Journal of Energy Research and Environmental Technology (JERET)
Print ISSN: 2394-1561; Online ISSN: 2394-157X; Volume 2, Number 5; April-June, 2015; pp. 385-388

http://www.krishisanskriti.org/jeret.html

Ethanol Production from Molasses and Sugarcane: Inoculum Effects and Costing

Naveet Kaushal[1] and R.P. Phutela[2]

[1]*Mata Gujri College*
[2]*PAU, Ludhiana*
E-mail: [1]leo.naveet@gmail.com

Abstract—*Molasses and sugarcane juice were evaluated for ethanol production using Saccharomyces cerevisiae and S bayanus. The best performance was achieved with S cerevisiae strain G @ 10% inoculum where 15^{0}B molasses wort produced 6.9% (v/v) ethanol (FE 98.4% and ECF 0.46 ml $^0B^{-1}$) while 18^{0}B sugarcane juice yielded 8.9% (v/v) ethanol (FE 98.8% and ECF 0.49 ml $^0B^{-1}$). Analysis of the costing based on best ECF values revealed cost of production at 39.15 INR and 43.43 INR for 1 litre ethanol from molasses and sugarcane respectively.*

Keywords: *Ethanol, molasses, sugarcane juice, Saccharomyces cerevisiae, S bayanus, fermentation efficiency (FE), ethanol convertibility factor (ECF), cost of production.*

1. INTRODUCTION

Ethanol or bioethanol as it may be called, is the largest volume fermentatively produced organic solvent of immense utility as chemical feedstock, fuel supplement and gasoline exlender[2]. Of late, a sudden escalation of interest is seen for using this biofuel in automotive engines due to alarmingly dwindling reserves of petroleum and zeal to go pollution-free from poisonous gases and particles emanating from petrofuels. Alternatively, bioethanol is an ecofriendly fuel in the form of its mixture with gasoline[8] for the present day petrol engines. Brazil is the first sustainable biofuel economy and world's largest and most successful bioethanol producer from sugarcane at the lowest cost @ US $ 0.27 litre^{-1},while the USA produces it @ 0.32 litre^{-1} from corn[5].India produces ethanol from molasses, barley, wheat and corn, primarily for bottling of liquor in more than 200 distilleries and almost none for the biofuel[11]. The Punjab state with annual 4.77 million tonnes sugarcane production and 9.7% sugar recovery has a potential for bioethanol production[1]. Eventually, the production process needs to be upgraded and economized by using efficient fermenting yeast cultures vis-à-vis comparing sugarcane juice and molasses in terms of cost. Martin et al[13] characterized several agricultural and agroindustrial lignocellulosic residues to be used as raw materials for ethanol production while Beatriz Palmrola et al[4] devised method for pretreatment of barley husk for bioethanol production. Periyasamy et al[15] optimized fermentation conditions to achieve the maximum 53% bioethanol yield from sugar molasses. The present paper reports findings of comparison of three yeast cultures for fermentation of sugarcane juice and molasses and computation of cost of production of ethanol.

2. MATERIALS AND METHODS

2.1 Yeast culture

Three yeast cultures namely, *Saccharomyces cerevisiae* (including strain G isolated from a local brewery waste and the other commercial preparation) and *S bayanus* (sourced from tea institute, Sri lanka) were employed in the present studies. These were propagated and maintained on glucose yeast extract (GYE) agar slants (ingredients litre^{-1} : D-glucose 10g, peptone 5g, yeast extract 5g, agar 15g, pH 5.5) by fortnightly transfers, incubation at 28 ± 2^{0}C and storage at 4^{0}C. Molasses and sugarcane juice were the media for preparation of inoculum broth and fermentation experimentation.

2.2 Inoculum

The yeast inocula were prepared in sugarcane juice and molasses wort, each at 10^{0}B TSS. The juice/wort was just boiled, cooled, inoculated with a bit of yeast culture and incubated at 28±2^{0}C for 24h on a 200 rpm rotary shaker. As for as possible, only fresh inocula was used for fermentation trials.The storage, if it any was done at 4^{0}C which never exceeded 16 h in any case. The viable cell count of the inoculum (cfu/ml) was determined by the plate count technique on GYE agar in 100 mm dia glass petridishes.

2.3 Fermentation

The fermentation trials were conducted with molasses wort (adjusted at 15, 20 and 25^{0}B TSS) and sugarcane juice (18^{0}B native TSS) in aliquots of 400 ml in 550 ml capacity glass bottles. The yeast inoculum was added according to experimental plan and incubated for fermentation at 28±2^{0}C in stationary condition. The bubbling out of CO_2 was a sign of active fermentation while its cessation indicated completion of the process.

