

Investigations on the Kinetics of Acid Catalyzed Hemicellulose Hydrolysis Derived from Waste Rice Straw for the Production of Bio-Ethanol

Anuradha Mukherjee¹, Tamal Mandal², Amit Ganguly³,
Prem Sunder Ghosh² and Pradip K. Chatterjee^{3*}

¹NSHM Knowledge Campus, Durgapur - 713212, West Bengal, India;
anuradha0061@gmail.com¹

²National Institute of Technology, Durgapur - 713209, West Bengal, India;
tamal_mandal@yahoo.com, premrock999@gmail.com

³CSIR Central Mechanical Engineering Research Institute, Durgapur - 713209, West Bengal, India;
pradipcmeri@gmail.com, amitganguly022@gmail.com

Abstract

Objectives: To understand the monophasic kinetic behavior of hemicellulose hydrolysis and determination of thermodynamic parameters involved in dilute acid pretreatment by tartaric acid.

Methods/Statistical Analysis: Biomass samples from thatched roofs were collected and analyzed for cellulose, hemicellulose and lignin through Neutral Detergent Fibre (NDF), Acid Detergent Fibre (ADF) and Acid Detergent Lignin (ADL) methods. The ground biomass was hydrolysed with tartaric acid, saccharified applying cellulase, xylanase at different time/agitation speed for obtaining reducing sugar and finally fermented for ethanol yield. The kinetics of hemicellulose hydrolysis was critically studied in the present work. **Findings:** The maximum yield of xylose and reducing sugars after hydrolysis and saccharification at optimum conditions were 134 mg/g and 494.8 mg/g using tartaric acid as hydrolyzing media. *Pichia stipitis* was very effective for conversion of pentoses and hexoses during fermentation. The maximum ethanol yield of 25.1% using tartaric acid was obtained after fermentation. Overall, the present works depicts the introduction of a new hydrolytic agent, tartaric acid and a comprehensive view of the complete bio processing of waste rice straw to bioethanol. The amount of ethanol yield was from 17% to 25.1%. It was noticed that the ethanol content was directly proportional with the increase in age of the samples. An increasing trend of ethanol with time was visible until 9th hour. High ethanol yield was obtained in this study since *Pichia stipitis* strain could utilize both hexose sugars and pentose sugars together. **Applications/Improvements:** Thatched rice straw roof can be better utilized for ethanol making with natural degradation of lignin due to aging instead of increasing environment pollution using significantly less synthetic enzymes.

Keywords: Bioethanol, Hydrolysis, Kinetics, Thermodynamics, Waste Rice Straw

1. Introduction

Increased utilization of fossil fuels, owing to rapid industrialization has eventuated a hike in the price of resources and resulted in inadequate supply of nonrenewable energy resources across the globe¹. Over utilization, the nonrenewable source of energy to cope with socioeconomic growth at present has led to deadly circumstances like global warming. To overcome this

situation, a more preferred renewable substitute is a biofuel which is derived from lignocellulosic biomass². Processing of reducing sugars by fermentation results in the formation of bioethanol, which acts as an inexhaustible ecofriendly alternative source. Lignocellulosic biomass is an efficacious feedstock which constitutes major benefits such as environmentally friendly, inexhaustibility, carbon neutrality and cheap³. Furthermore, second-generation biofuels are not only competent to overcome the harmful

* Author for correspondence

environmental effects and the rivalry between “food vs. fuel” but also has enhanced energy efficiency in contrast to first generation biofuels⁴. In India, boiled rice being the staple food authorizes a primary fraction of the desired diet. FAO statistics approve the yearly production of rice to be 650 million tonnes as in 2007. According to Maiorella (1985), around 11.5 kg of rice straw is correlated with each kilogram of rice grain produced. In the recent past, the term global warming has assumed new importance across the globe. In order to destroy rice straw by burning a boost in air pollution is noticed; hence, if this abundant waste component is utilized for the production of bioethanol it may highly reduce the stress on food crops. The present work aims at the production of bioethanol via high biodegradation of waste rice straw acquired from thatched roofs. The process of formation of bio ethanol from lignocellulosic biomass fundamentally comprises of pretreatment, saccharification and fermentation. The rice straw generally comprises of cellulose (32-47%), hemicellulose (19-27%) and lignin (524%)^{5,6,7}. Process of fermentation when effectively implemented results in the conversion of cellulose and hemicellulose fraction of waste rice straw to bioethanol. Hemicellulose being a heteropolysaccharide is intricate in nature. It is the second most abundant material of lignocellulosic biomass prior to cellulose, which mainly comprises of pentoses, hexoses and uronic acids⁸. Fermentation is inhibited by a necessary ingredient, lignin. As a result, there is a need to develop an efficient pretreatment strategy to enhance delignification, an increment of porosity and thereby boosting up the yield of ethanol.

