Bioethanol production from tapioca stem using SSF method – optimization, kinetics and modeling

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Abstract : The simultaneous saccharification and fermentation (SSF) of the 226 white rose tapioca stems to ethanol were investigated in a batch reactor using cellulase enzyme and *Saccharomyces cerevisiae* respectively. Pretreatment of lignocellulosic biomass using sodium hydroxide is basically a delignification process, in which a significant amount of hemicellulose is solubilized as well Maximum reduction in lignin of 54.66% is achieved for 2.0% sodium hydroxide concentration, 90 min residence time at 120ºC.The effect of initial substrate concentration, pH, temperature and cellulase loading are identified as the major factors affecting ethanol production by ssf and these can be well studied by statistically designed experiments using central composite design. The validation of the statistical model and regression equation are conducted by taking initial substrate concentration of 50.20 g/l, pH of 5.58, temperature of 31.26°C and Cellulase Loading of 18.94 mg/g of substrate. Maximum ethanol production of 16.10 g/l corresponding to 56% of theoretical yield is obtained under optimum conditions. The Logistic model for cell growth, Leudeking-Piret model for substrate utilization kinetics and product formation kinetics are tested. All the experimental results are found to be in good agreement with the theoretical predictions and all the models presented in this work provide a good description of biomass, product and substrate concentrations. A better prediction of biomass concentrations with high R^2 values of 0.9786 was obtained using Logistic model. The simulation results are useful to predict the dynamics of substrate utilization and are well suited for ethanol production from sequential pretreated tapioca stem with a minimum error of 7.25%. The simulation results of product formation kinetics are in good agreement with the experimental data obtained from the production of ethanol with a minimum error of 8.88%.

Keywords: Ethanol, Tapioca stem, Alkaline hydrolysis, simultaneous saccharification and fermentation(ssf), kinetics, modelling.

Introduction

The world's ever-increasing demand for energy, inevitable depletion of fossil fuels and growing concerns over global warming have stimulated the exploration for alternative energy sources.Biomass-based Ethanol is one of the most promising alternatives to fossil fuels to power the transportation sector. the interest in biotechnology-based production of fuels tends to augment with the concern about exhaustion of fossil fuels and the increase in their price [1]. The use of petrol blended with20–24% ethanol is a standard practice in Brazil. Therefore, it is highly desirable for a country like India to use ethanol–petrol blend as transportation fuel to save valuable foreign exchange in importing crude oil as well as in reducing the environmental pollution caused by the vehicular emission. The most critical element for the success of bioethanol technology is the availability of celluloses at a nominal cost. Major R&D effort is required to produce cellulase with high yield and productivity [2,3]. Alternatively, thermo-tolerant high activity liquefying and saccharifying enzymes (a-amylase and glucoamylase) would be required for the development of cost-effective starch-based ethanol production in India [4, 5]. For the last two decades, ethanol production by the yeast *Sacchoromyces cerevisiae* has been studied extensively [6]. *S.cerevisiae* is capable of metabolizing few types of sugar such as glucose, fructose and sucrose [7,8]. Corn starch, an agricultural product, is a cheap substrate that is easily available in tropical countries like India. There are few reports available on fermentation of Corn starch hydrolysate by *S. cerevisiae*. Ethanol production from Corn starch requires the use of amylase and glucoamylase for the pretreatment of Corn starch before fermentation [9].

This study includes pretreatment techniques using sodium hydroxide adopted for the pretreatment of toapioca stem and its subsequent conversion to ethanol. The bioconversion of ethanol is attempted by Optimization of process parameters namely effect of substrate concentration, initial pH, temperature and cellulase loading on ethanol concentration by *Saccharomyces cerevisiae* using Central Composite Design (CCD) using Response Surface Methodology (RSM).

Materials and methods

Microorganisms and Culture conditions

Commercially available cellulase enzyme was obtained from SISCO Laboratories, Mumbai. The activity of cellulase was found to be 15 FPU/ml and it was used throughout the experimentation. The cellulase activity was measured by standard Mandel's method. The stock culture of selected strain of baker's yeast *Saccharomyces cerevisiae* was maintained on agar medium with a composition of yeast extract 10 g/l, peptone 20g/l, dextrose 20g/l and agar 20g/l at pH of 5.0 and 30ºC. The fermentation medium had the following composition per liter of distilled water: KH_2PO_4, 2.22g; NaH_2PO_4, 7.65g; $MgSO_4.7H_2O$, 0.34g; $(NH_4)_2SO_4$, 1.60g; Citric acid monohydrate, 9.24g; Tween 80, 0.22g; Urea, 0.30g; $FeSO_4.7H_2O$, 0.005g; $ZnSO_4.7H_2O$, 0.0014g; $MnSO_4.6H_2O$, 0.0016g; $CaCl_2$, 0.0019g; and known amount of tapioca stem substrate.

