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Simultaneous Saccharification and Fermentation (SSF) of Sugarcane Bagasse - Kinetics and Modeling

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Abstract**—**Simultaneous Saccharification and Fermentation (SSF) of sugarcane bagasse by cellulase and *Pachysolen tannophilus* MTCC *1077 were investigated in the present study. Important process variables for ethanol production form pretreated bagasse were optimized using Response Surface Methodology (RSM) based on central composite design (CCD) experiments. A 2^3 five level CCD experiments with central and axial points was used to develop a statistical model for the optimization of process variables such as incubation temperature (25–45°) X_1, pH (5.0–7.0) X_2 and fermentation time (24–120 h) X_3. Data obtained from RSM on ethanol production were subjected to the analysis of variance (ANOVA) and analyzed using a second order polynomial equation and contour plots were used to study the interactions among three relevant variables of the fermentation process. The fermentation experiments were carried out using an online monitored modular fermenter 2L capacity. The processing parameters setup for reaching a maximum response for ethanol production was obtained when applying the optimum values for temperature (32°C), pH (5.6) and fermentation time (110 h). Maximum ethanol concentration (3.36 g/l) was obtained from 50 g/l pretreated sugarcane bagasse at the optimized process conditions in aerobic batch fermentation. Kinetic models such as Monod, Modified Logistic model, Modified Logistic incorporated Leudeking – Piret model and Modified Logistic incorporated Modified Leudeking – Piret model have been evaluated and the constants were predicted.

Keywords**—**Sugarcane bagasse, ethanol, optimization, *Pachysolen tannophilus*.

I. INTRODUCTION

ETHANOL has been known for a long time, being perhaps the oldest product obtained through traditional biotechnology. Its current applications include potable, chemical, and fuel ethanol [1-2]. Ethanol can be made from a number of renewable feedstocks, including sugar crops such as sugarcane, starch containing grains such as corn, or lignocellulosic materials including agricultural residues, herbaceous crops, and wood [3]. Lignocellulosic materials constitute an abundant and cheap feedstock, but the processing techniques required for ethanol production are presently costly and extensive. The cost of ethanol produced from lignocellulosic materials with currently available technology and under the present economic conditions is not competitive with the cost of gasoline. Comprehensive process development and optimization are still required to make the process economically viable [4]. One of the major lignocellulosic materials to be considered in tropical countries is sugarcane bagasse, the fibrous residue obtained after extracting the juice from sugar cane (*Saccharum officinarum*) in the sugar production process [5]. Sugarcane bagasse is accumulated in large quantities at cane-to-sugar processing plants and consists approximately of 50% cellulose, 25% hemicellulose, and 25% lignin [6]. Lignin forms a protective shield around cellulose and hemicellulose, protecting the polysaccharides from enzymatic degradation. To convert the biomass into ethanol, the cellulose must be readily available for cellulase enzymes. Thus, by removing the lignin, the cellulose becomes vulnerable to enzymes and allows the yeast to convert the glucose into ethanol during fermentation. Therefore, a pretreatment must be applied to degrade the lignin in the sugarcane residue, decrease cellulose crystallinity, and increase the surface area for enzymatic activity [7]. Enzymatic hydrolysis is a promising way for obtaining sugars from lignocellulosic materials (because it has the advantages of reduced sugar loss through side-reactions, is milder and more specific), but the low enzymatic accessibility of the native cellulose is a key problem for biomass-to-ethanol processes [8-9].

The bagasse produced is traditionally utilized for in-house energy production. The cellulose conversion option that many currently favor is the Simultaneous Saccharification and Fermentation (SSF) process [3]. In this option, the cellulose hydrolysis and glucose fermentation steps are combined in a single vessel [10]. Since cellulase is inhibited by glucose as it is formed, rapid conversion of the glucose into ethanol by yeast results in faster rates, higher yields, and greater ethanol concentrations than possible for SHF. Furthermore, by combining the hydrolysis and fermentation steps in one vessel, the number of fermenters required is approximately one-half that for the SFR process. The presence of ethanol in the fermentation broth also makes the mixture less vulnerable to invasion by unwanted microorganisms [11]. In practice, yeast has shown higher yields and ethanol tolerance than bacteria. A great number of investigators have studied yeast xylose fermentations, notably with the organisms *Pachysolen*

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tannophilus, *Candida shehatae*, and *Pichia stipitis* [3]. The classical method of studying one variable at a time can be effective in some cases but it is useful to consider the combined effects of all the factors involved. The Response Surface Methodology (RSM), based on statistical principles, can be employed as an interesting strategy to implement process conditions that drive to optimal ethanol production from pretreated sugarcane bagasse by performing a minimum number of experiments. Thus, RSM experimental design is an efficient approach to deal with a large number of variables and there are several reports on application of RSM for the production of primary and secondary metabolites through microbial fermentation [12-13].

