International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2018; 6(5): 2023-2027

Received: 14-07-2018
Accepted: 18-08-2018

Sugandha Mahajan
Department of Microbiology,
Punjab Agricultural University,
Ludhiana, Punjab, India

Gurvinder Singh Kocher
Department of Microbiology,
Punjab Agricultural University,
Ludhiana, Punjab, India

Correspondence
Sugandha Mahajan
Department of Microbiology,
Punjab Agricultural University,
Ludhiana, Punjab, India

Standardization of sugar beet juice extraction by microwave heating and its fermentation for bioethanol production

Sugandha Mahajan and Gurvinder Singh Kocher

Abstract
Sugar beet juice was used as a substrate for bioethanol production owing to its high sucrose content as an alternative to first generation sucrose resources such as molasses, sugar cane juice etc. The juice of sugar beet, variety SZ-35, was extracted by microwave pre-heating treatment of succulents for 10 minutes giving total soluble solids (12.8 ± 0.74 ˚B). Two yeast strains, *Saccharomyces cerevisiae* KY069279 (isolated strain) and *Saccharomyces cerevisiae* D7 (commercial strain), were studied for their growth profile on sugar beet juice which revealed *Saccharomyces cerevisiae* KY069279 to have maximum growth rate (0.45 per hour) at 12 hours of shaking conditions. The selected yeast strain *Saccharomyces cerevisiae* KY069279 was further employed for the optimization of fermentation parameters (brix, inoculum size (%v/v) and DAHP supplementation (mg/100ml)) using RSM plan of Design Expert 10.0 software. The results were recorded in three responses viz. brix, reducing sugars and ethanol revealed 14.5˚B with inoculum size of 6%v/v, supplemented with 1mg/ml of DAHP was found to produce maximum ethanol (8.61%v/v) after 92 hours of fermentation at 25˚C with a fermentation efficiency of 92.7%.

Keywords: bioethanol, fermentation, microwave, *Saccharomyces cerevisiae* yeast, sugar beet

Introduction
Bioethanol is produced by fermenting carbohydrates present in the sugar or starchy crops. Currently, modern industrial bioethanol generation plants utilize juice or molasses from sugar crops and starch from cereal crops as their substrates. However, molasses possess alternate uses as source of industrial ethanol, potable ethanol and supplement in animal feed and are thus insufficient for meeting complete demand of bioethanol in the country. Hence, there is need to search for alternate first generation substrates that don't have food-fuel debate and can be easily fermented. Among such different energy crops sugar beet, sweet sorghum, fruit waste are few names that are potential candidates. Sugar beet has an immense endurance to a broad range of climatic deviations and the water and fertilizer requirement of the crop is 30-40% less in comparison with sugar cane whereas sugar content is almost comparable to that of sugarcane (Chakauya *et al* 2009) [13]. Biochemically, sugar beet contains enough amounts of sucrose (16 - 20%) like in sugarcane as it can be readily fermented by *Saccharomyces cerevisiae*. Direct processing of sugar beets in fermentation, without first having to go through sugar extraction and refinery, potentially lowers feedstock related costs for fermentative products. However, sucrose is present in its bound state in sugar beet causing its extraction a rate limiting step that involves pre-treatment processes such as diffuser, osmosis etc. The pre-treatment provided is relatively mild in comparison to that of enzymatic hydrolysis in case of starch and lignocellulosics. According to Berlowska *et al* (2017) [2], the medium obtained after enzymatic hydrolysis is a source of carbohydrates that can be metabolized by ethanol-synthesizing yeast to produce ethanol. Sugar beet molasses has also been earlier used as a feedstock for ethanol production (Dodic *et al* 2009) [5, 11]. The highest ethanol yield reported by Marx *et al* (2012) [8] for sugar beet was 0.49 g/g which corresponds to a fermentation efficiency of 96%. Sugar beet is thus a potential crop from which fuel alcohol can be accessed after a multi-tower pressure distillation plant. An exotic sugar beet variety SZ-35 was evaluated in the present study for optimization of pre-treatment followed by its ethanolic fermentation.

Materials & Methods
Sugar beet variety SZ-35 to be used as raw material for fermentation, was procured from Rana Sugars Limited, Buttar Seviyan, Amritsar, Punjab.

