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Simultaneous Saccharification and Fermentation of Tapioca Stem var. 226 White Rose to Ethanol by Cellulase Enzyme and *Saccharomyces cerevisiae*

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Abstract: The Simultaneous Saccharification and Fermentation (SSF) of tapioca stem var. 226 white rose to ethanol using cellulase enzyme and *Saccharomyces cerevisiae* were studied in a fermentor. The fermentation conditions were optimized by studying the effect of particle size, substrate concentration, pH and temperature. In this study, the sequential pretreated of tapioca stem using dilute acid and alkali showed significant hemicellulose and lignin degradation when compared with untreated tapioca stem. The optimum values of particle size, substrate concentration, pH and temperature were found to be 100 mesh size, 50 g/l, 5 and 35^{o}C respectively with the maximum ethanol concentration of 13.6 g/l. Logistic model for growth and Leudeking-Piret model for Substrate utilization kinetics were used for the production of ethanol and the model parameters were evaluated using experimental data.

Keywords: Ethanol, Tapioca stem, Acid hydrolysis, Alkaline hydrolysis, Simultaneous Saccharification and Fermentation (SSF), Substrate utilization kinetics.

1 Introduction

The bioconversion of abundant and renewable cellulosic biomass into ethanol as an alternative to petroleum is gaining importance due to the realization of diminishing natural oil and gas resources [1]. Lignocellulosic materials constitute a major part of available biomass in nature. However, lignocellulosic feedstock contains cellulose, hemicellulose and lignin which are more difficult to breakdown than starch [2]. In lignocellulosic feedstock both hemicellulose and cellulose components are sugar based chains that can be fermented into ethanol whereas lignin is a structural component to the plant that cannot be fermented into alcohol [3]. Therefore a pretreatment required to alter cellulosic biomass macroscopic and microscopic size and structure as well as its sub-microscopic chemical composition so that hydrolysis is of the carbohydrate fraction to monomeric sugars can be achieved more rapidly and with greater yields [4]. Physical, physico-chemical, chemical and biological processes have been used for pretreatment of Lignocellulosic materials. Among different pretreatments, acid and alkali pretreatments is the most popular process and to provide satisfactory conversion to ethanol [5].

Huge amounts of tapioca stems are currently burned or wasted. Utilization of these materials can significantly reduce the cost of raw materials for ethanol production. The conversion of Lignocellulosic materials to ethanol includes two processes: hydrolysis of cellulose in the Lignocellulosic materials to fermentable reducing sugars, and fermentation of the

sugars to ethanol. The hydrolysis is usually catalyzed by cellulase enzymes, and the fermentation is carried out by yeasts or bacteria. Removal of lignin and hemicellulose, reduction of cellulose cystallinity, and increase of porosity in pretreatment processes can significantly improve the hydrolysis [6].

Several different organisms have been proposed for use in fermenting sugars to ethanol, with different strains of the yeast, *Saccharomyces cerevisiae*, being the most widely used due to its robust growth rate and high ethanol tolerance [7]. In this study, hydrolysis of cellulosic feedstock is accomplished by cellulase enzyme. This enzymatic hydrolysis possesses the advantage that side reactions which convert some of the carbohydrates in cellulosic feedstock to non-fermentable sugars are virtually absent [8].

1.1 Kinetics and Modeling

The model employs rate equations for biomass (X), ethanol (P) and cellulose (S) to describe the fermentation process. The simplest types of product formation kinetics arise when there is a simple stoichiometric connection between product formation and substrate utilization of cell growth. In such fermentation, especially those involving secondary metabolites, significant product formation does not occur during the log phase where product formation is proportional to the growth rate of cells [9, 10]. The Logistic model is used for the growth kinetics which is represented by Eq. (1).

