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KINETICS AND MODELING OF PRE-TREATED CALOTROPIS GIGANTEA TO ETHANOL PRODUCTION BY DMC METHOD.

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ABSTRACT

The potential of microbial pretreatment of Calotropis gigantea by Fusarium oxy sporum to degrade lignin and facilitate fuel ethanol production was investigated under direct microbial conversion (DMC) method. Pretreatment of lignocellulosic biomass using sodium hydroxide is basically a delignification process, in which a significant amount of hemi cellulose is solubilized as well Maximum reduction in lignin of 54.66% is achieved for 2.0% sodium hydroxide concentration, 90 min residence time at 120^{o}C. The effect of initial substrate concentration, pH and temperature are identified as the major factors affecting ethanol production by DMC 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 33 g/l, pH of 5.52, temperature of 30.13°C. Maximum ethanol production of 9.3 g/l corresponding to 32% 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.

KEYWORDS: Ethanol, Calotropis gigantea, alkaline hydrolysis, direct microbial conversion (DMC), kinetics, modelling.

I. INTRODUCTION

Today the earth must deal with the consequences of global climate change and somehow meet expanding energy needs while limiting green house gas emissions. Energy consumption has increased steadily over the last century as the world population has grown and more countries have become industrialized. Fossil fuels especially, crude oil has been the major resource to meet the increased energy demand. Because the economy in many nations

depends on oil, the consequences of inadequate oil availability could be severe. Moreover, burning of fossil fuels causes the emissions of greenhouse gases, which is the major contributor to global warming. 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. The idea of using biofuel as an alternative to coal energy has existed since the industrial revolution. Of all biofuels, ethanol has been trusted as an alternative fuel for the future. Ethanol is a renewable fuel and is now widely used in the transportation sector with higher octane number as well as heat of vaporization [1-2]. Bio ethanol production has increased rapidly because many countries targeted towards reducing oil imports, boosting rural economies along with improving quality of air [3-4]. The use of corn for bio fuels raised debate over its potential interference with the food market. This gave rise to the use of non-food-based feed stocks such as agricultural and forest residues, municipal wastes, lingo cellulosic, and algal biomass for bio ethanol production. Unlike crude oil, biomass feed stocks are diverse in their composition. Hence, different conversion processes have been developed to produce a variety of bio fuels [5].

Ligno cellulose to ethanol conversion is a promising technology to supplement corn-based ethanol production [6-12].Conversion of agricultural waste into a value-added product can provide an environmentally sound method of disposal and avoiding simultaneous destruction of feeding and fruiting sites of boll weevils and other insects. However, the recalcitrant structure of lingo cellulosic materials necessitates a pretreatment step to break up the lingo cellulosic matrix, thus improving the accessibility of carbohydrates to hydrolytic enzymes for fermentable sugar production [13-16]. Pretreatment of lingo cellulosic biomass using sodium hydroxide is an alternative to sulphuric acid pretreatment. Alkali pretreatment is basically a delignification process, in which a significant amount of hemi cellulose is solubilized as well. The lignin content of raw Calotropis giganteais found to be 17.4% and is higher than most agricultural feed stocks such as corn cobs, wheat straw and switch grass, thus making the accessibility of cellulose polymers a challenge [17-18].This study includes pretreatment techniques using sodium hydroxide adopted for the pretreatment of Calotropis giganteaand its subsequent conversion to ethanol. The bioconversion of ethanol is attempted by Optimization of process parameters namely effect of substrate concentration, initial pH and temperature on

ethanol concentration by *Fusarium oxysporum* using Central Composite Design (CCD) using Response Surface Methodology (RSM).

MATERIALS AND METHODS

Microorganisms and Culture conditions

The fungal culture *Fusarium oxysporum* (MTCC 284) was obtained from IMTECH, Chandigarh, India. The stock culturewas maintained on potato sucrose agar medium with acomposition of scrubbed and diced potatoes 200g/l, sucrose20g/l and agar 20g/l at pH of 6.0 and 30oC. The production medium had the following composition per liter of distilled water: KH_2PO_4-2g; $MgSO_4$-0.3g; $CaCl_2$-0.3g; peptone-5g; yeast extract-3g; malt extract- 3g; $FeSO_4.H_2O$- 0.05g; $ZnSO_4.4H_2O$- 0.014g; $MnSO_4.4H_2O$- 0.016g; $CoCl_2$-2g; and known amount of Calotropis gigantea.