Physicochemical analysis of juice and its extraction
Healthy sugar beet succulents were washed with water which were then peeled off and shredded into finer strips. These cossettes/strips were then dipped in the water and thermal extraction of the juice was done via different methods which includes boiling, microwave heating at maximum power, autoclave heating at 10psi for 20 minutes and counter current mechanism till they became soft and pulpy. The optimized method was then used to extract the juice from the beets by filtering the pulp through the muslin cloth. After the filtration the beet juice was pasteurized at 62.8±2 °C for 30 minutes and then cooled to room temperature. The juice so obtained was also analysed for its physical and chemical properties. It was then stored in flasks after adding KMS at 0.01% (w/v) and kept overnight under refrigeration conditions i.e. at 4˚C before the initiation of the fermentation process.

Optimization of ethanolic fermentation parameters
Ethanol fermentation conditions viz. substrate concentration (˚B), inoculum size (%v/v) and Di-ammonium hydrogen orthophosphate (DAHP) supplementation (mg/100ml) were statistically optimized by Response Surface Methodology (RSM) using Design expert 10.0. RSM was adopted in the experimental design as it emphasizes the modeling and analysis of the problem in which response of interest is influenced by several variables and the objective is to optimize this response. A set of 20 experiments was carried out for three variables:
Total no. of experiments = 2no.of variables + 2 × no. of variables + central points

= 23 + 2 × 3 + 6 = 20

Lower and higher limits taken for the optimization process for substrate concentration (˚B), inoculum size (%v/v) and DAHP supplementation (mg/ml) were 10-15 ˚B, 6-10% (v/v) and 1-3 mg/ml respectively. The experimental design matrix in actual form of variables is given in Table 1. The second degree polynomial equation (Eq. 1) was calculated with the statistical software (Design Expert software 10.0) to estimate the response of the dependent variable.
The response function (y) was related to the coded variables (x_i, i=1, 2, and 3) by a second degree polynomial equation as given below:

$$y=b_0+b_1x_1+b_2x_2+b_3x_3+b_{12}x_1x_2+b_{13}x_1x_3+b_{23}x_2x_3+b_{11}x_1^2+b_{22}x_2^2+b_{33}x_3^2+\varepsilon \quad (1)$$

The variance for each factor was assessed and partitioned into linear, quadratic and interactive components. The coefficients of the polynomial were represented by b_0 (constant), b_1, b_2, b_3 (linear effects); b_{12}, b_{13}, b_{23} (interaction effects); b_{11}, b_{22}, b_{33} (quadratic effects); and ε (random error). The significance of all terms in the polynomial functions was assessed statistically using *F*-value at probability (P) of 0.05. The statistical analysis of the data and three-dimensional (3D) plotting were performed using Design Expert software 'DE-10'.
For each of the 20 runs, 350ml of beet juice was taken in 500ml capacity glucose bottles and inoculated with the selected yeast, *Saccharomyces cerevisiae* KY069279 and incubated as per the conditions of RSM for ethanolic fermentation. Samples were periodically drawn and analysed for ethanol and reducing sugars till the completion of the fermentation.

Results & Discussion
Physicochemical analysis of the raw sugar beet juice
It was observed that the raw juice contained 15–20 % of soluble solids. The overall range in respect of ˚Brix (4-17.5), total sugars (3.7-16.2), reducing sugars (0.02-2.67), total acidity (0.09-0.216), pH (4-5.5), sucrose (3.56-15.45) and juice recovery (100%) was observed in six different varieties of sugar beet viz SZ-35, Aranka, ST-1, ST-12, ST-14 and a local variety. From the above sugar beet varieties SZ-35 was selected for the physicochemical analysis of its raw juice as given in table 1.
Various researchers have studied the physico-chemical properties of sugar beet juice extracted from different varieties of sugar beet. Dziugan *et al* (2013) [7] recorded the chemical composition of the beet juice in which he reported the juice to have 5.5±0.2 pH, 3.4±0.4 g/kg reducing sugars, 5.6±0.4 g/kg nitrogen and 4.4±0.2 g/kg acetic acid. Wruss *et al* (2015) [14] studied the compositional characteristics of the beet root juice and reported that the main sugar in beetroot was sucrose with only small amounts of glucose and fructose.

Table 1: Physicochemical analysis of raw sugar beet juice (variety SZ-35)

Parameters	Sugar beet Juice
Brix (°B)	12.5±0.68
Total Sugars (g/100ml)	11.7±0.64
Reducing Sugars (g/100ml)	0.75±0.04
Sucrose (g/100ml)	11.2±0.61
pH	5.5±0.30
Acidity (%w/v)	0.22±0.01
Brix-Acid Ratio	56.8±3.12
Proteins (mg/ml)	0.2±0.01
Furfurals (g/100ml)	0.024±0.001
Hemicellulose (%w/v)	23±1.265
Lignin (%w/v)	0.6±0.033
Cellulose (%w/v)	29±1.575

