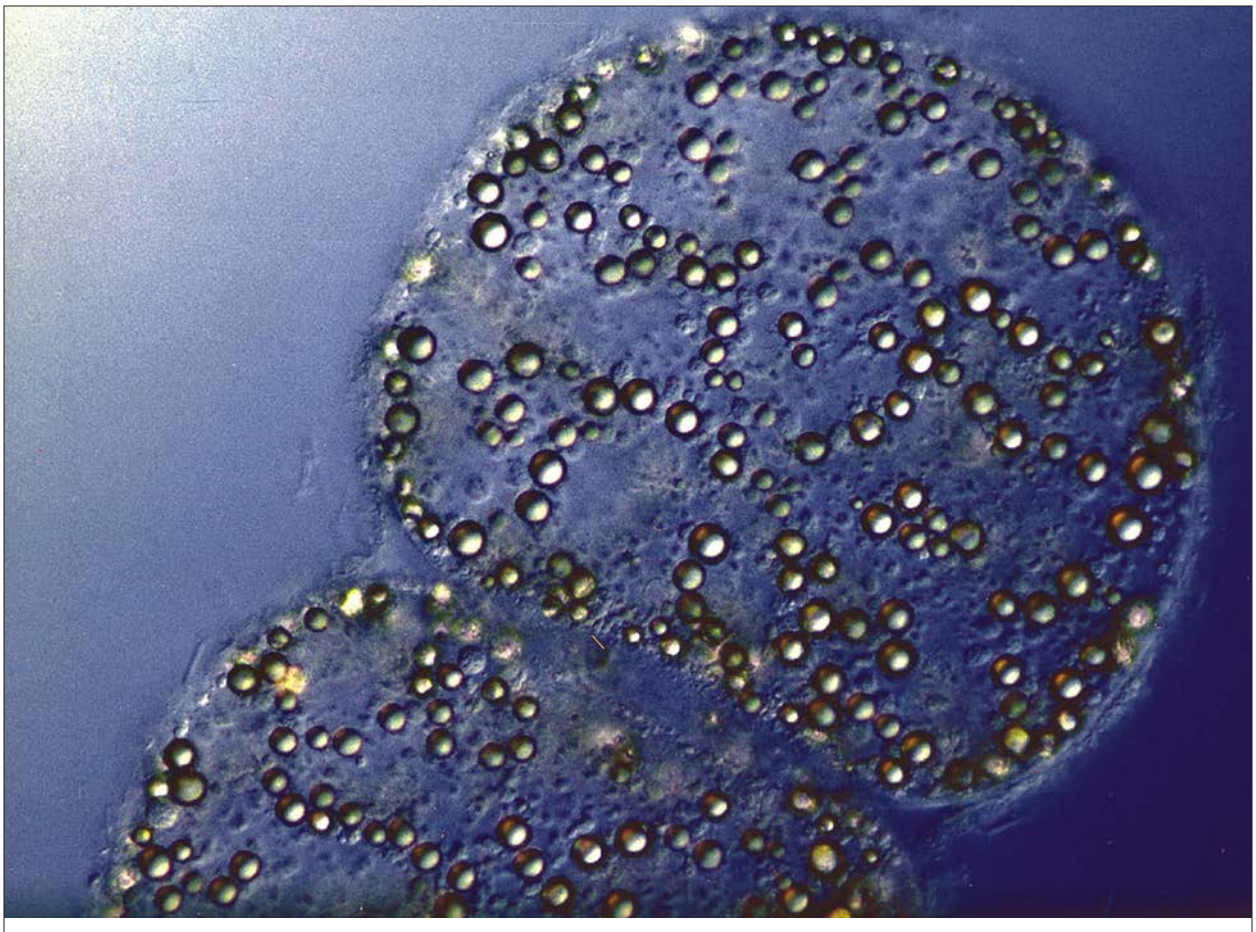

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Production and Optimization of Ethanol from Pretreated Sugarcane Bagasse using *Saccharomyces bayanus* in Simultaneous Saccharification and Fermentation

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ABSTRACT

The optimization of fermentation medium conditions for ethanol yield by *Saccharomyces bayanus* was investigated in simultaneous saccharification and fermentation process using pretreated sugarcane bagasse as carbon source. The FTIR spectrum of pretreated sugarcane bagasse was compared with standard cellulose library. The spectra of 1048 cm^{-1} for C-O stretch in cellulose and hemicellulose was observed in treated bagasse. The culture parameters, such as temperature, agitation, incubation time and yeast inoculum level, were optimized for enhancing ethanol yield by using central composite experimental design. The optimal level of each parameter for maximum ethanol yield by the yeast was determined. Predicted ethanol yield was highest (39.63 g L^{-1}) with the actual yield of (43.26 g L^{-1}) from 100 g L^{-1} treated bagasse at the temperature (37.5°C), agitation (100 rpm), incubation time (64 h) and yeast inoculum level (10%). The model computed for R^2 value was 0.9555% indicating that it was appropriate and could be useful to predict the levels of variables to achieve maximum ethanol yield. Validation of predicted results was done and the experimental values correlated well with that of predicted results.

