



Process Optimization for Ethanol Production from Sweet Sorghum Juice Using *Saccharomyces cerevisiae* Strain NRRL Y-2034 by Response Surface Methodology

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Abstract In the present study, ethanol production from sweet sorghum juice of variety, CSV19SS was optimized using response surface methodology with the help of statistical software Statgraphics Centurion XVI.I. To check storage stability, sweet sorghum juice was stored at 0 and -20°C and was analyzed for reducing sugars, total acidity, pH and total soluble sugars for a period of 75 days. Sweet sorghum juice was found to be stable under refrigeration conditions for about 75 days without any significant changes in the sugar profile and acidity of the juice. For optimization, effect of three factors i.e. inoculum size, agitation rate and temperature on three response variables i.e. ethanol content, total acidity and pH were studied. The surface plots for desirability and overlay plots were generated to determine the optimum response. Sweet sorghum juice inoculated with *Saccharomyces cerevisiae* NRRL Y-2034 yielded 8.83 % (v/v) ethanol with fermentation efficiency of 87.33 % under optimized conditions of temperature (30°C), agitation rate (50 rpm) and inoculum size (7.5 % v/v).

Keywords Sweet sorghum juice · Ethanol · Fermentation efficiency · *Saccharomyces cerevisiae*

Introduction

Biofuels are liquid fuels derived from plant materials which are entering the market at a fast pace. Biofuels provided 1.8 % of the world's transport fuel in 2008. Ethanol fuel is the most common biofuel worldwide and widely used in Brazil and USA. The importance of the bioethanol production increased many fold with the realization that air and underground water pollution can be reduced by replacing lead or methyl-tert-butyl ether (MTBE) of petroleum to bioethanol. MTBE is known as fuel oxygenate (Fischer et al. 2005) and used as a fuel additive to raise the octane number (standard measure of the performance of a motor). However it is highly soluble in water and is a possible human carcinogen (Belpoggi et al. 1995). Therefore, ethanol (an oxygenous biomass fuel) is considered as a predominant alternative to MTBE for its biodegradable nature, low toxicity, persistence and regenerative characteristic (Cassada et al. 2000).

Crop plants are one of the best sources of renewable energy which can be used as feedstock for biofuel production. Sweet sorghum is a revolutionary agricultural crop which has proven potential to help world's fuel dependency. Sweet sorghum [*Sorghum bicolor* (L.) Moench], is a C_4 graminaceous crop which has sugar rich stalks. It has a very good potential as an alternative feed stock for ethanol production. *Saccharomyces cerevisiae* (yeast) is a model eukaryotic organism, often used in research because it is easy to manipulate and culture. This yeast is also widely used in industrial applications to manufacture enzymes and proteins for beer, wine and bread, and because it

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metabolizes glucose to ethanol, is also the most commonly used microorganism for ethanol production which utilizes hexoses of the feed and converts them into ethanol. Sweet sorghum juice is assumed to be converted to ethanol at 85 % theoretical efficiency, or 54.4l ethanol per 100 kg fresh stalk yield (Rains et al. 1993). Wu et al. (2011) reported 90 % fermentation efficiency with a mixture of sweet sorghum juice and bagasse. Similarly, ethanol titers of 17–23 % (v/v) were reported when wheat and formulated mashes were used in fermentation process (Jones et al. 1994; Bayrock and Ingledew 2001). Kundiyana et al. (2010) reported maximum ethanol i.e. 79.6 g/l with sugar conversion efficiency of 95.6 % at pH 4.3 with no added urea.

Belloch et al. (2008) reported that most yeast strains can ferment juices of 20 °B with high efficiency batch fermentation showing 10–12 % (v/v) ethanol content. Reports about optimization of ethanol production from sweet sorghum juice using response surface methodology are virtually lacking. Therefore, the present work was aimed at optimization of cultural conditions like inoculum size, agitation rate and temperature for ethanol production by response surface methodology using statistical software Statgraphics Centurion XVI.I.

Materials and Methods

Sweet sorghum juice of variety CSV19SS was procured from sugarcane section, Department of Plant Breeding and Genetics PAU, Ludhiana. The juice was extracted using cane crusher and was stored at 0 and –20 °C separately in sampling bottles for analyzing TSS (°B) by refractometer, total acidity (AOAC 1999), reducing sugars (Miller 1959) and pH to check the storage stability after 0, 15, 30, 45, 60, 75 days.

Saccharomyces cerevisiae strain NRRL Y-2034 used in the present study was obtained from the industrial microbiology laboratory, Department of Microbiology, PAU, Ludhiana and was maintained on glucose yeast extract medium slants by subculturing at regular intervals and storage at 4 °C.

The initial Brix of sweet sorghum juice was measured using a hand refractometer. Triplicate flasks containing fifty ml of filtered juice were pasteurized at 63 °C for 30 min and were inoculated with 5 ml (v/v) of starter culture of *S. cerevisiae* strain NRRL Y-2034. The fermentation was carried out at 28 °C at 50 rpm. The fall in °Brix was noted at specific intervals with the help of hand refractometer. The observations were made till constant values of °Brix were obtained. The fermentation broth was analyzed for ethanol concentration (% v/v). Ethanol was estimated by the chemical oxidation method of Caputi and Wright (1969). The following relations were used for different parameters.

$$(a) \text{ Sugar utilized} = \text{Initial available sugars (\%)} \\ - \text{Residual sugars after fermentation (\%)}$$

$$(b) \text{ Fermentation efficiency (\%)} \\ = \frac{\text{Actual ethanol recovery (v/v)}}{\text{Theoretical ethanol recovery (v/v)}} \times 100$$

where, Theoretical ethanol recovery (v/v) = Total sugars \times 0.64.

