INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 8, No. 2, p. 106-114, 2016

RESEARCH PAPER OPEN ACCESS

Comparative study of three commercial strains of *Saccharomyces* for enhanced production of biofuel using high gravity molasses

Muzna Hashmi[1], Aftab Iqbal Shafi[2], Fariha Hasan[1], Abdul Hameed[1], Aamer Ali Shah[1*]

[1]*Deparment of Microbiology, Quaid-i-Azam University, Islamabad, Pakistan*

[2]*Department of Biochemistry, Quaid-i-Azam University, Islamabad, Pakistan*

Key words: Biofuel, *Saccharomyces cerevisiae*, High gravity molasses.

http://dx.doi.org/10.12692/ijb/8.2.106-114 Article published on February 20, 2016

Abstract

The potential of different commercial strains of *Saccharomyces cerevisiae* was studied for enhanced production of bioethanol under high gravity condition. Three strains i.e. Rossmoor, Saf-instant and Uvaferm-43, were compared in order to select best commercial yeast that could be utilized on industrial scale for production of bio-ethanol. Osmotic pressure is one of the main stress factors faced by microbial strains during fermentation process where high gravity sugarcane molasses is used as a substrate. Under optimized physicochemical parameters, osmotic (sugar) tolerance of all strains, i.e. Rossmoor, Saf-instant and Uvaferm-43, to high gravity molasses was determined, which came out as 15, 17 and 25% (w/v), respectively. Maximum ethanol yield by these strains was 6.5%, 7.5% and 9.3 % (v/v) with fermentation efficiency of 72.6%, 69.2% and 58.1% respectively. It is concluded from the present study that Uvaferm-43 is the best strain for industrial use which has the ability to produce maximum ethanol under stressful condition but more research should be done to enhance its fermentation efficiency.

* **Corresponding Author:** Aamer Ali Shah ✉ alishah@qau.edu.pk

Introduction

Depletion of fossil fuel resources, limited global supply of oil, energy crises and increasing CO_2 emission has increased the worldwide interest to substitute fossil fuels by some alternative fuel (Huang *et al.*, 2012). Today, the ecofriendly bioethanol utilization as a substituent for the petroleum based products, has attracted worldwide interest for its production at large scale because it can be used in current unmodified engines by blending it with gasoline in different proportions (Macedo, 1998; Hansen *et al.*, 2005).

In order to create more sustainable and economically viable system, it is more important to emphasize on cheaper ways to produce bioethanol to make it more favorable as compared to petroleum based products (Zabed *et al.*, 2014). Currently, ethanol producing industries are utilizing two main feedstock i.e. starch containing feedstock and sugar crops (Wilkie *et al.*, 2000; Mojović *et al.*, 2006; Balat *et al.*, 2009). More than 60% of ethanol is being produced from sugar crops i.e. sugarcane and rest of 40% is being produced from starchy grain, worldwide (Salassi, 2007). High cost ethanol production from sugarcane can be mainly attributed to more energy consumption during separation step conducted by distillation process due to low ethanol content in fermentation media (Zabed *et al.*, 2014). Therefore, efforts are made to enhance ethanol concentration in fermentation broth to reduce distillation cost (Bai *et al.*, 2004).

Use of high gravity medium is a promising and attractive process for enhanced bioethanol production which reduces the energy cost by showing significant improvements in the overall productivity (Bayrock *et al.*, 2001). This technique reduces labor cost, water consumption and distillation cost due to enhanced ethanol content in fermentation medium (Laopaiboon *et al.*, 2009). A high gravity medium imposes stressful conditions to the yeast cells (loss of viability) that are associated with reduction in fermentation rate due to incomplete fermentation. At higher sugar concentration the production of ethanol starts decreasing due to unfavorable osmotic pressure which also decreases sugar fermentation efficiency of microorganisms (Peña-Serna *et al.*, 2012). Thus, the selection and implementation of resistant yeast strains that efficiently ferment high sugar concentration, is very important. Therefore, these strains must be resistant to high sugar, ethanol as well as other stress factors (Pereira *et al.*, 2011).

The microorganisms that are usually used in industries cannot tolerate high ethanol and sugar concentrations thus limit the fermentation efficiency. Different physicochemical parameters like sugar concentration, pH, temperature and nutrients are needed to be optimized to determine the best condition at which maximum yield can be obtained from fermentation of substrate i.e. molasses (Basso *et al.*, 2011).

