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Article

Kinetics of Acid Catalyzed Hydrolysis of Water Hyacinth Biomass for Bioconversion of Lignocellulosic Xylose to Xylitol

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Abstract: Lignocellulosic biomass is rich in sugar polymers which can be efficiently hydrolysed to obtain fermentable C5 sugars for their bioconversion in to xylitol. The effect of various parametric conditions on xylose yield and their relation with the activation energy and rate of hydrolysis reaction was studied where the maximum yield of xylose obtained from dilute acid hydrolysis of fresh water hyacinth [*Eichhornia crassipes* (Mart.) Solms] was 77.4% at 3 hr soaking time with 50 ^{o}C soaking temperature at 160 rpm agitation using 3% dilute H_2SO_4 and boiling time of 20 min. A maximum of 33.5 g/l of xylitol was obtained during fermentation using *Pichia stipitis* with a yield of 0.63 g xylitol/g of xylose at 48 h fermentation.

Keywords: water hyacinth biomass, xylitol, acid catalyzed hydrolysis, detoxification, fermentation.

1. Introduction

Bioconversion of lignocellulosic materials to biofuels and biomaterials has gained extensive research interest during the last few decades due to the feasibility of this sustainable and eco-friendly process (Adsul et al., 2011). It is a promising feedstock for the bio-processing industries. A wide variety

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of lignocellulosic biomass are available on earth where up to 1.7–2.0 x 10^{11} ton (approx.) of biomass has been identified as an important source for alternative fuels and value added chemicals. The huge amount of residual plant biomass considered as "waste" can potentially be converted into different value-added products e.g. such as bio-ethanol, bio-methane, and other value added product (Kumar et al. 2016). However, only 6 x 10^9 ton (approx.) of biomass are currently used for food and non-food applications (Klass, 2004, Albuquerque et al., 2014). Amongst all the available lignocellulosic biomass proper selection of the biomass is a crucial step which depends on the availability of the biomass, growth rate, and use as food crop. Water hyacinth (*Eichhornia crassipes* [Mart.] biomass proves to be a promising feedstock based on all above conditions. It is considered to be the world's worst aquatic weed, which is rich in cellulose and hemicellulose content, from where monomeric sugars such as hexoses and pentose can be obtained from for further utilization in making several value added products. It is an aquatic, free floating, fast growing weed which adversely affects the biodiversity of ecosystem. It has higher hemicellulose content (~38.5%) from where fermentable xylose can be obtained by hydrolysis to produce Xylitol ($C_5H_{12}O_5$). It is a value added product that has attracted the worldwide interest due to its huge importance in Food and Pharmaceutical industries. It is a 5-Carbon sugar polyhydroxy alcohol, a sugar substitute for diabetic patients and a non-toxic, harmless food with many other beneficial roles to mankind (Iqbal et al., 2013). It is also known as wood sugar (Mudaliyar et al., 2011) and a low calorie sweetener having huge health benefits like low glycemic rate (Chen et. al, 2010), anticancerous and antiviral features and anticariogenic properties. It is also used as a nutriceuticals which maintains low plasma glucose and insulin levels and inhibits autoimmune diseases (Usvalampi, 2013). It is naturally present in some fruits and vegetables in very low concentration (Chaudhary et al., 2013). Xylitol is produced industrially by direct chemical hydrogenation of D-xylose using Raney-Nickel catalyst under high temperature (80-140 ^oC) and pressure (upto 50 atm) which follows extensive purification from non-specific reduction products (Chen et. al, 2010). Both extraction of xylitol from natural resources such as fruits and vegetables and also industrial process which requires specialized equipments are quite expensive. Several extensive studies have been made to find out the alternative routes which involve biotechnological process where lignocellulosic biomass is hydrolysed by dilute acid to obtain xylose sugar for microbial conversion to xylitol.