Hemicellulose mainly comprises of xylan, made up of xylose units. Hemicellulose on depolymerization produces xylose, which undergoes fermentation and produces Bioethanol effectively⁹⁻¹¹. Furthermore, the hemicellulose portion of the biomass is quite productive for the formation of a number of byproducts such as furfural, furfuryl alcohol, xylitol¹² and bio plastics derived furans⁹. The rice straw residues, when pretreated with dilute acid, undergo changes like the increase in porosity, etc. with almost no or minimal changes in cellulose and lignin fraction.

In this work, the following factors such as 1. Optimal use of dilute acid pretreatment technology with tartaric acid, 2. Improvement of Bioethanol production by optimizing xylose and reducing sugar yield through series of experiments, 3. Analysis on kinetic modeling of hemicellulose hydrolysis for gathering more detailed

perception of the intricate chemical reactions¹³⁻¹⁵.

The study of the kinetics of hemicellulose hydrolysis is of utmost importance for the appropriate design of equipment and in depth mechanistic understanding of the procedure.¹⁶, established a model on hemicellulose hydrolysis, which includes a two-step process, found to have a first order dependence on reactant concentration. On the other hand, the reaction rate constants are interrelated along with Arrhenius temperature.

Investigations on the hemicellulose hydrolysis of lignocellulosic biomass have been reported earlier¹⁷⁻¹⁹. The overall purpose of this work was to understand the monophasic kinetic behaviour of hemicellulose hydrolysis and determination of thermodynamic parameters involved in dilute acid pretreatment by tartaric acid, which was implemented by performing a number of experiments.

2. Materials and Methods

2.1 Biomass Feedstock and Compositional Analysis

Waste rice straw samples were collected from thatched roofs from an adjoining village at Durgapur existing over there for a lengthy time interval (six months, one year, three years, four years approximately). The collected samples experienced the sun drying for a week and decisively the amount of leftover moisture was eliminated in a tray dryer for a period 4 h kept at 60°C. The dried samples were subjected to grind prior to storage in airtight containers. The finely ground samples were then applied for conducting experiments. Primary constituents such as cellulose, hemicellulose and lignin were investigated for the biomass. Evaluation of hemicellulose and lignin was executed by Neutral Detergent Fibre (NDF), Acid Detergent Fibre (ADF) and Acid Detergent Lignin (ADL) methods²⁰. Anthrone test was applied for quantification of actual cellulose²¹.

The finely ground samples of rice straw were combined with a 10ml concentration of tartaric acid (10% loading) and then incubated at different time intervals, variable agitation speed and at distinct soaking temperatures. The samples after incubation were regulated by moist heat (autoclaved at 15 psi, 121°C, for 5 minutes). In order to eliminate the unhydrolysed material, all the pre-treated samples were filtered applying what man's filter paper No. 1. The xylose-containing hydrolysate was collected for

the estimation of xylose content applying Phloroglucinol assay by Trinder Method. The optical density of xylose solution was calculated in UV spectrophotometer, and the absorbance was noted at 554 nm.

2.2 Saccharification

The pretreated samples having the optimum yield of xylose and reducing sugar were dealt with saccharification applying cellulase from *Trichoderma reesei* and xylanase from *Trametes versicolor*. After adding the enzyme in the media, the conical flasks were incubated at 50°C, for a period of 60 h at (125-130) rpm. Sampling was performed at an interval of 6 h each and investigated for the existence of reducing sugars and pentoses applying various analytical techniques to optimize the time taken to reach the saturation point. Assessment of reducing sugar in the hydrolysate was accomplished applying DNS method²² and the evaluation of pentose sugars (xylose) in the hydrolysate was performed by modified Tollen's method²³. Further bio processing was performed for the selected samples with optimized reducing sugar and xylose yield.