In the present study, the potential use of sugarcane bagasse for ethanol fermentation using cellulase and yeast *Pachysolen tannophilus* MTCC *1077 was investigated. The influence of process variables such as incubation temperature, initial pH and fermentation time on ethanol production from pretreated sugarcane bagasse was studied using CCD experiments. Knowledge based approaches such as Artificial Neural Network (ANN) has been successfully applied for the purpose of simulation on the same experimental data used for RSM. Various kinetic models such as Modified Logistic model (growth kinetics), Modified Logistic incorporated Leudeking – Piret model (product formation kinetics) and Modified Logistic incorporated Modified Leudeking – Piret model (substrate utilization kinetics) have been evaluated for the better prediction of experimental data.

II. MATERIAL S AND METHODS

A. Materials

Sugarcane bagasse sample was obtained from M.R.K. Sugar Mills Ltd. Sethiyathope, Tamilnadu, India. The bagasse sample was made into 100 mesh (0.15mm) fine powder by use of laboratory blender at 3000 rpm. Sample was preserved in a sealed plastic bag at 4°C to prevent any possible degradation or spoilage. Pure cellulose powder was used in reference of cellulose estimation and fermentation tests. The control and pretreated bagasse samples were analyzed for cellulose content using Anthrone reagent at 630 nm in UV/Visible spectrophotometer ELICO BL 198 [14]. The estimated cellulose content of steam pretreated sample was 420 mg/g bagasse.

B. Microorganisms and Culture Conditions

Commercially available cellulase enzyme (ONOZUKA R–10) was obtained from HIMEDIA Laboratories, Mumbai. The activity of enzyme was found to be 15 FPU/ml and it was used throughout the experimentation. The cellulase activity was measured by standard Mandel's method [15]. Yeast strain *Pachysolen tannophilus* MTCC *1077was obtained from Microbial Culture Collection and Gene Bank (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, INDIA. Culture was maintained on yeast extract agar medium. After three days incubation at 25°C the agar slants were stored at 4°C.The liquid medium for the growth of inoculum for yeast was yeast extract – glucose nutrient medium composed of 3g/l of yeast extract, 1g/l of sodium chloride, 10g/l of glucose, 2g/l of potassium dihydrogen phosphate, 0.2g/l of calcium chloride, 1.7g/l of magnesium sulphate.

Inocula were grown aerobically in 250 ml Erlenmeyer flasks containing the above mentioned medium at 25°C in an Environmental Shaker (Remi Scientific) at 200 rpm for 24 h. Active cells were centrifuged in a clinical centrifuge (1200 rpm), washed with sterile water, and were used as inoculum. Fermentations for ethanol production were conducted aerobically in an online monitored modular fermenter 2L capacity with a working volume of 1000ml medium. Samples were withdrawn periodically (12h interval) for the analysis of cellmass, ethanol and residual sugar concentrations.

C. Pretreatment

Various pretreatment techniques such as steam autoclaving in an autoclave at 15 psi (121°C) for 60 minutes, dilute sulphuric acid (1% v/v) with steam autoclaving for about 20 minutes, concentrated sulphuric acid (50% v/v) with steam autoclaving for about 20 minutes, sodium hydroxide (1 % and 10% w/v) with steam autoclaving for about 20 minutes, sodium chlorite (1.5 g/g bagasse) with steam autoclaving for about 240 minutes was adopted for the pretreatment of bagasse samples. The treated samples were collected and filtered in crucibles followed by washed with distilled water under suction. Finally it was dried at room temperature before fermentation [16-18].