Raw material preparation

Tapioca stem, an abundant agricultural by-product, obtained from local farmers in Allivilagam, Nagai district, Tamilnadu, India was used as raw material in this study. After collection, the tapioca stems were crushed into small pieces and air-dried at 50ºC-55ºC in hot air oven. The dried stems were milled in a laboratory ball mill and screened through 100 mesh size was used for the production of ethanol.

Results and discussions

Effect of Sodium hydroxide pretreatment on Tapioca Stem

The effect of sodium hydroxide pretreatment on percentage hemicellulose solubilization and lignin reduction is studied by varying the alkali concentration from 1.0% (w/v) to 2.0% (w/v) and residence time from 30 min to 90 min keeping the temperature constant at 120°C. After sodium hydroxide pretreatment of tapioca stem, the solids are analyzed for cellulose, hemicellulose and lignin contents and the results are compared with raw tapioca stem & is given in Table 1which shows that the lignin content of pretreated tapioca stem decrease with increasing residence time and alkali concentration. The data in Table 1 are graphically represented and shown in Fig.1 The percentage lignin reduction after sodium hydroxide pretreatment ranged from 3.07% (30 min, 1.0%, 120°C) to 12.29% (30 min, 2.0%, 120°C), 13.10% (60 min, 1.0%, 120°C) to 35.98% (60 min, 2.0%, 120°C) and 34.02% (90 min, 1.0%, 120°C) to 54.66% (90 min, 2.0%, 120°C). Lignin is a three-dimensional complex aromatic polymer which forms and sheath surrounding cellulose and hemicellulose, stiffening and holding together the fibers of polysaccharides. Since it is a major barrier limiting the accessibility of carbohydrates to hydrolytic enzymes, its reduction is crucial to the improvement of plant biomass digestibility. Reducing the lignin content of the biomass helps to expose the highly ordered crystalline structure of cellulose and facilitates substrate access by hydrolytic enzymes. Maximum reduction in lignin of 54.66% is achieved for 2.0% sodium hydroxide concentration, 90 min residence time at 120°C. Results from this study are comparable to those data given in literature [10].These results suggest that, the application of alkaline solutions leads to removal of lignin barrier, disruption of structural linkages, reduction of cellulose crystallinity, and decrease in polymerization degree of carbohydrates.

Table 1. Composition of Sodium hydroxide Pretreated Tapioca stem

S.No	Time (min) Concentration (% w/v), Temperature (°C)	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Hemicellulose solubilization (%)	Lignin Reduction (%)
1	30,1.0,120	57.45	20.04	16.87	0.79	3.07
2	30,1.5,120	59.79	19.34	16.26	4.26	6.55
3	30,2.0,120	61.49	19.15	15.26	5.19	12.29
4	60,1.0,120	61.25	19.11	15.12	5.39	13.10
5	60,1.5,120	65.89	17.45	13.45	13.61	22.70
6.	60,2.0,120	69.36	16.48	11.14	18.42	35.98
7.	90,1.0,120	68.84	17.02	11.48	15.74	34.02
8.	90,1.5,120	72.11	16.55	8.78	18.07	49.54
9.	90,2.0,120	74.34	15.27	7.89	24.41	54.66

Raw tapioca stem composition: Cellulsoe – 56.40%, Hemicellulose – 20.20% and Lignin 17.40%. Composition percentages are on dry weight basis (% w/w).