The goal of the current study was to optimize various physicochemical parameters for different commercial yeast strains for enhanced production of ethanol. It also helped to determine the tolerance level among them so that most efficient strain could be offered to those industries, which are producing bioethanol from sugarcane molasses.

Materials and methods

Sample collection and source of microbial strains

Three different strains of *Saccharomyces cerevisiae*, Rossmoor, Saf-Instant gold and Lallemand Uvaferm-43 that are already being used in different industries, were considered in this study (Bechem *et al.*, 2007; Schmidt *et al.*, 2011; Sultana *et al.*, 2013). The effect of stress caused by high gravity molasses on all three strains was compared. Sugarcane molasses sample was obtained from Murree brewery, Rawalpindi, Pakistan, with 498 g/l total sugar content.

Inoculum preparation

Dried yeast was rehydrated in sterilized distilled water (1:10 w/v) and allowed to stand at 35-40°C for 10 min (minutes). Rehydrated yeast was then inoculated in diluted molasses [30% (w/v) (1.080 specific gravity)] to carry out fermentation process for

ethanol production.

Optimization of physicochemical parameters

Several physicals and chemical factors impose stress over microbes in fermentation medium during industrial application, their optimization is essential in order to obtain enhanced ethanol yield.

Effect of pH and temperature

The effect of pH on ethanol production was studied by adjusting pH of the reaction medium in the range 4.0-5.2, in 3 liters Erlenmeyer flasks, containing 500ml molasses solution and 2% inoculum. All the flasks were incubated at 30°C for 72 hrs (hours) under static condition. For determining the effect of temperature on ethanol production, the experiment was set in a similar fashion and flasks were incubated at different temperatures ranges 28-36°C for 72 hrs under static condition.

Effects of nutrient supplementation

The experiment was set in the same manner as previous experiment to determine the effect of nitrogen and phosphorous supplements. The effect of various concentrations was studied by adding urea and DAP (Di-ammonium phosphate) in range 0.4-1.5gm/l and 0.3-1.1gm/l, respectively in fermentation media. All flasks were adjusted with optimized pH and incubated at optimized temperature of inoculated yeast strain, for 72 hrs under static condition.

Effect of sugar concentration

Molasses was diluted and used in various concentrations such as 18-58% having specific gravity in the range of 1.050-1.150 that was adjusted with the help of gravity hydrometer. The sugar concentration was estimated from 9-29%, in experimental flasks. Sugar content of molasses then determined by dinitrosalysilic acid (DNS) method (Miller, 1959). All the flasks were incubated at optimized pH, temperature and nutrient supplements, under static condition for 72 hrs. Fermentation efficiency (%) of each strain at different sugar concentrations was also determined (Jayasundara *et al.*, 2008).

Analytical methods

Ethanol estimation

All the samples were distilled to analyze ethanol concentration by High performance liquid chromatography (HPLC). Ethanol was quantified by HPLC (Waters 1525) with RI detector (Waters 2410) with IC-PAK™ Ion Exclusion [50 A° 7µM (300 × 7.8mm)] column by using H_2SO_4 (0.5mM) as the mobile phase and injection volume of 20µl at a flow rate of 0.5ml/min. Breeze 2 software was used for analysis and interpretation.

Results and discussion

It has been widely observed during industrial processes that microbes encounter several stress conditions, which adversely affect their metabolism and production of desired compound. High ethanol content is desired in fermentation broth to reduce the water consumption and distillation cost; therefore, there is an extreme need to determine the best conditions required for maximum efficiency of fermenting microbes. In most distilleries, final ethanol content cannot be achieved up to expected value because of ethanol intolerance in microbial strains, which might be intensified by acidity and temperature (Dorta *et al.*, 2006). In ethanol industry, microbes mostly face stress conditions like high ethanol content, high osmotic pressure, hydrogen ions imbalance and temperature which must be monitored for maximum ethanol production (Basso *et al.*, 2011).

During this study, three different commercial strains having different ethanol tolerance were used. Rossmoor was not considered as good ethanol tolerant strain whereas Saf-instant and Uvaferm-43 were tolerant to ethanol concentration up to 10 and 16% (v/v), respectively (Bechem *et al.*, 2007). High ethanol content has also been reported to affect yeast physiology including enzymatic inactivation and growth inhibition, hence decreased the number of living cells which in turn reduced ethanol production (Basso *et al.*, 2011).