Globally xylitol production is expanding with eventually great demand in global market of more than 125 x 10^3 tons/year with higher value ($ 4.50-5.50 per kg for bulk purchase by phamaceuticals and food companies and $ 20.00 per kg in supermarket). Xylitol has a 12 % share of total polyols market (Kelloway et al., 2014, Nigam et al., 1995, Koutinas et al., 2014, Franceschin et al., 2011) which is approved for food use in more than 50 countries. Recently considerable attention has been drawn to the bioconversion of xylose into xylitol from lignocellulosic hydrolysate which involves hydrolysis,

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detoxification and fermentation using xylose fermenting yeast. Hydrolysis is a crucial step in the biochemical conversion of lignocellulosic biomass to fermentable sugars, which aims at separating and providing easier access to the main biomass components (cellulose, hemicellulose), eventually removing lignin, preserving the hemicellulose, reducing the cellulose crystallinity and increasing the porosity of the material. Dilute acid hydrolysis is mostly used because it effectively solubilizes hemicelluloses to xylose and other sugar monomers. Dilute acid pretreatment process, is inexpensive which avoid corrosion and also it is easy to perform (Pereira et al., 2011). Detoxification is an important step for removing or decreasing the concentration of inhibitors. Some of the methods to remove these inhibitors are use of activated charcoal, ion exchange resins, $Ca(OH)_2$, laccase enzyme, recombinant strains and adaptation of microbial strains, CaO treatment and over liming. Activated charcoal, over liming, neutralization, sulphite treatment, treating with ion-exchange resins and extraction with organic solvents reduce the ionization properties of inhibitory compounds (Wyman et al., 2005b).

The kinetics of hemicellulose hydrolysis is extremely important for understanding the complex reactions, process development and sugar recovery. The reaction rate of hydrolysis depends on a number of variables like temperature, acid concentration, time, substrate concentration and substrate composition. Kinetic modeling of hemicellulose hydrolysis by dilute acids has already been studied to improve the understanding of the complex reactions (Aguilar et al., 2002, Saeman, 1945). Hemicellulose hydrolysis model was proposed to follow a first order dependence on reactant concentration by Seamen (Saeman, 1945). In order to maximize xylose yield while avoiding the degradation reaction, the kinetic models based on dilute sulphuric acid pre-treatment have to be studied extensively.

The objective of the present research work is to study the effect of parametric conditions such as soaking time, soaking temperature, agitation speed, boiling time and acid concentration on the activation energy, reaction rate and frequency factor during dilute acid catalyzed hydrolysis of water hyacinth biomass in the production of xylitol.

2. Materials & Methods

2.1. Biomass Preparation

Fresh water hyacinth was collected from a natural unpolluted pond and thoroughly washed several times under tap water to remove adhering dirt. The leaf and petiole portion of the plant was taken and cut into pieces of size 1 cm (approx), ground and made a paste for further investigations. After washing, the leaf, petiole and root portion was chopped into small pieces, dried at 80 ^{o}C for 8 h and ground to fine powder for biomass characterisation. The cellulose, lignin and hemicellulose fraction of water hyacinth biomass were analyzed in laboratory using Acid detergent fibre (ADF), Neutral detergent

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Fibre (NDF) and Acid detergent Lignin (ADL), standard detergent extraction method (Robertson et al., 1981).

2.2. Fourier Transform Infrared Spectroscopy (FTIR)

Fourier Transform Infrared Spectroscopy (FTIR) was used on dilute acid treated and untreated water hyacinth biomass (WHB) using a Schimadzu spectrophotometer (Japan), to investigate the structural and chemical changes in biomass which affects the absorption spectra during transmittance of spectral bands. The analysis was carried out using 3 mg of dried biomass sample which was dispersed on 300 mg of spectroscopic grade potassium bromide (KBr) and subsequently pressed into disc at 10 MPa for 3 min. An average of 25 scan were done with resolution of 4 cm^{-1} within a range of 500 - 4500 cm^{-1}.

2.3. Preparation of Hemicellulose Acid Hydrolysate

8 g of fresh water hyacinth paste (~1 g dried biomass) was mixed with 30 ml of 1%, 3% & 5% dilute sulphuric acid respectively in 100 ml conical flask for a soaking time of 1, 2, 3 and 4 h, with an agitation speed of 130, 160 and 190 rpm, at different temperatures ranging from 30, 40, 50 and 60 ^{o}C. After soaking, the samples were boiled at 100 ^{o}C in heating mantle for 5, 10, 15, 20, 25 and 30 min respectively. Finally the hydrolysate was filtered using Whatman paper no.1 to remove the unhydrolysed material and solid residue. The filtrate was collected for further experimental analysis.