Actual ethanol production (% v/v) was determined from the standard curve using absorbance values at 600 nm

$$\text{For total acidity} = \text{Assuming 1ml 0.1 N.NaOH} \\ = 0.0075 \text{ g tartaric acid.}$$

$$\text{Total acidity (g/l tartaric acid)} \\ = \frac{\text{Volume of alkali used} \times \text{Normality of alkali used} \times 75 \times 100}{\text{Volume of sample}}$$

For optimization, the effect of three factor variables i.e. inoculum size, agitation rate, and temperature on three response variables pH, total acidity and ethanol content was studied using software Statgraphics Centurion XVI.I as per Table 2 and the least significant factor affecting the response variable was selected. The three dimensional plots and contour plots according to the fitted model were drawn using software. response surface methodology was used for multiple regression analysis of the experiment and F-test was employed to evaluate the statistical significance of quadratic polynomial equation. Coefficient of correlation (r), and coefficient of determination (R²) were employed to evaluate the performance of regression equation.

Ethanol content, pH and total acidity were determined using sets of conditions given in Table 2. The sets of experiments were conducted in triplicate by taking the above mentioned parameters in combination. The ethanol content in the juice was measured after 12 days of fermentation and efficiency was calculated as described above.

Results and Discussion

Shelf Life Study of Sweet Sorghum Juice

Mature plants of sweet sorghum variety CSV19SS were collected from the field and juice was extracted from the stem using cane crusher. The data of various parameters like reducing sugars, total acidity, pH and total soluble sugars (°B) analyzed during the study are presented in Table 1.

Table 1 indicates that there is 15.61 % enhancement in the reducing sugar level of sweet sorghum juice, when stored at 0 °C and 14.31 % enhancement when juice was

Table 1 Compositional analysis of sweet sorghum juice stored at 0 and −20 °C

Day(s)	Reducing sugars (mg/ml)		Total acidity (%)		pH		Total soluble sugars (°B)	
	Storage temperature		Storage temperature		Storage temperature		Storage temperature	
	0 °C	−20 °C	0 °C	−20 °C	0 °C	−20 °C	0 °C	−20 °C
0	0.58 ± 0.003	0.58 ± 0.003	0.93 ± 0.006	0.93 ± 0.003	5.27 ± 0.006	5.27 ± 0.003	20 ± 0.033	20 ± 0.033
15	0.64 ± 0.01	0.70 ± .0057	0.80 ± 0.008	0.77 ± 0.008	5.32 ± 0.003	5.37 ± 0.003	20 ± 0.034	20 ± 0.033
30	0.69 ± 0.003	0.75 ± 0.005	0.67 ± 0.005	0.60 ± 0.006	5.43 ± 0.008	5.50 ± 0.003	19.9 ± 0.033	19.9 ± 0.057
45	0.74 ± 0.008	0.81 ± 0.006	0.78 ± 0.006	0.60 ± 0.003	5.36 ± 0.003	5.50 ± 0.005	19.9 ± 0.033	19.8 ± 0.033
60	0.80 ± 0.005	0.86 ± 0.0057	0.91 ± 0.005	0.60 ± 0.005	5.25 ± 0.003	5.50 ± 0.006	19.9 ± 0.034	19.8 ± 0.033
75	0.85 ± 0.005	0.91 ± 0.008	0.91 ± 0.003	0.60 ± 0.005	5.25 ± 0.003	5.50 ± 0.006	19.9 ± 0.034	19.8 ± 0.033

Average of triplicate data ± values indicate standard error

Table 2 Cultural parameters by optimized by response surface methodology for ethanol production from sweet sorghum juice inoculated with *S. cerevisiae* NRRL Y-2034

Sr. no.	Factor variables			Response variables				Fermentation efficiency (%)
	Inoculum size (%)	Agitation rate (rpm)	Temperature (°C)	Total acidity (%)	pH	% Ethanol		
						v/v	w/v	
1	7.5	50	30	0.60	5.02	8.83	7.06	87.33
2	10	50	30	0.66	4.97	8.45	6.76	83.58
3	5	50	30	0.69	5.02	8.50	6.80	84.07
4	10	50	25	0.64	4.95	7.10	5.68	70.22
5	5	50	25	0.64	4.94	7.04	5.63	69.63
6	5	100	30	0.93	3.37	7.44	5.95	73.59
7	7.5	100	30	0.88	3.88	7.47	5.97	73.88
8	10	100	30	0.95	3.33	8.37	6.69	82.78
9	7.5	75	30	0.75	4.49	8.54	6.83	84.47
10	10	75	30	0.75	4.49	7.49	5.99	74.08
11	10	50	20	0.98	3.39	7.01	5.60	69.33
12	5	100	25	0.97	3.43	6.98	5.58	69.04
13	7.5	75	25	0.94	3.42	8.40	6.72	83.08
14	5	75	20	0.82	4.20	7.39	5.91	73.09
15	5	75	30	0.70	4.45	7.79	6.23	77.05
16	10	75	20	0.85	3.99	7.81	6.24	77.25
17	7.5	50	20	0.76	4.58	7.58	6.06	74.97
18	5	50	20	0.79	4.61	7.43	5.94	73.49
19	7.5	100	20	0.66	4.68	7.45	5.96	73.68
20	10	100	25	0.87	4.10	7.85	6.28	77.64
21	5	100	20	0.76	4.58	7.05	5.64	69.73
22	10	100	20	0.80	4.18	7.15	5.72	70.72

Bold values signifies the name of strain of *Saccharomyces cerevisiae*

Cultural conditions: volume of sweet sorghum juice, 50 ml; fermentation period, 12 days; adjusted brix, 20 °B; pH 5.5; average of triplicate data

stored at −20 °C for 75 days. There was no significant change in total acidity at 0 °C whereas it decreased by 0.33 % during storage at −20 °C after 75 days. The pH of the juice stored at 0 °C varied between 5.25 and 5.43 whereas at −20 °C, there was slight increase in pH values from 5.27 to 5.50. The total soluble sugar level of the fresh

juice was 20 °B which remained almost constant i.e. 19.9 and 19.8 °B at 0 and −20 °C after 75 days of storage. Ratnavathi et al. (2010) reported the TSS value of sweet sorghum juice in the range of 16–23 °B. There was no noticeable change in the sugar profile in refrigerated juices. Wu et al. (2011) reported that the juice stored at room

temperature, showed an average decrease of pH from 4.7 to 3.8 in one week whereas the pH increased from 4.7 to 5.1, when stored in refrigerator. Ronghou and Shen (2008) reported that pH 5.0 is suitable for ethanol fermentation instead of 3.5.