Physicochemical parameters were optimized to

determine the potential of each strain for maximum production of ethanol from sugarcane molasses. The normal pH of the available molasses was found to be 5.2, whereas the low pH is considered as the best factor to minimize the contamination of lactic acid bacteria during fermentation process and also enhance membrane permeability and microbial enzyme activity for fermentation process (Rault *et al.,* 2009). Due to these reasons, the molasses dilutions were set at pH range 4.0-5.2 to determine optimized pH for all of the three strains. Maximum ethanol yield was determined as 7.2% and 6.8% (v/v) at pH 4.4, when molasses was fermented by Uvaferm-43 and Saf-instant strains, respectively. Rossmoor strain showed better fermentation at pH 4.6, but ethanol yield determined by this strain (i.e. 6.2% (v/v)) was much lower as compared to other two strains (Fig.1.a). These results were comparable to previous researches when pH range from 4.0 to 4.6 was considered best for the fermentation of molasses by *S. cerevisiae* strains (Patrascu *et al.*, 2009; Periyasamy *et al.*, 2009; Mukhtar *et al.*, 2010).

Table 1. Enhanced production of bioethanol by using different specific gravity of molasses having different concentration of sugar.

Specific gravity	Total sugar before fermentation (% w/v)	Theoretical yield of Ethanol (% v/v)	Actual yield of Ethanol (% v/v)			Fermentation Efficiency (%)		
			Rossmoor ±S.D	Saf-instant ±S.D	Uvaferm-43 ±S.D	Rossmoor ±S.D	Saf-instant ±S.D	Uvaferm-43 ±S.D
1.050	9	5.6	4.1±0.08	4.2±0.04	4.8±0.08	72.6±1.4	75.6±1.0	85.1±1.7
1.060	11	7.0	4.8±0.12	5.3±0.08	5.7±0.12	68.5±2.3	75.2±1.4	81.9±2.1
1.070	13	8.3	5.5±0.04	6.2±0.16	6.9±0.08	66.4±0.5	74.4±2.4	82.8±1.2
1.080	15	9.6	6.5±0.12	7.1±0.08	7.2±0.16	67.3±2.1	73.9±1.0	75.0±2.0
1.090	17	10.8	6.4±0.12	7.5±0.04	7.8±0.08	59.1±1.1	69.2±0.5	71.6±0.9
1.100	19	12.1	6.1±0.08	7.0±0.08	8.1±0.08	50.1±0.6	57.5±0.8	66.6±0.8
1.110	21	13.4	5.4±0.04	6.7±0.04	8.5±0.08	40.4±0.3	50.3±0.4	63.2±0.7
1.120	23	14.7	4.3±0.08	6.1±.08	8.7±0.08	29.2±0.5	41.4±0.6	59.1±0.6
1.130	25	16.0	3.6±0.04	5.6±0.16	9.3±0.08	22.9±0.2	35.0±1.2	58.1±0.6
1.140	27	17.2	3.4±0.08	5.4±0.08	8.5±0.12	19.6±0.4	31.2±0.5	49.5±0.8
1.150	29	18.5	2.4±0.08	4.7±0.12	7.7±0.08	12.9±0.4	25.6±0.8	41.4±0.5

S.D= Standard deviation.

The fermentation performance of molasses at different temperature revealed that higher temperature condition adversely affected the process and ethanol concentration dropped down significantly. The maximum ethanol production was observed at 34°C when Uvaferm-43 and Saf-instant yeast were used as fermenting microbes and ethanol yield was determined as 7.6% and 6.9% (v/v), respectively. Similarly, Rossmoor strain showed better fermentation at 32°C but produced only 6.3% (v/v) of ethanol in the fermentation media (Fig.1.b). Similar results have been shown by previous researchers who determined the temperature range between 30 to 35°C was best for maximum ethanol yield by *S. cerevisiae* (Periyasamy *et al.*, 2009; Mukhtar *et al.*, 2010). At higher temperature, decrease in ethanol yield might be attributed to protein denaturation which hinders enzyme's catalytic activity or cause death of yeast cells (Dhaliwal *et al.*, 2011).

