g/L), sugar conversion coefficient (47 w/w%) and bioethanol total yield per cultivated area (1239 L/ha) than other ethanologenic microorganisms such as bacterium *Zymomonas mobilis* (32 g/L, 46 w/w% and 1145 L/ha, respectively) (Khalil *et al.*, 2015). The fermentation time could be 3-4 times shorter than that of conventional fermentation technology when immobilized yeast *Saccharomyces cerevisiae* CICC 1308 is involved in the production of ethanol from sweet sorghum stalk juice. Using this strain in a 5 L bioreactor, the fermentation time is reported to be between 11 and 13 hours (Mei *et al.*, 2009; Liu and Shen, 2008). Sweet sorghum can be treated in a similar way as sugar cane, including the use of industrial yeast like *Saccharomyces cerevisiae* Y940 for fermentation. This strain can completely convert a concentration of 140 g/L of sugar into ethanol within 8 hours. Total sugar concentration (which is the sum of sucrose, glucose and fructose during the maturation period) lower than 140 g/L can result in lower efficiency compared to sugar cane and increased production costs for ethanol industry (Fernandes *et al.*, 2014). *Saccharomyces cerevisiae* TISTR 5048 can be used efficiently for bioethanol production from sweet sorghum juice by fed-batch and repeated-batch fermentation (Laopaiboon *et al.*, 2007; Laopaiboon and Laopaiboon, 2012). This high ethanol-producing yeast strain is suitable for bioethanol fermentation under normal gravity more than under very high gravity conditions (Laopaiboon *et al.*, 2008), with several advantages over other ethanol-producing microorganisms: (i) high ethanol concentrations; (ii) high ethanol tolerance; (iii) high robustness; and (iv) high resistance to toxic inhibitors (Choonut *et al.*, 2014).
Some of the isolated *Saccharomyces cerevisiae* yeast strains used in bioethanol fermentation from sweet sorghum juice are as follows: NP01 and DBKKU Y-53.
Saccharomyces cerevisiae NP01 (isolated from dried starter used for Thai rice wine making) is considered a robust ethanol-producing strain because of its ability to produce high ethanol concentrations under very high gravity fermentation (Laopaiboon *et al.*, 2008), and to tolerate up to 12% (v/v) ethanol without loss of cell viability (Phukoetphim *et al.*, 2017). *Saccharomyces cerevisiae* DBKKU Y-53 (isolated from soil and plant samples) is a thermotolerant ethanol-producing yeast capable of growth and ethanol production from sweet sorghum juice at high temperatures (37°C and 40°C). Using a high potential thermotolerant yeast strain like DBKKU Y-53 in the ethanol fermentation at high temperatures brings a number

of advantages, such as: (i) increased rate of fermentation; (ii) decreased risk of contamination by mesophilic microorganisms; (iii) reduced cost of the cooling system; and (iv) use of simultaneous saccharification and fermentation coupled with a continuous stripping system for ethanol recovery (Nuanpeng *et al.*, 2016).

Table 2 presents different growth conditions for inoculum preparation, based on the yeast type and strain involved in the bioethanol fermentation from sweet sorghum juice.

Table 2. Growth conditions of different *Saccharomyces cerevisiae* yeast strains

Yeast strain	Strain type	Culture medium (g/L)	Shaking rate (rpm)	Growth temp. (°C)	Time (h)	pH	Reference
CAT-1	Industrial	Sweet sorghum juice (40), $(NH_4)_2SO_4$ (0.14), $MgSO_4 \cdot 7H_2O$ (0.4), KH_2PO_4 (0.2), K_2SO_4 (0.8), $MnSO_4 \cdot H_2O$ (0.0005), $ZnSO_4 \cdot 7H_2O$ (0.0007)	150	30	12	4.5	Larnaudie *et al.*, 2016
CICC 1308	Industrial	Glucose or Sucrose (50), Yeast extract (5), Peptone (5), KH_2PO_4 (1.25), $MgSO_4 \cdot 7H_2O$ (0.5)	150	30	20–24	5.0	Mei *et al.*, 2009
ATCC 24858	Industrial	Yeast extract (3), Malt extract (3), Peptone (5), Glucose (10)	150	30	24	6.2	Luo *et al.*, 2014
ATCC 7754	Industrial	Yeast extract (3), Malt extract (3), Peptone (5), Glucose (10)	—	30	48	6.0	Khalil *et al.*, 2015
TISTR 5048	Industrial	Yeast extract (3), Malt extract (3), Peptone (5), Glucose (10)	100	30	18	4.8	Bunphan *et al.*, 2015
NP01	Isolated	Yeast extract (3), Malt extract (3), Peptone (5), Glucose (10)	100	30	15	4.9	Laopaiboon *et al.*, 2009
DBKKU Y-53	Isolated	Yeast extract (3), Malt extract (3), Peptone (5), Glucose (10), Ethanol (40)	100	35	72	5.5	Nuanpeng *et al.*, 2016