D. Fermentation

Batch experiments were conducted as per the central composite experimental design for ethanol production in a fermenter (APPLIKON Biotech ADI 1025, Holland), with 2 L capacity, equipped with flat blade impeller, oxygen and pH electrodes, temperature and dO_2 (dissolved oxygen) probe. The equipment also monitored temperature, agitation speed, gas purging flow rate, pumping rates, antifoam addition, dO_2 and the vessel level. All processing parameters were online monitored, with the aid of BioXpert Lite 1.00 software. The agitation speed (400±1 rpm) and dissolved oxygen, dO_2 (8±0.1 ppm) were kept constant during the experiments. Other parameters, like temperature, pH and fermentation time, were chosen as the most significant ones, considering the experimental design. After selecting those parameters, experiments were done in duplicate, for superior (+) and lower (-) levels of the experimental design, and in triplicate, for the central point (0). The process was conducted at the initial substrate concentration of 50g/l (pretreated sugarcane bagasse) with the addition of nutrient medium (without glucose) and 0.05 M Sodium phosphate buffer (pH 5.7) followed by sterilization for 15 min, at 15 psi (121°C). Cellulase dosage of 15 FPU/g bagasse was used for hydrolysis. For each experiment, 10ml of the inoculum was used, that is,10%(v/v) of the initial working volume (1L). Samples were withdrawn periodically (12h interval), centrifuged in a laboratory centrifuge at 1200 rpm, and the supernatants were analyzed for total sugars and ethanol conc.

E. Cell growth and Chemical Analysis

The sugarcane bagasse sample was analyzed for hemicellulose and Klason lignin content following the

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procedures described in NREL Standard Procedure (No.002). Cellmass was determined by direct optical density at 660 nm using SYSTRONICS colorimeter (420 – 820 nm). Total reducing sugar was measured by the Dinitrosalicylic acid (DNS) method using a UV/Visible spectrophotometer ELICO BL 198 at 510 nm [19]. Ethanol was estimated using NUCON 5765 Gas Chromatography (GC) with a Flame Ionization Detector (FID) and CHROMATOPAK (10% Carbowax 20M) column (3m length and 1/8 mm dia) using N_2 as the carrier gas at the rate of 20 µl per minute. The oven temperature was held at 80°C. The injector and detector temperature was maintained at 200°C. Ethanol concentration of the sample was obtained directly by using WINACDS software version 6.2.

F. Experimental design and Statistical Analysis

In the Central Composite Design (CCD), the total number of experimental combinations was $2^K + 2K + n_0$, where K is the number of independent variables and n_0 is the number of repetitions of the experiments at the central point, which indicated that 20 experiments were required for this procedure. The CCD contains a total of 20 experiments with five level full factorial design and replications of the central points and axial points (Table 2). The dependent variable selected for this study was ethanol concentration, Y (g/l). The independent variables chosen were incubation temperature (25 – 45°) X_1, pH (5.0 – 7.0) X_2 and fermentation time (24 – 120 h) X_3. A mathematical model, describing the relationships among the process dependent variable and the independent variables in a second-order equation, was developed [20]. Design-based experimental data were matched according to the following second-order polynomial equation (1).

$$Y = b_0 + \sum_{i=1}^{k} b_i x_i + \sum_{i=1}^{k} b_{ij} x_i^2 + \sum_{i_i<j}^{k} \sum_{j}^{k} b_{ij} x_i x_j + e \quad (1)$$

Where, *i*, *j* are linear, quadratic coefficients, respectively, while ‘b’ is regression coefficient, *k* the number of factors studied and optimized in the experiment and ‘e’ is random error.

The quality of fit of the second order equation was expressed by the coefficient of determination R^2, and its statistical significance was determined by *F*-test. The significance of each coefficient was determined using Student’s *t*-test. The student *t*-test was used to determine the significance of the parameters regression coefficients. The P-values (Probability value) were used as a tool to check the significance of the interaction effects, which in turn may indicate the patterns of the interactions among the variables. In general, larger magnitudes of *t* and smaller of P, indicates that the corresponding coefficient term is significant. The coefficients of the equation were determined by employing MINITAB software version 15. Analysis of variance (ANOVA) for the final predictive equation was done using the same software package. The response surface equation was optimized for maximum yield in the range of process variables using MATLAB software version 7.0.1. Isoresponse contour plots were obtained based on the effect of the levels of three parameters (at five different levels each) and their interactions on the yield of ethanol by keeping the other parameters at their optimal concentrations. From these contour plots, the interaction of one parameter with another parameter was studied. The optimum concentration of each parameter was identified based on the hump in the contour plots.