Optimization of Cultural Parameters

Table 2 indicates the effect of cultural parameters using response surface methodology of sweet sorghum juice inoculated with *S. cerevisiae* NRRL Y-2034. Best results were

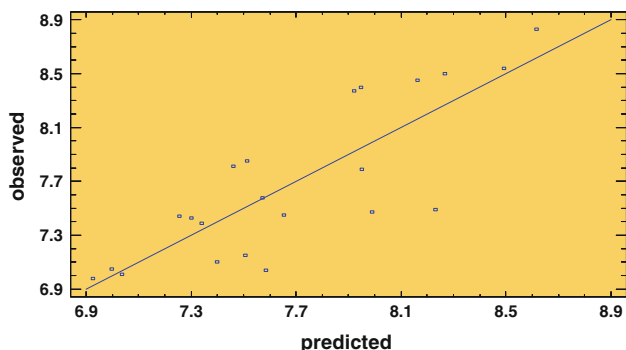


Fig. 1 Experimental versus predicted results of ethanol production (%)

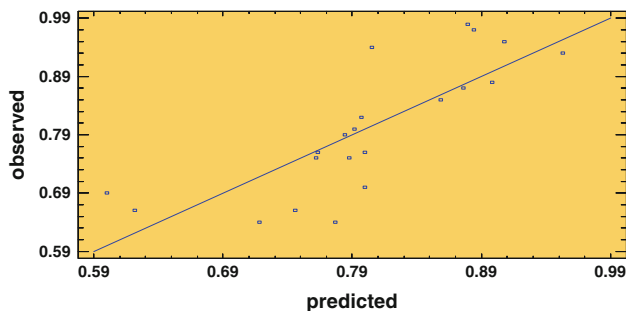


Fig. 2 Experimental versus predicted results of total acids (%)

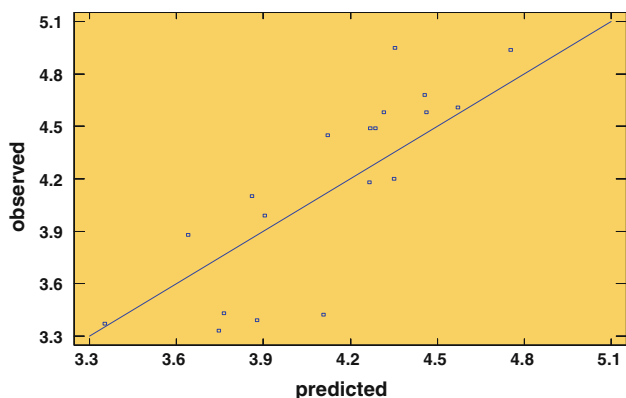


Fig. 3 Experimental versus predicted results of pH units: Ethanol content, %; total acids, %; agitation rate, rpm; temperature, °C; inoculum size, %

obtained at temperature of 30 °C at inoculum concentration of 7.5 % and agitation rate of 50 rpm which yielded 8.83 % ethanol with fermentation efficiency of 87.33 %. This was followed by 8.54 % ethanol with fermentation efficiency of 84.47 % at a temperature of 30 °C, inoculum concentration of 7.5 % and agitation rate of 75 rpm.

Effect of Cultural Conditions on Ethanol Content, Total Acids and pH

For appropriate fitting of each model, the analysis of variance (ANOVA) was carried out for ethanol production. This gives good correlation between input factors and their responses. Experimental results were close to predicted ones for ethanol production (Fig. 1), total acids (Fig. 2) and pH (Fig. 3). Each of the estimated effects and interactions (Tables 3, 4, 5, 6, 7 and 8), analysis of variance (Tables 5, 7 and 9) and regression coefficients (Table 10) for ethanol content, total acids and pH were determined. The straight line represents perfect agreement between experimental (observed) and calculated (predicted) values. The distance of each symbol from straight line corresponds to its deviation from related experimental values. Standard

Table 3 Statistical analysis for fitting of model

Model	Total acids	Ethanol content	pH
Transformation	None	None	None
Model df	9	9	9
P value	0.0491	0.0497	0.0223
Error df	12	12	12
Standard error	0.0886617	0.436415	0.40769
R ²	67.84	67.76	72.67
Adjusted R ²	43.72	43.58	52.18

Table 4 Estimated effects for ethanol content (%)

Effect	Estimate	Standard error	Variance inflation factor
Average + block	7.9471	0.301809	
A:inoculum size	0.20125	0.218208	1.0
B:agitation rate	-0.2725	0.218208	1.0
C:temperature + block	0.689746	0.213411	1.01359
AA + block	-0.803877	0.421944	1.01976
AB	0.385	0.251964	1.0
AC	0.08	0.251964	1.0
BB + block	-0.378877	0.421944	1.01976
BC + block	-0.355	0.251964	1.0
CC + block	0.400109	0.447347	1.0149

Standard errors are based on total error with 12 df

Table 5 Analysis of variance for ethanol content

Source	Sum of squares	df	Mean square	F-ratio	P value
A:inoculum size	0.162006	1	0.162006	0.85	0.3746
B:agitation rate	0.297025	1	0.297025	1.56	0.2355
C:temperature + block	1.9895	1	1.9895	10.45	0.0072
AA + block	0.691303	1	0.691303	3.63	0.0810
AB	0.444675	1	0.444675	2.33	0.1524
AC	0.0192	1	0.0192	0.10	0.7563
BB + block	0.153563	1	0.153563	0.81	0.3869
BC + block	0.378075	1	0.378075	1.99	0.1842
CC + block	0.152359	1	0.152359	0.80	0.3887
Total error	2.2855	12	0.190458		
Total (corr.)	7.08864	21			

Table 6 Estimated effects for total acids (%)