$$X = \frac{X_o e^{Kt}}{1 - \beta X_o \left(1 - e^{Kt}\right)} \quad \ldots (1)$$

where X_o is the initial biomass concentration (g/l), β and k are constants. The kinetics of ethanol formation was based on the Leudeking-Piret equation originally developed for the fermentation of gluconic acid. It is an unstructed model, which combines growth and non-growth associated contributions towards product formation. A carbon substrate such as cellulose is used to form cell material and metabolic products as well as for the maintenance of cell. Therefore, substrate consumption can be described by the following Eq. 2

$$\frac{dX}{dt} = \gamma_s = -\frac{1}{Y_{X/S}}\frac{dX}{dt} - \frac{1}{Y_{P/S}}\frac{dP}{dt} - m_s X \quad \ldots (2)$$

where $Y_{X/S}$ and $Y_{P/S}$ are the yield coefficient for the biomass and product, respectively and m_s is the specific maintenance coefficient. On simplifying, the above equation becomes,

$$\frac{dS}{dt} = \gamma_S = -\gamma\frac{dX}{dt} - \lambda X \quad \ldots (3)$$

where γ and λ are the parameters for growth and non-growth associated substrate consumption expressed in Eq. 4

$$\gamma = \left\{\frac{1}{Y_{P/S}} + \frac{\alpha}{Y_{P/S}}\right\} \qquad \lambda = \left[\frac{\beta}{Y_{P/S}} + m_S\right] \quad \ldots (4)$$

The overall objective was to obtain high yields of ethanol using sequential pretreated tapioca stem. Simultaneous saccharification and fermentation was done by using cellulase enzyme and *Saccharomyces cerevisiae*.

2 Materials and methods

2.1 Microorganisms and Culture conditions

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 30°C. 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.

2.2 Enzyme

Commercially available cellulase enzyme was obtained from SISCO Laboratories, Mumbai. The activity of cellulase was found to be 15 FPU/g of substrate and it was used throughout the experimentation. The cellulase activity was measured by standard Mandel's method [11].

2.3 Raw materials

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. The composition of the untreated tapioca stem was given in Table 1.

2.4 Preparation of raw substrate

After collection, the tapioca stems were crushed into small pieces and air-dried at 50°C in hot air oven. The dried stems were milled in a laboratory ball mill and screened through various mesh sizes.

The optimum mesh size which gives the maximum production of ethanol was used for further studies.

2.5 Sequential Pretreatment with dilute acid and dilute alkali

Ten grams samples of dried untreated tapioca stem were suspended in 80 ml of 1.25% (w/v) H_2SO_4 solution in a 250 ml beaker at 120°C for 17 min. After reaction, the residues were separated by centrifugation and washed extensively with water until neutral pH and dried at 55°C. Acid treated samples were then suspended in 20 ml of 2% (w/v) NaOH aqueous solution in a 100 ml beaker at 120°C for 90 min. The residues were separated by filtration in 100% polyester cloth, washed with water to remove residual alkali and dried at 55°C. The cellulose content was determined consecutively.

2.6 Batch fermentation studies

Batch fermentations were carried out in the 100ml fermentation medium containing known concentration of tapioca stem substrate. Cellulase loading 15 FPU/g of substrate concentration and 2% (v/v) inoculum medium was transferred to 100 ml production medium under sterile conditions. The flask was gently agitated on a shaker with a constant shaking rate at 150 rpm. Samples were taken from the solution at regular time intervals for the analysis of biomass concentration, cellulose concentration and ethanol concentration. Biomass concentration was determined by centrifuging the samples at 5000 rpm, dried and weighed. Ethanol concentration in the fermented broth was estimated using NUCON 5765 Gas Chromatography with a flame ionization detector. The cellulose content was measured by Anthrone reagent method [12] using Bio-Spectrophotometer (ELICO BL 198) at 630 nm. The hemicellulose and lignin content were also measured [12].