Key words: Ethanol, *Saccharomyces bayanus*, simultaneous saccharification and fermentation, sugarcane bagasse, central composite design

INTRODUCTION

During the last few decades, the excessive consumption and depletion of fossil fuels has lead to an increased demand for alternative renewable sources of fuels such as ethanol. Currently, ethanol for fuel market is mainly produced from the starch materials; however, will not be enough to meet the demand for fuel ethanol (Hahn-Hagerdal *et al*., 2006).

Lignocellulosic materials are cheap global renewable resources available in large quantities which include a wide variety of materials, such as agricultural residues, fruit and vegetable wastes, woods, municipal solid wastes, wastes from the pulp and paper industry, as well as herbaceous energy crops. The degradation of cellulosic material is gaining increasing research attention due to its worldwide availability and the immense potential for its transformation into sugars, alternative fuels and chemical feed stocks and in particular for the biofuel production (Cassman and Liska, 2007). Overall the steps involved in fuel ethanol production from lignocellulosic biomass consists of feedstock preparation, pretreatment, fractionation, enzymatic hydrolysis (saccharification), fermentation, product recovery and waste treatment (Saha, 2004).

Several researchers have reported pre-treatments (both acid and alkali) using cheaply available agricultural substrates such as rice straw (Sherief *et al.*, 2010), *Carica papaya* fruit waste (Akin-Osanaiye *et al.*, 2008) and empty fruit bunches (Kassim *et al.*, 2011) for bioethanol production. The hydrolysis of cellulose can be affected by the porosity of lignocellulosic biomass, by cellulose crystallinity and by lignin and hemicellulose content (Zhang and Cai, 2008). Pretreatment procedures are essential for removal of the hemicellulose and lignin, for reducing cellulose crystallinity and for increasing the porosity of the materials. Chemical pretreatments that remove lignin are more effective. Peracetic acid-treated biomass can be efficiently fermented to make ethanol (Teixeira *et al.*, 1999). Prehydrolysis using steam explosion and diluted acids constitute the most studied technologies. Both methods use high-temperature and high-pressure reactors and have added costs for fractionation procedures. The use of peracetic acid at relatively high temperatures, about 100°C, has been studied for improving biomass digestibility but little work has been done at ambient temperature (Holtzapple *et al.*, 1992). Peracetic acid has been recognized as a powerful oxidizing agent and is quite selective toward the lignin structure. It oxidizes aromatics in lignin generating dicarboxylic acids and their lactones (Lai and Sarkanen, 1968). In addition, there are no significant carbohydrate losses, furfural is not produced during the process and lignin is oxidized during the ensiling-type pretreatment. Hence, the substrate sugarcane bagasse was pretreated preferentially with peracetic acid prior to fermentation to ethanol.

Response Surface Methodology (RSM) is a statistical technique for the modelling and optimization of multiple variables which determines optimum process conditions by combining experimental designs with interpolation by first or second-order polynomial equations in a sequential testing procedure. This methodology has already been successfully applied for the optimization of enzymatic hydrolysis of several substrates including cellulose (Susana *et al.*, 2009). In the present study, an attempt was made to employ RSM to identify the optimum conditions for ethanol yield from pretreated sugarcane bagasse by analyzing the relationships among a number of parameters that affect the overall process.

Certain fermentation parameters such as inoculum, enzyme and substrate concentration besides optimum pH, temperature, time, agitation speed and others play important roles in obtaining good ethanol yield. In this study, sugarcane bagasse was pretreated by soaking in aqueous peracetic acid under relatively mild conditions. The effectiveness of the pretreatment of bagasse was then determined based on the enzymatic digestibility and the degree of delignification. Simultaneous Saccharification and Fermentation (SSF) was conducted to determine the ethanol yield and productivity of the pretreated sugarcane bagasse.

MATERIALS AND METHODS

Substrate and pretreatment: The sugarcane bagasse was collected from S.V. Sugar Industries, Tirupati, India. It was cut into small pieces and oven-dried at 60°C for 6 h. The dried biomass was ground and sieved to 2 mm mesh size and pretreated with peracetic acid (10% v/v) at 90°C for 90 min. The pretreated substrate was washed thoroughly with distilled water by passing through the nylon filter membrane until the pH comes to nearer to 6.0. The solid and liquid hydrolysates were separated for further analysis.