Effect	Estimate	Standard error	Variance inflation factor
Average + block	0.804928	0.0613153	
A:inoculum size	0.025	0.0443309	1.0
B:agitation rate	0.1325	0.0443309	1.0
C:temperature + block	-0.0339855	0.0433564	1.01359
AA + block	0.0638406	0.0857218	1.01976
AB	-0.0333333	0.0511889	1.0
AC	-0.0366667	0.0511889	1.0
BB + block	-0.0461594	0.0857218	1.01976
BC + block	0.186667	0.0511889	1.0
CC + block	-0.0521014	0.0908826	1.0149

Standard errors are based on total error with 12 df

Table 7 Analysis of variance for total acids

Source	Sum of squares	df	Mean square	F-ratio	P value
A:inoculum size	0.0025	1	0.0025	0.32	0.5832
B:agitation rate	0.070225	1	0.070225	8.93	0.0113
C:temperature + block	0.00483006	1	0.00483006	0.61	0.4483
AA + block	0.00435997	1	0.00435997	0.55	0.4708
AB	0.00333333	1	0.00333333	0.42	0.5272
AC	0.00403333	1	0.00403333	0.51	0.4875
BB + block	0.00227935	1	0.00227935	0.29	0.6001
BC + block	0.104533	1	0.104533	13.30	0.0033
CC + block	0.00258351	1	0.00258351	0.33	0.5770
Total error	0.0943308	12	0.0078609		
Total (corr.)	0.293295	21			

Table 8 Estimated effects for pH

Effect	Estimate	Standard error	Variance inflation factor
Average + block	4.10812	0.281944	
A:inoculum size	-0.15	0.203845	1.0
B:agitation rate	-0.74125	0.203845	1.0
C:temperature + block	0.0655435	0.199364	1.01359
AA + block	-0.182645	0.394172	1.01976
AB	0.248333	0.23538	1.0
AC	0.296667	0.23538	1.0
BB + block	0.332355	0.394172	1.01976
BC + block	-0.881667	0.23538	1.0
CC + block	0.289529	0.417902	1.0149

Standard errors are based on total error with 12 df

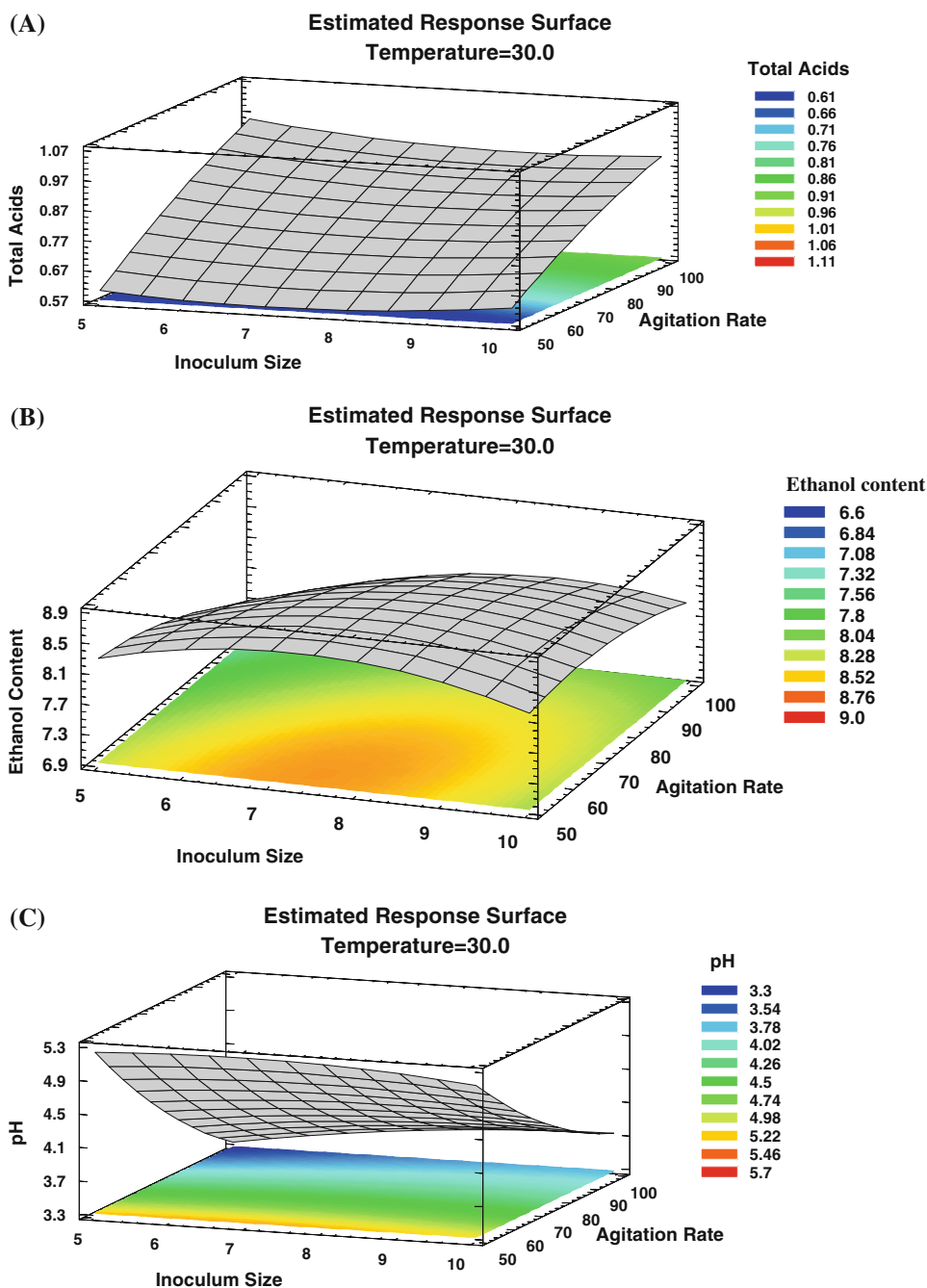
Table 9 Analysis of variance for pH

Source	Sum of squares	df	Mean square	F-ratio	P value
A:inoculum size	0.09	1	0.09	0.54	0.4760
B:agitation rate	2.19781	1	2.19781	13.22	0.0034
C:temperature + block	0.0179649	1	0.0179649	0.11	0.7480
AA + block	0.0356866	1	0.0356866	0.21	0.6514
AB	0.185008	1	0.185008	1.11	0.3122
AC	0.264033	1	0.264033	1.59	0.2315
BB + block	0.118166	1	0.118166	0.71	0.4156
BC + block	2.33201	1	2.33201	14.03	0.0028
CC + block	0.0797802	1	0.0797802	0.48	0.5016
Total error	1.99453	12	0.166211		
Total (corr.)	7.29913	21			