Optimization of thermal treatment for juice extraction
Thermal pre-treatment of cossettes carried out as explained in material and methods was analysed for the ˚Brix, time and the juice recovered, the results of which are given in table 2.
Statistically, it has been observed from the above data, that microwave pre-treatment method for sugar beet juice extraction gave maximum juice recovery of 100% (including volume of water taken for extraction) in minimum time period of 10 minutes. The extracted juice was recorded to have 12.8°B±0.74 which was significantly higher among all the juice extraction methods. Standardization of time in microwave pre-treatment method for sugar extraction from sugar beet juice was also done by taking different times of treatment, as shown in the table 3.
Since, no significant difference between the total soluble solids of juice was observed among treatments B, C and D i.e. 12.5°B, 12.67°B and 12.8°B respectively, treatment B was considered to be best from economic point of view.
Traditional production of raw sugar beet juice is characterized by water extraction from sugar beet cossettes. Sugar beet is sliced and mixed with hot water in the juice-making machine or diffusion at about 70°C, then sugar from beet tissue enters the water and raw juice is manufactured (Mohammad 2013)

[9]. Duraisam *et al* (2017) [6] also reported the extraction of sugar (sucrose) from sugar beet using hot water in a multi-step process. Wruss *et al* (2015) [14] gave the compositional characteristics of sugar beet juice of seven different varieties and reported that the sugar composition was similar in all the varieties with an average sugar content of 7.7%, consisting of 95% sucrose. The microwave extraction that gave 12.5°B have not been reported earlier though Dhanraj (2014) [4] observed 16°B in a concentrate which will need to be diluted prior to fermentation.

Table 2: Optimization of thermal pre-treatment of sugar beet (SZ-35) for juice extraction

Juice Extraction Methods	Sugar beet-Water Ratio*	Time Taken (min.)	Juice Recoverey (%)**	Brix (°B)
Boiling	1:1.5	180	60	6±0.33
Microwave	1:1	10	100	12.8±0.74
Autoclave (10psi)	1:1	20	93	7.8±0.43
Counter current mechanism	1:0.8	180	86	11±0.61
$CD_{5\%}$	0.11	12.91	10.78	1.01

$$\text{*Sugar beet-Water Ratio} = \frac{\text{Amount of sugar beet taken (kg)}}{\text{Amount of water added (litre)}}$$

$$\text{**Juice recovery (\%)} = \frac{\text{Volume of extracted juice (ml)}}{\text{Weight of sugar beet taken (kg)}} \times 100$$

Optimization of fermentation parameters

Fresh sugar beet juice chaptalized with cane sugar (having 14.5 ˚B) was taken in 500 ml capacity glucose bottles (350 ml working volume) for each of the 20 combinations according to the RSM plan and responses were studied in terms of ethanol production (%v/v), brix (˚B) and reducing sugars (%) at the end of fermentation process. Table 4 revealed the 20 combinations of three fermentation factors along with their responses for *S. cerevisiae* KY069279, respectively.

Table 3 represents that in run 17 maximum ethanol production of 8.61% (v/v) with the fermentation efficiency of 90% was achieved at a temperature of 25 ˚C with the initial brix of 15 ˚B, inoculum size of 6% (v/v) and DAHP supplementation @100mg/100ml. Further, insignificantly low reducing sugars (0.02%) with final 0°B was recorded which indicated it to be an efficient combination. By keeping constraints at a 'target' final brix of 0˚B, inoculum size 'in range', DAHP supplementation and reducing sugars to 'minimum' and ethanol content to 'maximum'; a solution was provided by RSM as demonstrated in Table 4. Results indicated that when sugar beet juice with 14.5 ˚B, 6% (v/v) inoculum size was supplemented with 100mg/100ml DAHP and fermented at 25°C lead to 8.61% (v/v) ethanol content with a desirability of 98.7% for the ethanol production as shown in Table 5. On the basis of desirability, solution at serial number one was selected for the validation of sugar beet juice fermentation.

