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SIMULTANEOUS SACCHARIFICATION AND FERMENTATION (SSF) OF PRETREATED SUGARCANE BAGASSE USING CELLULASE AND *Saccharomyces cerevisiae* - KINETICS AND MODELING

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Abstract: Optimization of process variables in the bioconversion of pretreated sugarcane bagasse using cellulase and *Saccharomyces cerevisiae* by Simultaneous Saccharification and Fermentation (SSF) was investigated in the present study. A 2^3 five level Central Composite Design (CCD) experiments with central and axial points were used to develop a statistical model for the optimization of process variables, e.g. incubation temperature , pH and fermentation time. Data obtained from Response Surface Methodology (RSM) on ethanol production were subjected to the analysis of variance (ANOVA) and analyzed using a second order polynomial equation and the contour plots were used to study the interactions among three relevant variables of the fermentation process. Experiments were carried out using an online monitored modular fermenter of 2 L capacity under aerobic condition. The processing parameters setup for reaching a maximum response for ethanol production (4.80 g/l) was obtained from 50 g/l pretreated sugarcane bagasse when applying the optimum values for temperature (35°C), pH (5.5) and fermentation time (114 h). Various 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: *Optimization, response surface methodology (RSM), simultaneous saccharification and fermentation (SSF), ethanol, Saccharomyces cerevisiae*

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1. Introduction

Cellulosic biomass is an alternate source of energy since it is renewable and available throughout the globe in large quantities. Ethanol, as an alternate energy, is one of the largest volumes that can be produced from cellulosic biomass. Ethanol was formerly used mainly to increase the octane level and improve the emissions quality of gasoline, but today is increasingly being used as a partial or total replacement for gasoline in cars and other road vehicles. Therefore, with the expectation of supply shortfalls in future from non-renewable fossil fuels, the production of fermentatively produced bio-alcohols from low-cost biomass such as cellulosic wastes to meet energy demands is an attractive alternative [1]. Cellulosic biomass for ethanol production needs pretreatment via liquefaction and saccharification. Over the past decades, emphasis has been placed on enzymatic hydrolysis of cellulosic biomass to fermentable sugar. However, the process has been hampered by economic problems such as high costs of biomass pretreatment and cellulase production [2].

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. Sugarcane bagasse is accumulated in large quantities at cane-to-sugar processing plants and consists approximately of 50% cellulose, 25% hemicellulose, and 25% lignin [3, 4]. 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 [5]. 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). However, low enzymatic accessibility of the native cellulose is a key problem for biomass-to-ethanol processes [6, 7].

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. In this option, the cellulose hydrolysis and glucose fermentation steps are combined in a single vessel. Since cellulase is inhibited by glucose as it is formed [8], rapid conversion of the glucose into ethanol by yeast results in faster rates, higher yields, and greater ethanol concentrations than possible for SSF. The presence of ethanol in the fermentation broth also makes the mixture less vulnerable to invasion by unwanted microorganisms. In practice, yeast has shown higher yields and ethanol tolerance

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than bacteria [9].

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. 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 [10, 11]. In the present study, the potential use of sugarcane bagasse for ethanol fermentation using cellulase and yeast *Saccharomyces cerevisiae* 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 (for growth kinetics), Modified Logistic incorporated Leudeking-Piret model (for product formation kinetics) and Modified Logistic incorporated Modified Leudeking-Piret model (for substrate utilization kinetics) have been evaluated.

2. Materials and Methods

2.1. 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 [12]. The estimated cellulose content of steam pretreated sample was 420 mg/g bagasse.

2.2. 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 [13]. Yeast strain *Saccharomyces cerevisiae* was 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 (12 h interval) for the analysis of cellmass, ethanol and residual sugar concentrations.

2.3. Steam pretreatment

Steam pretreatment was carried out for the milled sugarcane bagasse in an autoclave at 15 psi (121°C) for about 60 minutes. The treated sample was collected and filtered in crucibles followed by washed with distilled water under suction. Finally it was dried at room temperature before fermentation [14, 15].