Yeast strain and preparation of inoculum: The inoculum of the ethanol tolerant and flocculating *Saccharomyces bayanus* kindly provided by Dr. Roberto Ambrosoli, university of Turin, Italy, was prepared by transferring the organism maintained on MGYPA medium (Malt extract 3;

Glucose 10; Yeast extract 5; Peptone 5; Agar 24 g L^{-1}) into 250 mL flask with 100 mL of the basal medium without agar but having 36 g L^{-1} glucose. The yeast was cultured at 33°C on an orbital shaker for 12 h. The inoculum concentration was about 1.5×10^8 yeast cells mL^{-1} and the amount of inoculum level added was according to the response design model (v/v) to the SSF medium.

Simultaneous saccharification and fermentation (SSF): SSF reaction mixtures contained peracetic acid pretreated substrate 10% (w/v), crude cellulase (15 IU) and yeast inoculum and Mary Mandel's mineral salts solution (Jeffries, 1996) to make up the volume to 100 mL. The pH was adjusted to 5.1 with 0.05 M citrate buffer. Reactions were carried out in 250 mL Erlenmeyer conical flasks with 100 mL working volume on an orbital shaker. Samples were aseptically drawn at regular intervals for the analysis of ethanol content.

FTIR spectrum of pretreated sugarcane bagasse: A Perkin Elmer Spectrum 1 FTIR was used. The samples were used in the form of Potassium Bromide (KBR) discs which were prepared by grinding 1 mg sample/100 mg pre-dried KBR. The spectra were recorded in the range of 450-4000 cm K^{-1}.

Ethanol quantification using gas chromatography: Agilent Systems Model 6890 was used and the conditions were as follows: Graphitized packed column 5% carbowax 20 M phase, matrix 80/120 carbopack-B, length 6 ft (1.83 m)×2 mm I.D. x 1/4" O.D. Nitrogen was used as carrier gas with flow rate of 20 mL min^{-1} and eluted compounds were detected by Flame Ionization Detector (FID). Hydrogen was used as fuel gas, with flow rate 40 mL min^{-1}; along with air at a flow rate of 400 mL min^{-1}. Secondary butyl acetate was used as internal standard (Antony, 1984).

Experimental design and optimization: A factorial, Central Composite Design (CCD) for four factors with replicates at the centre point and star points were used in the investigation. The variables used were temperature (X_1), agitation (X_2), fermentation time (X_3) and inoculum level (X_4) each at low (-1) and high (+1) coded levels. The actual levels of the variables for CCD experiments were selected based on the initial levels as the center points. A total of 30 experimental trails that included 16 trails for factorial design, 8 trails for axial points (two for each variable) and 6 trails for replication of the central points were performed. The response value of ethanol yield (Y g L^{-1}) is the average of triplicate.

Statistical analysis: The experimental data were analyzed according to the response surface regression procedure to fit the fallowing second-order polynomial equation in which the level of significance (p-value) of all coefficients was <0.05:

$$Y=A_0+A_1X_1+A_2X_2+A_3X_3+A_4X_4+A_5X_1^2+A_6X_2^2+A_7X_3^2+A_8X_4^2+A_9X_1X_2+A_{10}X_1X_3+A_{11}X_1X_4+A_{12}X_2X_3+A_{13}X_2X_4+A_{14}X_3X_4 \quad (1)$$

In the above equation where, Y is the predicted response, A_0 is the intercept, A_1-A_4 are the linear coefficients, A_5-A_8 are the quadratic coefficients, A_9-A_{14} are the cross product coefficients and Xi are the coded independent variables.

The statistical software package Design-expert® (version 8.0.6.1; stat-ease, Inc., Minneapolis, USA) was used for regression analysis of experimental data and to plot response surface. ANOVA

was used to estimate the statistical parameters. Optimization of the reaction parameters for maximum ethanol yield was obtained through the software package of design expert.

RESULTS

FT-IR analysis of pretreated sugarcane bagasse: Spectral characteristics of pretreated sugarcane bagasse were done by FT-IR spectroscopy. The bagasse sample pretreated with peracetic acid at 90°C for 90 min was used for this study. The Fig. 1 shows the IR spectrum of extracted cellulose using peracetic acid pretreatment method by FTIR. The IR spectrum is similar with standard library of cellulose (Kondo, 1997). The IR spectrum shows the typical adsorption of cellulose backbone at 1600 cm^{-1}. From the hydrogen bonded OH stretching at 4000-2995 cm^{-1}, is due to the H bonded OH groups and the stretching frequency of the -OH group as well as intramolecular and intermolecular hydrogen bonds (Richard, 2002) and the C = C stretch having weak intensity is made at 2359.97 cm^{-1} frequency. The wide band between 3,500 and 3,000 cm^{-1} for all of the samples are due to OH stretching vibrations of alcohols and phenols (Singh *et al.*, 2005). The stretching frequency of 3435.33 cm^{-1} is observed due to -OH bond whose intensity is strong and broad. The absorption peak region at 1629.97 cm^{-1} is not as intense as that seen for C = O. It is variable and may be fairly small in symmetrical, or nearly symmetrical cases and indicates the occurrence of alkene C = C bond which is weak in intensity. Multiple sharp, medium peaks were observed at 1571.74 cm^{-1} with aromatic C = C stretch vibration. The pattern of peaks varies depending upon the substitution pattern. The peak at 1151.87 cm^{-1} confirmed the presence of tertiary alcoholic bond (C-O) which is medium. The absorption peak at 1115.10 cm^{-1} indicates the aromatic C-H in plane deformation and typical for syringyl units that indicate the occurrence of secondary alcohols and shows the C = C stretching. The frequency of 1048.37 cm^{-1} is seen for