According to Rankovic *et al* (2009) [11], the fermentation course of sugar beet juice was carried out by *Saccharomyces cerevisiae* to have a final concentration of 10g per $1000cm^3$ that is 1×10^8 cells per cm^3 that produced the highest ethanol of 8.28% (v/v) with the starting sugar content of 13% (v/v) after 48 hours of incubation. Zabed *et al* (2014) [15] studied dried yeast of *Saccharomyces cerevisiae, S. diastaticus, Kluyveromyces marxianus, Pichia kudriavzevii, Escherichia coli* strain KO11 and *Klebsiella oxytoca* strain P2 and *Zymomonas mobilis* for ethanol production from sugar juices. Among these ethanol producing microorganisms, *S. cerevisiae* is the most attractive choice in fermentation due to its greater efficiency in sugar conversion to alcohol. Also its capability of producing flocs during growth makes it easier to settle or suspend on need and high tolerance to ethanol. Moreover, fermentation of some crop juices containing sucrose employs this yeast for its ability to hydrolyze sucrose into glucose and fructose with invertase enzyme. Elsewhere, Wang *et al* (2004) [13] suggested that the glucose concentration should not be too high in the fermentation medium (sugar beet juice) if the desired fermentation efficiency of the sugar is to be reached, since inhibition of glucose can occur. Furthermore, when fermentable sugars are used as adjuncts in some cases, it is suggested that high levels of glucose could yield this inhibitory effect.

Hence, 14.5°B of sugar beet juice, DAHP supplementation at 113-200 mg/100ml and inoculum range of 6-7% (v/v) were found to be optimum for sugar beet juice ethanol production.

In the literature it has been recorded that the highest ethanol yield obtained in this study was 0.49 gg-1 correspond to a fermentation efficiency of 96%. A kilogram of tropical sugar beet juice will yield approximately 400 ml of thick juice or 87.2 g of sugar. This translates to an ethanol yield of 110.5 L per kg of tropical sugar beet roots (Marx *et al* 2012) [8].

Table 3: Effect of microwave pre-treatment duration (min.) on recovery of sugar (°B) for sugar beet variety SZ-35

Treatments	Time (min.)	Brix (°B)
A	5	8.3
B	10	12.5
C	15	12.67
D	25	12.8
CD @ 5% (Brix)	1.21	

*The wort volume was 1litre/kg of sugar beet

Table 4: Experimental response profile for optimization of fermentation process parameters in fresh sugar beet juice using *Saccharomyces cerevisiae* KY069279

	Factor 1	Factor 2	Factor 3	Response 1	Response 2	Response 3
Run	Inoculum size (%v/v)	DAHP (mg/100ml)	Brix (˚B)	Final Brix (˚B)	Ethanol (%v/v)	Reducing sugars (g/100ml)
1	8	0.2	12.5	0	5.54	0.005
2	8	0.2	12.5	0	6.5	0.02
3	11.3636	0.2	12.5	0	5.9	0.022
4	6	0.3	10	0	3.7	0.027
5	8	0.368179	12.5	0	4.09	0.095
6	8	0.2	12.5	0	5.7	0.01

7	10	0.3	10	0	6.7	0.005
8	8	0.2	16.7045	0	7.36	0.062
9	8	0.2	12.5	0	6.3	0.007
10	4.63641	0.2	12.5	0	5.82	0.023
11	10	0.1	10	0	2.17	0.15
12	8	0.0318207	12.5	0	5.4	0.078
13	8	0.2	12.5	0	5.9	0.004
14	8	0.2	8.29552	0	3.7	0.03
15	8	0.2	12.5	0	6.2	0.015
16	10	0.1	15	0	7.32	0.065
17	6	0.1	15	0	8.61	0.02
18	6	0.3	15	0	4.32	0.145
19	10	0.3	15	0	6.08	0.052
20	6	0.1	10	0	5.12	0.03
Model F-value					23.81	48.35
p-value*					<0.0001	<0.0001
Predicted R^2 value					0.7235	0.8621
Adjusted R^2 value					0.9153	0.9573

*Incubated at 25 ˚C

*p-value <0.05 and **R2 value of >0.75 indicates good fitness of the model

Table 5: Numerical optimization by RSM using *S. cerevisiae* KY069279 of data presented in table 4

Name	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
A:inoculum size	minimize	6	10	1	1	3
B:DAHP	minimize	0.1	0.3	1	1	3
C:Brix	is in range	10	15	1	1	3
Final Brix	none	0	0	1	1	3
Ethanol	maximize	2.17	8.61	1	1	3
Reducing Sugars	minimize	0.004	0.15	1	1	3

Solutions

Number	Inoculum size	DAHP	Brix	Final Brix	Ethanol	RS	Desirability	
1	6	0.1	14.524	0.00	8.610	0.012	0.987	Selected
2	6	0.1	14.545	0.00	8.626	0.012	0.986	
3	6	0.1	14.495	0.00	8.588	0.011	0.986	
4	6	0.1	14.565	0.00	8.641	0.012	0.986	
5	6	0.1	14.474	0.00	8.572	0.011	0.986	