3 Results and Discussion

3.1 Effect of pretreatment on composition of Tapioca stem

Fig 1 represents the composition profile of untreated, acid treated and acid-alkali treated tapioca stem particles. The composition profile changes in cellulose, hemicellulose and lignin after pretreatment was measured to evaluate the pretreatment performance. Fig 1 shows that high cellulose content of 89.49 % was achieved for acid-alkali treated particles. From Table 1, 91.60% of the hemicellulose was reduced after sequential pretreatment. An advantage of largely removing hemicellulose during pretreatment is the reduced possibility of inhibiting enzymatic hydrolysis and subsequent fermentation by *Saccharomyces cerevisiae* [13]. 70% losses were obtained for lignin after alkaline pretreatment. The purpose of the alkaline pretreatment was delignification. The amount of weight lost following chemical pretreatment of residue was due to lignin removal which is represented in Table 1 [14].

Table 1 Chemical composition of sequential pretreated tapioca stem particles.

Component	Composition of untreated tapioca stem(%) (w/w)	Mass recovered after acid pretreatment (g)	Losses after acid treatment (%) (w/w)	Composition of acid treated tapioca stem(%) (w/w)	Mass recovered after alkaline pretreatment (g)	Losses after alkaline treatment (%) (w/w)	Total losses after sequence of treatments (%) (w/w)	Composition of acid and alkali treated tapioca stem (%) (w/w)
Cellulose	56.40	52.76	6.45	65.30	51.30	2.80	9.00	89.49
Hemicell-ulose	20.20	9.54	52.75	11.81	1.70	82.10	91.60	2.98
Lignin	17.40	16.47	5.45	20.39	3.40	79.40	80.50	5.99
Others	6.00	2.02	66.89	2.50	0.60	70.00	90.20	1.54

3.2 Scanning electron microscopic (SEM) observations of tapioca stem particles

The internal structure of tapioca stem was observed using SEM analysis to study the effects of sequential pretreatment and possible disruption of the cell wall. Fig 2, Fig 3 and Fig 4 shows the morphological structure of untreated, acid treated and acid-alkali treated samples. Fig 2 clearly indicates that all the components are tightly packed like thick mat which represents the crystalline structure of cellulose. After acid treatment, the particles structure was slightly modified which is indicated in Fig 3. Small holes in the internal structure clearly indicate the partial degradation of hemicellulose structure which significantly improves the cellulose hydrolysis. When treated with dilute acid; most of the hemicellulose is hydrolyzed leaving a porous structure of primarily cellulose and lignin that is more accessible to enzymatic action [15]. The acid pretreatment also results in enlargement of the inner surface area of substrate particles, accomplished by partial solubilization and/or degradation of hemicellulose and lignin. This leads the fractionation of the three components and opening of cellulose structure [16]. The internal structures of acid treated particles were further modified when treated with dilute alkali. The breakdown of crystalline structure of cellulose and the increase in porosity was clearly seen from Fig 4. Sodium hydroxide besides converting lignin to its more soluble sodium derivative, removes low molecular weight carbohydrate components from the cell wall structure, thereby causing an enlargement of the pores in the lignocellulosic substrate and an improvement in the accessibility to reagents [17]. It was concluded that sequential pretreatment with dilute acid and alkali to remove hemicellulose and lignin can enhance the hydrolysis of cellulose.

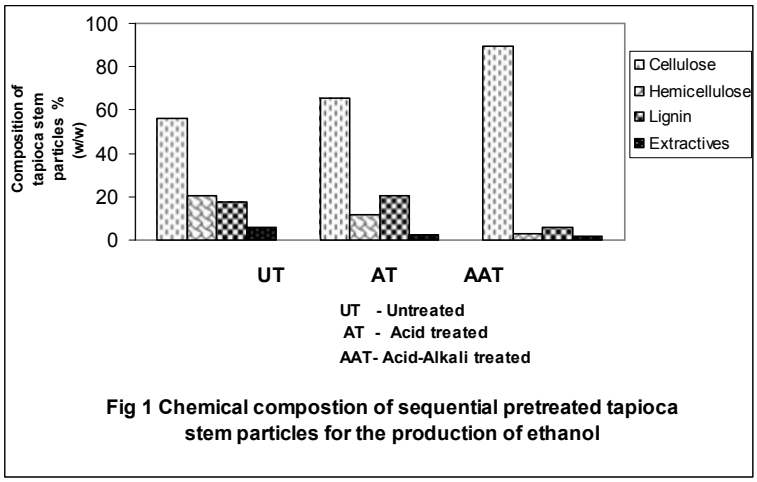

Fig 1 Chemical compostion of sequential pretreated tapioca stem particles for the production of ethanol