Effects of Process Variables, Yeast Strain, Nutrient Supplementation and Feeding Systems on the Final Ethanol Concentration

The fermentation kinetic, ethanol yield and final ethanol concentration could be affected by process parameters such as fermentation temperature (from 30 to 40°C), juice solid concentration (from 6.5 to 26%) and yeast load (from 0.5 to 2 g/L) during the fermentation of sweet sorghum juice. The juice solid concentration has significant inverse effects on the ethanol yield and final ethanol concentration but a slight effect on the fermentation kinetic (Luo *et al.*, 2014).

Nutrient supplementation and aeration are the essential factors for promoting ethanol production efficiency in terms of ethanol concentration and ethanol productivity from sweet sorghum juice under very high gravity conditions (Deesuth *et al.*, 2015; Deesuth *et al.*, 2012; Suwanapong *et al.*, 2013). Both sufficient nitrogen and optimal aeration during very high gravity fermentation can affect the activity of alcohol dehydrogenase (which catalyzes the reduction of acetaldehyde to ethanol during the final step of alcoholic fermentation) resulting in high levels of ethanol production (Deesuth *et al.*, 2016).

Yeasts can tolerate ethanol at various concentrations in order to achieve high-levels of ethanol production. Ethanol tolerance depends on the yeast strain used and growth medium composition. In fed-batch fermentation from sweet sorghum juice, apart from nitrogen supplementation, feeding time and feeding rate are the key parameters to improve ethanol production efficiency under very high gravity conditions. In batch mode, yeast extract supplementation stimulates yeast growth, leading to an increase in ethanol production and reduced fermentation time (Phukoetphim *et al.*, 2017).

Fed-batch fermentation can improve the efficiency of ethanol concentration and product yield in comparison with batch fermentation where the kinetic parameters for ethanol production depend on the initial yeast cell and sugar concentrations (Laopaiboon *et al.*, 2007).

Basavaraj *et al.* (2013) presented a sweet sorghum ethanol production scenario where the sweet sorghum stalks were crushed and separated into juice (which was fermented into bioethanol and blended into transport fuel) and bagasse (which was used internally as fuel in the ethanol production process). The indicators used in the financial feasibility assessment have shown that an input of 22.3 tons per kiloliter of sweet sorghum resulted in a recovery of 45 liters of ethanol per ton of stalks and an output of 40 kiloliters of ethanol per day.

Larnaudie *et al.* (2016) studied the conversion of sweet sorghum to ethanol, in which the process stages were divided into: reception of raw material, cleaning, sugar extraction, juice clarification and concentration, syrup fermentation, ethanol recovery, and effluent treatment (Figure 1). An industrial yeast strain *Saccharomyces cerevisiae* CAT-1 was used for the fermentation process (with a operation time of 24 h, at 30°C). The results showed that under very high gravity fermentation of concentrated sweet sorghum juice (226 g/L of total sugar concentration) was obtained the maximum bioethanol concentration of 97.8 g/L.