Conclusion

Microwave thermal pre-treatment for 10 minutes at maximum power was optimized for the production of sugar beet juice giving total soluble solids 12.5±0.68˚B. The pre-treated juice (diluted to 6°B) subjected to growth kinetics showed *Saccharomyces cerevisiae* KY069279 was best in terms of growth rate (0.45 h^{-1}) and consumption of sugars (2.785g/100ml). Ethanolic fermentation of sugar beet juice by *S. cerevisiae* KY069279 was standardized under Response Surface Methodology (RSM) using the statistical software “Design Expert 10.0” for Degree Brix (˚B), inoculum size and DAHP supplementation as fermentation parameters incubated at 25˚C under 20 different combinations. RSM results revealed that the brix of 14.5˚B, an inoculum size of 6% (v/v) of *S. cerevisiae* KY069279 and DAHP supplementation @ 100.0 mg/100ml were optimum for sugar beet fermentation. Optimized parameters were validated for 10 litres successfully with fermentation efficiency of 95.6% in yeast strain *S. cerevisiae* KY069279. A sugar concentration of 14.5˚B produced an ethanol level of 9.45 % (v/v) for KY069279 and was optimized to be best for sugar beet ethanol production. The maximum ethanol yield obtained from the sugar beet juice is 67.9g/kg with the production efficiency of 96.57%. It may thus be concluded from the present research that the sugar beet crop has a good potential for ethanol production with 96.6% of fermentation efficiency. It can also be used for blending purposed in petrol as a biofuel. The fermentation of sugar beet juice by *Saccharomyces cerevisiae* KY069279 (isolated strain) may prove to be a sustainable supplement to molasses for bioethanol production in India.

Acknowledgement

The authors are thankful to Rana Sugars Limited for providing us with sugar beet to make this research work possible.

References

1. Balat M, Balat H. Recent trends in global production and utilization of bioethanol fuel. Appl Energy. 2009; 86:2273-82.
2. Berlowska J, Przybyiska K, Balcerek M, Cieciura W, Borowski S, Kregiel D. Integrated bioethanol fermentation/anaerobic digestion for volrization of sugar beet pulp. Energies. 2017; 10(1255):1-16.
3. Chakauya E, Beyene G, Chikwamba RK. Food production needs fuel too: perspectives on the impact of biofuels in southern Africa. *S* Afr J Sci. 2009; 105:174-81.
4. Dhanraj NB. Cost effective method for production of ethanol from sugar beet and its estimation by modified dichromate method. Central European J of Bio. 2014; 3(1):9-12.
5. Dodic S, Popov S, Dodic J, Rankovic J, Zavargo Z, Mucibabic RJ. Bioethanol production from thick juice as intermediate of sugar beet processing. Biomass Bioenerg. 2009; 33:822-27.

6. Duraisam R, Salelgn K, Berekete AK. Production of beet sugar and bioethanol from sugar beet and its baggase: A Review. Int J Engin Trends Technol (IJETT). 2017; 43(4):222-33.
7. Dziugan P, Balcerek M, Przybylska KP, Patelski P. Evaluation of the fermentation of high gravity thick sugar beet juice worts for efficient bioethanol production. Biotechnol Biofuels. 2013; **6**:158-68.
8. Marx S, Brandling J, Gryp P. Ethanol production from tropical sugar beet juice. African J Biotechnol. 2012; 11(54):11709-20.
9. Mohammad S, Miladi M, Nateghi L, Yousefi M. Laboratory testing of three samples of major sugar beet sources of Iran for manufacturing sugar. Euro J Exp Bio. 2013; 3(6):44-48.
10. Nigam PS, Singh A. Production of liquid biofuels from renewable resources. Progress Energy Combustion Science. 2010; 37(1):1-17.
11. Rankovic J, Dodic J, Dodic S, Popov S, Mucibabic RJ. Thin juice from sugar beet processing as medium for bioethanol production. Proceedings of Faculty of technology. 2009; 19:44-51.
12. Swain KC. Biofuel production in India: Potential, prospectus and technology. J Fundamentals Renewable Energy Resources. 2014; 4(1):1-4.
13. Wang D, Xu Y, Hu J, Zhao G. Fermentation Kinetics of Different Sugars by Apple Wine Yeast Saccharomyces cerevisiae. J Inst Brew. 2004; 110(4):340-46.
14. Wruss J, Waldenberger G, Huemer S, Uygun P, Lanzerstorfer P, Muller U. *et al*. Compositional characteristics of beetroot products and beet root juice prepared from seven seven beet root varieties grown in Upper Austria. J Food Composition and Analysis. 2015; 42:46-55.
15. Zabed H, Faruq G, Sahu JN, Azirun MS, Hashim R, Boycel AN. Bioethanol Production from Fermentable Sugar Juice. Scientific World J. 2014; 2014:1-11.