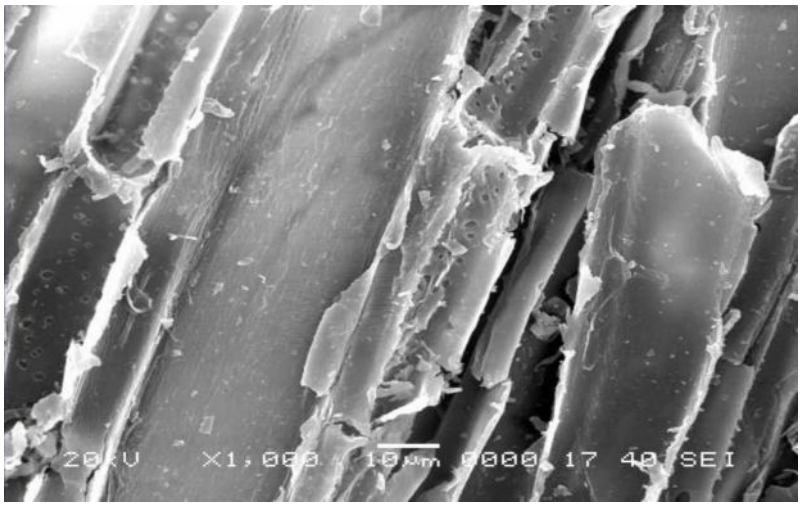

Fig 2 Morphological structure of untreated tapioca stem.

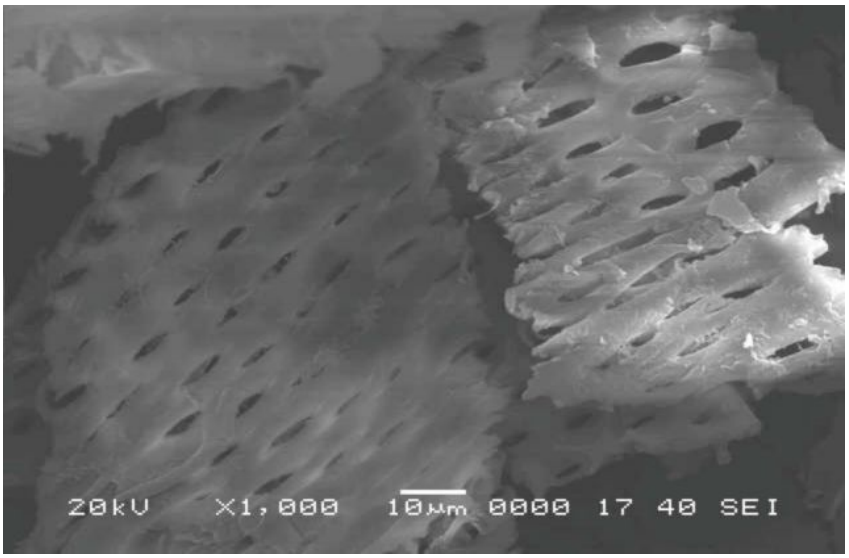

Fig 3 Morphological structure of acid treated tapioca stem.