G. Artificial Neural Network (ANN) and Modeling

A number of design parameters affect performance and these parameters include the choice of activation function and training algorithm, training parameters such as learning rate and momentum, number of hidden layers, number of neurons in each hidden layer, initial weights, and training duration. In general, feed-forward neural networks with one hidden layer containing a sufficiently large number of hidden neurons have been shown to be capable of providing accurate approximations to any continuous nonlinear function [21-22]. The choice of design parameters for a neural network is thus often the result of empirical rules combined with trial and error as detailed. The configuration of the two neural networks developed in this work were 3-5-1 structure: three input neurons are incubation temperature (°C), initial pH and fermentation time (h)-five neurons in one hidden layer-one output neuron and are determined after brief experimentation. To avoid the problem of overtraining, the data set comprising 20 experimental runs is split into two categories: a training set comprising 17 experimental runs is used to optimize the weights of the two neural networks and a validation set comprising 3 experimental runs is used to evaluate their predictive capability (Table 2). Because empirical models like neural networks do not extrapolate data well, data for network training should be selected carefully if the best results are to be achieved. In this study the data selected for network training covered the lower and upper bounds of the one output neurons (y_1).

III. RESULTS AND DISCUSSION

A. Evaluation of pretreatment techniques

It has long been recognized that some form of pretreatment is necessary to achieve reasonable rates and yields in the enzymatic hydrolysis of biomass. This has generally been attributed to the crystallinity of cellulose, the lignin-hemicellulose sheath that surrounds the cellulose, and the lack of available surface area for enzymes to attack. A number of different pretreatment methods, such as dilute sulphuric acid (1% v/v), concentrated sulphuric acid (50% v/v), sodium hydroxide (1% and 10% w/v), sodium chlorite (1.5 g/g bagasse) and steam autoclaving have been extensively investigated. Figure 1 shows the comparison of the percentage reduction in bagasse composition after 20 minute treatment. All of the components and total weight decreased gradually with increasing pretreatment time. The amount of weight lost following chemical pretreatment of residue was due to lignin removal. Greater weight loss equals more lignin loss and the percent weight lost was used to compare pretreatment effects on lignin removal. It was observed that there is a drastic reduction in percentage cellulose, hemicellulose and lignin content of 45.98% (w/w), 48.15% and 44.22% respectively obtained after 15 minute treatment by concentrated sulphuric acid treatment. And there is a 59.12% increase in total sugars

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is observed when treated with concentrated acid after 20 minute which shows that the polymers are converted into monomer and dimers of sugars. There is a significant reduction in percentage cellulose, hemicellulose and lignin content of 9.26%, 18.19% and 20.75% respectively obtained after 20 minute treatment by 10% (w/v) sodium hydroxide treatment.

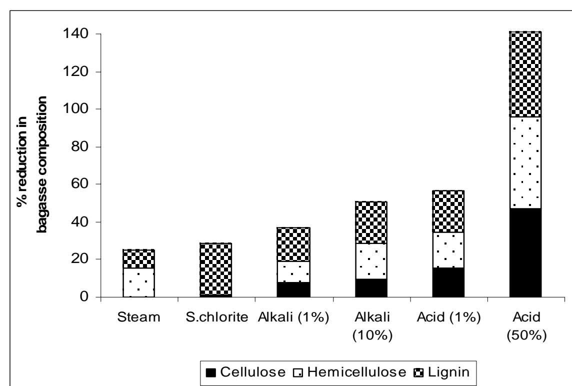

Fig.1 Comparative chart of the percentage reduction in bagasse composition after various pretreatments

There is an 8.97% increase in total sugars is observed when treated with concentrated acid at 20 minute which shows that the more cellulose polymers and hemicellulose are converted into monomer and dimers of sugars. There is a considerable reduction in percentage lignin content of 27.19% obtained after 240 minute treatment by sodium chlorite leaving native cellulose and hemicellulose unaltered. And there is an 11.82% increase in total sugars is observed when treated with sodium chlorite for 240 minutes which shows that the only a less amount of hemicellulose are converted into monomer sugars. There is a considerable reduction in percentage hemicellulose and lignin content of 14.79% and 8.93% respectively obtained after 20 minute treatment leaving native cellulose unaltered. And there is an 3.76% increase in total sugars is observed when treated with steam for 20 minute which shows that only hemicellulose are hydrolyzed to simple sugars. It is seen from the treatment results the cellulose content is not changed even when the steam treatment time increased upto 20 minutes and also the hemicellulose and lignin content is significantly reduced by about 16.58 and 10.23% (in comparison with untreated bagasse) respectively. Generally steaming of biomass in the 120–200°C temperature range leads to increased enzymatic digestibility, as a result of increasing pore size and the partial hydrolysis of hemicelluloses.