Molasses contained most of the nutrients important for yeast growth; however, nitrogen and phosphorous sources were still required for efficient fermentation. For better fermentation, urea was commonly added in fermentation media as nitrogen, whereas DAP (Di-ammonium hydrogen phosphate) as phosphorus plus nitrogen source. Nitrogen was important for amino acid synthesis, while phosphate had major role in glycolytic pathway during fermentation and also involved in nucleic acid synthesis thus played vital role in yeast replication (Mukhtar *et al.*, 2010). In this report, Rossmoor, Saf-instant and Uvaferm-43 yielded maximum ethanol i.e. 6.5%, 7.2% and 7.7%, respectively in the presence of 0.7gm/l of urea (Fig.2.a). Further increase in urea neither enhanced

nor reduced ethanol production by Rossmoor and Uvaferm-43 but reduced ethanol yield was observed in case of Saf-instant when urea concentration was increased. Addition of DAP (0.6gm/l) enhanced ethanol yield up to 6.5%, 7.1% and 7.6% when Rossmoor, Saf-instant and Uvaferm-43 were used respectively, (Fig.2.b) but further increase in DAP reduced ethanol yield by all of the three strain. All of three strains produced maximum amount of ethanol when both urea and DAP were used in combination (Fig.2.c). It was found that both Rossmoor and Saf-instant produced same amount of ethanol when urea was added either alone or in combination with DAP which indicated that they did not have phosphate requirement. Only urea alone was sufficient to meet their nutrient requirement to obtain maximum ethanol production. On the other side, the ethanol production was maximum in case of Uvaferm-43 when urea and DAP was used in combination which revealed requirement of phosphates for proper fermentation by Uvaferm-43 strain. In previous studies, 2 g/l of urea addition has been shown to enhance the ethanol concentration up to maximum level when fermentation was carried out at 35°C (Nofemele *et al.*, 2012). Other researches has been shown that addition of urea and DAP both played important role to enhance ethanol yield; whereas Saf-instant yeast showed similar increase in ethanol yield when either urea of DAP was added (Mukhtar *et al.*, 2010).