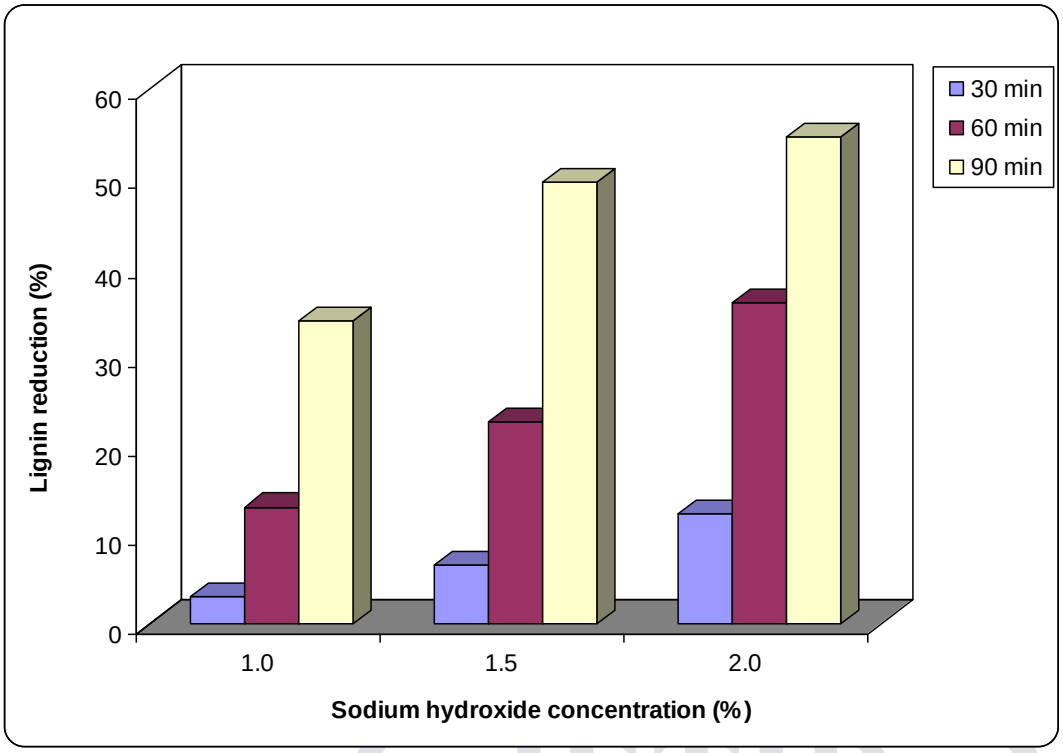

Fig 1. Percentage Lignin reduction for sodium hydroxide pretreatment on topioco stem

Statistical Optimization of Process Parameters for Simultaneous Saccharification and Fermentation (SSF)with *Cellulase Enzyme* and *Saccharomyces cerevisiae*

Response surface methodology is very effective and popular tool to optimize the parameters having equal importance and influence each other in the process [11]. The factors affecting the simultaneous saccharification and fermentation of sequentially pretreated tapioca stem with *cellulase enzyme* and *Saccharomyces cerevisiae*is studied using central composite design experiments. The initial substrate concentration (A) g/l, pH (B), temperature (C) °C and cellulase loading (D) mg/ g of substrate are chosen as the independent variables as shown in Table 2. Ethanol concentration (Y) is chosen as the dependent output variable. An orthogonal 2^4 full factorial central composite design with eight star points (α=2) and seven replication at the center point, all in duplicates, resulting in a total of 31 experiments are used to optimize the chosen key variables for the production of ethanol by SSF in a batch reactor.Thirty one experiments based on central composite design are carried out with different combination of variables and the results are presented in Table 3. The data obtained from the five level central composite design matrix are used to develop models in which each dependent variable (Ethanol concentration ,Y) is obtained as the sum of the contributions of the independent variable through second order polynomial equation and interaction terms. The regression equation coefficients are calculated and the data is fitted to a second order polynomial equation. The response, Y (Ethanol concentration) by *cellulase enzyme* and *Saccharomyces cerevisiae* can be expressed in terms of the following regression equation 1:

$$Y = 14.8857 + 0.1321A - 0.2329B - 1.3637C + 0.2679D - 0.2651A^2 - 1.4151B^2 - 1.1026C^2 - 0.3026D^2 - 0.2394AB - 0.1931AC - 0.4756AD + 0.7294BC - 0.5231BD + 0.0056CD \quad \ldots (1)$$

The results of multiple linear regressions conducted for the second order response surface model are given in Table 3. The significance of each coefficient is determined by Students t-test and P-values are listed in Table 4. The coefficient of determination (R^2) is calculated to be 0.9517 for ethanol production, this implies that 93.67% of experimental data of the ethanol production is compatible with the data predicted by the model (Table 3) and only 6.33% of the total variations are not examined by the model. Besides the linear effect of the ethanol concentration Y, g/l, the response surface method also gives an insight about the parameter's quadratic and combined effects. In this case C, B^2, C^2 and BC are significant model terms.

The effect of temperature is found to be highly significant (p=0.000) on ethanol production It is found from the coefficient C, the ethanol production is high at 30-35°C, further increase in temperature gave less ethanol yield. The coefficient of the interaction terms of pH and temperature is found to be highly significant. The interaction coefficients of AB. AC and CD are less significant when compared to the coefficients AD, BC, and BD. ANOVA of the regression model for ethanol yield demonstrated that the model is significant due to a very high F value and a very low probability value. The graphical representations of the regression equation called the surface contour plot are obtained using the same software package.