B. *Optimization of process variables in ethanol fermentation*

The experimental results associated to the processing set up of each independent variable are listed in Table 1. Five level central composite design matrix and the experimental responses of the dependent variable (ethanol conc.) are listed in Table 2. The regression equation coefficients were calculated and the data is fitted to a second-order polynomial equation. The response, Y (ethanol concentration) by *P. tannophilus*, can be expressed in terms of the following regression equation (2):

$$Y = 3.4584 - (0.2212x_1) - (0.2657x_2) + (0.4144x_3) - (0.5128x_1^2) - (0.3784x_2^2) - (0.4208x_3^2) + (0.1838x_1x_2) + (0.0983x_1x_3) + (0.0485x_2x_3) \quad (2)$$

Besides the linear effect of the ethanol concentration, Y g/l, the response surface method also gives an insight about the parameters quadratic and combined effects. The analyses were done by using both Fisher's *F*- test and Student *t*-test statistical tools. The regression coefficient, *t* and P values for all the linear, quadratic and combined effects with a 95% significance level are given in the Table 3. It shows that the regression coefficients of the all linear and quadratic coefficients of *X1, X2* and *X3* were significant at < 1% level (p

TABLE I
CODES AND ACTUAL LEVELS OF THE INDEPENDENT VARIABLES FOR DESIGN OF EXPERIMENT

Independent variables	Symbols	Coded levels				
		– 1.682	– 1	0	+1	+1.682
Temp (°C)	X_1	25	30	35	40	45
pH	X_2	5	5.5	6.0	6.5	7.0
Fermentation time (h)	X_3	24	48	72	96	120

TABLE II
FIVE LEVEL CCD AND THE EXPERIMENTAL RESPONSES OF DEPENDENT VARIABLE, Y

Run No.	Coded levels			Real variables			Ethanol conc. (g/l)		
	x_1	x_2	x_3	X_1	X_2	X_3	Exp	Pred RSM	Pred ANN
1	-1.000	1.000	-1.000	30.0	6.5	48.0	1.54	1.55	1.58
2	1.682	0.000	0.000	43.4	6.0	72.0	1.62	1.63	1.60
3	0.000	0.000	0.000	35.0	6.0	72.0	3.46	3.45	3.48
4	0.000	0.000	0.000	35.0	6.0	72.0	3.46	3.45	3.48
5	0.000	0.000	0.000	35.0	6.0	72.0	3.46	3.45	3.48
6	1.000	1.000	1.000	40.0	6.5	96.0	2.32	2.40	2.36
7	0.000	0.000	0.000	35.0	6.0	72.0	3.46	3.45	3.48
8	-1.682	0.000	0.000	26.6	6.0	72.0	2.34	2.37	2.32
9	0.000	0.000	-1.682	35.0	6.0	31.6	1.47	1.57	1.59
10	0.000	0.000	0.000	35.0	6.0	72.0	3.46	3.45	3.48
11	1.000	-1.000	-1.000	40.0	5.5	48.0	1.58	1.54	1.60
12	0.000	0.000	0.000	35.0	6.0	72.0	3.46	3.45	3.48
13	1.000	-1.000	1.000	40.0	5.5	96.0	2.52	2.46	2.49
14	-1.000	-1.000	-1.000	30.0	5.5	48.0	2.67	2.54	2.70
15	0.000	-1.682	0.000	35.0	5.2	72.0	2.74	2.83	2.81
16	1.000	1.000	-1.000	40.0	6.5	48.0	1.34	1.28	1.36
17	-1.000	1.000	1.000	30.0	6.5	96.0	2.29	2.28	2.32
18	0.000	1.682	0.000	35.0	6.9	72.0	1.98	1.94	1.21
19	0.000	0.000	1.682	35.0	6.0	112.3	3.01	2.96	3.09
20	-1.000	-1.000	1.000	30.0	5.5	96.0	3.07	3.08	3.11

X_1(incubation temperature, °C) is calculated as: $X_1 = 35 + x_1 (5)$
X_2 (initial pH) is calculated as: $X_2 = 6.0 + x_2 (0.5)$
X_3 (fermentation time, h) is calculated as: $X_3 = 72 + x_3 (24)$

< 0.001) and the interaction coefficients of X_1X_3 and X_2X_3 were less significant ($p < 0.005$). The statistical significance of the ratio, between the mean square variation, due to regression, and the mean square residual error, was tested using analysis of variance (ANOVA). ANOVA is a statistical technique that subdivides the total variation of a set of data into component associated to specific sources of variation. The regression equation obtained form the ANOVA shows (Table 4) that the R^2 (coefficient of determination) was 0.995 (a value > 0.75 indicates fitness of the model). This is an estimate of the fraction of overall variation in the data accounted by the model, and thus the model is capable of explaining 99.5% of the variation in the response. The 'adjusted R^2' is 0.991, which indicates that the model is good (for a good statistical model, the R^2 value should be in the range of 0 to 1.0, and the nearer to 1.0 the value is, the more fit the model is deemed to be). ANOVA of the regression model for ethanol yield demonstrated that the model was significant due to a very high *F*-value of 436.5 and a very low probability value (P model >F – 0.001).