Raw material preparation

Calotropis gigantea, obtained from Allivilagam, Nagai district, Tamilnadu, India was used as raw material in this study. After collection, the Calotropis gigantea were crushed into small pieces and air-dried at 50^{o}C-55^{o}C in hot air oven. The dried materials were milled in a laboratory ball mill and screened through 100 mesh sizewas used for the production of ethanol.

RESULTS AND DISCUSSIONS

Effect of Sodium hydroxide pretreatment on Calotropis gigantean

The effect of sodium hydroxide pretreatment on percentage hemi cellulose 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^{o}C. After sodium hydroxide pretreatment of Calotropis gigantea, the solids are analyzed for cellulose, hemi cellulose and lignin contents and the results are compared with raw Calotropis gigantea and is given in Table 1which shows that the lignin content of pretreated Calotropis gigantea 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^{o}C) to 12.29% (30 min, 2.0%, 120^{o}C), 13.10% (60 min, 1.0%, 120^{o}C) to 35.98% (60 min, 2.0%, 120^{o}C) and 34.02% (90 min, 1.0%, 120^{o}C) to 54.66% (90 min, 2.0%, 120^{o}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^{o}C. Results from this study are comparable to those data given in literature [19].

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 Calotropis gigantea

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

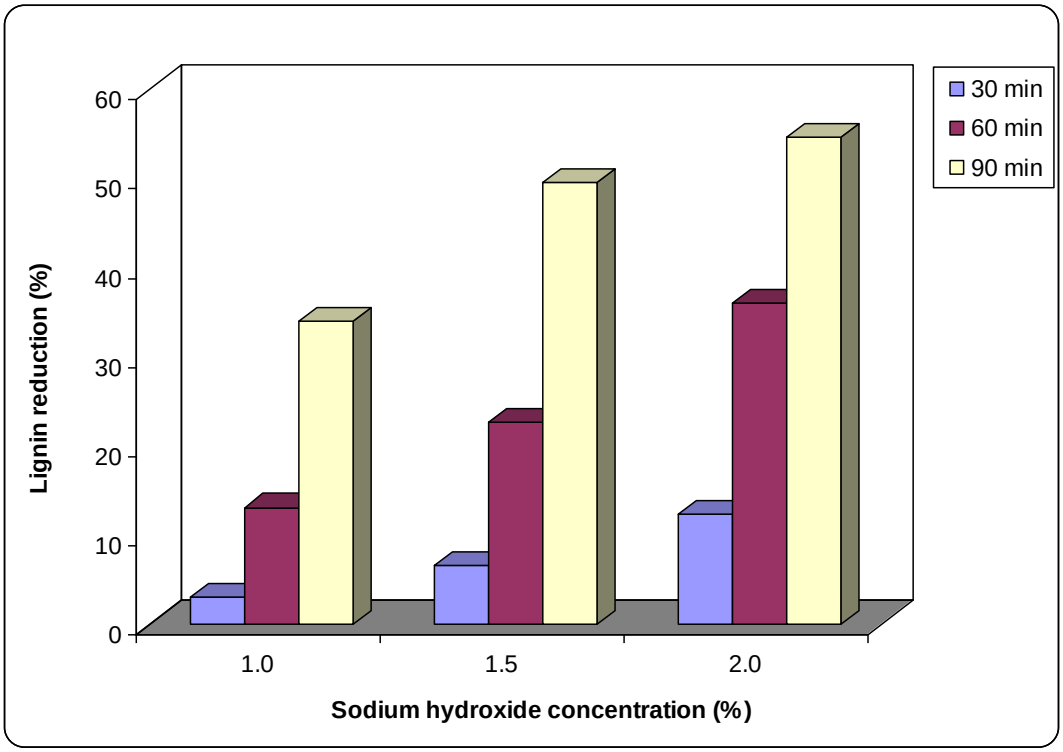

Figure1. Percentage Lignin reduction for sodium hydroxide pretreatment on Calotropis gigantea