The optimum range for different values of the test variables are obtained from the circular or elliptical nature of the contours. The circular nature of the contour signifies that the interactive effects between tests are not significant and the optimum values of the test variables can be easily obtained. Fig 2,3,4,5,6 and 7shows the response surface contour plot for the production

of ethanol and interactive effects of substrate concentration, pH, temperature and cellulase loading on ethanol production. It is evident from the elliptical nature of the contours that the interaction between the individual variables is negligible. From all the figures, it is observed that the lower and higher levels of all the variables did not result in higher ethanol yields. Fig.2 shows that the ethanol concentration decreases with increases in substrate concentration at high temperature. The optimum values of variables obtained from regression equations for the production of ethanol is given in Table 6. Experiment is performed under the above optimized conditions in the fermentor and the experimental values are given in table7. Maximum ethanol production of 16.10 g/l corresponding to 56% of theoretical yield is obtained under optimum conditions. This value agrees closely with the values obtained from the response surface analysis confirming that the RSM using statistical design of experiments can be effectively used to optimize the process parameters and to study the importance of interactive effects of the test variables in the production of ethanol.

Table 2 Range and levels of the independent variables selected for the production of ethanol by DMC

Independent variable	Range and level				
	- 2	**-1**	**0**	**+1**	**+2**
Initial Substrate concentration (g/l), A	30	40	50	60	70
Initial pH, B	4	5	6	7	8
Temperature (°C),C	25	30	35	40	45
Cellulase loading (mg/g of substrate) (D)	5	10	15	20	25

Table 3. Orthogonal and real values of the independent variables along with observed and predicted responses for the production of ethanol by SSF

Run No	Orthogonal values				Ethanol concentration (g/l)	
	A	**B**	**C**	**D**	**Experimental**	**Predicted**
1	-1	-1	-1	-1	12.30	12.30
2	1	-1	-1	-1	15.30	14.38
3	-1	1	-1	-1	13.30	11.90
4	1	1	-1	-1	12.05	13.02
5	-1	-1	1	-1	8.30	8.49
6	1	-1	1	-1	9.20	9.79
7	-1	1	1	-1	11.00	11.01
8	1	1	1	-1	12.44	11.36
9	-1	-1	-1	1	14.00	14.82
10	1	-1	-1	1	15.10	15.00
11	-1	1	-1	1	13.02	12.33
12	1	1	-1	1	12.00	11.55
13	-1	-1	1	1	12.10	11.03
14	1	-1	1	1	9.30	10.44
15	-1	1	1	1	10.80	11.46
16	1	1	1	1	10.00	9.91
17	-2	0	0	0	13.00	13.56
18	2	0	0	0	14.30	14.09
19	0	-2	0	0	10.20	9.69
20	0	2	0	0	7.90	8.76
21	0	0	-2	0	12.50	13.20
22	0	0	2	0	8.10	7.75
23	0	0	0	-2	12.50	13.14
24	0	0	0	2	14.50	14.21
25	0	0	0	0	14.20	14.89
26	0	0	0	0	15.00	14.89
27	0	0	0	0	14.70	14.89
28	0	0	0	0	15.50	14.89

29	0	0	0	0	15.20	14.89
30	0	0	0	0	14.60	14.89
31	0	0	0	0	15.00	14.89

Table 4 Analysis of Variance (ANOVA) of quadratic model for the production of ethanol by SSF

Sources	Sum of square	df	Mean square	F	P – Value Prob > F
Model	143.24	14	10.23	11.72	< 0.0001
A-Initial Substrate Concentration	*0.42*	*1*	*0.42*	*0.48*	*0.4992*
B-pH	*1.30*	*1*	*1.30*	*1.49*	*0.2409*
C-Temperature	*44.64*	*1*	*44.64*	*51.13*	*< 0.0001*
D-Cellulase loading	*1.72*	*1*	*1.72*	*1.97*	*0.1805*
AB	*0.92*	*1*	*0.92*	*1.05*	*0.3217*
AC	*0.60*	*1*	*0.60*	*0.68*	*0.4213*
AD	*3.62*	*1*	*3.62*	*4.15*	*0.0598*
BC	*8.51*	*1*	*8.51*	*9.75*	*0.0070*
BD	*4.38*	*1*	*4.38*	*5.02*	*0.0407*
CD	*5.063E-004*	*1*	*5.063E-004*	*5.799E-004*	*0.9811*
A^2	*1.86*	*1*	*1.86*	*2.13*	*0.1652*
B^2	*54.55*	*1*	*54.55*	*62.49*	*< 0.0001*
C^2	*33.06*	*1*	*33.06*	*37.86*	*< 0.0001*
D^2	*2.43*	*1*	*2.43*	*2.79*	*0.1158*
Residual	13.10	15	0.87		
Lack of Fit	*12.02*	*10*	*1.20*	*5.60*	*0.0355*
Pure Error	*1.07*	*5*	*0.21*		
Cor Total	156.33	29			