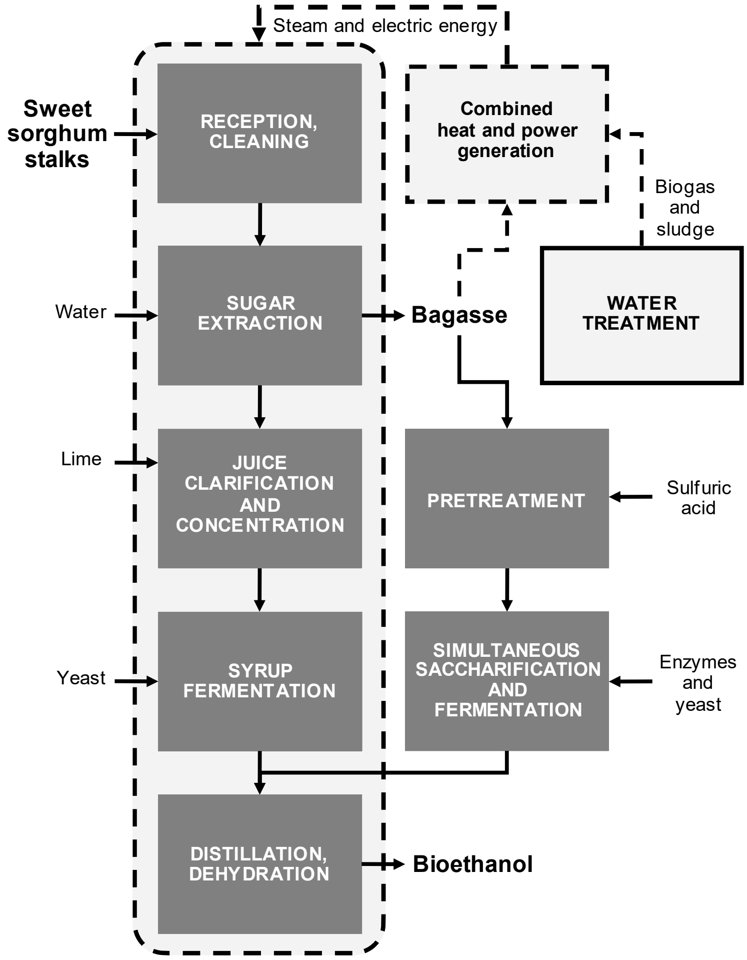

Figure 1. Block flow diagram of the ethanol production from sweet sorghum stalks (*source*: Larnaudie *et al.*, 2016; Gnansounou *et al.*, 2005)

Mei *et al.* (2009) indicated that the optimum conditions for ethanol production from sweet sorghum stalk juice (Liaotian No.1 variety) by immobilized yeast strain *Saccharomyces cerevisiae* CICC 1308 were found to be an initial total sugar concentration of 22.88%, supplement rate of $(NH_4)_2SO_4$ of 0.244%, and particles stuffing rate of 25.15%, resulting in a maximum predicted ethanol yield of 93.83%. Shen et al. (2011) reported that fermentation temperature of 33°C, pH of 4.5, particles stuffing rate of 25% and the initial sugar concentration of 218.1 mg/mL could be selected as suitable conditions for ethanol fermentation from sweet sorghum stalk juice (Ethanol-Sweet No.2 variety) by immobilized yeast *Saccharomyces cerevisiae* CICC 1308, obtaining the final ethanol concentration of 90.34 mg/mL at the end of fermentation of 9 h.

Luo *et al.* (2014) analyzed the effects of the process variables on the final ethanol concentration during the fermentation of sweet sorghum juice (Sugar Drip variety, with total sugar concentration of 94.5 g/L) using yeast *Saccharomyces cerevisiae* ATCC 24858 and reported that at the fermentation temperature of 35°C, yeast solid concentration of 1 g/L and juice solid concentration of 13%, the predicted final ethanol concentration was 49.48 g/L after 72 h of fermentation.

Davila-Gomez *et al*. (2011) evaluated the bioethanol production from sweet sorghum juice of three improved cultivars (Río, Della and M81E) by *Saccharomyces cerevisiae* ATCC 24858 at 30°C for 48 h. The results showed that Rio and Della varieties yielded significantly higher ethanol production (56.36 and 52.84 mL/L, respectively) compared to M81E (35.78 mL/L), with respect to the total sugar concentration in each juice (112.96, 102 and 70.34 g/L, respectively).