2.3 Fermentation

Pichia stipitis (NCIM 3500) was utilized for conducting fermentation as it is competent of using both xylose (pentoses) and glucose (hexoses). The inoculated flask was incubated at a BOD incubator temperature being maintained at 30 °C on a rotating shaker at an agitation speed of 125 rpm. There after incubating for 20 hrs, the broth was kept on centrifuge tubes, and all centrifuge tubes were centrifuged at 10000 rpm for 10 minutes. After centrifugation, the cell pellets produced at the bottom of the tube were aseptically washed and suspended in sterile distilled water under pre-sterilized Laminar Air Flow (LAF) hood. Fermentation media (Ammonium chloride; 0.5 g/l, Potassium dihydrogen phosphate; 2.0 g/l, Magnesium sulphate heptahydrate; 0.5 g/l, Yeast extracts; 1.5 g/l, Calcium chloride dihydrate; 0.1 g/l, Ferric chloride dihydrate; 0.1 g/l, Zinc sulphate heptahydrate 0.001 g/l) was prepared and autoclaved at 121.1 °C, 1.5 psi for 20 minutes.

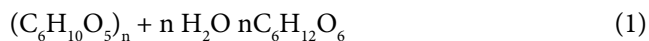
The hydrolysate acquired after saccharification by filtering the biomass with a muslin cloth and then Whatmann's filter paper 1, and it were subsidized into the media prior to sterilization. The medium was inoculated with 10% inoculum of *Pichia stipitis* at pH

5.0 after sterilization under LAF or aseptic condition and incubated at 30°C and 150 rpm. Sampling was executed generally and then centrifuged for 15 minutes and tested for the presence of ethanol, reducing sugars and xylose. Ethanol evaluation was executed by potassium dichromate method with the support of the spectrophotometer. The assessment of reducing sugar present in the hydrolysate of biomass performed using DNS method²² and the estimation of xylose was done by modified Tollen's method.

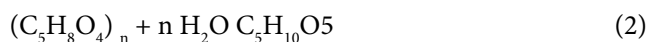
2.4 Governing Equation

The structural arrangement of hemicellulose is quite intricate, as hemicellulose is a complex heteropolysaccharide. The included solid substrate droops within the fluid catalyst. Overall reaction study involving of catalyst is initiated to pursue the respective steps: 1. The protons undergo diffusion over the thin liquid film within the biomass matrix, 2. The oxygen atom situated in contact to the heterocyclic ether bonds within the sugar monomers undergoes protonation 3. Ether bonds undergo segregation 4. Evolvement of intermediary carbonation, 5. Mixing up of carbonation in water thereby forming a solution, 6. Reformation of protons in accordance to the co-formation of sugar polymer, oligomers and monomers depending on the position of the ether bonds, 7. Reaction products undergo diffusion into the liquefied phase, if it gives concession depending on their form and size, 8. Reiteration from the second step²⁴.

The characterized representation of reactions involved during the formation of hexose sugars (glucose, galactose and mannose) by hemicellulose hydrolysis is shown as in Equation (1).



Equation (1) depicts the gain in a mass of about 11.1% by weight, nevertheless, the demonstration of the stoichiometry posterior to the addition of water to pentose sugar molecules at the time of hemicellulose hydrolysis can be represented as follows:



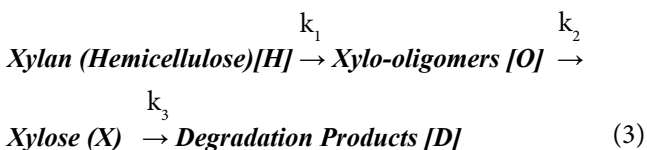
$(C_5H_8O_4)_n$ is a chained heterocyclic polymer fundamentally made up of arabinan and xylan. $(C_5H_{10}O_5)_n$ is the equivalent pentose sugar produced after

hemicellulose hydrolysis. Xylose is the fundamental hemicellulosic monomeric sugar. In the time of occurrence of the fermentation process for the production of Bioethanol by waste rice straw, xylose serves as a primary component for carbon and energy needed for optimum growth of the microorganisms. As a result, effective use of hemicellulosic fraction at the time of hemicellulose hydrolysis would depend significantly on the persuasive conversion procedure of the heterocyclic polymer into xylose.