R-Squared -0.9162 Adj R-Squared-0.8380 Pred R-Squared-0.747140

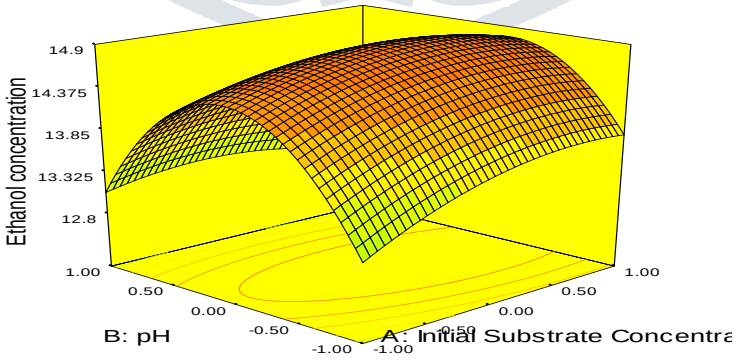

Fig 2 Response surface contour plot showing interactive effect of substrate concentration and pH on the production of ethanol by SSF

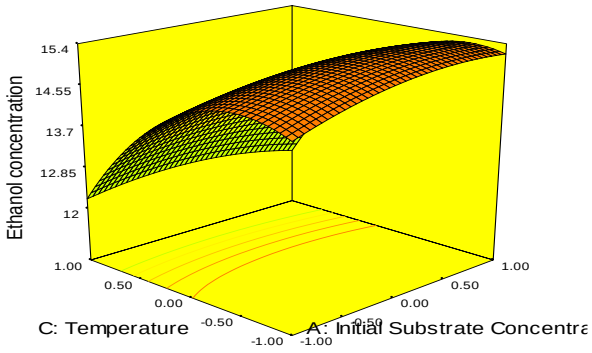

Fig 3 Response surface contour plot showing interactive effect of Substrate concentration and Temperature on the production of ethanol by SSF

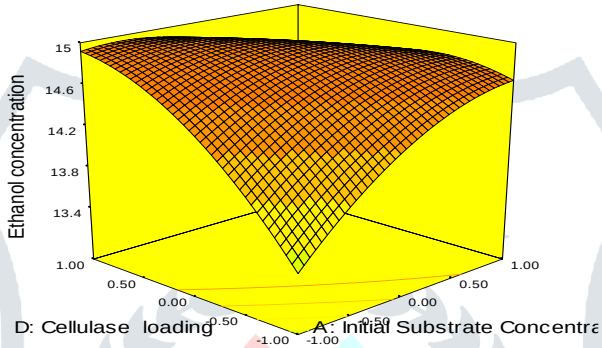

Fig 4 Response surface contour plot showing interactive effect of Substrate concentration and Enzyme loading on the production of ethanol by SSF

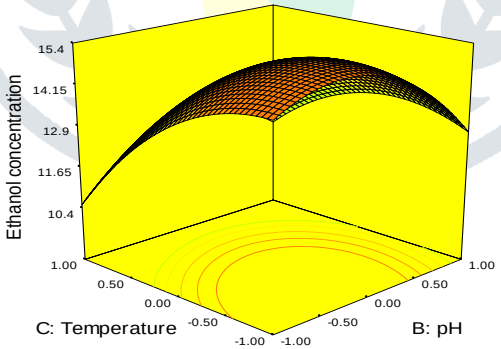

Fig 5 Response surface contour plot showing interactive effect of pH and Temperature on the production of ethanol by SSF

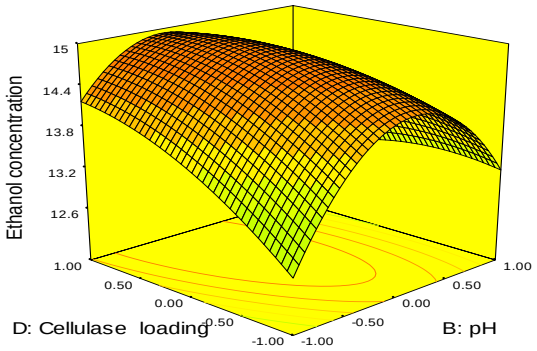

Fig 6Response surface contour plot showing interactive effect of pH and Enzyme loading on the production of ethanol by SSF