Statistical Optimization of Process Parameters for Direct Microbial Conversion (DMC) Of Sequential Pretreated Calotropis gigantea to Ethanol by *Fusarium oxysporum*

Response surface methodology is very effective and popular tool to optimize the parameters having equal importance and influence each other in the process [20]. The initial substrate concentration (X_1) g/l, initial pH (X_2) and temperature (X_3) °C are chosen as the independent variables as shown in Table 2. Ethanol concentration (Y) is chosen as the dependent output variable. An orthogonal 2^3 full factorial central composite design with six star points (α=1.682) and six replication at the center point, all in duplicates, resulting in a total of 20 experiments are used to optimize the chosen key variables for the production of ethanol by DMC in a batch reactor.

Batch experiments are conducted as per the central composite design matrix for ethanol production in 250 ml Erlenmeyer flasks for 8 days. Confirmatory experiments are performed in a 2 litre fermentor (APPLIKON Biotech with BIOCONSOLE ADI 1025 controller, Holland), using optimum values of process variables obtained from response surface methodology. The fermentor is equipped with flat blade impeller, oxygen and pH electrodes, temperature and dissolved oxygen probe. The equipment is also monitored gas purging flow rate, pumping rates, antifoam addition and the vessel level. All processing parameters are online monitored, with the aid of BioXpert Lite 1.00 software. Samples are withdrawn periodically, centrifuged in a laboratory desktop centrifuge at 1200 rpm, the residue are analyzed for biomass concentration and the supernatants are analyzed for cellulose & ethanol concentrations.

Twenty 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 three 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 *Fusarium oxysporum* can be expressed in terms of the following regression equation (1):

$$Y = 8.60 - 0.24X_1 - 0.1388X_2 - 0.47X_3 - 0.31X_1^{\,2} + 0.3651X_2^{\,2} - 0.4897X_3^{\,2} + 0.250X_1X_2 + 0.064000X_1X_3 - 0.2650X_2X_3 \quad \ldots (1)$$

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 3. The goodness of fit of the model is checked by the determination coefficient (R^2). The value of R = 0.981 closer to one indicates that the correlation best predicts the performance the system and the values obtained by the correlation closely agrees with the experimental results. 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 are done by using both fisher's f-

test and student t-test statistical tools. The P values are used as a tool to check the significance of each of the coefficients, which in turn, may indicate the patterns of the interaction among the variable. Larger the magnitude of t and smaller the value of P indicate that the corresponding coefficient is more significant. In this case X_1, X_3, X_1^2, X_2^2, X_3^2 are significant model terms.

The effect of temperature is found to be highly significant ($p<0.001$) on ethanol production. It is found from the coefficient of X_3, the ethanol production is high at 30-35°C. Further increase in temperature gave less ethanol yield. Exposure to lower temperatures is known to give a high ethanol concentration. The squared effect of level of all parameters is also found to be significant. The coefficient of the interaction terms of substrate concentration and temperature ($p<0.001$) is found to be highly significant.

Table.4 shows the analysis of variance (ANOVA) summary of model for the production of ethanol. ANOVA is required to test the significance and adequacy of the model. The mean squares are obtained by dividing the sum of squares of each of the two sources of variations the model and the error variance, by the respective degrees of freedom. The fishers variance ratio, F value is the ratio of the mean square owing to regression to the mean square owing to error. It is the measure of variation in the data about the mean. Here, the ANOVA of these regression model demonstrates that the model is highly significant, as is evident from the calculated F value (57.89).

The graphical representations of the regression equation called the surface contour plot were obtained using the Minitab 15 software package. The response surfaces can be used to predict the optimum range for different values of the test variable 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 2-4 shows the response surface plot for the production of ethanol and interactive effects of initial substrate concentration, initial pH and temperature on ethanol production. It is evident from the circular nature of the contours that the interaction between the individual variables is negligible.