Equation (2) illustrates that the adjoining water molecules culminated at a 13.6 % gain in weight⁹. Due to the huge cost of enzymes; the viability of bio processing by enzyme catalysis is not appreciable. So in order to make the bio processing cost effective, hemicellulose hydrolysis is performed by tartaric acid, which is readily available and cheap.

The Monophasic model of hemicellulose hydrolysis can be understood²⁵ as represented by Equation (4). The demonstration of the modeling of the kinetics of hemicellulose hydrolysis can be elucidated by consecutive first-order reactions: starting from the conversion of xylan to xylose, posterior to the derogation of xylose to furfuryl and other inhibitory byproducts.

Where, k_1 , k_2 & k_3 are the reaction rate constants of the respective reactions. For the evaluation of kinetic parameters, the governing model equation involved to fit the experimental data can be represented by Equation (4)-(7):



$$\frac{d[H]}{dt} = k_1[H] \quad (4)$$

$$\frac{d[O]}{dt} = k_1[H] - k_2[O] \quad (5)$$

$$\frac{d[X]}{dt} = k_2[O] - k_3[X] \quad (6)$$

$$\frac{d[D]}{dt} = k_3[X] \quad (7)$$

Where $[H]$ = the concentration of hydrolysing xylan, $[O]$ = the concentration of intermediate xylose oligomers and $[X]$ = concentration of xylose respectively. Resolving the differential equation having the initial conditions

at $t = 0$, $[O] = 0$, $[X] = 0$, the standardized solutions of the differential equation are analyzed as represented by Equation 8-10

$$H = H_0 e^{-k_1 t} \quad (8)$$

$$O = H_0 \frac{k_1}{(k_2 - k_1)[e^{-k_1 t} - e^{-k_2 t}]} \quad (9)$$

$$X = H_0 \frac{k_1 k_2}{(k_2 - k_1)(k_3 - k_1)} [e^{-k_1 t} - e^{-k_3 t}] - H_0 \frac{k_1 k_2}{(k_2 - k_1)(k_3 - k_2)} [e^{-k_2 t} - e^{-k_3 t}] \quad (10)$$

Where k_1 = the kinetic rate constant for hydrolysing xylan, k_2 = kinetic parameter involved during formation of oligomers, k_3 = kinetic parameter involved during the degradation of xylose to furfural derivatives or inhibitory products.

3. Results and Discussion

3.1 Rice Straw Component Analysis

The percentage of hemicellulose and lignin was determined for different sample ages acquired from old thatched roofs²⁰. Figure 1 represents the amount of total cellulose, hemicellulose, lignin and ash content that existed within the biomass. Lignin content decreased with an increase in sample age, which may result in increased yield of sugars by solubilisation. In contrast to the other samples, the amount of lignin (10.82%) was found to be least in four years-old sample.

Figure 2 depicts the variation of hemicellulose with time; it was visible that the hemicellulose content of the rice straw samples increases with time, which may be due to the degradation of lignin with a hike in age of the samples.

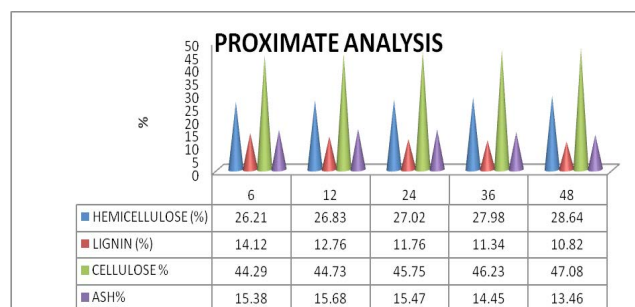


Figure 1. Change in the constituents of rice straw samples with age of biomass.