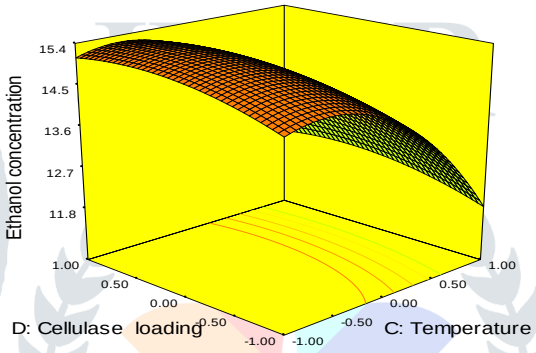

Fig 7 Response surface contour plot showing interactive effect of temperature and Enzyme loading on the production of ethanol by SSF

Table 5 Optimum values of variables obtained from regression equations for the production of ethanol by SSF

Parameter	Optimum value for Ethanol Production
Substrate Concentration (g/l)	50.20
pH	5.58
Temperature (°C)	31.26
Cellulase Loading (mg/g of substrate)	18.94
Ethanol Concentration (g/l)	15.55

Table 6. Production of ethanol by SSF under optimized conditions

S.No	Time (h)	Concentration (g/l)		
		Substrate	Biomass	Ethanol
1	0	48.7	1.1	0
2	6	47.1	1.5	1.60
3	12	41.3	2.0	4.50

4	18	30.7	5.0	7.00
5	24	25.0	6.9	9.20
6	30	20.2	8.2	11.30
7	36	15.0	9.6	13.20
8	42	10.2	10.2	14.80
9	48	7.9	10.5	15.90
10	54	7.6	10.7	16.10

Logistic Growth Model

The most widely used unstructured models to describe cell growth are the Monod kinetic model and the Logistic equation. Verlhurst in 1844 and Pearl and Reed in 1920 contributed to a theory which included an inhibiting factor to population growth. Assuming that inhibition is proportional to x^2, they used

$$\frac{dx}{dt} = kx(1-\beta x) \quad x(0) = x_o \qquad \ldots (2)$$

Where x is the biomass concentration (g/l), k is the rate constant (h^{-1}), and β is the Logistic constant. The Logistic curve is sigmoidal and leads to a stationary population of size $x_s = 1/\beta$. Eq. (2) is a Riccati equation which can be easily integrated to give the Logistic curve.

$$x = \frac{x_o e^{kt}}{1-\beta x_o\left(1-e^{kt}\right)} \qquad \ldots (3)$$

Where x_o is the initial biomass concentration (g/l) and t is time (h). The advantage of this model for ethanol fermentation is that it provides the exponential phase and endogenous metabolic phase accurately[12].

Product Formation Kinetics

The kinetics of product formation was based on the Leudeking-Piret equations. This model was originally developed for the formation of lactic acid by *Lactobacillus delbrucckii.* The classic study of Leudeking and Piret on the lactic acid fermentation by *Lactobacillus delbrucckii* indicated product formation kinetics which combined growth-associated and non-growth-associated contributions:

$$r_{f_p} = \alpha_{LP} r_{f_x} + \beta_{LP} x \qquad \ldots (4)$$

where r_{f_p} is the product formation rate, r_{f_x} is the biomass growth rate, α_{LP} and β_{LP} are the kinetic parameter of Leudeking-Piret model respectively.

This two parameter kinetic expression, often termed Leudeking-Piret kinetics, has proved extremely useful and versatile in fitting product formation data from much different fermentation. According to this model, the product formation rate depends upon both the instantaneous biomass concentration, x and growth rate, dx/dt, in a linear manner.The product formation constants α and β may vary with fermentation conditions.

$$\frac{dp}{dt} = \alpha \frac{dx}{dt} + \beta x \qquad \ldots (5)$$

Integration Eq. (5) with x given by Eq. (2) gives

$$p(t) - p_o - \beta\left(\frac{x_s}{k}\right)\left[1-\frac{x_o}{x_s}\left(1-e^{kt}\right)\right] = \alpha\left[x(t)-x_o\right] \qquad \ldots (6)$$

Substrate Utilization Kinetics

Substrate consumption depends on the magnitude of three sink terms, the instantaneous biomass growth rate, the instantaneous product formation rate and a biomass maintenance function. The substrate consumption rate can be modeled using Leudeking-Piret like equation that neglects the amount of carbon substrate used for product formation and maintenance constant, the model equation becomes:

$$-\frac{ds}{dt}=\frac{1}{Y_{x/s}}\frac{dx}{dt} \quad \ldots (7)$$

Integrating Eq. (9) with two initial conditions, $x=x_o(t=0)$ and $s=s_o(t=0)$ gives Eq. (10).

$$s=s_o-\frac{1}{Y_{x/s}}(x-x_o) \quad \ldots (8)$$

where $Y_{x/s}$ and $Y_{p/s}$ are the yield coefficient for the biomass and product respectively

Data analysis and Modeling

The kinetics of ethanol production by simultaneous saccharification and fermentation using *Saccharomyces cerevisiae* was studied under optimum process conditions obtained from CCD using RSM and modeling is attempted using different kinetic models. The kinetic parameters for biomass growth, substrate consumption and ethanol formation are evaluated by using Eq. (3), (6) and Eq. (8) with the experimental data.