The response surfaces can be used to predict the optimum range for different values of the test variables and the major interactions between the test variables can be identify from the circular or elliptical nature of the contours. The circular nature of the contours signify that the interactive effects between the test variables are not significant and optimum values of the test variables can be easily obtained. Figs. 2 – 4 show the isoresponse contour plots of the interactive effect of incubation temperature, initial pH and fermentation time on ethanol production. The response values for the variables can be predicted form these plots. The effect of incubation temperature and pH on ethanol production, while other variable (fermentation time) was fixed at central level (72 h), is shown in Fig. 2. According to Fig. 2, the contours around the stationary point were elliptical and it became elongated more and more along the temperature axis, which meant that a small change of the response value would require a small move along the temperature axis.

It was evident that the ethanol concentration steadily decreased with increasing incubation temperature upto 45°C and at low pH level. While at high temperature, the increase in the response value was negligible with as the pH value was increased. So a lower temperature and lower pH value enhance the ethanol yield. The significant interaction between incubation temperature and initial pH were apparent not only from the elliptical nature of the contour plot, but also from the low probability value (Table 3). The other pair of the independent variables incubation temperature and fermentation time shows a less interactive effect (Fig. 3) while keeping the third independent variable, initial pH at 6.0. From Fig. 3, it was evident that the interactive effects between the test variables were less significant not only from the circular nature of the contour plot and also from the probability value (P - 0.004). Then the optimum values of the test variables can be easily obtained form this type of circular contour plot.

Fig. 4 show the similar effect, that the variables initial pH and fermentation time show a less interactive effect in the ethanol fermentation while keeping the third variable incubation temperature as constant at 35°C and found that the test variables were less significant. The results show that as the values of process variables increased, the yield also increased but only up to the midpoint of range of variables and thereafter the yield decreased even though the values of variables increased. The ethanol yield is significantly affected by incubation temperature and initial pH than other pair of variables in the ethanol fermentation by SSF process. The matching quality, of the data obtained by the model proposed in equation (2), was evaluated considering the correlation coefficient, R^2, between the experimental and modeled data. The mathematical adjust of those values generated a R^2 = 0.995, revealing that the model would explain very well 99.5% of the overall effects and only 0.5% was not explained. In ANN modeling the R^2 value between the experimental and predicted responses is determined as 0.953, revealing that the model could not explain only 4.7%. The increase in the number of experimental points in training the data set improved the network's performance. From equations derived by differentiating Equation 2, the optimum values for the independent variables obtained were incubation temperature 32°C, pH 5.6 and fermentation time 110 h. Based on the model, the optimal working conditions were obtained to attain high ethanol yield.

TABLE III
RESULTS OF REGRESSION ANALYSIS AND CORRESPONDING T AND P VALUE OF SECOND ORDER POLYNOMIAL MODEL

Term Constant	Regression coefficient	Std. deviation	*t*-statistics	*P*-value
Intercept	3.4584	0.02993	115.569	< 0.001
X_1	– 0.2212	0.01985	– 11.141	< 0.001
X_2	– 0.2657	0.01985	– 13.381	< 0.001
X_3	0.4144	0.01985	20.874	< 0.001
X_1X_1	– 0.5128	0.01933	– 26.530	< 0.001
X_2X_2	– 0.3784	0.01933	– 19.578	< 0.001
X_3X_3	– 0.4208	0.01933	– 21.773	< 0.001
X_1X_2	0.1838	0.02594	7.083	< 0.001
X_1X_3	0.0963	0.02594	3.710	0.004
X_2X_3	0.0488	0.02594	1.879	0.090