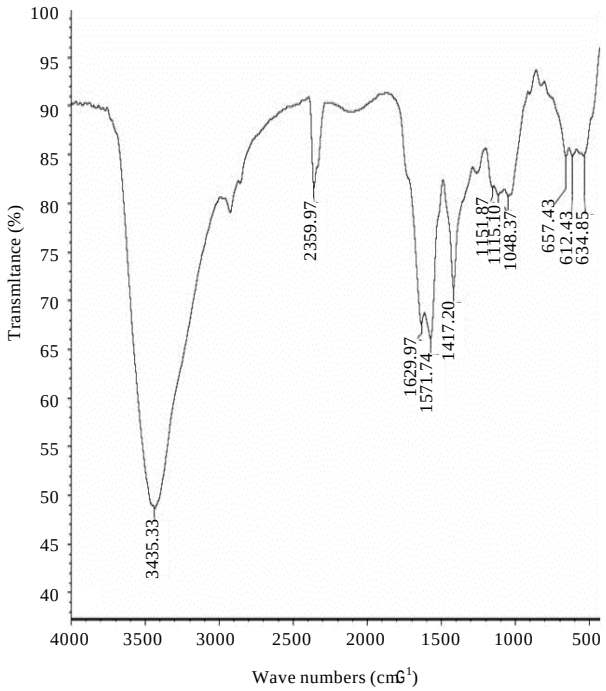

Fig. 1: FTIR spectrum of peracetic acid pretreated sugarcane bagasse

C-O stretch and the bond intensity is strong. The spectra of 1048 cm^{-1} for C-O stretch in cellulose and hemicellulose. The band at 657.43 cm^{-1} is due to C-S vibration of sulfonic group. The band at 655 cm^{-1}, characteristic of lignosulfonate (Nada *et al*., 1998), appears at 650 cm^{-1} for LS8 and at 618-650 cm^{-1} for LS9 and LS10. The presence of sulfur has been confirmed by elemental analysis.

Table 1: Coded and actual values of the factors in central composite design (CCD)

Factor	Name	Units	Low level	Middle level	High level	Low coded level	Middle coded level	High coded level
A	Temperature	°C	30	37.5	45	-1	0	1
B	Agitation	rpm	50	103.3	200	-1	0	1
C	Time	h	36	65.87	92	-1	0	1
D	Inoculum	%	5	10.67	15	-1	0	1

A, B, C and D represents the process parameters denoted as X_1, X_2, X_3 and X_4, respectively, for regression equation

Table 2: Experimental design with coded values of variables and experimental and predicted responses of the Central Composite Design matrix (CCD) model

Std	A: Temperature (°C)	B: Agitation (rpm)	C: Time (h)	D: Inoculum (%)	Actual ethanol yield (g L^{-1})	Predicted ethanol yield (g L^{-1})
1	-1	-1	-1	-1	14.02	13.33
2	1	-1	-1	-1	11.14	9.98
3	-1	1	-1	-1	10.08	10.41
4	1	1	-1	-1	10.68	12.18
5	-1	-1	1	-1	16.11	17.94
6	1	-1	1	-1	13.07	13.07
7	-1	1	1	-1	14.09	15.51
8	1	1	1	-1	12.86	15.76
9	-1	-1	-1	1	20.20	21.20
10	1	-1	-1	1	11.04	11.86
11	-1	1	-1	1	14.48	16.72
12	1	1	-1	1	10.42	12.50
13	-1	-1	1	1	24.38	25.12
14	1	-1	1	1	10.68	14.26
15	-1	1	1	1	16.06	21.13
16	1	1	1	1	12.46	15.39
17	-2	0	0	0	11.03	8.13
18	2	0	0	0	2.32	0.95
19	0	0	0	0	43.26	39.63
20	0	2	0	0	8.35	2.18
21	0	0	0	2	32.78	32.86
22	0	0	2	0	35.75	29.58
23	0	0	0	2	39.32	32.86
24	0	0	0	0	39.10	39.43
25[a]	0	0	0	0	39.12	39.63
26[a]	0	0	0	0	39.12	39.63
27[a]	0	0	0	0	39.12	39.63
28[a]	0	0	0	0	39.12	39.63
29[a]	0	0	0	0	39.12	39.63
30[a]	0	0	0	0	39.12	39.63