The logistic model for microbial growth for its validity is tested using MATLAB 7.1 software. The logistic constants are obtained from the same tool. Kinetic parameter values obtained are then used to simulate the profiles of biomass, product and substrate concentration during fermentation. Logistic model predictions are carried out by solving the differential equations by Runge Kutta's numerical integration using ode solver in MATLAB 7.1 and the results are given in Table 7. Fig 8 shows that there is an excellent agreement between the experimental data and the simulation results, and the Logistic model appeared to provide adequate representation of growth and fermentation kinetics of *Saccharomyces cerevisiae*. A summary of model parameters are tabulated in Table 9. For each set of experimental data and for each of the variables x(t), p(t) and s(t), the error between the predicted and experimental values are calculated. A better prediction of biomass concentrations with high R^2 values of 0.9786wasobtained using Logistic model and it is most suited for ethanol production using sequential pretreated tapioca stem as substrate.

The Leudeking-Piret model for substrate utilization kinetics and product formation kineticare tested by graphical method using solver in MS Excel. Model predictions are carried out by solving the differential equations by Runge Kutta's numerical integration using ode solver in MATLAB 7.1 and the results are given in Table 8. Fig 9 shows the comparison of simulation results derived from substrate utilization kinetics, and the experimental data obtained for the production of ethanol using *Saccharomyces cerevisiae*utilizing sequential pretreated tapioca stem substrate. Better substrate utilization kinetics is obtained using Leudeking-Piret model. The simulation results are useful to predict the dynamics of substrate utilization and are well suited for ethanol production from sequential pretreated tapioca stem with a minimum error of 7.25% .Fig 10 shows the comparison of simulation results derived from product formation kinetics, and the experimental data obtained for the production of ethanol. The simulation results of product formation kinetics is in good agreement with the experimental data obtained from the production of ethanol with a minimum error of 8.88%.

Fermentation is very complex process, and it is often very difficult to obtain a complete picture of what is actually going on in a particular fermentation. All of the experimental results are found to be in good agreement with the theoretical predictions. The models presented in this work provide a good description of biomass, product and substrate concentrations.

Table 7 Experimental and predicted concentration of biomass, substrate and ethanol by *Saccharomyces cerevisiae*

S. No	Time (h)	Biomass Concentration (g/l)		Substrate Concentration (g/l)		Ethanol Concentration (g/l)	
		Experimental	Predicted	Experimental	Predicted	Experimental	Predicted
1	0	1.1	1.10	48.7	48.7	0.00	0.00
2	6	1.5	1.98	47.1	47.03	1.60	1.48
3	12	2	3.32	41.3	44.94	4.50	3.75
4	18	5	5.04	30.7	32.42	7.00	6.70
5	24	6.9	6.82	25	24.49	9.20	9.83
6	30	8.2	8.32	20.2	19.07	11.30	12.54
7	36	9.6	9.35	15	13.22	13.20	14.56
8	42	10.2	9.97	10.2	10.72	14.80	15.95
9	48	10.5	10.32	7.9	9.47	15.90	16.91
10	54	10.7	10.51	7.6	8.63	16.10	17.61

Substrate Concentration	-	50.20g/l
pH	-	5.58
Temperature	-	31.26°C
Cellulase Loading	-	18.94 mg/g of substrate

Table 8 Model parameters for ethanol production from sequential pretreated tapioca stem.

Logistic Model			Leudeking-Piret Model				
			Substrate Utilization Kinetics		Product Formation kinetics		
$k(h^{-1})$	β (l/g)	R^2	$Y_{X/S}$	Error%	α_{LP}	β_{LP}	Error%
0.1138	0.0934	0.9786	0.2396	7.25	1.62	0.00648	8.88