Guigou *et al*. (2011) evaluated the bioethanol production from the extracted juice of three sweet sorghum varieties (M81, Topper and Theis) with total sugar concentration of 121, 174 and 140 g/L, respectively. The cultures were incubated with dry baking yeast (*Saccharomyces cerevisiae*) at 30°C for 24 h, resulting in a higher ethanol concentration for Topper (77 g/L) and Theis (72 g/L) than for M81 (46 g/L). Khalil et al. (2015) used the sugar-rich juice of five sweet sorghum varieties (GK-coba, Mn-1054, Ramada, Mn-4508 and SS-301) to obtain bioethanol by two microorganisms (*Saccharomyces cerevisiae* ATCC 7754 and *Zymomonas mobilis* ATCC 29191). The results showed that the juice of sweet sorghum SS-301 variety had the highest total sugar content of 19.12% (initial sugar concentration of 143 g/L), compared to GK-coba variety of 16.87% (initial sugar concentration of 132 g/L) and Mn-4508 variety of 17.43% (initial sugar concentration of 136 g/L). The highest bioethanol concentration (39.2 g/L) was achieved with SS-301 variety (inoculated with the mixed-culture of both microorganisms at 1:1 ratio and incubated at 30°C for 96 h), compared to GK-coba variety (35.3 g/L) and Mn-4508 variety (36.6 g/L). Juices from four sweet sorghum varieties (BRS 506, BRS 508, BRS 509 and BRS 511) were evaluated by Fernandes et al. (2014) for fuel ethanol production using yeast *Saccharomyces cerevisiae* Y940 at 35°C (with the initial pH of the fermentation juice adjusted to 4.8). The results showed that the juice from sweet sorghum BRS 508 variety (with total sugar concentration of 162 g/L) was successfully fermented within 8 h, reaching a final ethanol concentration of 72.3 g/L, similar to sugarcane juice (73.2 g/L). Although the sweet sorghum BRS 511 variety had the highest content of total sugar in the juice (191 g/L), its ethanol concentration reached the lowest value (67.8 g/L) during the alcoholic fermentation.

Bunphan *et al.* (2015) compared four genotypes of sweet sorghum (KKU40, Theis, BJ248 and SPV1411) for juice content and ethanol production. The stalks of sweet sorghum varieties were crushed (using a sugarcane three-roller crusher) to extract juice which was fermented (at 30°C) by a highly ethanol-tolerant yeast strain *Saccharomyces cerevisiae* TISTR 5048, without adjustment of pH (juice pH was 4.8), in order to obtain ethanol. Total sugar content of the sweet sorghum juice (mean of 163.7 g/L) was highly correlated with sucrose concentration (mean of 130.70 g/L). The accumulation of ethanol after 36 h (mean of 66.2 g/L) and 48 h (mean of 70.4 g/L) of fermentation was similar among the four sweet sorghum varieties, with SPV1411 genotype reaching the highest concentration at 48 h (76.4 g/L).

Laopaiboon *et al*. (2007) demonstrated the efficiency of bioethanol production from sweet sorghum stalk juice (Keller variety) supplemented with small amount of nitrogen source (0.5% ammonium sulphate). The results showed that under optimum conditions (initial yeast cell and sugar concentration of 1.0×10^8 cells/mL and 24 °Bx, respectively), the ethanol concentration produced in batch fermentation (operated at 30°C under static condition) by *Saccharomyces cerevisiae* TISTR 5048 was 100 g/L. Fed-batch fermentation increased the efficiency of ethanol production in terms of ethanol concentration by approximately 18% (120 g/L), compared with batch mode.

Laopaiboon and Laopaiboon (2012) investigated the fermentation of sweet sorghum juice (KKU40 variety) with total sugar concentration of 240 g/L (corresponding to 24 °Bx of total soluble solids in the juice) by *Saccharomyces cerevisiae* TISTR 5048 (commercial high ethanol-producing yeast strain) and *Saccharomyces cerevisiae* NP01 (osmotolerant yeast strain isolated from Chinese yeast cake), using corncob as the support material for cell immobilization in order to produce bioethanol. In batch fermentation (operated at 30°C under static condition), *Saccharomyces cerevisiae* TISTR 5048 immobilized on $6 \times 6 \times 6$ mm^3 particle size of corncobs gave higher ethanol concentration (102.39 g/L) than immobilized cells on $12 \times 12 \times 12$ mm^3 particle size of corncobs (101.58 g/L). In repeated-batch fermentation, under the same immobilization condition (on $6 \times 6 \times 6$ mm^3 corncobs), the ethanol concentration obtained from *Saccharomyces cerevisiae* TISTR 5048 (97.19 g/L) was significantly higher compared with that from yeast strain *Saccharomyces cerevisiae* NP01 (90.75 g/L).