Table 10 Regression coefficients for ethanol content, total acids and pH

Coefficients	Estimate for ethanol content	Estimate for total acids	Estimate for pH
Constant	5.67858	1.05694	6.30394
A:inoculum size	0.693902	-0.014942	-0.256493
B:agitation rate	0.0524152	-0.00847754	0.0185591
C:temperature	-0.248634	0.0037029	-0.107475
AA	-0.0643101	0.00510725	-0.0146116
AB	0.00308	-0.000266667	0.00198667
AC	0.0032	-0.00146667	0.0118667
BB	-0.000303101	-0.0000369275	0.000265884
BC	-0.00142	0.000746667	-0.00352667
CC	0.00800217	-0.00104203	0.00579058

Fig. 4 Surface plots showing effect of inoculum size and agitation rate on total acids **a**, ethanol content **b** and pH **c** at constant temperature 30 °C units: Ethanol content, %; total acids, %; agitation rate, rpm; temperature, °C; inoculum size, %

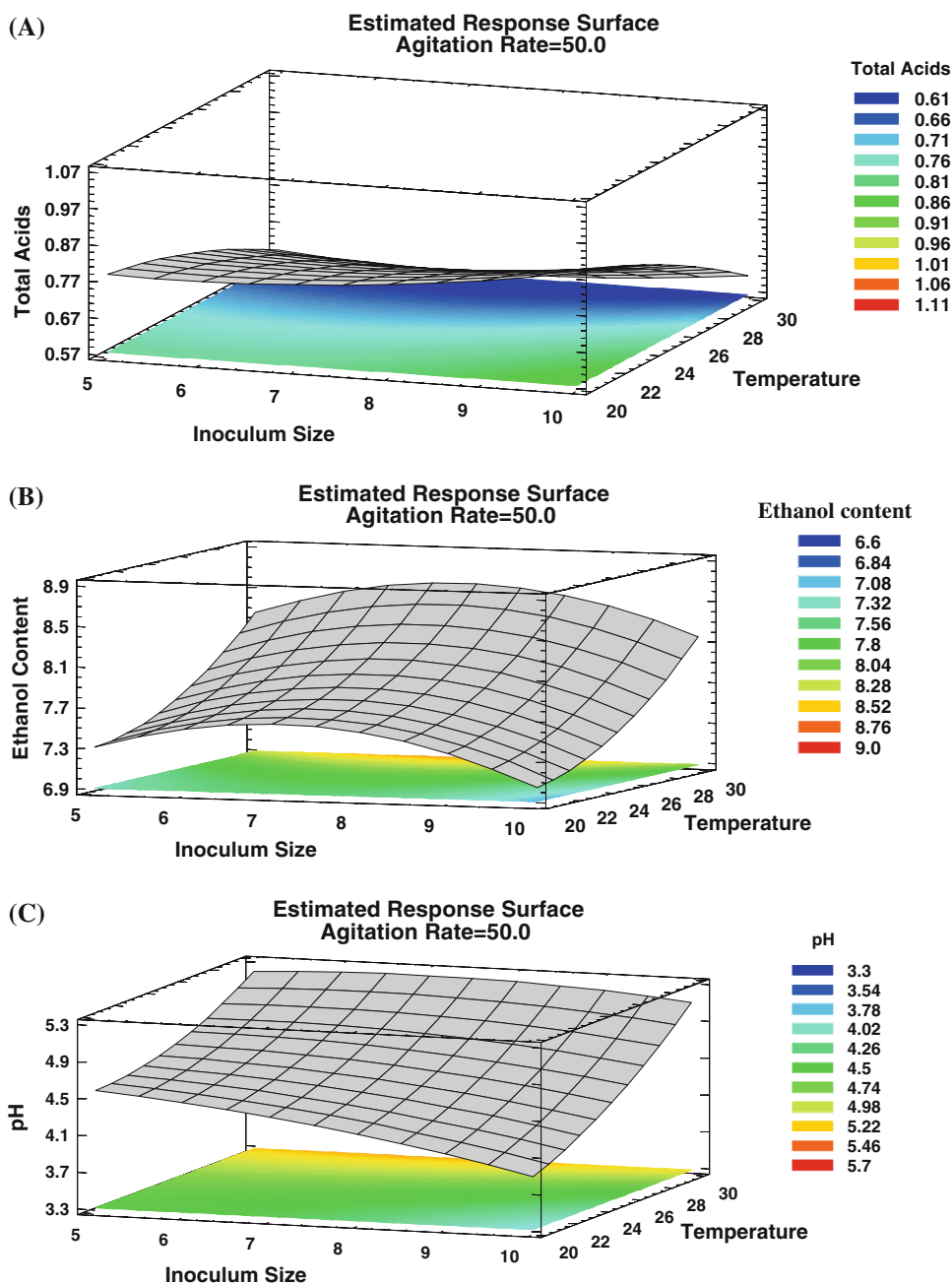


error of each of the effects is shown which measures their sampling error. The largest variance inflation factor (VIF) equals 1.01976 for all three responses. For a perfectly orthogonal design, all of the factors would equal to 1. Factors of 10 or larger were usually interpreted as indicating serious confounding amongst the effects. Block effects were included in the model to reduce the magnitude of experimental error.