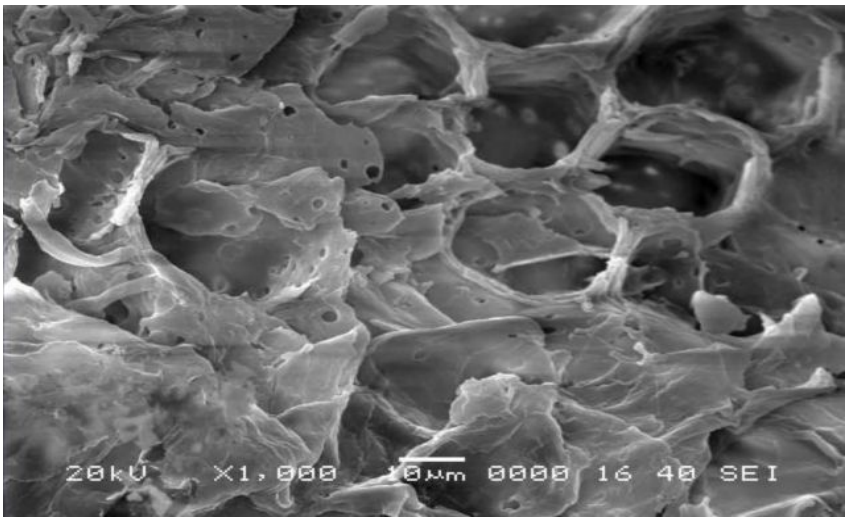

Fig 4 Morphological structure of acid-alkali treated tapioca stem.

3.3 Effect of Particle size on the production of ethanol

The effect of particle size on the production of ethanol was carried out in 100 ml fermentation medium by varying the mesh size namely 60, 100 and 150 respectively with 30g/l substrate concentration, inoculum concentration of 2 %(v/v) and cellulase concentration of 1.5% (v/**v).** Fig. 5 indicates the effect of particle size of sequential pretreated ball milled tapioca stem particles on the production of ethanol. Maximum ethanol concentration was obtained in 2 days when the mesh size was 100 which have the average diameter of 0.197 mm. It was concluded that maximum ethanol concentration of 8.02 g/l and easy access of microorganisms to cellulose was achieved at 100 mesh size particles.

3.4 Effect of substrate concentration on the production of ethanol

The effect of substrate concentration on ethanol concentration was studied by varying the substrate from 30 g/l to 70 g/l, keeping all the other parameters constant at temperature 30^{o}C and pH 5.0. The results are shown in Fig 6. The rate of ethanol concentration increased with increase in initial substrate concentration up to 50 g/l with a maximum yield of 8.6 g/l and the yield decreased with further increase in the substrate concentration. The decreased conversion may be due to insufficient amount of enzyme and microorganism.