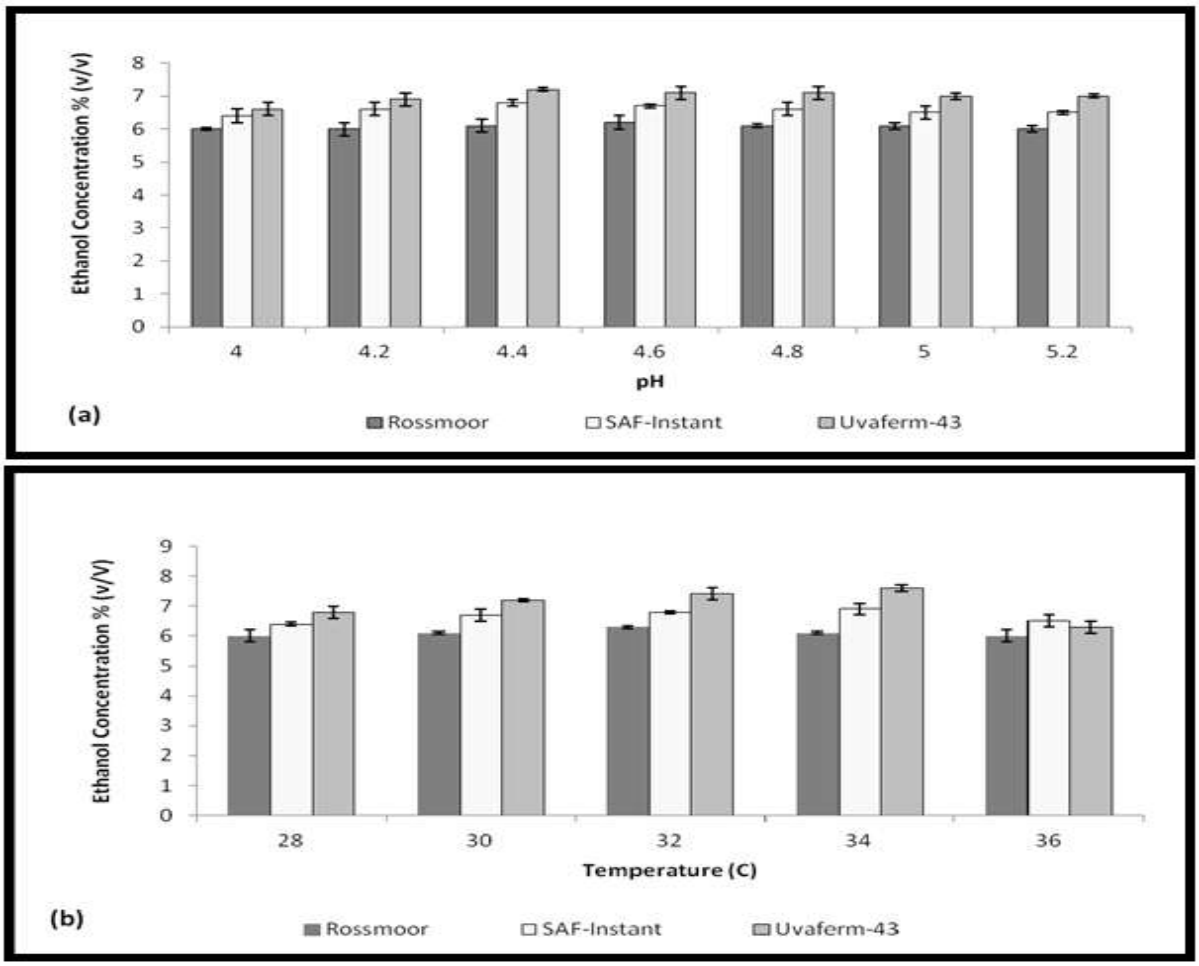

Fig. 1. Comparison among three strains for maximum ethanol production at different: (a) pH, (b) Temperature.

Now efforts are being done to use very high gravity molasses in industry to enhance ethanol yield which reduces the distillation cost but one of the major problems faced by industry is intolerance of yeast strains against high osmotic stress created due to high sugar concentration. Another problem faced by industry is the reduction in fermentation efficiency with increased osmotic stress under high gravity

conditions (Pratt *et al.*, 2003). Due to these reasons, there is an immense importance to use those yeast strains in industry which might have ability to tolerate high osmotic stress to produce maximum ethanol with high fermentation efficiency. The increased sugar concentration in the medium also enhanced actual ethanol yield; therefore, the determination of the maximum amount of sugar which can be efficiently converted in to ethanol is very important (Bai *et al.*, 2004). It was noted in current study that maximum ethanol yield obtained by Rossmoor, Saf-instant and Uvaferm-43 was 6.5%, 7.5% and 9.3% at sugar concentrations of 15%, 17% and 25% (w/v), respectively (Table.1); however, further increase in sugar concentration reduced the ethanol production. During the process of fermentation, more sugar concentration in fermentation media yielded more ethanol; however, there was a certain limit of sugar tolerance for each strain beyond which the strain couldn't perform efficiently. Some researchers in their studies revealed that under optimized condition, *S. cerevisiae* strain produced 6.7% (v/v) of ethanol when 30% (w/v) of sugar was present in fermentation media (Periyasamy *et al.*, 2009). In another study maximum ethanol production was determined as 7.7% (v/v) from 16% (w/v) sugar containing fermentation media (Arshad *et al.*, 2008).