The ANOVA table partitions the variability in responses into separate pieces for each of the effects. It tests the

statistical significance of each effect by comparing the mean square against an estimate of the experimental error. In case of ethanol, two effects have *P* values less than 0.05, indicating that they are significantly different from zero at the 95.0 % confidence level. The *R*² statistic indicates that the model as fitted explains 67.7583 % (Table 3) of the variability in ethanol content. The adjusted *R*² statistic is 43.577 % (Table 3). The standard error of the estimate shows the standard deviation of the residuals to be 0.436415. The mean absolute error (MAE) of 0.259102 is the average value of the residuals.

Fig. 5 Surface plots showing effect of inoculum size and temperature on total acids **a**, ethanol content **b** and pH **c** at constant agitation rate of 50 rpm units: Ethanol content, %; total acids, %; agitation rate, rpm; temperature, °C; inoculum size, %



The regression equation of the fitted model for ethanol is:

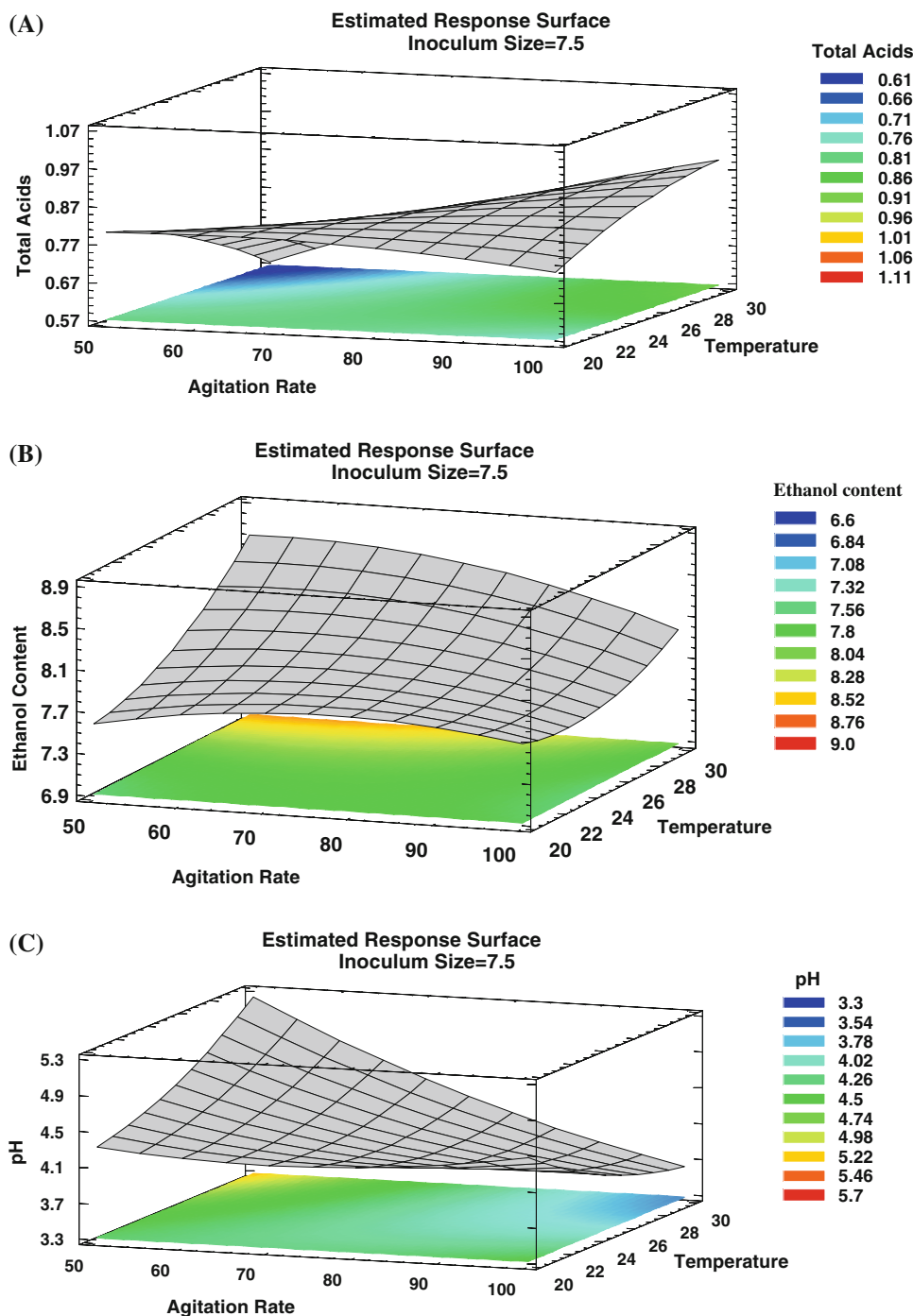
$$\begin{aligned}
 &5.67858 + 0.693902 * A + 0.0524152 * B \\
 &- 0.248634 * C - 0.0643101 * A^2 \\
 &+ 0.00308 * A * B + 0.0032 * A * C \\
 &- 0.000303101 * B^2 - 0.00142 * B * C \\
 &+ 0.00800217 * C^2
 \end{aligned}$$

where *A* inoculum size, *B* agitation rate, *C* temperature, * complex conjugate, ^ circumflex.

In case of acids, two effects have *P* values less than 0.05, indicating that they are significantly different from zero at the 95.0 % confidence level. The *R*² statistic indicates that the model as fitted explains 67.8376 % (Table 3) of the variability in total acids. The adjusted *R*² statistic is 43.7158 % (Table 3). The standard error of the estimate shows the standard deviation of the residuals to be 0.0886617.

The mean absolute error (MAE) of 0.0499308 is the average value of the residuals.

Fig. 6 Surface plots showing effect of agitation rate and temperature on total acids **a**, ethanol content **b** and pH **c** at constant inoculum size of 7.5 % units: Agitation rate, rpm; temperature, °C; inoculum size, %



The regression equation of the fitted model for total acids is:

$$\begin{aligned}
 &1.05694 - 0.014942 * A - 0.00847754 * B \\
 &+ 0.0037029 * C + 0.00510725 * A^2 \\
 &- 0.000266667 * A * B - 0.00146667 * A * C \\
 &- 0.0000369275 * B^2 + 0.000746667 * B * C \\
 &- 0.00104203 * C^2
 \end{aligned}$$

where A inoculum size, B agitation rate, C temperature, * complex conjugate, ^ circumflex.