Ariyajaroenwong *et al*. (2012) investigated the ethanol production from sweet sorghum juice (KKU40 variety) containing 230 g/L of total sugar without nutrient supplementation, using sweet sorghum stalks as a low cost substrate for *Saccharomyces cerevisiae* NP01 immobilization. The results showed that in repeated-batch fermentation (at least eight successive batches) carried out at 30°C under the optimum conditions (size of sorghum stalk pieces of $6 \times 6 \times 6$ mm^3 and initial cell concentration for the immobilization of 1.0×10^8 cells/mL), the average ethanol concentration was 99.28 g/L.

Laopaiboon *et al*. (2009) investigated the efficiency of ethanol production from sweet sorghum juice (KKU40 variety) under very high gravity fermentation (carried out in batch mode at 30°C under static condition) by yeast *Saccharomyces cerevisiae* NP01 and reported that when sucrose was used to adjust the total soluble solids from 18 to 28 °Bx (corresponding to the total sugar concentration of 280 g/L), the maximum ethanol concentration was 120.68 g/L. Suwanapong *et al*. (2013) used a low-cost nitrogen supplement (dried spent yeast) to improve ethanol production from sweet sorghum stalk juice (KKU40 variety) by *Saccharomyces cerevisiae* NP01 under very high gravity condition (with total sugar concentration of 280 g/L) and showed that at 21 g/L of dried spent yeast addition on single batch ethanol fermentation (operated at 30°C) resulted the highest ethanol concentration of 107 g/L.

Deesuth *et al*. (2012) optimized the bioethanol production from sweet sorghum juice extracted from its stalks (KKU40 variety) by *Saccharomyces cerevisiae* NP01 under very high gravity condition (270 g/L of total sugar concentration) and reported that under the optimum nutrient supplementation (0.01 g/L of zinc (Zn), 0.05 g/L of magnesium (Mg), 0.04 g/L of manganese (Mn) and 9 g/L of yeast extract) for the ethanol fermentation (operated in batch mode at 30°C), the ethanol concentration was 120.58 g/L compared to the control fermentation without nutrient supplementation (93.45 g/L). Deesuth *et al*. (2015) investigated the ethanol production (at 30°C in batch mode) from sweet sorghum juice (KKU40 variety) by *Saccharomyces cerevisiae* NP01 under nutrient supplementation and/or aeration conditions. The results showed that under high gravity condition (200 g/L of total sugar), the addition of nitrogen supplements (yeast extract of 6 g/L or dried spent yeast of 9 g/L) did not increase the ethanol concentration (93.4–94 g/L). Under very high gravity condition (280 g/L of total sugar), the supplementation of nitrogen (13.5 g/L of dried spent yeast) and trace elements (0.01 g/L of zinc, 0.05 g/L of magnesium and 0.04 g/L of manganese) coupled with small amount of aeration supply (at 0.05 vvm-volume of air per volume of liquid per minute, for 12 h) improved the ethanol concentration (126.3 g/L), compared to the fermentation under the same supplementation without aeration (114.8 g/L) and fermentation under no supplementation and no aeration (108 g/L). Deesuth *et al.* (2016) optimized the aeration rate and aeration time for high levels of ethanol production from sweet sorghum stalk juice (KKU40 variety) under very high gravity fermentation by *Saccharomyces cerevisiae* NP01. The concentrated juice (containing 65 °Bx of total soluble solids) was diluted to a total sugar concentration of 280 g/L and used in the batch mode fermentation carried out at 30°C. The results showed that under the optimal aeration conditions (aeration rate of 0.31 vvm and aeration time of 12 h), the ethanol concentration was 127.8 g/L.