2.4 Inoculum parameters

Three yeast cultures as indicated above were evaluated for ethanol production @ 5% inoculum in molasses wort. Three inoculum sizes; 5, 7.5 and 10% of *S. cerevisiae* strain *G* were used for comparing fermentation efficiency in molasses and sugarcane juice.

2.5 Analysis

1. TSS was determined using a brix hydrometer calibrated with standard glucose solution and expressed as ^{0}B.
2. Total sugars were estimated by phenol-sulphuric acid method of Dubois et al[7].
3. Ethanol was determined by chemical oxidation method of Caputi and Wright[6].
4. Percent fermentation efficiency (FE) was calculated from the formula: (Actual ethanol produced / theoretical ethanol production) × 100.
5. A new term- ethanol convertibility factor (ECF) is coined to facilitate quick appraisal of fermentative production of alcohol in a given medium. Expressed as ml (or g) ethanol $^0B^{-1}$ (g^{-1} TSS) of the substrate, the ECF can determine quantity of ethanol produced from a given quantity of the substrate. Alternatively, quantity of the substrate can also be found to produce a specific quantity of ethanol, say 1 litre ethanol may be produced from:

   i. Molasses (kg) : 10^2/ (ECF × ^{0}B molasses).
   ii. Sugarcane (kg) : 10^4/ (ECF × ^{0}B juice× % juice recovery).
   Higher units of measurement may be applied for bigger quantities.

These formulae have been derived as follows:

Let ECF : x ml g^{-1} TSS for a given source TSS to produce 1000 ml ethanol : 1000/x g or 1/x kg.

Molasses

Let brix : y^0 B (% TSS, w/w)

$\therefore$ 1/x kg TSS: 1/x × 100/y kg

: 10^2/ xy kg

Hence quantity of molasses required to produce 1litre ethanol: 10^2/ (ECF × ^{0}B) kg

Sugarcane

Let juice brix: z ^{0}B (%TSS, w/v)

$\therefore$ 1/x kg TSS : 1/x ×100/z l

: 100/xz l

Let juice recovery from sugarcane: r% (v/w)

$\therefore$100/xz l juice : 100/xz × 100/r kg sugarcane

: 10^4/xzr kg

Hence quantity of sugarcane required to produce 1litre ethanol: 10^4/(ECF × ^{0}B × % juice recovery).

3. RESULTS AND DISCUSSION

3.1 Comparison of yeast cultures

The yeast cultures- *Saccharomyces cerevisiae* strain *G*, *S. cerevisiae* (commercial) and *S. bayanus* were compared for alcohol production in 15, 20 and 25^{0}B molasses worts. The process monitored for maximum 6 days, until completion of fermentation, revealed fall of TSS to the lowest values, 2-3^{0}B in 4-6 days in different treatments (Table 1). The highest 7.8% (v/v) ethanol was produced by *S cerevisiae* strain *G* in 20^{0}B wort which corresponds to the maximum ethanol convertibility factor (ECF) 0.39 ml g^{-1} TSS. Although *S bayanus* gave the highest fermentation efficiency (FE) 82.1% with 15^{0}B wort, yet its ECF 0.35 ml g^{-1} TSS was lower than several such values presented in Table 1. Reference of literature cannot draw a parallel among performance of different yeast cultures, but a variable trend of ethanol production in these strains is always evident. Out of four strains of *Zymomonas mobilis,* two performed optimally on 15% sugar while the remaining two were better suited to ferment 20% sugar wort[9]. Another report mentions *S cerevisise* as better ethanol producer than *Z mobilis* at 15% or more sugar solution[3]. The maximum rate of sugar utilization by *S. cerevisiae* to target the highest FE 99.46% as compared to the lowest of *S.carlsbergensis* is reported[14].