The R^2 statistic indicates that the model as fitted explains 72.6744 % (Table 3) of the variability in pH. The adjusted R^2 statistic is 52.1802 % (Table 3). The standard error of the estimate shows the standard deviation of the residuals to be 0.40769. The mean absolute

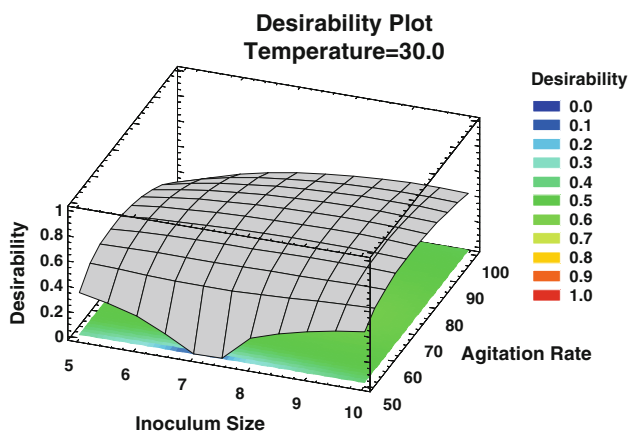


Fig. 7 Surface plot for desirability showing the effect of inoculum size and agitation rate on total acids (%), ethanol content (%) and pH at constant temperature 30 °C units: Agitation rate, rpm; temperature, °C; inoculum size, %

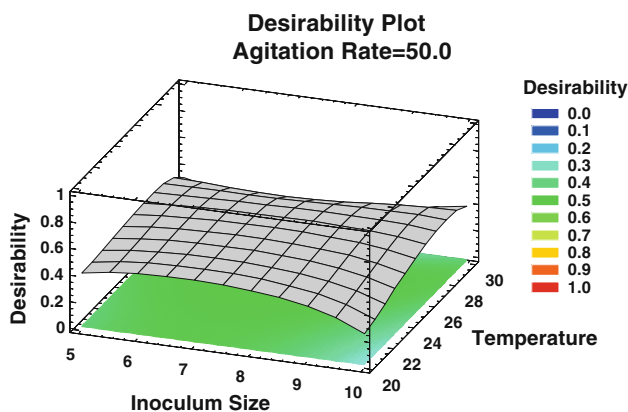


Fig. 8 Surface plot for desirability showing the effect of inoculum size and temperature on total acids (%), ethanol content (%) and pH at constant agitation rate of 50 rpm units: Agitation rate, rpm; temperature, °C; inoculum size, %

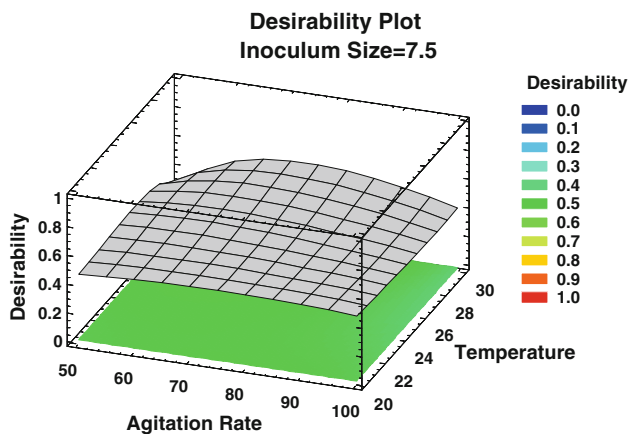


Fig. 9 Surface plot for desirability showing the effect of agitation rate and temperature on total acids (%), ethanol content (%) and pH at constant inoculum size of 7.5 % units: Ethanol content, %; total acids, %; agitation rate, rpm; temperature, °C; inoculum size, %

error (MAE) of 0.250949 is the average value of the residuals.

The regression equation of the fitted model for pH is:

$$\begin{aligned}
 &6.30394 - 0.256493 * A + 0.0185591 * B \\
 &- 0.107475 * C - 0.0146116 * A^2 \\
 &+ 0.00198667 * A * B + 0.0118667 * B * C \\
 &+ 0.000265884 * B^2 - 0.00352667 * B * C \\
 &+ 0.00579058 * C^2
 \end{aligned}$$

where *A* inoculum size, *B* agitation rate, *C* temperature, * complex conjugate, ^ circumflex.

Response Surface Methodology for Two Variable Interaction Studies

The surface plots for different interaction of any two independent variables, while holding the third variable constant, on ethanol production were generated using software. The graphical representation provides a method to visualize the relationship between the response and experimental levels of each variable in order to deduce the optimum conditions.

A direct correlation was found between inoculum size and agitation rate and graphs were plotted to understand their effect on ethanol production at fixed temperature 30 °C. The response of experimental levels of each variable was examined and it was observed that with the increase of agitation rate from 50, 75 and 100 rpm and inoculum size from 5, 7.5 and 10 %, total acids varied in the range of 0.62–0.74 %, ethanol content in range of 8.52–9.0 % and pH in range of 5.0–5.3 as shown in Fig. 4a, b and c.

Figure 5a, b and c showed the surface plots of inoculum size and temperature at fixed agitation rate of 50 rpm for total acids, ethanol content and pH. Increase in inoculum size from 5, 7.5 and 10 % and temperature from 20, 25 30 °C resulted in variation of total acids in the range of 0.62–0.73 % and ethanol content from 8.52 to 9.0 %. The pH was found to be in the range of 5.1–5.3.

Figure 6a, b and c showed the surface plots of agitation rate versus temperature at fixed inoculum size of 7.5 % for total acids, ethanol content and pH. Increase in agitation rate of 50, 75, and 100 rpm and temperature from 20, 25 30 °C resulted in variation of total acids in the range of 0.62–0.73 % and ethanol content from 8.52 to 9.0 %. The pH was found to be in the range of 5.1–5.3.