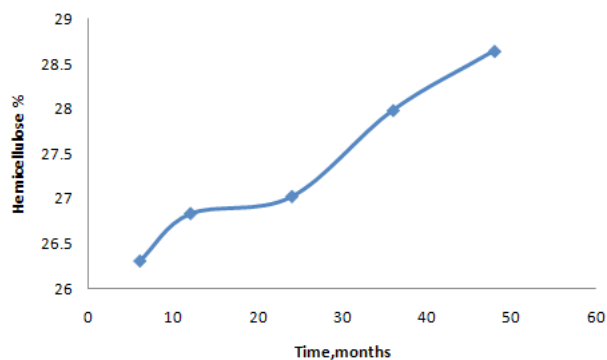


Figure 2. Variation of percentage of hemicelluloses with time.

A significant amount of research on hemicellulose hydrolysis from waste rice straw has not been performed till the date. The present work focuses on better understanding of the kinetic modeling of waste rice straw during the transformation of hemicellulose to xylose sugar. Xylose is the major constituent produced after effective pretreatment of waste rice straw by tartaric acid. After the process of pretreatment, the xylose-containing hydrolysate was collected from diverse pretreatments. Varied parameters such as the concentration of acid, soaking time, treatment time, treatment temperature and agitation speed; all of these affect the yield of xylose. These were examined, and few schematics of variations are represented in Figures 3 and Figure 4 respectively.

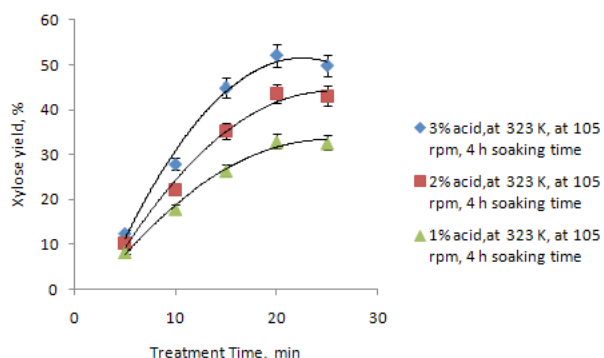


Figure 3. Variation of xylose yield with treatment time and acid concentration.

Tinder method was utilized for the estimation of xylose yield and to determine the xylose yield % the yield of xylose was divided by the amount of dry biomass, which was generally equal to 1 g as shown in Equation 11

$$\text{xylose yield \%} = \frac{\text{xylose yield}}{\text{amount of biomass used}} \times 100 \quad (11)$$

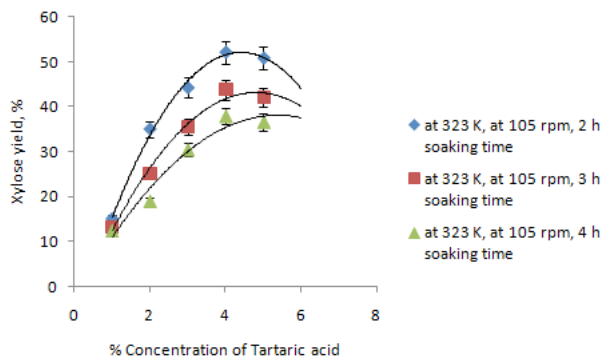


Figure 4. Variation of xylose yield with different concentration of tartaric acid.

A soaking time of 4 h proved to be efficient for performing out of the experiments for hemicellulose hydrolysis. With a variation in the % of tartaric acid, an increase in xylose yield at various treatment times was observed as represented in Figure.3, without changing the other parameters. The highest amount of xylose derived from waste rice straw was calculated to be 52.1% at a treatment temperature of 323K, concentration of tartaric acid was calculated to be 3%, treatment time 20 minutes, agitation speed of 105 rpm and soaking time of four hour. Figure 4 depict an increment in the concentration of tartaric acid, which increased xylose yield considerably up to 4%. Then a gradual drop in the yield of xylose was observed this cause might be due to the creation of furfurals or inhibitory end products.

During the bio-conversion of hemicellulose to xylose, the monophasic kinetic model of wood cellulose¹⁶ undergoing hydrolysis is anticipated to follow first-order kinetics. The equivalent rate equation can be represented as in Equation 12

$$\frac{dC}{dT} = KC \quad (12)$$

Where C denotes the concentration of xylose at any time interval, t is the time taken and K represents the rate constant for conversion of hemicellulose to xylose. The rate constant can be determined by integration technique as in Equation 13

$$K = \frac{\ln\left(\frac{c_2}{c_1}\right)}{t_2 - t_1} \quad (13)$$

Where, c_1 and c_2 denotes the concentrations of xylose at t_1 and t_2 time consequently.