Std = Standard run order, [a] Central value

Central composite design (CCD) experiments: The experimental design for the four variables, i.e., temperature (30-45°C), agitation (50-200 rpm), fermentation time (36-92 h) and yeast inoculum level (5-15% v/v) were studied for measuring the ethanol yield. The design was applied for selection range of each variable (maximum and minimum) as shown in Table 1. Total 30 experiments were deigned by the model and performed (Table 2).

The experimental results associated with the processing set up of each independent variable are listed in Table 1. Generally the parameters that predominantly affect the ethanol yield in a fermentation process are temperature, agitation, fermentation period and inoculum level of yeast culture. To study the combined effects of these factors, experiments were conducted at different combinations of these parameters using statistically designed experiments. For response surface methodology based on the CCD, 30 experimental runs with different combinations of four factors were carried out. For each run, the experimental responses along with the predicted responses calculated from the regression equation (Eq. 2) are presented in Table 2. Six experiments (run No. 25-30) were replicated at the center point to verify any change in the estimation procedure as a measure of the precision property. The maximum response was obtained in run number 19.

The coefficients of the response surface model as given in Eq. 1 were evaluated. A second-order polynomial equation (Eq. 2) was derived to represent the ethanol yield by SSF as a function of the independent variables tested:

$$Y = 39.63 - 2.27(X_1) - 0.44(X_2) + 1.87(X_3) + 1.86(X_4) - 9.01(X_1^2) - 9.14(X_2^2) - 3.45(X_3^2) - 2.63(X_4^2) + 1.28(X_1X_2) - 0.37(X_1X_3) - 1.49(X_1X_4) + 0.12(X_2X_3) - 0.39(X_2X_4) - 0.17(X_3X_4) \quad (2)$$

where, Y is the predicted response (ethanol yield g L^{-1}) and X_1, X_2, X_3 and X_4 are coded values of incubation temperature, agitation, incubation time and inoculum level of yeast culture, respectively. The regression equation was used to calculate the predicted responses given in Table 2. A comparison of the predicted values with the experimentally obtained actual values indicated that these data are in reasonable agreement (Fig. 2).

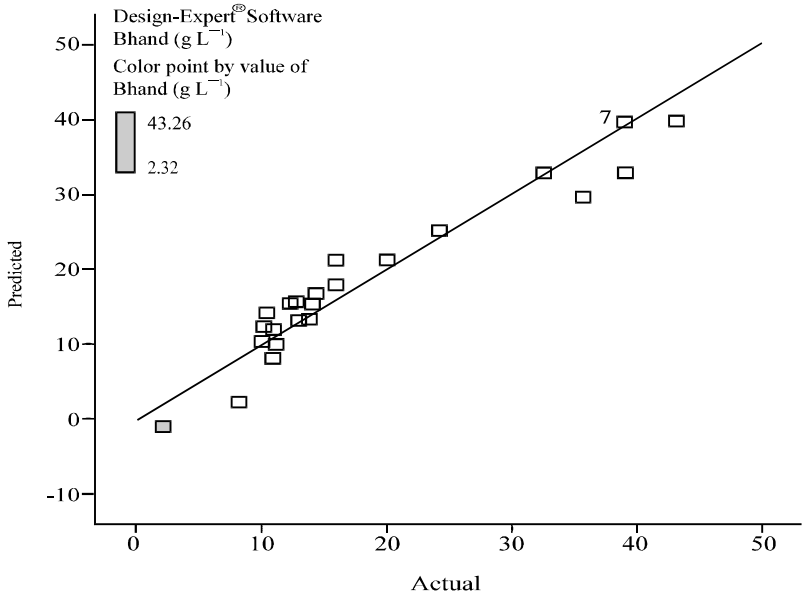

Fig. 2: Parity plot showing the distribution of actual experimental and predicted values of ethanol yield

Table 3: Analysis of variance (ANOVA) for quadratic model for ethanol yield (g L^{-1})