2.4. Fermentation

Batch experiments were conducted as per the CCD 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 (dissolved oxygen) probe. The equipment also monitored temperature, agitation speed, gas purging flow rate, pumping rates, antifoam addition, DO 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 DO (8±0.1 ppm) were kept constant during the experiments. 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 (1 L). Samples were withdrawn periodically (12 h interval), centrifuged in a laboratory desktop centrifuge at 1200 rpm, and the supernatants were analyzed for total sugars and ethanol concentrations.

2.5. Cell growth and chemical analysis

The sugarcane bagasse sample was analyzed for hemicellulose and Klason lignin content following the 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 [16]. 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.

2.6. 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 . The dependent variable selected for this study was ethanol concentration, Y (g/l). The independent variables chosen were incubation temperature X_1, pH X_2 and fermentation time X_3. A mathematical model, describing the relationships among the process dependent variable and the independent variables in a second-order equation, was developed [17]. Design-based experimental data were matched according to Equation 1 .

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

where, i, j are linear, quadratic coefficients, respectively, x_i, x_j are the dimensionless value of an independent variables, 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.

2.7. ANN modeling

Knowledge-based approaches such as ANN have been successfully applied to modeling and control of various biological processes in recent years. ANN represents the nonlinearities better than the RSM does. ANN cannot produce a model equation similar to RSM but it works as human brain and it estimates the response based on the trained data in the investigated range. The first step in implementing a neural network modeling approach is to design the topology of the network. 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 [18, 19].

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 . Since 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).

3. Results and Discussion

3.1. 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 concentration) 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 *S.cerevisiae*, can be expressed in terms of the following regression Equation 2:

$$\begin{aligned} Y = 3.4584 - 0.2212x_1 - 0.2657x_2 + 0.4144x_3 - 0.5128x_1^2 \\ -0.3784x_2^2 - 0.4208x_3^2 + 0.1838x_1x_2 + 0.0983x_1x_3 \\ +0.0485x_2x_3 \end{aligned} \qquad (2)$$

Table 1: Codes and actual levels of the independent variables for design of experiment

Independent variables	Symbols	Coded levels				
		1.682	1	0	+1	+1.682
Temperature (°C)	X_1	26.6	30	35	40	43.4
pH	X_2	5.2	5.5	6	6.5	6.9
Fermentation time (h)	X_3	31.6	48	72	96	112.3

Table 2: Five level factorial central composite design and the experimental responses of dependent variable, Y (ethanol concentration, g/l)

Run No.	Coded levels			Real variables			Ethanol conc. (g/l)		
	x_1	x_2	x_3	X_1	X_2	X_3	Exp	Predicted	
								(RSM)	(ANN)
1	0	0	0	35	6	72	3.9	3.92	3.95
2	1	-1	-1	40	5.5	48	1.54	1.62	1.6
3	-1	-1	1	30	5.5	96	3.54	3.58	3.61
4	1	1	-1	40	6.5	48	1.37	1.43	1.45
5	0	0	0	35	6	72	3.9	3.92	3.95
6	0	-1.682	0	35	5.2	72	3.08	2.98	3.18
7	-1	-1	-1	30	5.5	48	2.91	2.85	3.12
8	0	0	0	35	6	72	3.9	3.92	3.95
9	1.682	0	0	43.4	6	72	2.2	1.95	1.95
10	0	0	1.682	35	6	112.3	3.96	3.75	4.13
11	-1.682	0	0	26.6	6	72	2.62	2.7	2.68
12	-1	1	1	30	6.5	96	2.55	2.58	2.6
13	-1	1	-1	30	6.5	48	1.78	1.7	1.71
14	0	0	0	35	6	72	3.9	3.92	3.95
15	1	1	1	40	6.5	96	2.75	2.92	2.88
16	1	-1	1	40	5.5	96	2.78	2.96	2.92
17	0	0	0	35	6	72	3.9	3.92	3.95
18	0	0	0	35	6	72	3.9	3.92	3.95
19	0	1.682	0	35	6.9	72	2.04	1.97	2.01
20	0	0	-1.682	35	6	31.6	1.84	1.88	1.89

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 linear term X_3, and all quadratic coefficients of X_1, X_2 and X_3 were significant at < 1% level ($p < 0.001$ for all) and the interaction coefficients were of 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).