3.2 Activation energy and frequency factor

Figure 5 represents the change of $\ln k$ versus $1/T$ for various tartaric acid concentration for 4 h soaking time with agitation speed of 100 rpm. The straight lines signify that the graph during hydrolysis follows Arrhenius rate equation as represented by Equation 14

$$K = Ae^{(-E_A/RT)} \quad (14)$$

'A' depicts the frequency factor which depends on the concentration of the reacting medium involved. The variation of frequency factor, as shown in Figure 6, with the soaking time has been studied, which shows an increase with the increase in the concentration of tartaric acid. 'E_A' represents the activation energy and 'T' the treatment temperature at Kelvin's scale. 'R' signifies the molar gas constant. From the slope and intercept of Figure 4 the value of $(-E_a/R)$, and $\ln A$ can be calculated. The estimated value of activation energy at different concentration of tartaric acid (%) is tabulated as in Table 1.

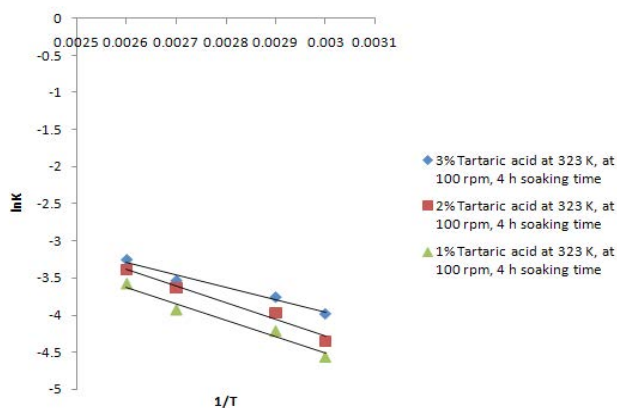


Figure 5. Represents the Arrhenius plot of $\ln k$ vs. $1/T$ with variation in tartaric acid concentration.

Table 1. Thermodynamic parameters of hemi-celluloses hydrolysis for 3% catalyst concentration at 100 rpm for a soaking time 4 h

Concentration of tartaric acid	Activation energy (E _a) (kJmol ⁻¹)	ΔH (kJ mol ⁻¹)				-ΔS (kJ mol ⁻¹)				-ΔG (kJ mol ⁻¹)			
		ΔH ₃₂₃	ΔH ₃₄₃	ΔH ₃₆₃	ΔH ₃₈₃	ΔS ₃₂₃	ΔS ₃₄₃	ΔS ₃₆₃	ΔS ₃₈₃	-ΔG ₃₂₃	-ΔG ₃₄₃	-ΔG ₃₆₃	-ΔG ₃₈₃
1%	11.677	8.892	8.826	8.66	8.493	0.9691	0.9669	0.9666	0.9662	304.12	322.82	342.21	361.56
2%	14.54	11.855	11.689	11.523	11.356	0.9589	0.9606	0.9612	0.9613	297.86	298.58	337.39	356.82
3%	15.58	12.895	12.563	12.563	12.396	0.9582	0.9585	0.9584	0.9577	296.60	316.20	335.33	354.23

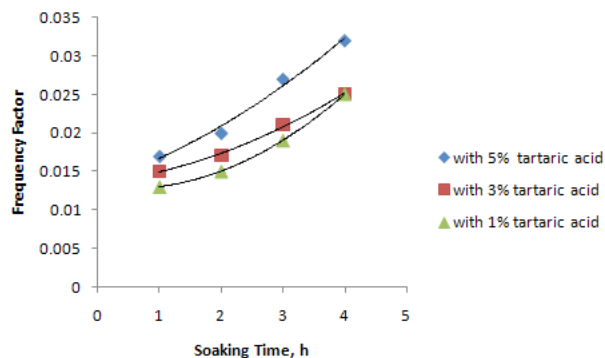


Figure 6. Variation of frequency factor with soaking time at different concentration of tartaric acid.