2.4. Concentration and Detoxification of Acid Hydrolysate

To increase the amount of sugars in hydrolysate of water hyacinth, concentration of the hydrolysate was carried out by YAMATO Rotary Evaporator (RE-300). To the concentrated hydrolysate calcium oxide is added with stirring, until the pH of the hydrolysate reaches 10.0. It was then incubated for half an hour followed by centrifugation (3000 rpm, 20 min) and filtered, and again made the pH back to 6. After over liming, 3.5% (w/v) of activated charcoal was added to the hydrolysate and stirred for 1 h. The mixture was again centrifuged (3000 rpm, 10 min) and vacuum filtered. Sugar and phenolics were estimated before and after detoxification process. The treated hydrolysate was then used for the fermentation studies.

2.5. Microorganisms and Inoculums Preparation for Fermentation

Pichia stipitis (NCIM 3500) and *Candida shehatae* (NCIM 3497) were used for pentose fermentation obtained by the courtesy of National Collection of Industrial Microorganisms (NCIM), Pune, India. The stock culture was maintained on Yeast extract, Peptone, Xylose (YPX) agar slants containing (g/l) yeast extract, 10 ; peptone, 20 ; Xylose, 30 and agar, 25, pH: 5.0 and stored at 4° C. The

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medium used for inoculums preparation contained (g/L): yeast extract 10, peptone 20, D glucose 10. To prepare the inoculums, a 250 ml Erlenmeyer flask containing 50 ml medium was inoculated from a fresh culture plate and incubated at 30° C on a rotary shaker at 125 rpm for 20 h and the broth was centrifuged at 10,000 for 10 minutes to obtain the pellets. The cell pellet was washed and suspended in sterile distilled water.

2.6. Fermentation

The detoxified hydrolysate was supplemented with (g/l) NH_4Cl 0.5, KH_2PO_4 2.0, $MgSO_4 \cdot 7H_2O$ 0.5, yeast extract 1.5, $CaCl_2 \cdot 2H_2O$ 0.1, $FeCl_3 \cdot 2H_2O$ 0.1, $ZnSO_4 \cdot 7H_2O$ 0.001 was inoculated with 10% (v/v) inoculums of *P. stipites and C.shehatae* at pH 5.0 and incubated at 30 ^{O}C with 150 rpm of agitation. Samples were withdrawn at regular intervals of time and centrifuged for 10 min. The supernatant was used to determine the xylitol and residual sugar concentration.

2.7. Analytical Methods

The total reducing sugars, released after acid hydrolysis were estimated by the Dinitro Salicyclic Acid (DNS) method (Miller, 1959) and Xylose sugar was determined by the Phloroglucinol assay (Eberto et al., 1979) using Phloroglucinol (1 ,3,5-trihydroxybenzene) as the chromogenic reagent, where the concentration of pentose phosphate pathway metabolites are reduced during xylose fermentation when acetic acid is present. HCl - hydrolyzes the glycosidic bonds to form two monosaccharides. The fermentation inhibitors (furans and phenolics) were analyzed by spectroscopic analysis. Phenolics estimation was carried out by Folincio calteus method (Singleton et al., 1965).

2.8. Xylitol Estimation

Xylitol and the sugars present in the fermented broth were estimated by high performance liquid chromatography (HPLC) fitted with thermo scientific HI chrome column (CECIL, UK) and RI detector using acetonitrile-water (75:25) as mobile phase at a flow rate of 1 ml/min at 30 °C.

2.9. Kinetics of Acid Hydrolysis of Hemicellulose

The kinetics of hemicellulose hydrolysis is extremely important to understand the complex reactions occurring during hydrolysis of the xylan complex in hemicellulose, process development, optimisation and sugar recovery. The reactions using dilute acid are very complex, mainly because the substrate is in a solid phase and the catalyst in a liquid phase and the chemical composition and structural orientation of hemicellulose makes the hydrolysis reaction very complex. The reaction rate of hydrolysis depends on a number of variables like operating temperature, acid concentration, time of reaction, substrate concentration and substrate composition.