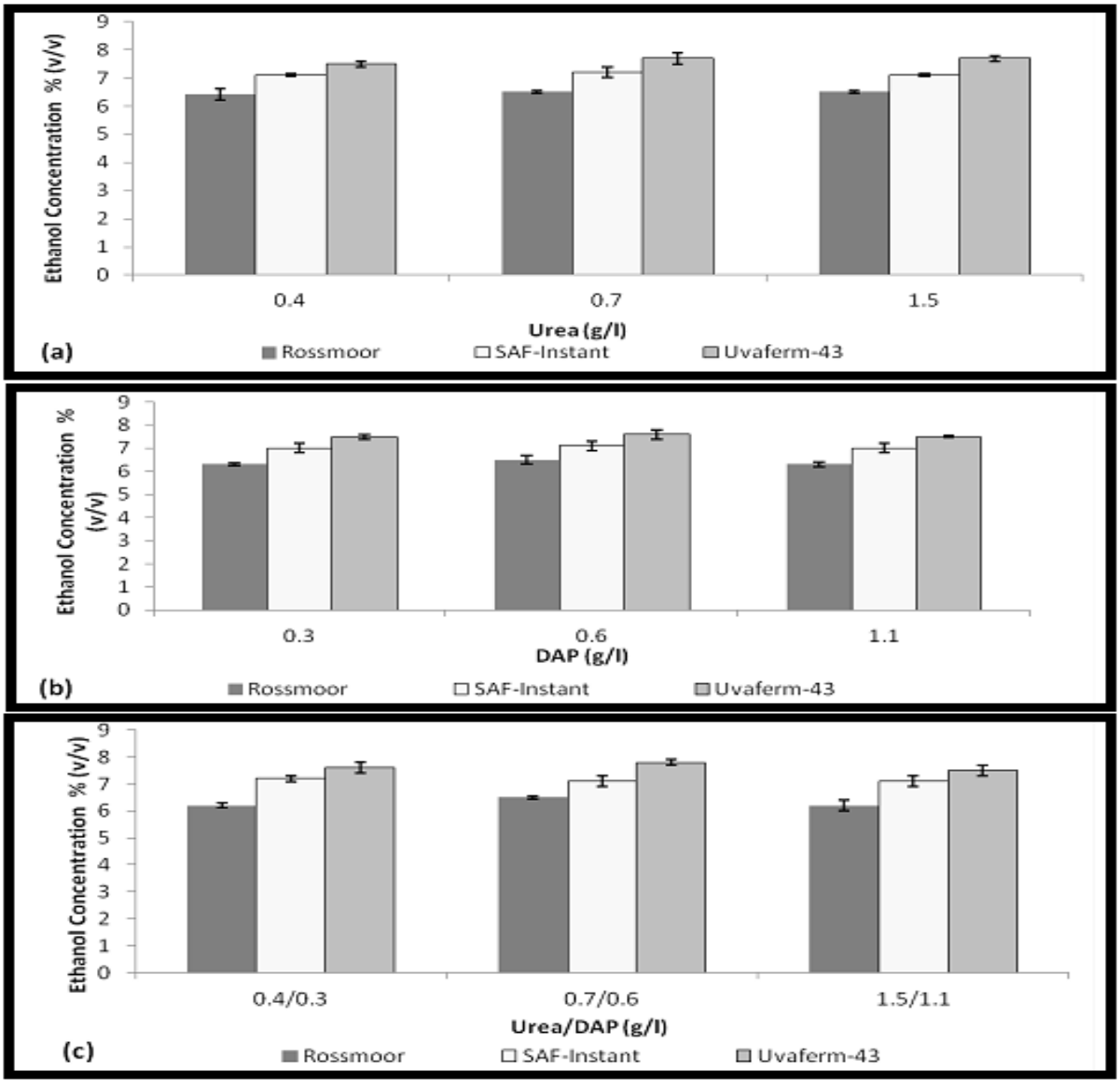

Fig. 2. Effect of nutrient supplement on enhanced production of bioethanol in the presence of (a) Only urea(g/l); (b) only DAP (g/l); and (c) both urea and DAP (g/l).

Fermentation efficiency of all the three strains to determine their ability to convert maximum of sugar into ethanol was also calculated. It was observed that Rossmoor was not a good ethanol tolerant strain and showed maximum fermentation efficiency of 72.6% with 4.1% (v/v) ethanol yield when 9% (w/v) sugar was present in media. The maximum ethanol yield obtained by Rossmoor was 6.5% (v/v) from 15% (w/v) sugar containing media; but the increased sugar concentration reduced the fermentation efficiency to 67.3% (Table.1). Due to low final ethanol yield, this strain was not considered as good option for industrial operations which has made the fact clear that osmotic tolerance is not an important factor for any strain unless it doesn't has the ability of high ethanol tolerance.

In comparison to Rossmoor strain, Saf-instant strain was considered as better option for enhanced ethanol production. Although it showed maximum fermentation efficiency of 74.4% with 4.2% (v/v) ethanol yield from 9% (w/v) sugar containing media, but high gravity molasses was required to use in order to increase ethanol yield which in turn reduce distillation cost. Saf-instant strain showed maximum ethanol yield of 7.5% (v/v) when 17% (w/v) sugar containing medium was used and fermentation efficiency during these conditions was calculated as 69.2% (Table.1). This strain can be considered as a better fermenting microbe to use a considerable amount of sugar present in molasses as compared to Rossmoor; however, to reduce energy cost during distillation process, much higher ethanol concentration is still required in fermentation medium.