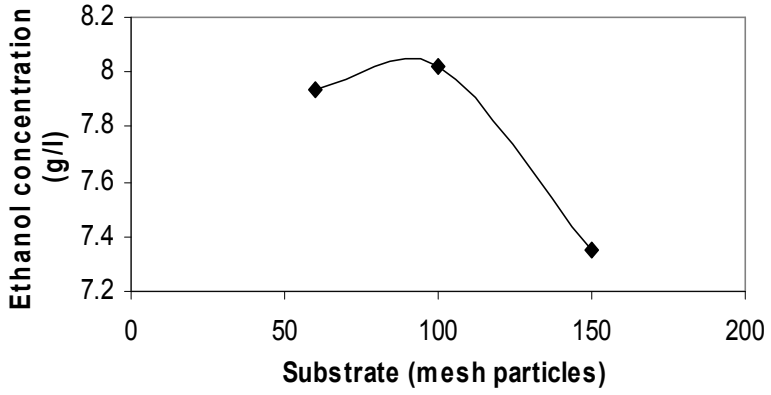

Fig 5 Effect of particle size on ethanol concentration

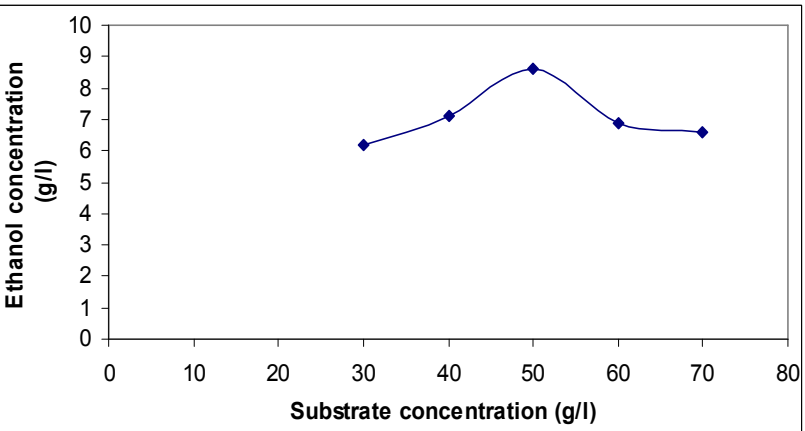

Fig 6 Effect of substrate concentration on ethanol production

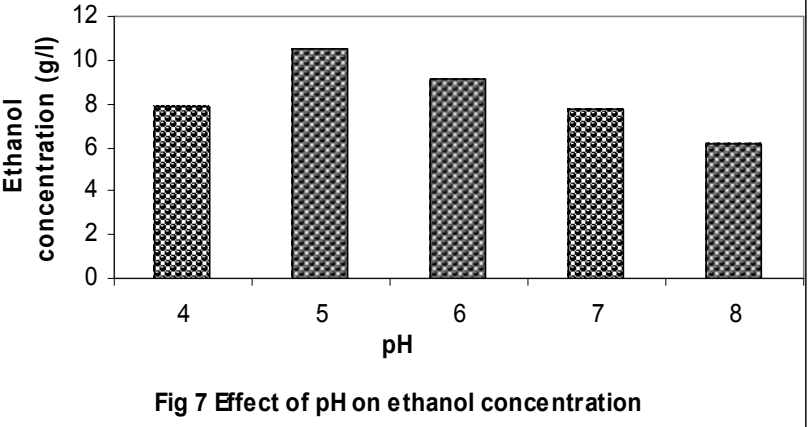

Fig 7 Effect of pH on ethanol concentration

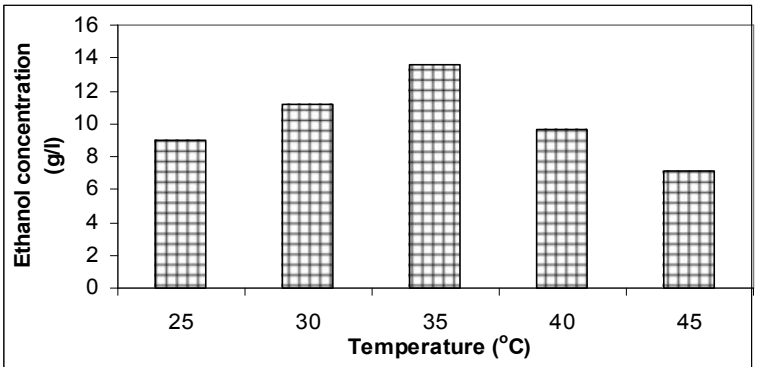

Fig 8 Effect of temperature on ethanol concentration

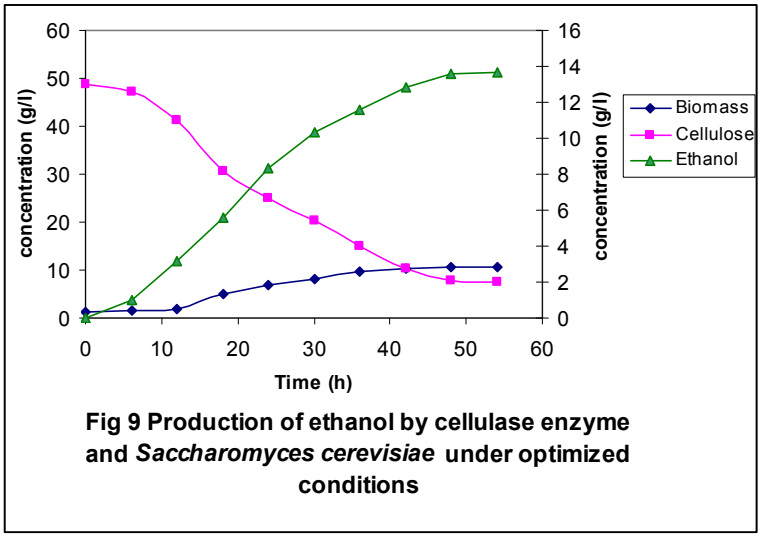

Fig 9 Production of ethanol by cellulase enzyme and *Saccharomyces cerevisiae* under optimized conditions