3.2 Evaluation of substrates

Molasses and sugarcane juice were evaluated for ethanol production by *S cerevisiae* strain *G* (found best in the preceeding experiment). The molasses worts at 15 and 20^{0}B were inoculated @ 5, 7.5 and 10% (inoculum with viable cell count 4.6 ×10^7 cfu ml^{-1}) and the fermentation was carried out for maximum 6 days. The 15^{0}B wort with 10% inoculum produced the 6.9% (v/v) ethanol, equivalent to the highest ECF 0.46ml $^0B^{-1}$ and it accounted for the maximum FE 98.4% (Table2). The alcoholic fermentation of sugarcane at 18^{0}B yielded the maximum 8.9% (v/v) ethanol with 10% inoculum and it stood for the highest values of ECF 0.49 ml $^0B^{-1}$ and FE 98.8%. It is inferred that the 10% inoculum size was optimum for obtaining the maximum ethanol production in all the treatments studied, more precisely with 15^{0}B molasses wort and 18^{0}B sugarcane juice. Alcoholic FE 88% is reported from 20^{0}B sugar wort using *S cerevisiae* MK-1 strain inoculum having 10^7-10^8 cells ml^{-1}yeast population[10,11]. Similarly, 9.5% (w/v) ethanol was produced from 22.5% sugar wort of molasses by maintaining yeast cell population at > 10^9 ml^{-1} in continuous fermentation tower[12].

3.3 Cost of production

Variability exists in the sugar content of commercial consignments of molasses and sugarcane. The molasses is found to contain 40-50% sugar. Variation in sugarcane are reflected on two counts i.e. juice recovery and sugar conc. in the juice. The juice recovery from sugarcane varies between 40-47% while the juice may have TSS 18-22^{0}B. Consequently, the cost of ethanol production from these substrates varies. In the present studies, the cost of production calculations have been based on the best ECF values i.e. 0.46 for molasses and 0.49 for sugarcane juice (Table 2). Accordingly, one needs 4.35 kg molasses of 50^{0}B to produce 1 litre ethanol to cost 39.15 Indian rupees (INR) as shown in Table 3. Sugarcane with the highest 47% juice recovery at 22^{0}B can produce alcohol @ 43.43 INR litre^{-1}. It is pertinent to indicate her that these calculations of cost of production are based solely on the cost of basic raw materials. The actual cost ought to be higher when expenditure on processing, energy, capital, taxation and several such factors are accounted for. Inspite of all these intricacies, fermentative ethanol production is a step in right earnest; after all it is a renewable cleaner fuel.

Table 1: Comparison of alcohol production from molasses by species of *Saccharomyces*

Species[1]	**Initial conc. in wort**		**Days to complete fermentation**	**Residual conc.**		**Ethanol produced**	**ECF**	**FE**
	TSS (^{0}B)	Total sugars (%)		TSS (^{0}B)	Total sugars(%)	(%, v/v)	(ml g^{-1} TSS)	(%)
S.cerevisiae G	15	12.6	4	2	1.6	5.6	0.37	79.5
	20	17.6	5	2	1.7	7.8	0.39	77.6
	25	23.3	6	2	1.8	7.3	0.29	53.1
S.Cerevisiae (commercial)	15	12.6	4	3	2.3	4.5	0.30	68.3
	20	17.4	5	3	2.5	7.3	0.36	76.6
	25	23.3	6	3	2.9	4.5	0.18	34.5
S.bayanus	15	12.6	4	3	2.7	5.2	0.35	82.1
	20	17.4	5	2	1.8	5.4	0.27	54.1
	25	23.3	6	2	1.8	6.2	0.25	45.1

C.D (Ethanol produced, p 0.05) : Species 0.3, TSS 0.16, Species x TSS 0.28.

1. Inoculum size: 5%, Viable cell count of inocula (cfu ml^{-1}): *S.cerevisiae G* : 4.3 x10^6, *S.cerevisiae* (commercial) : 4.8 x 10^6 and *S.bayanus* : 1.3 x 10^7.

Table 2: Effect of inoculum size of *Saccharomyces cerevisiae G* on alcohol production.

Species[1]	**Inoculum Size**[1] **(%)**	**Initial conc. in wort**		**Days to complete fermentation**	**Residual conc.**		**Ethanol produced**	**ECF**	**FE**
		TSS (^{0}B)	Total sugars (%)		TSS (^{0}B)	Total sugars (%)	(%, v/v)	(ml g^{-1} TSS)	(%)
Molasses	5.0	15	12.6	5	2	1.7	6.0	0.40	85.2
	7.5			5	2	1.6	6.7	0.45	95.4
	10.0			6	2	1.6	6.9	0.46	98.4
	5.0	20	17.4	4	2	1.6	7.2	0.36	71.4
	7.5			5	2	1.7	7.6	0.38	75.8
	10.0			6	2	1.8	8.2	0.41	81.9
Sugar cane Juice	5.0	18	16.7	5	3	2.5	8.2	0.46	90.7
	7.5			4	3	2.5	8.4	0.47	93.0
	10.0			4	3	2.6	8.9	0.49	98.8

C.D (Ethanol produced, p 0.05) Molasses: TSS 0.28, Inoculum size NS, TSS X Inoculum size 0.17.