Source	Sum of squares	df	Mean square	F-value	p-value Prob>F
Model	4853.99	14	346.71	22.99	<0.0001*
A-Temperature	123.72	1	123.72	8.20	0.0118
B-Agitation	3.39	1	3.39	0.22	0.6422
C-Time	59.33	1	59.33	3.93	0.0659
D-Inoculum	59.41	1	59.41	3.94	0.0658
AB	26.24	1	26.24	1.74	0.2070
AC	2.30	1	2.30	0.15	0.7015
AD	35.91	1	35.91	2.38	0.1437
BC	0.24	1	0.24	0.016	0.9008
BD	2.44	1	2.44	0.16	0.6931
CD	0.48	1	0.48	0.032	0.8609
A^2	2270.24	1	2270.24	150.52	<0.0001*
B^2	1277.85	1	1277.85	84.72	<0.0001*
C^2	182.21	1	182.21	12.08	0.0034
D^2	146.38	1	146.38	9.70	0.0071
Residual	226.25	15	15.08		
Lack of Fit	191.15	7	27.31	6.22	0.0098
Pure Error	35.09	8	4.39		
Cor Total	5080.23	29			

Ethanol yield, g L^{-1}: R-squared = 0.9555, Adj R-squared = 0.9139, Pred. R-squared = 0.5026, C.V% = 17.44

Statistical significance of the respective model equations was checked using F test Analysis of Variance (ANOVA) using Design Expert software and the results are shown in Table 3. ANOVA of the quadratic regression model suggests that the model is significant with a computed F value of 22.99 and a p>F value lower than 0.001. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise. The Coefficient of Variation (CV) indicated the degree of precision with which the treatments were compared. A lower value for the coefficient of variation suggests higher reliability of the experiment, and in this case, the obtained CV value of 17.44% demonstrates a greater reliability of the trials.

The precision of a model could be checked by the determination coefficient (R^2) and correlation coefficient (R). In the present study, coefficient of determination (R^2) of the model was 0.9555, which further indicates that the model is suitable to adequately represent the real relationships among the selected reaction variables and indicating that 95.55% of the sample variation is attributed to the factors and only 4.55% can occur due to chance. Here, the value of R (0.9139) for (Eq. 2) indicated a close agreement between the experimental results and the theoretical values predicted by the model equation. The value of determination coefficient indicates that ~5% of the total variations in the ethanol yield were not satisfactorily explained by the model (Eq. 2). This unexplained value of response (~5%) was presented in terms of residual error in the ANOVA table (Table 3). Besides the relationship between the actual experimental values and predicted values (Fig. 2) showed that plotted points cluster around the diagonal line, indicating good fitness of the model.

The p-values are used as tool to check the significance of each coefficient which also indicates the interaction strength between each independent variable. The smaller the p-values, the more evidence that indicate support for rejecting the null hypothesis (Cui *et al.*, 2006). Table 3 also gives the p-values of each of the parameters and their quadratic and interaction terms. The significance

of individual variables can be evaluated from their p-values, the more significant terms having a lower p-value. Values of "Prob>F" less than 0.0500 indicate model terms are significant. In this case A, A^2, B^2, C^2, D^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. There were no significant interactions between the parameters. The lack of fit is presented as significant ($p<0.05$), but the model remained significant (p-value with 0.0098). The "Lack of Fit F-value" of 6.22 implies the Lack of Fit is significant. There is only a 0.98% chance that a "Lack of Fit F-value" this large could occur due to noise (Design-Expert,® Software, 2011).

Response suraface plots: Response surface curves were plotted to understand the interaction effects of variables and for identifying the optimal levels of each parameter for attaining ethanol yield. The response surfaces can be used to predict the optimum range of different variables and the major interactions between the tested variables can be identified from the circular or elliptical nature of the contours. The Fig. 3a-d represents the response surfaces obtained for the interaction effects of tested variables. The data presented in the response plots indicated that the ethanol yield increased with the interaction of temperature, agitation, incubation time and yeast inoculum level.

Regardless of temperature, the maximum ethanol yield was obtained at or near the middle level of incubation time (64 h) and variations in incubation time did not affect the temperature optima between 36 and 39°C, confirming the lack of interaction between these parameters (Fig. 3a). The actual ethanol yield was 43.26 g L^{-1}, with the predicted ethanol yield of about 39.63 g L^{-1} at 37.5°C with an incubation time of about 64 h.

The effect of temperature and inoculum level along with the variables such as agitation and incubation time on ethanol yield was presented in Fig. 3b. Increase in ethanol yield was observed at agitation speed of about 100 rpm with the incubation time of about 64 h. The ethanol yield was high with agitation and incubation time of 100 rpm and 64 h, respectively.

The interaction effects plotted for agitation speed and incubation time showed that there are no significant interactions between these variables that affect ethanol yield (Fig. 3c). However, this confirmed that optimal time range lied between 60 and 64 h and the optimal agitation speed was between 80 and 100 rpm.