As the pH increases, the ethanol concentration decreases. This finding is in consistence with Mollision, 1993. The optimum pH 5.52 favors cell reproduction and growth of fungal culture. Lower pH levels ensure that the fungal functions under minimal internal stress and therefore

can ferment sequential pretreated tapioca stem into ethanol more efficiently. Moreover, the growth of harmful bacteria is retarded by acidic solution. It can therefore be concluded that a slightly acidic initial pH of 5.52, is optimal for fungal fermentation.

Ethanol production increases with increase in temperature and reaches a maximum value of 8.6 g/l at 30.13°C and thereafter decreases with further increase in temperature. This shows that the fungal cells are structurally sound and are capable of healthy and efficient reproduction. The growth rate of fungal culture *Fusarium oxysporum* is found to be high and able to utilize the substrate completely at all the temperatures. At low temperatures fungi tend to be less sensitive to the toxic effects of high alcohol concentration. It is assumed that the high temperature put a stress on the fungi as it reproduced. With reproduction slower and less efficient, there is less fungi to consume the available substrate. Excessive high temperatures may disrupt enzyme and membrane functions of the microorganism, resulting in stuck fermentation. This is in good agreement with work reported by other workers [21,22].

From Fig shows that the ethanol concentration decreasing with increasing substrate concentration. Maximum ethanol concentration is observed at an initial substrate concentration of 30 g/l. Similar results have also been observed for other lingo cellulosic such as softwood, weeds and bagasse [23].

The second – degree polynomial regression equation (1) is solved and the optimum values are obtained using the same software package. The optimum values of the test variables and the corresponding maximum ethanol production (8.64 g/l) in coded units as X_1= 0.3, X_2 =-0.5 and X_3= -1.001 given in appendix and the results are given in table 5.

Batch experiment is performed under the above optimized conditions and the experimental values are given in table 6. Maximum ethanol production of 9.3 g/l corresponding to 32% of theoritical 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 individual, cumulative and 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				
	- 1.682	**-1**	**0**	**+1**	**+1.682**
Initial Substrate concentration (g/l), X_1	10	20	30	40	50
Initial pH, X_2	4	5	6	7	8
Temperature (°C),X_3	28	30	32	34	36

Table 3 Central composite design matrix of orthogonal values along with observed responses for ethanol production by DMC

Exp No	Orthogonal Values			Response (Ethanol Concentration) (g/l)	
	X_1	X_2	X_3	Experimental	Predicted
1	0.00000	0.00000	0.00000	8.6	8.604
2	1.00000	1.00000	1.00000	8.0	7.927
3	-1.00000	1.00000	1.00000	7.9	8.101
4	0.00000	0.00000	-1.68179	6.5	6.451
5	1.00000	1.00000	-1.00000	7.0	7.191
6	0.00000	0.00000	0.00000	8.6	8.604
7	0.00000	0.00000	1.68179	8.1	8.025
8	-1.00000	-1.00000	-1.00000	6.1	6.261
9	0.00000	1.68179	0.00000	7.9	7.803

10	0.00000	-1.68179	0.00000	7.4	7.374
11	0.00000	0.00000	0.00000	8.6	8.604
12	0.00000	0.00000	0.00000	8.6	8.604
13	0.00000	0.00000	0.00000	8.6	8.604
14	0.00000	0.00000	0.00000	8.6	8.604
15	1.00000	-1.00000	1.00000	8.1	8.272
16	1.00000	-1.00000	-1.00000	7.5	7.386
17	-1.68179	0.00000	0.00000	7.4	7.339
18	-1.00000	1.00000	-1.00000	7.2	7.115
19	1.68179	0.00000	0.00000	8.2	8.138
20	-1.00000	-1.00000	1.00000	7.5	7.396

X_1 -Initial Substrate Concentration (g/l)

X_2 -Initial pH

X_3 - Temperature (°C)