Uvaferm-43 was the most ethanol tolerant strain having ability to tolerate 16% (v/v) ethanol. It was observed that, like Saf-instant, it showed maximum fermentation efficiency of 85.1% and produced 4.8% (v/v) ethanol from the molasses dilution containing 9% (w/v) sugar. An important finding of uvaferm-43 was its stable fermentation efficiency at much higher sugar concentration where the efficiency of Saf-instant was declined. It was also examined that, it not only had the ability to tolerate much higher ethanol concentration but also higher sugar concentration was also favorable for them. Uvaferm-43 yielded up to 9.3% (v/v) of ethanol with fermentation efficiency of 58.1% when 25% (w/v) sugar was present in fermentation media (Table.1) Previous researches has shown 8.4% (v/v) of actual ethanol yield with fermentation efficiency of 75% (Fadel *et al.*, 2013) and 7.6% (v/v) ethanol with 76% fermentation efficiency (Nofemele *et al.*, 2012). Although low fermentation efficiency is not a major issue for those distilleries where distillery waste is used for biogas production but new investigations should be done to increase its fermentation efficiency so that maximum of the sugar could be utilized for the production of ethanol.

Conclusion

Among three industrial strains of *saccharomyces cerevisiae* used in this study, Uvaferm-43 was determined as the best strain. It not only enhanced the ethanol yield thus helping in reduction of distillation cost but it's fermentation efficiency also remains stable at higher sugar concentration making this strain more favorable for industrial application of bioethanol production from sugarcane molasses.

References

Arshad M, Khan Z, Shah F, Rajoka M. 2008. Optimization of process variables for minimization of byproduct formation during fermentation of blackstrap molasses to ethanol at industrial scale. Letters in Applied Microbiology **47,** 410-414. https://dx.doi.org/10.1111/j.1472-765X.2008.02446.x

Bai F, Chen L, Anderson W, Moo-Young M. 2004**.** Parameter oscillations in a very high gravity medium continuous ethanol fermentation and their attenuation on a multistage packed column bioreactor system. Biotechnology and Bioengineering **88,** 558-566. https://dx.doi.org/10.1002/bit.20221

Balat M, Balat H. 2009. Recent trends in global production and utilization of bio-ethanol fuel. Applied Energy **86,** 2273-2282.

https://dx.doi.org/10.1016/j.apenergy.2009.03.015

Basso LC, Rocha SN, Basso TO. 2011. Ethanol production in Brazil: the industrial process and its impact on yeast fermentation. Biofuel Production-recent developments and prospects. InTech Europe, p. 85-101.

Bayrock D, Ingledew WM. 2001. Application of multistage continuous fermentation for production of fuel alcohol by very-high-gravity fermentation technology. Journal of Industrial Microbiology and Biotechnology **27,** 87-93.

Bechem E, Omoloko C, Nwaga D, Titanji V. 2007. Characterization of palm wine yeasts using osmotic, ethanol tolerance and the isozyme polymorphism of alcohol dehydrogenase. African Journal of Biotechnology **6(14),** 1715-1719.

Dhaliwal SS, Oberoi HS, Sandhu SK, Nanda D, Kumar D, Uppal SK. 2011. Enhanced ethanol production from sugarcane juice by galactose adaptation of a newly isolated thermotolerant strain of *Pichiakudriavzevii*. Bioresource Technology **102,** 5968-5975.
https://dx.doi.org/10.1016/j.biortech.2011.02.015

Dorta C, Oliva-Neto P, De-Abreu-Neto M, Nicolau-Junior N, Nagashima A. 2006. Synergism among lactic acid, sulfite, pH and ethanol in alcoholic fermentation of *Saccharomyces cerevisiae* (PE-2 and M-26). World Journal of Microbiology and Biotechnology **22,** 177-182.

Fadel M, Keera AA, Mouafi FE, Kahil T. 2013. High level ethanol from sugar cane molasses by a new thermotolerant *Saccharomyces cerevisiae* strain in industrial scale. Biotechnology Research International 2013, 3.
https://dx.doi.org/10.1155/2013/253286

Hansen AC, Zhang Q, Lyne PW. 2005. Ethanol diesel fuel blends-a review. Bioresource Technology **96,** 277-285.
http://dx.doi.org/10.1016/j.biortech.2004.04.007

Huang F, Ragauskas AJ. 2012. Dilute H_2SO_4 and SO_2 pretreatments of Loblolly pine wood residue for bioethanol production. Industrial Biotechnology **8,** 22-30.
https://dx.doi.org/10.1089/ind.2011.0018