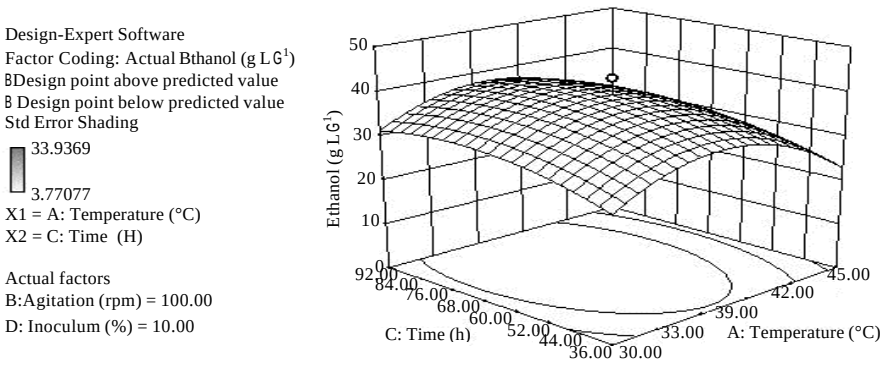

Fig. 3a: Response surface plot of ethanol yield as a function of temperature and incubation time at 100 rpm agitation and 10% inoculum

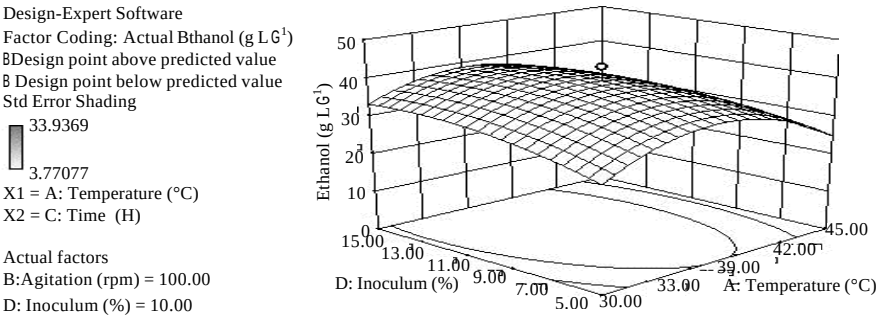

Fig. 3b: Response surface plot of ethanol yield as a function of temperature and inoculum level at 100 rpm agitation and 64 h incubation time

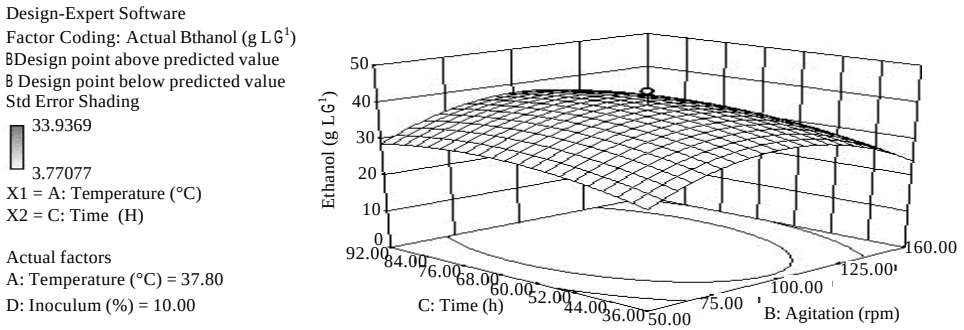

Fig. 3c: Response surface plot of ethanol yield as a function of agitation and incubation time at 10% inoculum level and 37.5°C

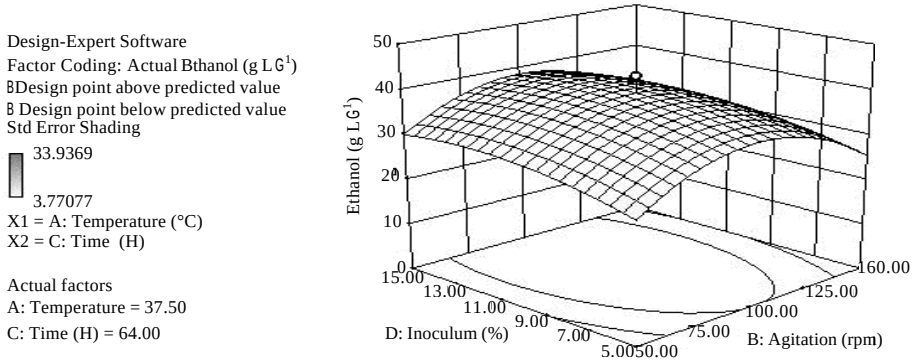

Fig. 3d: Response surface plot of ethanol yield as a function of agitation and inoculum level at 64 h incubation time and 37.5°C

The Fig. 3d depicts interactive effect of agitation and inoculum level on ethanol yield. Increase in inoculum level up to 10% and agitation up to 100 rpm improved ethanol yield after 64 h with temperature optima at 37.5°C.