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

Sources	Sum of Squares	df	Mean Squares	F Value	P – Value Prob > F
Model	10.12	9	1.12	57.89	<0.0001
A – Initial substrate	0.77	1	0.77	39.71	<0.0001
B – Initial pH	0.22	1	0.22	11.43	0.0070
C - Temperature	2.99	1	2.99	153.99	<0.0001
AB	0.55	1	0.55	28.38	0.003
AC	0.031	1	0.031	1.61	0.2334
BC	0.011	1	0.011	0.58	0.4642
A^2	1.35	1	1.35	69.43	<0.0001
B^2	1.86	1	1.86	95.59	<0.0001
C^2	3.36	1	3.36	172.86	<0.0001
Residual	0.19	10	0.019		
Lack of Fit	0.19	5	0.039		
Pure Error	0.000	5	0.000		
Cor Total	10.31	19			

R-Squared -0.9812 Adj R-Squared-0.9642 Pred R-Squared-0.8340

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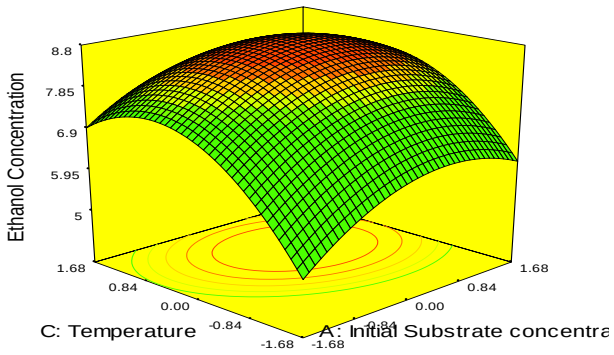

Fig 2 Response surface contour plot showing interactive effect of substrate concentration and temperature on the production of ethanol by DMC

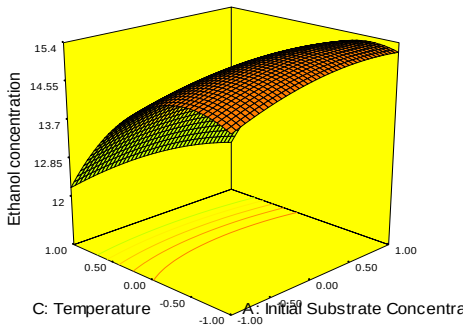

Fig 3 Response surface contour plot showing interactive effect of substrate concentration and temperature on the production of ethanol by DMC

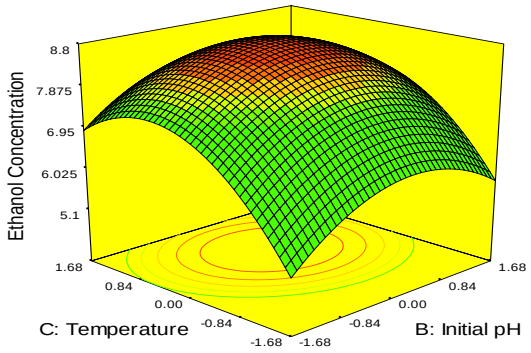

Fig 4 Response surface contour plot showing interactive effect of pH and temperature on the production of ethanol by DMC

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

Parameter	Optimum Value for Ethanol production
Substrate Concentration (g/l)	33.0
pH	5.52
Temperature(^{o}C)	30.13

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Table 6 Production of ethanol by DMC under optimized conditions

S.No	Time (h)	Concentration (g/l)		
		Substrate	Biomass	Ethanol
1	0	27.0	1.0	0
2	24	25.10	1.30	0.39
3	48	23.90	1.80	0.85
4	72	19.30	2.90	2.10
5	96	15.80	3.90	2.90
6	120	12.60	5.10	4.20
7	144	8.00	6.90	6.73
8	168	4.80	7.70	8.81
9	192	4.50	7.90	9.00

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.

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

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[24].

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

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

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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:

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

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

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

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 direct conversion using *Fusarium oxysporum*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 6. Fig 6 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 *Fusarium oxysporum*

. A summary of model parameters are tabulated in Table 7. 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 0.985 wasobtained using Logistic model and it is most suited for ethanol production using sequential pretreated Calotropis giganteaas substrate.