Table 3: Results of regression analysis and corresponding t and P value of second order polynomial model for optimization of ethanol production

Term Constant	Regression coefficient	Standard deviation	t-statistics	P-value
Intercept	3.9246	0.05952	65.933	< 0.001
X_1	0.2231	0.03949	5.648	< 0.001
X_2	0.2980	0.03949	7.544	< 0.001
X_3	0.5554	0.03949	14.064	< 0.001
X_1X_1	0.5639	0.03845	14.667	< 0.001
X_2X_2	0.5108	0.03845	13.287	< 0.001
X_3X_3	0.3906	0.03845	10.161	< 0.001
X_1X_2	0.24	0.0516	4.651	< 0.001
X_1X_3	0.1525	0.0516	2.955	0.014
X_2X_3	0.035	0.0516	0.678	0.513
$R^2 = 0.987$; Adjusted $R^2 = 0.974$				

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 0.951 (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 95.1% of the variation in the response. The 'adjusted R^2' is 0.907, 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 an F-value of 32.74 and a very low probability value (P model > F – 0.001).

Table 4: ANOVA for the fitted quadratic polynomial model for ethanol production

Sources of variation	Sum of squares	Degrees of freedom (DF)	Mean square (MS)	F-value	P-value
Regression	15.6195	9	1.7355	81.48	< 0.001
Linear	6.1051	3	2.035	95.54	< 0.001
Square	8.8578	3	2.9525	138.62	< 0.001
Interaction	0.6567	3	0.2188	10.28	0.002
Residual Error	0.213	10	0.0213	-	-
Lack-of-Fit	0.213	5	0.0426	-	-
Pure Error	0	5	0	-	-
Total	15.8325	19	-	-	-

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. Figures 1-3 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 (96 h), is shown in Figure 1. According to Figure 1, the contours around the

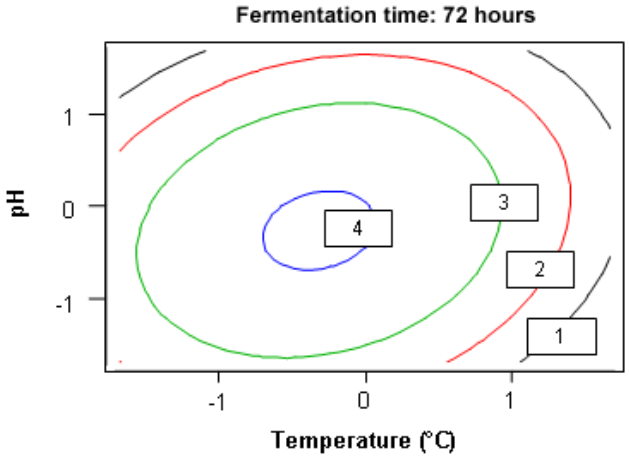

Figure 1: Isoresponse contour plot for the effect of incubation temperature versus initial pH on ethanol production

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 (P value is 0.028; since the P value for the interaction effects were < 5% level) (Table 3). The other pair of the independent variables incubation temperature and fermentation time shows a less interactive effect (Figure 2) while keeping the third independent variable, initial pH at 6.0. From Figure 2, 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 high probability value (P-0.520). Then the optimum values of the test variables can be easily obtained form this type of circular contour plot. Figure 3 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.

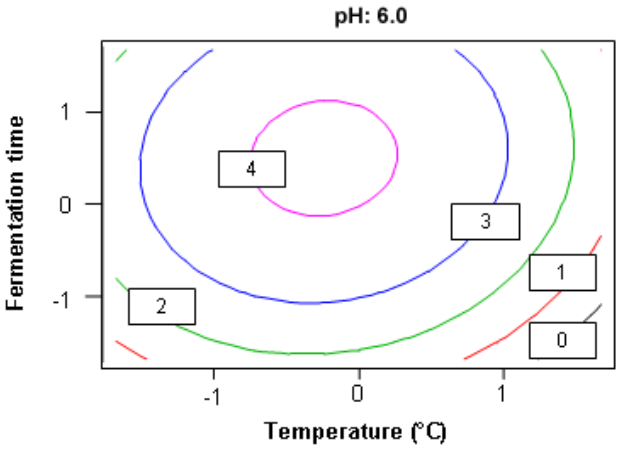

Figure 2: Isoresponse contour plot for the effect of incubation temperature versus fermentation time on ethanol production

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 = 0.95, revealing that the model would explain very well 95% of the overall effects and only 5% was not explained. In ANN modeling the R^2 value between the experimental and predicted responses is determined as 0.985, revealing that the model could not explain only 1.5%. 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. Response analysis revealed the maximum ethanol concentration (4.80 g/l) by *S. cerevisiae* could be achieved at the optimum process conditions.