Model validation: To confirm the validity of the statistical experimental strategies a conformation experiment with a duplicate set was performed at the specified condition (Temperature: 37.5°C, agitation: 100 rpm, incubation time 64 h and inoculum level of 10%) demonstrated that the ethanol yield (38.96 g L^{-1}) was closer to the predicted value (39.46 g L^{-1}). This result indicated that there is good correlation between the experimental and predicted values of ethanol yield indicating a good fit of model.

DISCUSSION

Lignocellulosic biomass cannot be saccharified by enzymes to high yield without a pretreatment mainly because the lignin in plant cell walls forms a barrier against enzymatic attack. An ideal pretreatment reduces the lignin content and crystallinity of the cellulose and increases the surface area. Teixeira *et al.* (1999) have investigated the use of peracetic acid at ambient temperatures as a pretreatment method for hybrid poplar and sugarcane bagasse. Peracetic acid is very lignin selective and no significant carbohydrate losses occurred. According to their studies, the enzymatic hydrolysis of the cellulose increased from 6.8% (untreated) to a maximum of about 98% (pretreated) at a 21% peracetic acid pretreatment. Hence, in the present study, lignocellulosic biomass such as sugarcane bagasse was pretreated with peracetic acid.

It was envisaged that increase in the ethanol yield by *S. bayanus* using pretreated sugarcane bagasse substrate by the optimization of process parameters. Based on the results obtained from the SSF experiments on sugarcane bagasse, most significant influence was exhibited by parameters such as incubation temperature and agitation on ethanol yield. However, the other parameters were found to have little significance. These results were comparable with those obtained by Wilkins *et al.* (2007) who reported that ethanol production from simultaneous saccharification and fermentation of citrus peel waste by *S. cerevisiae* was the greatest when the fermentation temperature and pH were adjusted to 37°C and 6.0, respectively. Uma *et al.* (2010) have investigated enzymatic hydrolysis of sugarcane bagasse and ethanol fermentation using three different methods.

The *S. cerevisiae* is reported to grow well within the temperature range of 28-40°C. The increase in temperature accelerates the inhibition effect of the residual sugar and ethanol concentration on cell activities, thereby lowering both cell density and ethanol yields (Phisalaphong *et al.*, 2005). The deleterious effect of higher temperatures on ethanol yield can be attributed to the denaturation of ribosomes and enzymes and problems associated with the fluidity of membranes (McMeckin *et al.*, 2002). At incubation temperatures lower or higher than optimum, less ethanol production is observed in the present study.

In the present study, the agitation is the second most influencing parameter on ethanol yield. The agitation speed of 100 rpm was found to be optimal for ethanol yield. Low and high agitation speed have little influence on ethanol yield. According to the studies of Arisra *et al.* (2008) the agitation rate of 50 rpm was suitable for ethanol production by mixed culture of *S. cerevisiae* and *Candida tropicalis* in batch ethanol fermentation. Agitation could be beneficial to the growth and performance of the microorganism cells by improving the mass transfer characteristics with respect to substrates, products and oxygen. Thus, agitation results in a better mixing of the fermentation broth, helping to maintain a concentration gradient between the interior and the exterior of the cells. Such a concentration gradient works in both directions; through better diffusion it helps to maintain a satisfactory supply of sugars and other nutrients to the cells, while it facilitates the removal of gases and other byproducts of catabolism from the microenvironment of the cells.

Khongsay *et al.* (2010) have reported that *Saccharomyces cerevisiae* gave better ethanol yield at temperature of 37°C with an agitation of 100 rpm on sweet sorghum stem juice fermentation.

Apart from the optimal conditions of temperature and agitation, the ideal incubation time and yeast inoculum level were 64 h and 10%, respectively, in order to obtain maximal ethanol yield. The optimized yield obtained on pretreated sugarcane bagasse substrate is reasonably good, however, further improvements are needed in the pretreatment conditions in order to attain maximum sugar production from bagasse as well as optimization of other fermentation parameters for higher ethanol yield.

CONCLUSION

Optimization of cultural conditions for fermentation is a significant concern in developing a suitable bioprocess for ethanol yield. The present study using RSM based on CCD established an efficient second order polynominal model to describe the ethanol yield by peracetic acid treated sugarcane bagasse fermentation. The high similarity between the experimental value and the predicted ones ($R^2 = 0.9555$) suggested that the model was good fit. The actual experimental ethanol yield (43.26 g L^{-1}) correlated well with the predicted one (39.63 g L^{-1}). This indicated the reliability of the model employed and the success of RSM in optimizing the fermentation conditions for higher ethanol yield from bagasse by SSF process. The results are encouraging as a cheap biomass resource like sugarcane bagasse could be used for ethanol production by SSF.

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