The Leudeking-Piret model for substrate utilization kinetics and product formation kineticare tested by graphical method. 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 6. Fig 7 shows the comparison of simulation results derived from substrate utilization kinetics, and the experimental data obtained for the production of ethanol using *Fusarium oxysporum* utilizing sequential pretreated Calotropis gigantean 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 Calotropis gigantean with a minimum error of 7.73% .Fig 8 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 9.36%.

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.

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Table 6 Experimental and predicted concentration of biomass, substrate and ethanol by *Fusarium oxysporum*

S. No	Time (h)	Biomass Concentration (g/l)		Substrate Concentration (g/l)		Ethanol Concentration (g/l)	
		Experimental	Predicted	Experimental	Predicted	Experimental	Predicted
1	0	1.00	1.00	27.00	27.00	0.00	0.00
2	24	1.30	1.58	25.10	24.92	0.39	0.37
3	48	1.80	2.38	23.90	22.04	0.85	0.92
4	72	2.90	3.38	19.30	18.43	2.10	1.70
5	96	3.90	4.48	15.80	14.46	2.90	2.72
6	120	5.10	5.54	12.60	10.66	4.20	3.97
7	144	6.90	6.43	8.00	7.48	6.73	5.41
8	168	7.70	7.09	4.80	5.10	8.81	6.98
9	192	7.90	7.54	4.50	3.48	9.00	8.63

Table 7 Model parameters for ethanol production from pretreated Calotropis gigantea

Logistic Model			Leudeking-Piret Model				
			Substrate Utilization Kinetics		I		ın kinetics
$k(h^{-1})$	β (l/g)	R^2	$Y_{X/S}$	Error%	α_{LP}	β_{LP}	Error%
0.2242	0.1208	0.9854	0.2779	7.73	0.1591	0.009	9.36

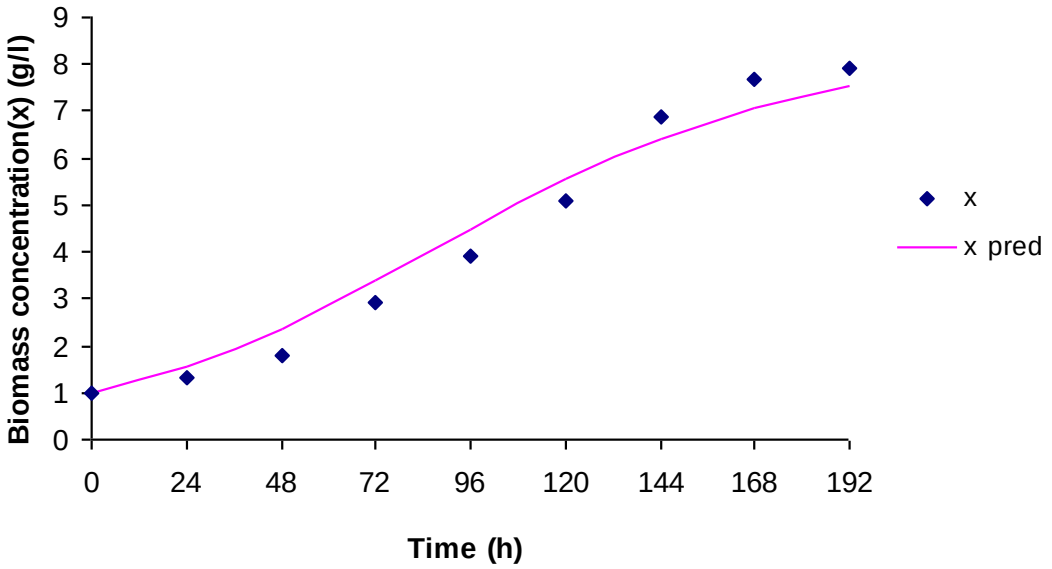

Fig 5 Comparison between experimental and predicted microbial growth(x) for *Fusarium oxysporum*