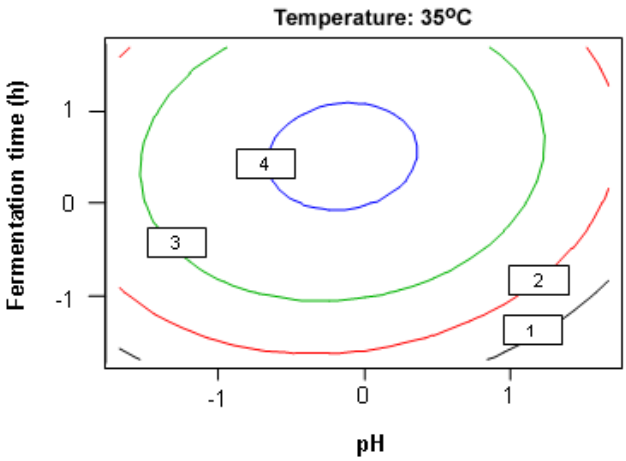

Figure 3: Isoresponse contour plot for the effect of initial pH versus fermentation time on ethanol production

3.2. *Kinetics and modeling*

3.2.1. *Modified Logistic model (growth)*

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 3

$$\frac{dX}{dt} = \mu_0 X_t \tag{3}$$

where μ_0 is a constant defined as the initial specific growth rate and X_t is the cellmass concentration at time t. The logistic model equation implies that the growth rate increases with increase in cellmass concentration and is independent of the substrate concentration. In reality the growth of cell is governed by a hyperbolic relationship and the logistic Equation 4 is given by

$$\frac{dX}{dt} = \mu_0 \left[1 - \frac{X_t}{X_{\max}}\right] X_t \tag{4}$$

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 [20, 21], as Equation 5

$$\frac{dX}{dt} = \mu_0 \left[1 - \left(\frac{X_t}{X_{\max}}\right)^r\right] X_t \text{ ;for } r > 0 \tag{5}$$

when r = 0 will be a complete inhibition of cell growth; r = 1 Equation 5 reduces to logistic model Equation 4; 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, gives Equation 6

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

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 modified logistic model and it was most suited for ethanol production with the minimum average error of 4.56%.

Table 5: ANOVA for the fitted quadratic polynomial model for ethanol production

Model parameters	Models			
	Monod	Modified Logistic	Modified Logistic incorporated Leudeking Piret	Modified Logistic incorporated Modifed Leudeking Piret
[1]μ_0	0.11	0.11	-	-
[2]μ_m	0.2	-	-	-
[3]K_s	0.54	-	-	-
[4]r	-	0.59	0.56	0.76
[5]α	-	-	0.424	0.502
[6]β	-	-	0.024	0.021
[7]γ	-	-	7.885	8.315
[8]η	-	-	0.063	0.012
Avg. error (%)	4.95	3.13	6.88	7.11
R^2	0.976	0.981	0.985	0.988

[1]Initial specific growth (h^{-1})
[2]Maximum specific growth rate (h^{-1})
[3]Substrate affinity constant (g/l)
[4]Inhibitory effect index
[5]Non-growth associated constant for substrate
[6]Substrate consumption constant (g substrate / g biomass h)
[7]Growth associated constant (g product / g biomass)
[8]Non-growth associated constant (g product / g biomass h)

3.2.2. *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

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

where, α is non-growth associated constant for substrate and β is substrate consumption constant (g substrate / g biomass h). 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 5.69%.

3.2.3. *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

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

where, γ is the growth associated constant (g product / g biomass) and η is the non-growth associated constant (g product / g biomass h). 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 (Equation 8) and is well suited for ethanol production from pretreated sugarcane bagasse with a minimum average error of 6.82%.

4. 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 (4.80 g/l) was obtained from 50 g/l of pretreated sugarcane bagasse at the optimized conditions (incubation temperature 35°C, initial pH 5.5 and fermentation time 114 h) by using yeast strain *S.cerevisiae*. 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.

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