3.6 Effect of temperature on the production of ethanol

Effect of temperature was studied by varying the temperature from 25°C to 45°C at 50 g/l substrate concentration and a pH of 5.0**.** Fig 8 shows the effect of temperature on ethanol production by cellulase enzyme and *Saccharomyces cerevisiae*. The ethanol concentration was found to be high at 35°C and thereafter decreases with further increase in temperature. When the temperature increased above 40°C, a significant contamination was observed during the experiments. Fig 9 indicates the production of ethanol, biomass concentration and substrate consumption under optimized conditions. As time increases the ethanol and biomass concentration also increases whereas the cellulose concentration decreases with increase in time. The maximum ethanol concentration of 13.6 g/l, the maximum biomass concentration of 10.7 g/l was obtained with a maximum substrate utilization of 7.6 g/l. The yield factor Yx/s was found to be 0.239.

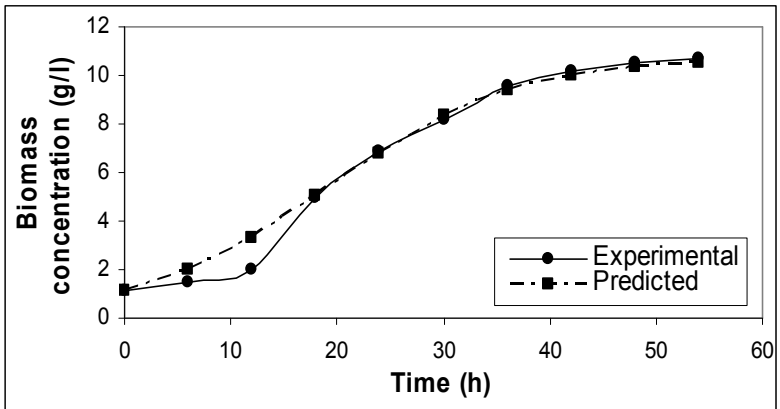

Fig 10 Comparison between experimental results and logistic growth model

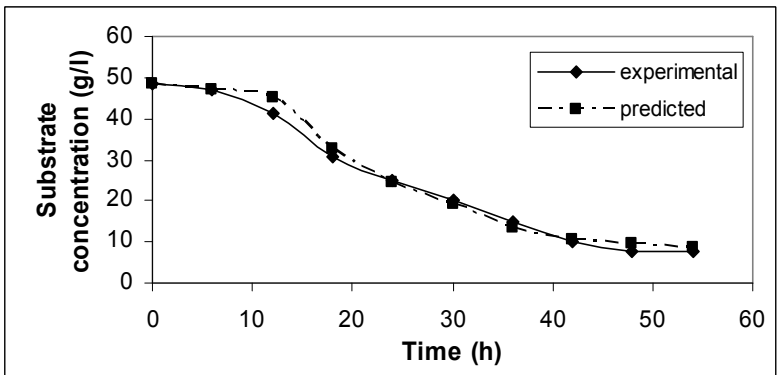

Fig 11 Comparison between experimental results and substrate utilization kinetics

3.7 Kinetic modeling for the production of ethanol

The kinetic data obtained were used to evaluate the model parameters in the Logistic growth model and Substrate utilization kinetics. The model parameter values obtained were then used to simulate the model to predict the concentration of biomass and substrate. Fig 10 & Fig 11 shows the experimental and predicted concentration of biomass and substrate data for Logistic growth model and Substrate utilization kinetics. The kinetic parameter values of k and β for Logistic growth model were found to be 0.114 and 0.093 respectively with the regression coefficient of 0.9786. The parameter values of γ and λ for Substrate utilization kinetics were found to be 0.232 and 0.289 respectively. Both models are a reasonable representation of the fermentation process.