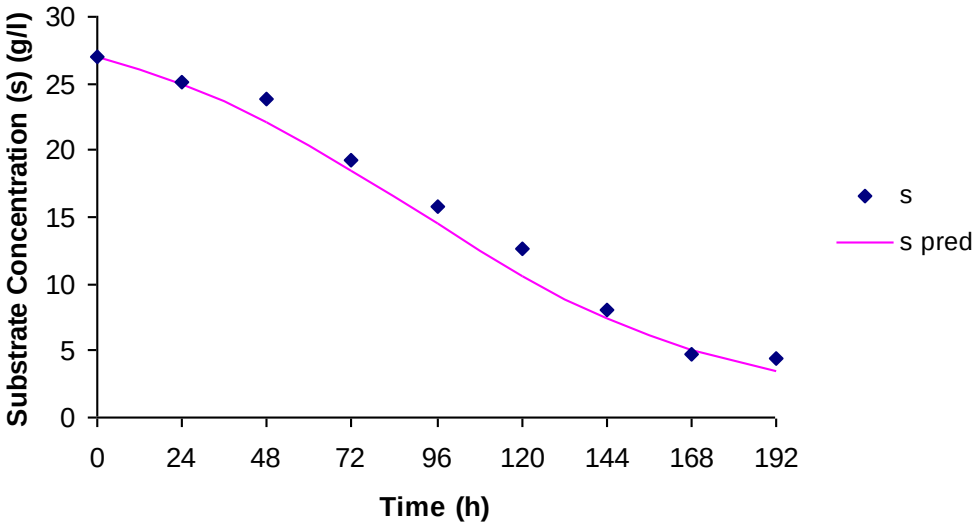

Fig 6.Comparison between experimental and predicted substrate(s) consumption for *Fusarium oxysporum*

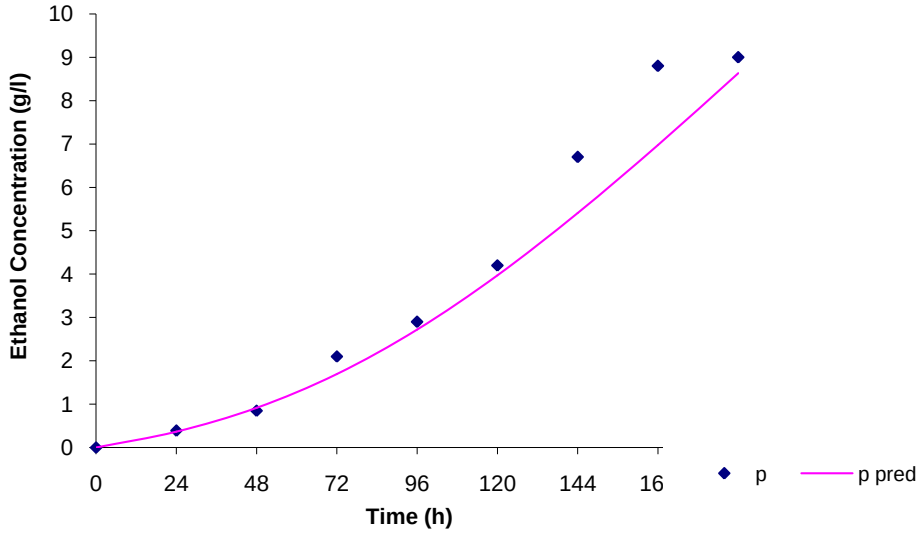

Fig 7Comparison between experimental and predicted product (p) concentration for *Fusariumoxysporum*

CONCLUSIONS

Bioethanol production from sequential pretreated Calotropis giganteais studied by direct conversion *by Fusarium oxysporum.* 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 120^{o}C. 54.66% of lignin reduction and 24.41% solubilization of hemi cellulose are achieved for 2.0% sodium hydroxide concentration, 90 min residence time at 120^{o}C.A full factorial central composite design using response surface methodology is employed for ethanol production from alkali pre-treated Calotropis giganteain the direct conversion process by *Fusarium oxysporum* instead of using conventional optimization techniques. Under these optimized conditions, the predicted response for ethanol production is 8.64 g/l, and the observed experimental value is 9.3 g/l corresponding to 32% of the theoretical yield by DMC. The kinetics of ethanol production by direct conversion using *Fusarium oxysporum*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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