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To consider, all the responses simultaneously for optimization, surface plots were generated for each response. The

Fig. 10 Overlay plots showing the effect of inoculum size and agitation rate on total acids (%), ethanol content (%) and pH at constant temperature 30 °C units: Ethanol content, %; total acids, %; agitation rate, rpm; temperature, °C; inoculum size, %

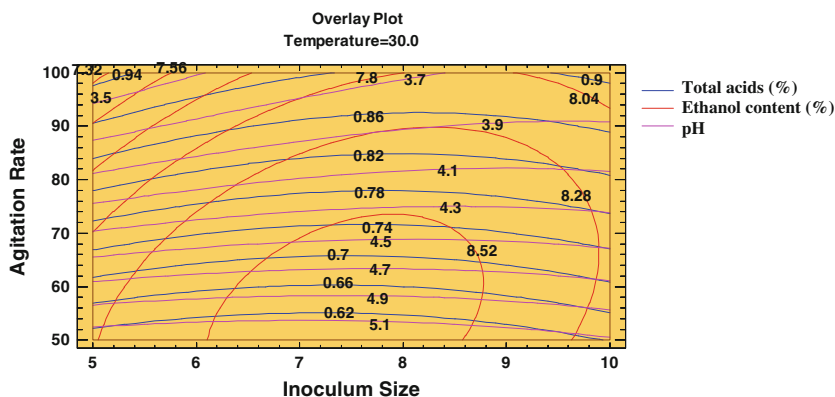


Fig. 11 Overlay plots showing the effect of inoculum size and temperature on total acids (%), ethanol content (%) and pH at constant agitation rate of 50 rpm units: Ethanol content, %; total acids, %; agitation rate, rpm; temperature, °C; inoculum size, %

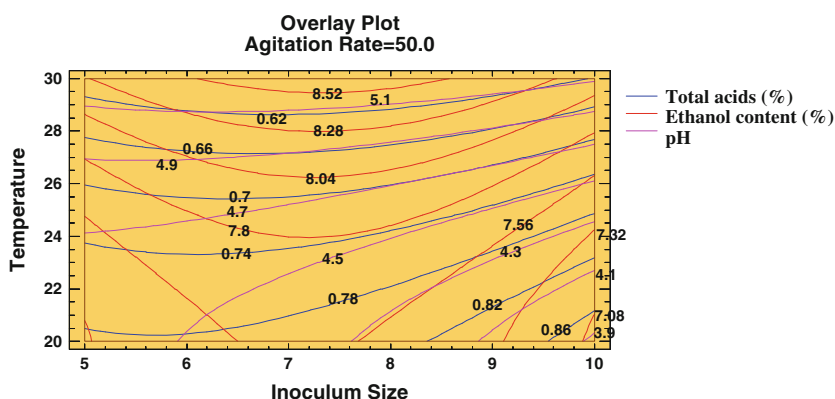
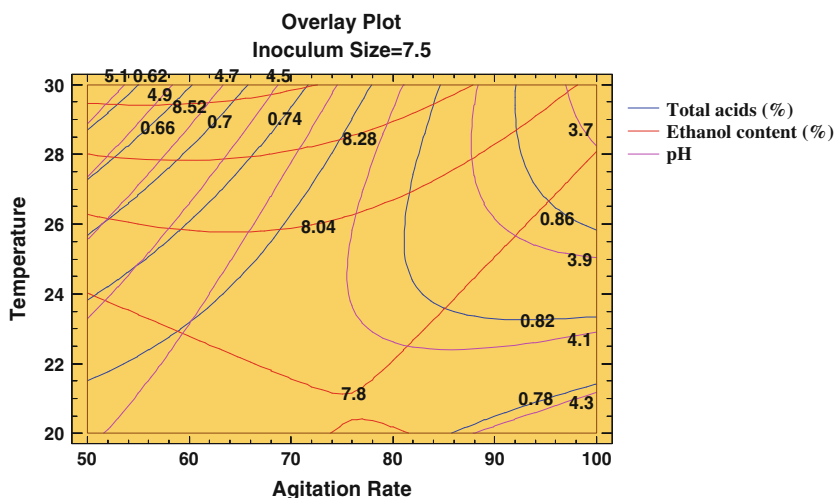


Fig. 12 Overlay plots showing the effect of agitation rate and temperature on total acids (%), ethanol content (%) and pH at constant inoculum size of 7.5 % units: ethanol content = %, total acids = %, agitation rate = rpm, temperature = °C and inoculumsize =%



most desired combinations were selected for maximum ethanol production from desirability surface plots (Figs. 7, 8, 9). To get maximum ethanol production, surface plots were overlaid with the targets mentioned above. Overlay plots describing the optimum conditions are shown in Figs. 10, 11 and 12. Hence, inoculum size of 7.5 %, agitation rate of 50 rpm and fermentation temperature of 30 °C were found to be the best parameters for optimum

responses i.e. total acidity, ethanol content and pH values in sweet sorghum juice inoculated with strain *S. cerevisiae* NRRL Y-2034.

Jin et al. (2011) reported that Hinshelwood model could give an adequate fit to the dynamics of ethanol fermentation with immobilized *S. cerevisiae* growing on sweet sorghum stalk juice of initial reducing sugar concentrations in the range from 85 to 156 g/l. Jovana et al. (2011)

reported optimized bioethanol production from intermediates of sugarbeet processing by response surface methodology and reported that the ethanol production from sugarbeet processing intermediates is technically possible.

Conclusion

From the above studies, it is suggested that sweet sorghum juice can be stored under refrigeration conditions (0 and -20°C) for 75 days without any significant changes in sugar content, acidity and pH.

The results from optimization studies for ethanol production from sweet sorghum juice using *S. cerevisiae* strain NRRL Y-2034 showed maximum ethanol concentration of 8.85 % (v/v) with fermentation efficiency of 87.35 % under optimized conditions of temperature (30°C), agitation rate (50 rpm) and inoculum size (7.5 % (v/v)). Hence, sweet sorghum juice was found to be a good substrate for ethanol production.

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