4 Conclusions

Ethanol could be produced efficiently by simultaneous saccharification and fermentation of tapioca stem var. 226 white rose using cellulase enzyme and *Saccharomyces cerevisiae.* The sequential pretreatment of tapioca stem using dilute acid and dilute alkali was carried out before SSF to modify the internal structure of cellulose in tapioca stem. The optimum values of particle size, substrate concentration, pH and temperature were found to be 100 mesh size, 50 g/l, 5 and 35^{o}C respectively with the maximum ethanol concentration of 13.6 g/l. The kinetic parameters were determined using Logistic growth model & Substrate utilization kinetics and both the models fit the experimental data very well.

References

1. Sudha rani K, Swamy M V, Seenayya G., Production of ethanol from various pure and natural cellulosic biomass by *Clostridium thermocellum* strains SS21 and SS22, Proc Biochem., 1998, 33(4), 435–440.
2. Erika Felix, David R Tilley., Integrated energy, environmental and financial analysis of ethanol production from cellulosic switchgrass, Energ., 2009, 34, 410–436.
3. Van Zessen E, Weismann MRR, Bakker HW, Elbersen JH Reith, Denvil H., Lignocellulosic-ethanol: a second opinion. Report no: 2GAVE-03.11, Apelsdoorn, The Netherlands, 2003.
4. Nathan Mosier, Charles Wyman, Bruce Dale, Richard Elander, Lee Y Y, Mark Holtzapple, Michael Ladisch., Features of promising technologies for pretreatment of lignocellulosic biomass, Bioresour Technol., 2005, 96, 673–686.
5. Zhu J Y, Pan X J., Woody biomass pretreatment for cellulosic ethanol production: Technology and energy consumption evaluation, Bioresour Technol., 2010, 101, 4992–5002.
6. McMillan J D., Pretreatment of lignocellulosic biomass. In: Himmel M E, Baker J O, Overend R P. (Eds). Enzymatic conversion of biomass for fuels production. American Chemical Society, Washington, DC, 1994, 192–324.
7. Ingledew W M., The biochemistry of alcohol production. In: Lyons T P, Kelsall D R, Murtagh J E. The alcohol Text book. Nottingham, UK: Nottingham University Press, 1995, 55–79.
8. Ann C Wilkie, Kelly J Riedesel, John M Owens., Stillage characterization and anaerobic treatment of ethanol stillage from conventional and cellulosic feedstocks, Biomass Bioenerg., 2000, 19, 63–102.
9. Bailey J E, Ollis D F., "Biochemical Engineering Fundamentals", McGraw-Hill, New York.1986
10. Zand H, Blazej M, Bales V & Markos J., A kinetic model for gluconic acid production by Aspergillus nier. Chem Pap, 2004, 58: 23-28.
11. Mandel M, Andreotti R, Roche C., Measurement of saccharifying cellulase. Biotechnol Bioeng Symp, 1976, 6:21–33.
12. Sadasivam S, Manickam A., "Biochemical Methods" 2nd edn., New Age International Publishers, New Delhi, 1996
13. Lynd L R., Overview and evaluation of fuel ethanol from cellulosic biomass, Annual Review of Energ Environ., 1996, 21, 403–465.
14. Wyman C E., Ethanol production from lignocellulosic biomass: Overview; Handbook of Bioethanol: Production and Utilization, Wyman C E (ed), Taylor & Francis, Washington, DC, 1, 1996, 11-12.
15. Janusz Szczodrak, Jan Fiedurek., Technology for conversion of lignocellulosic biomass to ethanol, Biomass Bioenerg., 1996, 10(5/6), 367–375.
16. Ashok Pandey, Carlos R Soccol, Poonam Nigam, Vanete T Soccol., Biotechnological potential of agro- industrial residues I: Sugarcane bagasse, Bioresour Technol., 2000, 74, 69-80.
17. Marton R, Granzow S., Ethanol-alkali pulping, Tappi., 1982, 65, 103–106.

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