TABLE IV
ANOVA FOR THE QUADRATIC POLYNOMIAL MODEL FOR ETHANOL PRODUCTION

Sources of variation	Sum of squares	Degrees of freedom (DF)	Mean square (MS)	*F*-value	*P*-value
Regression	11.3909	9	1.26566	235.10	< 0.001
Linear	3.9778	3	1.32594	246.29	< 0.001
Square	7.0499	3	2.34996	436.50	< 0.001
Interaction	0.3632	3	0.12108	22.49	< 0.001
Residual Error	0.0538	10	0.00538	–	–
Lack-of-Fit	0.0538	5	0.01077	–	–
Pure Error	0.0000	5	0.0000	–	–
Total	11.4448	19	–	–	–

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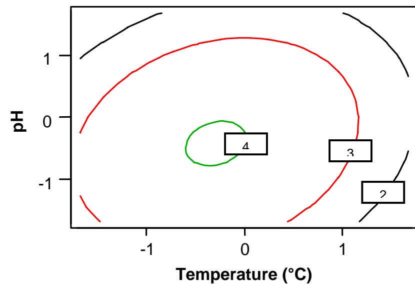

Fig. 2 Isoresponse contour plot for the effect of incubation temperature versus initial pH on ethanol production

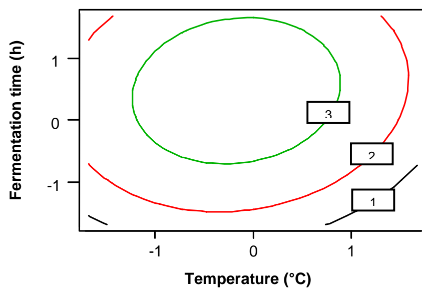

Fig. 3 Isoresponse contour plot for the effect of incubation temperature versus fermentation time on ethanol production

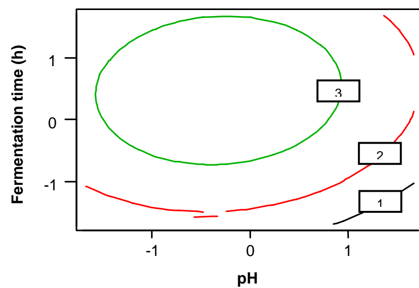

Fig. 4 Isoresponse contour plot for the effect of initial pH versus fermentation time on ethanol production

Response analysis revealed the maximum ethanol concentration (3.36 g/l) by *P. tannophilus* could be achieved at the optimum process conditions.

C. *Kinetics and Modeling*

A comprehensive mechanistic kinetic model has been derived based on the mechanism of ethanol production from lignocellulosic materials by using yeast strains [23-24]. The validity of the proposed model under different experimental conditions has been tested. The cellmass, product formation and substrate utilization kinetics using *P. tannophilus* with different parameters were studied.

Monod and Modified Logistic model (growth)

Monod model (Eqn. 3) relates the growth rate to the concentration of a single growth-controlling substrate [$\mu = f(s)$] via two parameters, the maximum specific growth rate (μ_{max}), and the substrate affinity constant (Ks). Since growth is a result of catabolic and anabolic enzymatic activities, these processes, i.e., substrate utilization or growth-associated product formation, can also be quantitatively described on the basis of growth models.

$$\mu = \frac{\mu_{\max} S}{K_S + S} \tag{3}$$

Under optimal growth conditions and when the inhibitory effects of substrates and product play no role, the rate of cell growth is given by equation (4)

$$\frac{dX}{dt} = \mu_0 X \tag{4}$$

where μ_o is a constant defined as the initial specific growth rate. The logistic model equation implies that the growth rate increases with increase in cellmass concentration and is independent of the substrate concentration. The logistic equation utilized to describe the kinetics of several polysaccharides fermentation systems. A modified form of logistic equation is used to describe the cell growth kinetics by introducing an index of the inhibitory effect 'r' which accounts for the deviation of growth from the exponential relationship [23], as equation (5)

$$\frac{dX}{dt} = \mu_0 \left[1 - \left(\frac{X}{X_{\max}}\right)^r\right] X \tag{5}$$

when r = 0 will be a complete inhibition of cell growth; r = 1 equation (5) reduces to logistic model equation (5); r ranges between 0 and 1 equation (5) describes a higher degree of inhibition compared to logistic growth; r >1 the growth lies between exponential and logistic patterns. Equation (5) was rearranged and integrated by using partial fraction method with the initial conditions, $X=X_0$ (t=0) gives equation (6)

$$X_t = \frac{X_m^r e^{\mu_0 rt}}{1 - \frac{X_0^r}{X_m^r (1 - e^{\mu_0 rt})}} \tag{6}$$

The model parameter values were evaluated using MATLAB software version 7.0.1 program and are shown in Table 5. A better prediction of cellmass concentrations was obtained using the monod and modified logistic model and were most suited for ethanol production with the minimum average error of 4.56 % and 6.99% respectively.