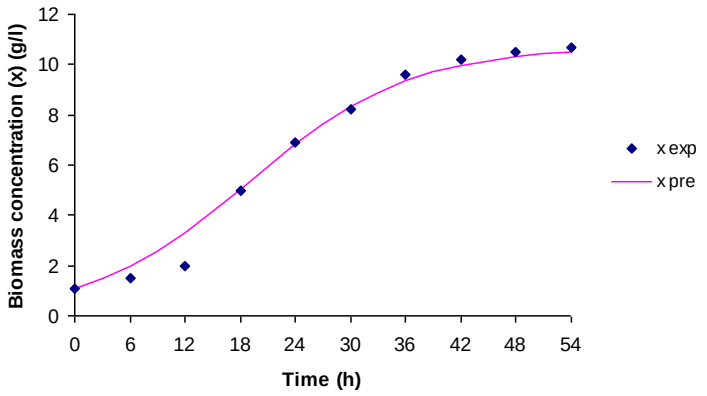

Fig 8Comparison between experimental and predicted microbial growth for *Saccharomyces Cerevisiae*

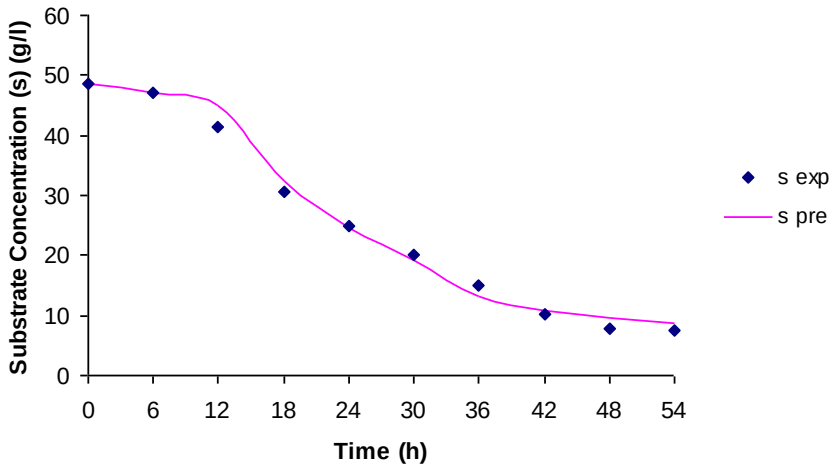

Fig 9Comparison between experimental and predicted substrate consumption for *Saccharomyces Cerevisiae*

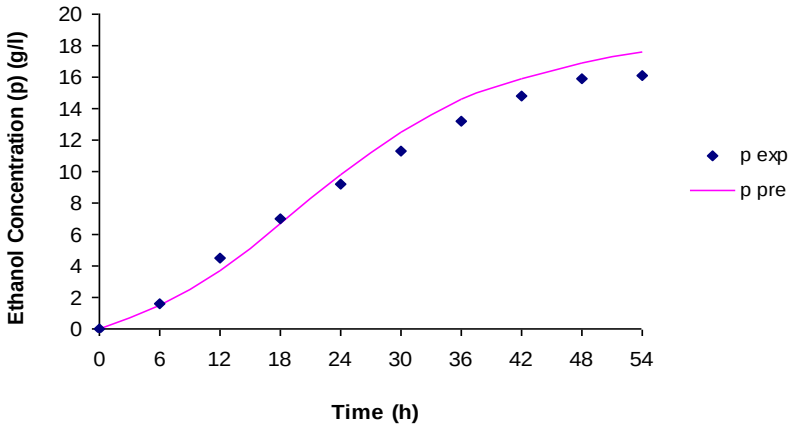

Fig 10Comparison between experimental and predicted product concentration for *Saccharomyces Cerevisiae*

Conclusions

Bioethanol production from sequential pretreated tapioca stem is studied by direct conversion *by Saccharomyces cerevisiae*. The effect of sodium hydroxide pretreatment on percentage hemicellulose solubilization and lignin reduction is studied by varying the concentration from 1.0% (w/v) to 2.0% (w/v) and residence time from 30 min to 90 min keeping the temperature constant at 120ºC. 54.66% of lignin reduction and 24.41% solubilization of hemicellulose are achieved for 2.0% sodium hydroxide concentration, 90 min residence time at 120ºC.A full factorial central composite design using response surface methodology is employed for ethanol production from alkali pre-treated tapioca stem in the direct conversion process by *Fusarium oxysporum* instead of using conventional optimization techniques. Under these optimized conditions, the predicted response for ethanol production is 15.55 g/l, and the observed experimental value is 16.10 g/l corresponding to 56% of the theoretical yield by DMC. The kinetics of ethanol production by direct conversion using *Saccharomyces cerevisiae*is studied under optimum process conditions obtained from CCD using RSM and modeling is attempted using different kinetic models. The Logistic model for cell growth, Leudeking-Piret model for substrate utilization kinetics and product formation kinetics are tested. All the experimental results are found to be in good agreement with the theoretical predictions and all the models presented in this work provide a good description of biomass, product and substrate concentrations.

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