Jayasundara J, Phutela R, Kocher G. 2008. Preparation of an alcoholic beverage from tea leaves. Journal of the Institute of Brewing **114,** 111-113.
https://dx.doi.org/10.1002/j.20500416.2008.tb00314.x

Laopaiboon L, Nuanpeng S, Srinophakun P, Klanrit P, Laopaiboon P. 2009. Ethanol production from sweet sorghum juice using very high gravity technology: Effects of carbon and nitrogen supplementations. Bioresource Technology **100,** 4176-4182.
http://dx.doi.org/10.1016/j.biortech.2009.03.046

Macedo IDC. 1998. Greenhouse gas emissions and energy balances in bio-ethanol production and utilization in Brazil (1996). Biomass and Bioenergy **14,** 77-82.
http://dx.doi.org/10.1016/S0961-9534(97)00038-X

Miller GL. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Analytical Chemistry **31,** 426-428.
https://dx.doi.org/10.1021/ac60147a030

Mojovic L, Nikolic S, Rakin M, Vukasinovic M. 2006. Production of bioethanol from corn meal hydrolyzates. Fuel **85,** 1750-1755.

Mukhtar K, Asgher M, Afghan S, Hussain K, Zia-ul-Hussnain S. 2010. Comparative study on two commercial strains of *Saccharomyces cerevisiae* for optimum ethanol production on industrial scale. BioMed Research International 2010, 3-4.
https://dx.doi.org/10.1155/2010/419586

Nofemele Z, Shukla P, Trussler A, Permaul K,

Singh S. 2012. Improvement of ethanol production from sugarcane molasses through enhanced nutrient supplementation using *Saccharomyces cerevisiae*. Journal of Institute of Brewing and Distillation **3,** 29-35.

Patrascu E, Rapeanu G, Bonciu CA, Hopulele T. 2009. Bioethanol production from molasses by different strains of *Saccharomyces cerevisiae*. International Symposium Euro-aliment, p. 9-10.

Pena-Serna C, Castro-Gil C, Pelaez-Jaramillo CA. 2012. Evaluation of ethanol production from two recombinant and a commercial strains of *saccharomyces cerevisiae* (fungi: ascomycota) in sugar-cane molasses and rejected-banana juice from uraba (antioquia), colombia. Actualidades Biologicas **34,** 21-31.

Pereira FB, Guimarães PM, Teixeira JA, Domingues L. 2011. Robust industrial Saccharomyces cerevisiae strains for very high gravity bioethanol fermentations. Journal of Bioscience and Bioengineering **112,** 130-136. http://dx.doi.org/10.1016/j.jbiosc.2011.03.022

Periyasamy S, Venkatachalam S, Ramasamy S, Srinivasan V. 2009. Production of bio-ethanol from sugar molasses using *Saccharomyces cerevisiae*. Modern Applied Science **3,** 32.

Pratt PL, Bryce JH, Stewart GG. 2003. The effects of osmotic pressure and ethanol on yeast viability and morphology. Journal of the Institute of Brewing **109,** 218-228. https://dx.doi.org//10.1002/j.2050-0416.2003.tb00162.x

Salassi ME. 2007. Economic Feasibility of Ethanol Production from Sugar Crops. Louisiana Agriculture, p. 6.

Schmidt SA, Dillon S, Kolouchova R, Henschke PA, Chambers PJ. 2011. Impacts of variations in elemental nutrient concentration of Chardonnay musts on Saccharomyces cerevisiae fermentation kinetics and wine composition. Applied Microbiology and Biotechnology **91,** 365-375. https://dx.doi.org/10.1007%2Fs00253-011-3197-3

Sultana S, Akhtar N, Asif HM. 2013. Phytochemical screening and antipyretic effects of hydro-methanol extract of Melia azedarach leaves in rabbits. Bangladesh Journal of Pharmacology **8,** 214-217.

Wilkie AC, Riedesel KJ, Owens JM. 2000. Stillage characterization and anaerobic treatment of ethanol stillage from conventional and cellulosic feedstocks. Biomass and Bioenergy **19,** 63-102. http://dx.doi.org/10.1016/S0961-9534(00)00017-9

Zabed H, Faruq G, Sahu JN, Azirun MS, Hashim R, Nasrulhaq Boyce A. 2014. Bioethanol production from fermentable sugar juice. The Scientific World Journal 2014, 2. http://dx.doi.org/10.1155/2014/957102