Modified Logistic incorporated Leudeking – Piret model (product formation)

Modified Logistic incorporated Leudeking – Piret model was developed by rearranging and integrating the Leudeking – Piret model with two initial conditions, $X=X_0$ (t=0) and $P = P_0$ (t=0) gives equation (7)

$$P_t = P_0 + \alpha\left\{\left[\frac{X_0^r e^{\mu_0 rt}}{1-\frac{X_0^r}{X_m^r}\left(1-e^{\mu_0 rt}\right)}\right]^{1/r} - X_0^r\right\} + \frac{\beta X_m^r}{\mu_0}\ln\left[1-\frac{X_0^r}{X_m^r}\left(1-e^{\mu_0 rt}\right)\right] \quad (7)$$

The model parameter values were evaluated using MATLAB program and are presented in Table 5. The simulation result of the Modified Logistic incorporated Leudeking – Piret model is in good agreement with the experimental data obtained from the pretreated sugarcane bagasse and the minimum average error of 7.01 %.

Modified Logistic incorporated Modified Leudeking – Piret model (substrate utilization)

The substrate utilization kinetics is the modified form of the Leudeking – Piret model which can be used for substrate utilization kinetics. Substrate consumption depends on the magnitude of three sink terms, the instantaneous cellmass growth rate, the instantaneous product formation rate and a cellmass maintenance function. The Modified Logistic incorporated Modified Leudeking – Piret model was developed by rearranging and integrating the Modified Leudeking – Piret model with two initial conditions, $X=X_0$ (t=0) and $S= S_0$ (t=0) gives equation (8)

$$S_t = S_0 - \gamma\left\{\left(\frac{X_0^r e^{\mu_0 rt}}{1-\frac{X_0}{X_m^r\left(1-e^{\mu_0 rt}\right)}}\right)^{1/r} - X_0^r\right\} - \frac{\eta X_m^r}{\mu_0}\ln\left[1-\frac{X_0}{X_m^r}\left(1-e^{\mu_0 rt}\right)\right] \quad (8)$$

The model parameter values shown in Table 5 are then used to simulate the experimental data of substrate concentration at any time during the entire course of fermentation. Better substrate utilization kinetics is obtained using the Modified Logistic incorporated Modified Leudeking – Piret model (Eqn. 8) and is well suited for ethanol production from pretreated sugarcane bagasse with a minimum average error of 7.32%.

IV. Conclusion

Based on the present study, it is evident that the use of statistical optimization tools, response surface methodology (RSM), has helped to locate the optimum levels of the most significant parameters for ethanol production, with minimum effort and time. Maximum ethanol concentration (3.36 g/l) was obtained from 50 g/l of pretreated sugarcane bagasse at the optimized conditions (incubation temperature 32°C, initial pH 5.6 and fermentation time 110 h) by using yeast strain *P. tannophilus*. Modified logistic model, Modified Logistic incorporated Leudeking – Piret model and Modified Logistic incorporated Modified Leudeking – Piret model were attempted for representing the batch growth kinetics, product formation kinetics and substrate utilization kinetics respectively. The results of the process simulation from the various models using the experimental data were compared and found to predict more accurately during the entire course of fermentation.

TABLE IV
MODEL PARAMETERS FOR ETHANOL PRODUCTION

Model paramet-ers	Models			
	Monod	Modified Logistic	Modified Logistic incorporated Leudeking – Piret	Modified Logistic incorporated Modifed Leudeking – Piret
μ_0	0.12	0.12	–	–
μ_m	0.23	–	–	–
K_s	0.52	–	–	–
r	–	0.540	0.610	0.720
α	–	–	0.387	0.543
β	–	–	0.021	0.025
γ	–	–	6.523	8.340
η	–	–	0.059	0.011
Avg. error (%)	4.56	6.99	7.01	7.32
R^2	0.993	0.986	0.996	0.980

μ_0 = Initial specific growth (h^{-1}), μ_m= Maximum specific growth rate (h^{-1}), K_s = Substrate affinity constant (g/l), r = Inhibitory effect index, α = Non-growth associated constant for substrate, β = Substrate consumption (g substrate / g biomass h), γ = Growth associated constant (g product / g biomass), η = Non-growth associated constant (g product / g biomass h)

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