Thani *et al.* (2017) also optimized the process variables (aeration rate and recycle ratio) of a continuous ethanol fermentation (at 30°C) with a cell recycling system from sweet sorghum stem juice (KKU40 variety) by *Saccharomyces cerevisiae* NP01. To prepare the ethanol production medium, the concentrated juice (containing total soluble solids of 68 °Bx) was diluted with distilled water to obtain a total sugar concentration of 230 g/L before supplementation with yeast extract (6 g/L). The results revealed that under the optimum conditions (aeration rate of 0.25 vvm and recycle ratio of 0.625), the ethanol concentration was 99.28 g/L.

Phukoetphim *et al.* (2017) investigated the improvement of ethanol production from sweet sorghum juice (KKU40 variety) by nutrient supplementation and alternative feeding systems (batch and fed-batch fermentations performed at 30°C) using yeast strain *Saccharomyces cerevisiae* NP01. The results showed that under high gravity condition (at 200 g/L of total sugar) in the batch fermentation without yeast extract, the highest ethanol concentration was 90 g/L. Under very high gravity condition (at 280 g/L of total sugar), the ethanol concentration in the fed-batch fermentation (at feeding time of 9 h and feeding rate of 40 g sugar/h) resulted in a similar value (112.9 g/L) compared to batch fermentation with 9 g/L of yeast extract supplementation (112.5 g/L).

Nuanpeng *et al*. (2016) tested a newly thermotolerant yeast *Saccharomyces cerevisiae* DBKKU Y-53 (isolated from soil and plant samples) for ethanol production from sweet sorghum juice and reported that the maximum ethanol concentrations produced by this ethanol-producing yeast at high temperatures (37°C and 40°C), under optimum conditions (pH of 5.5, sugar concentration of 250 g/L, yeast cell concentration of 1.0×10^8 cells/mL and without nitrogen supplementation) were 106.82 g/L and 85.01 g/L, respectively, compared to industrial yeast strain *Saccharomyces cerevisiae* SC90 (91.59 g/L and 78.69 g/L, respectively).

Given the above information, we can conclude that ethanol production from sweet sorghum juice supplemented with small amount of nitrogen source (0.5% ammonium sulphate) can achieve a concentration of 100 g/L when industrial yeast strain *Saccharomyces cerevisiae* TISTR 5048 is used in batch fermentation at 30°C under optimum conditions (initial yeast cell and sugar concentration of 1.0×10^8 cells/mL and 24 °Bx, respectively). Fed-batch mode can increase the efficiency of ethanol production in terms of ethanol concentration by approximately 18% (120 g/L).

High levels of ethanol concentration (126.3 g/L) from sweet sorghum juice can be obtained under very high gravity fermentation (total sugar concentration of 280 g/L) carried out at 30°C in batch mode by *Saccharomyces cerevisiae* NP 01 (isolated strain) using nitrogen supplementation (13.5 g/L of dried spent yeast) and trace elements (0.01 g/L of zinc, 0.05 g/L of magnesium and 0.04 g/L of manganese) coupled with small amount of aeration supply (at 0.05 vvm, for 12 h). Under very high gravity fermentation, the supplementation of nitrogen (3.45 g/L of urea) and optimal aeration conditions (aeration rate of 0.31 vvm and aeration time of 12 h) can improve the ethanol concentration up to 127.8 g/L.

Conclusions

This study has provided a comprehensive overview on the bioethanol production from stalk juice of different sweet sorghum varieties by industrial and isolated *Saccharomyces cerevisiae* yeast strains capable of maximizing ethanol yields under a wide range of fermentation conditions. The optimum conditions for ethanol production depend on several factors such as total sugar concentration, yeast cell concentration and nutrient supplementation, in order to achieve high ethanol yields in the fermentation process. Yeast strains with high ethanol-tolerance can produce higher ethanol concentrations especially during very high gravity fermentation of concentrated sweet sorghum juices (with high total sugar concentrations). Nutrient supplementation coupled with fed-batch fermentation of sweet sorghum juices can increase the efficiency of ethanol production in terms of ethanol concentration.

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