To study the thermodynamics of the hemicellulose hydrolysis reaction, diverse parameters were as well determined. Using the information obtained from the slope of the Arrhenius plot under different reaction conditions the value of Energy of Activation (EA) was calculated. The change in enthalpy of the process was calculated with the help of the Equation 15 below.

$$\Delta H = E_A - R T \quad (15)$$

Where, ΔH is the difference in enthalpy of activation energy. Tabulated values of activation enthalpy estimated for different concentration of tartaric acid for a soaking time of 4 h at 100rpm is shown in Table 1.

Eyring equation was utilized for the determination of entropies of activation (ΔS) against the measured values of enthalpy and rate constant.

$$\frac{\Delta S}{R} = \ln k_b T \left(K e^{\frac{\Delta H}{RT}} \right) \quad (16)$$

Where, ΔS represents the entropy of activation, K the reaction rate constant at the time of generation of xylose.

K_b represents the Boltzmann's constant; h the Planck's constant, R is the Universal Gas Constant and T the treatment temperature.

Gibb's free energy (ΔG) was determined using the following relationship as shown in Equation (17).

$$\Delta G = \Delta H - T\Delta S \quad (17)$$

Table 1 illustrates the calculated values of ΔG , ΔH , and ΔS during hemi-celluloses hydrolysis of waste rice straw biomass.

3.3 Fermentation

The process of fermentation was carried out for waste rice straw samples found to have different sample ages (one year, two years three years, four years approximately). The percentage ethanol content was estimated by spectrophotometer. Schematics for the difference of percentage ethanol content at varied time intervals was observed as represented in Figure 7. It was noticeable that the ethanol content was directly proportional with the increase in age of the samples. An increasing trend of ethanol with time was visible until 9th hour, after that the amount of ethyl alcohol gradually decreased, this may be due to the formation of inhibitory products or other furfuryl derivatives.

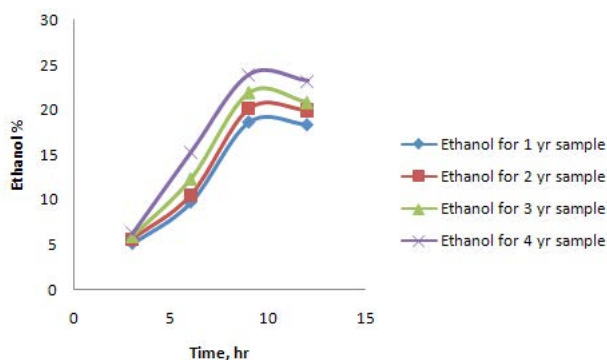


Figure 7. Effect of the age of waste rice straw on ethanol yield.

4. Conclusions

The experiment was designed for gathering detailed knowledge about the bio processing and equipment design. Monophasic kinetic modelling of hemicellulose hydrolysis of waste rice straw by tartaric acid proved to be

quite effective. The process of conversion of hemicellulose to xylose is found to follow first-order reaction kinetics, so Arrhenius equation was chosen in order to describe the hydrolysis step. The determined values of kinetic and thermodynamic parameters extensively demonstrated that the process parameters (treatment time, the concentration of catalyst (acid or alkali), soaking time, soaking temperature, and agitation speed) confirm to be important in improving the yield of xylose at the time of hydrolysis. Fermentation of the samples with highest xylose yield was taken for further studies. The best yield of xylose and reducing sugars at optimum conditions were 134 mg/g and 94.8 mg/g using tartaric acid. Fermentation by *Pichia stipitis* proved to be very efficient for the transformation of pentoses and hexoses. After fermentation, the maximum yield of ethanol was estimated to be 25.1% *Pichia stipitis* was capable of improving the production of ethanol. Overall, the present works depict a comprehensive view of the complete bio processing of waste rice straw to Bioethanol.