Sugarcane juice: Inoculum size NS.

1. Viable cell count : 4.6 x 10^7 cfu ml^{-1}.

Table 3: Projected cost of one litre ethanol production.

Substrate	**Juice recovery**[1]**(%)**	**TSS**[1] **(^{0}B)**	**Substrate required**[2]**(kg)**	**Cost**[3] **(INR)**
Molasses		40	5.43	48.87
		44	4.94	44.46
		47	4.63	41.67
		50	4.35	39.15
Sugarcane	40	18	28.34	62.35
		20	25.51	56.12
		22	23.19	51.02
	44	18	25.77	56.69
		20	23.19	51.02
		22	21.08	46.38
	47	18	24.12	53.06
		20	21.71	47.76
		22	19.74	43.43

1. Assumed values for different grades of molasses and sugarcane juice.
2. Calculated from ECF of molasses (0.46) and sugarcane juice (0.49).

Based on market price (Jan-Aug 2009) of molasses (900 INR qtl^{-1}) and sugarcane (220 INR qtl^{-1})

4. ACKNOWLEDGEMENT

This work was carried under the guidance of senior microbiologist R.P Putela in the campus premises of PAU, Ludhiana

REFERENCES

[1] Anonymous, *Package of practices for Kharif crops of Punjab*. Punjab Agricultural University, Ludhiana, India, 2005.
[2] Bajaj, B. K. and Yousuf, S., Selection and Characterization of yeast for desirable fermentation characteristics. *Indian J. Microbiol*., 2001, **41,** 107-110.
[3] Bansal, R. and Singh, R. S., A comparative study on ethanol production from molasses using *Saccharomyces cerevisiae* and *Zymomonas mobilis*. *Indian J. Microbiol*., 2003, **43**, 261-265.
[4] Beatriz, P. A., Mats, G. and Guido, Z., Pretreatment of barley husk for bioethanol production. *J. Chem. Technol. Biotechnol*., 2005, **80,** 85-91.
[5] Budny, D. and Sotero, P., Brazil institute special report: The global dynamics of biofuels. 2007. (http://en. Wikipedia.org/wiki/ethanol_fuel_in_brazil).
[6] Caputi, A. and Wright, D., Collaborative study of the determination of ethanol in wine by chemical oxidation. *J. Assoc. Off. Anal. Chem*., 1969, **52**, 85-88.
[7] Dubois, M., Gills, K. A., Hamilton, J. K., Roberts, P. A. and Smith, F.,Colorimetric method for determination of sugars and related substances. *Anal. Chem*., 1956, **28**, 350-356.
[8] Hansen, A. C., Qin, Z., Peter,W. L. Lyne., Ethanol diesel fuel blends-a review. *Bioresource Technol*., 2005, **96**, 277-285.
[9] Karunakaran, T., Gunasekaran, P. and Kasthuribai, M., Fermentation pattern of *Zymomonas mobilis* strains on different substrates- a comparative study. *J. Biosciences*, 1986, **10**, 181-186.
[10] Kaur, U., Studies on alcohol fermentation at higher substrate concentration. M. Sc. Thesis, Punjab Agricultural University, Ludhiana, India, 1995.
[11] Kaur, M. and Kochher, G. S., Ethanol production from molasses and sugarcane juice by an adapted strain of *Saccharomyces cerevisiae*. *Indian J. Microbiol*., 2002, **42**, 255-260.
[12] Kida, K., Morimura, S., Thong, Y. and Thong, Y. L., Production of ethanol from molasses by flocculating yeast for use as an alternative energy source. *Seibutsu- Kogaku- Kaushi*, 1997, **75**, 15-34. (Ref. CAB Abstract, CD-ROM, AN 970310673).
[13] Martin, C., Lopez, Y., Plasencia, Y. and Hernandez, E., Characterization of agricultural and agroindustrial residues as raw material for ethanol production. *Chem. Biochem. Eng*., 2006, **20** (**4**), 443-447.
[14] Okunowo, W. O., Okotore, R. O., Akinniyi, P. and Suntoki, A. O., The alcoholic fermentation efficiency of indigenous yeast strains of different origin on orange juice. *African J. Biotechnol.*, 2005, **4**, 1290-1296.
[15] Periyasamy, S., Venkatachalam, S., Ramasamy, S., and Srinivasan, V., Production of bioethanol from sugar molasses using *Saccharomyces cerevisiae. Modern Appl. Sci*., 2009, **3** (**8**), 32-37.