5. References

- Demirbas A. Biomass resource facilities and biomass conversion processing for fuels and chemicals. *Energy Conversion and Management*. 2001; 42(11):1357-78. Crossref
- Stocker M. Biofuels and biomass-to-liquid fuels in the biorefinery: Catalytic conversion of lignocellulosic biomass using porous materials. *Angewandte Chemie International Edition*. 2008; 47(48):9200-11. Crossref
- Patel AK, Singhanian RR, Pandey A. Biofuels from biomass. *Novel Combustion Concepts for Sustainable Energy Development*: Springer; 2014. p. 25-44.
- Valentine J, Clifton-Brown J, Hastings A, Robson P, Allison G, Smith P. Food vs. fuel: The use of land for lignocellulosic 'next generation' energy crops that minimize competition with primary food production. *GCB Bioenergy*. 2012; 4(1):1-19. Crossref
- Zamora R, Sanchez CJ. Production of an acid extract of rice straw. *Acta Cientifica Venezolana*. 1994; 46(2):135-9.
- Garrote G, Domí H, Parajo JC. Autohydrolysis of corncob: Study of non-isothermal operation for xylooligosaccharide production. *Journal of Food Engineering*. 2002; 52(3):211-8. Crossref
- Saha BC. Hemicellulose bioconversion. *Journal of Industrial Microbiology and Biotechnology*. 2003; 30(5):279-91. Crossref
- Rafiqul I, Sakinah AM. Processes for the production of xylitol: A review. *Food Reviews International*. 2013; 29(2):127-56. Crossref
- Zhang M, Eddy C, Deanda K, Finkelstein M, Picataggio

- S. Metabolic engineering of a pentose metabolism pathway in ethanogenic *Zymomonas mobilis*. *Science*. 1995; 267(5195):240-3. Crossref
10. Ho NW, Chen Z, Brainard AP. Genetically engineered *Saccharomyces* yeast capable of effective cofermentation of glucose and xylose. *Applied and Environmental Microbiology*. 1998; 64(5):1852-9.
 11. Ingram L, Gomez P, Lai X, Moniruzzaman M, Wood B, Yomano L, et al. Metabolic engineering of bacteria for ethanol production. *Biotechnology and Bioengineering*. 1998; 58(2-3):204-14. Crossref
 12. Horitsu H, Yahashi Y, Takamizawa K, Kawai K, Suzuki T, Watanabe N. Production of xylitol from D-xylose by *Candida tropicalis*: Optimization of production rate. *Biotechnology and Bioengineering*. 1992; 40(9):1085-91. Crossref
 13. Lee Y, Iyer P, Torget R. Recent progress in bioconversion of lignocellulosics. *Recent Progress in Bioconversion of Lignocellulosics*. 1999.
 14. Jacobsen SE, Wyman CE, editors. Cellulose and hemicellulose hydrolysis models for application to current and novel pretreatment processes. 21st Symposium on Biotechnology for Fuels and Chemicals; 2000.
 15. Wyman CE, Dale BE, Elander RT, Holtzapple M, Ladisch MR, Lee Y. Coordinated development of leading biomass pretreatment technologies. *Bioresource Technology*. 2005; 96(18):1959-66. Crossref
 16. Saeman JF. Kinetics of wood saccharification-hydrolysis of cellulose and decomposition of sugars in dilute acid at high temperature. *Industrial & Engineering Chemistry*. 1945; 37(1):43-52. Crossref
 17. Elander R, Hsu T. Processing and economic impacts of biomass delignification for ethanol production. *Applied Biochemistry and Biotechnology*. 1995; 51(1):463-78. Crossref
 18. Pessoa Jr A, Mancilha I, Sato S. Acid hydrolysis of hemicellulose from sugarcane bagasse. *Brazilian Journal of Chemical Engineering*. 1997; 14(3). Crossref
 19. Dumitriu S. Polysaccharides: Structural diversity and functional versatility. CRC Press; 2004. Crossref
 20. Goering HK, Van Soest PJ. Forage fiber analyses (apparatus, reagents, procedures, and some applications). *USDA Agr Handb*. 1970.
 21. Updegraff DM. Semimicro determination of cellulose in biological materials. *Analytical Biochemistry*. 1969; 32(3):420-4. Crossref
 22. Miller GL. Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry*. 1959; 31(3):426-8. Crossref
 23. Trinder P. Micro-determination of xylose in plasma. *Analyst*. 1975; 100(1186):12-5. Crossref
 24. Aguilar R, Ramirez J, Garrote G, Vazquez M. Kinetic study of the acid hydrolysis of sugar cane bagasse. *Journal of Food Engineering*. 2002; 55(4):309-18. Crossref
 25. Li X, Converse AO, Wyman CE. Characterization of molecular weight distribution of oligomers from autocatalyzed batch hydrolysis of xylan. *Applied Biochemistry and Biotechnology*. 2003; 107(1-3):515-22. Crossref