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The overall catalytic reaction proceeds in the following steps: (i) diffusion of the protons through the thin liquid film into the biomass matrix, (ii) protonation of the oxygen atom present on the heterocyclic ether bonds between the sugar monomers, (iii) dissociation of the ether bonds, (iv) formation of carbocation intermediates, (v) solvation of the carbonations in water, (vi) regeneration of protons and simultaneous co-generation of sugar polymer, oligomers and monomers depending on the locations of the ether bonds, (vii) diffusion of the reaction products out of the biomass matrix into the bulk phase depending on their form and size, (viii) repetition from the second step (Aguilar et al., 2002). The stoichiometric representation of the reactions for the formation of five carbon sugar molecules in the hemi-cellulose matrix on addition of water is shown as:

$$(C_5H_8O_4)_n + n\,H_2O \longrightarrow nC_5H_{10}O_5$$

$(C_5H_{10}O_5)_n$ is the corresponding pentose sugar monomer obtained from hetero-cyclic polymer hemi-cellulose $(C_5H_8O_4)_n$ hydrolysis. Xylose is the main hemi-cellulosic monomeric sugar. For the production of xylitol, xylose serves as the carbon and energy source for the microbes during the fermentation. The hemi-cellulose hydrolysis reactions were also modeled as a consecutive homogeneous pseudo-first order reactions which follow hydrolysis of the xylan to xylose, followed by the degradation of xylose to furfurals once the monomeric sugar molecules diffuses out from the solid matrix into the bulk acid solution. Due to the structural complexities of the hemicellulose fibres a more networked model was prepared by Kobayshi and Sakai (1956) (Kobayshi and Sakai, 1956).

2.10. Hydrolysis Model and Governing Equation

Hemicellulose hydrolysis Models:

As proposed by Saeman in 1945 (Saeman, 1945) the simplest model describing a monophasic hemicellulose hydrolysis based on a two-step first-order is shown in Fig 1. According to this approach, hemicellulose is hydrolyzed to xylose, which in turn breaks down to degradation products in a second reaction:

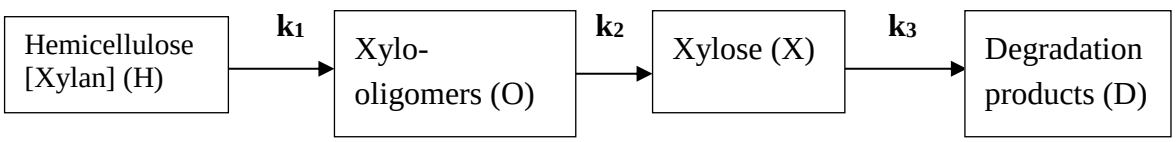

Fig 1. Schematic diagram of monophasic hydrolysis model of hemi-cellulose.

The variation of each component can be theoretically explained by the following set of equations:

$$\frac{dH}{dt} = -k_1[H] \qquad (1)$$

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$$\frac{dO}{dt} = k_1[H] - k_2[O] \quad (2)$$

$$\frac{dX}{dt} = k_2[O] - k_3[X] \quad (3)$$

$$\frac{dD}{dt} = k_3[X] \quad (4)$$

The above differential equations can be solved analytically using the initial conditions at *t= 0, O= 0* and *X=0*. The time dependent H, O and X may be presented as

$$H = H_0\, e^{-k_1 t} \quad (5)$$

$$O = \frac{k_1 H_0}{(k_2 - k_1)}\left[\, e^{-k_1 t} - e^{-k_2 t}\,\right] \quad (6)$$

$$X = \frac{H_0\, k_1 k_2}{(k_2 - k_1)(k_3 - k_1)}\left[e^{-k_1 t} - e^{-k_3 t}\right] - \frac{H_0\, k_1 k_2}{(k_2 - k_1)(k_3 - k_2)}\left[e^{-k_2 t} - e^{-k_3 t}\right] \quad (7)$$

Here, 'O' denotes concentration of Xylo-Oligomers, 'X' denotes concentration of Xylose, 'k_1' denotes kinetic constant of hemicellulose (xylan) hydrolysis, 'k_2' is the rate constant for oligomer formation and 'k_3' is the rate constant for formation of inhibitory products such as furfural, furan etc.

3. Results

3.1. Characterisation of Water Hyacinth Biomass (WHB)

The composition of water hyacinth biomass (WHB) in the root, leaf and stem (petiole) of the plant is analysed separately and illustrated in Fig 2(a). From the analysis it was found that cellulose and hemicellulose content is very high in leaf and petiole/stem of water hyacinth plant whereas lignin content is very high in root. Therefore, leaf and petiole of the biomass was taken for the experiment discarding the root. Again an uniform mixture of the dried powder of leaf and stem (1g of leaf and 1 g of the stem were mixed) was analysed and shown in Fig 2(b).

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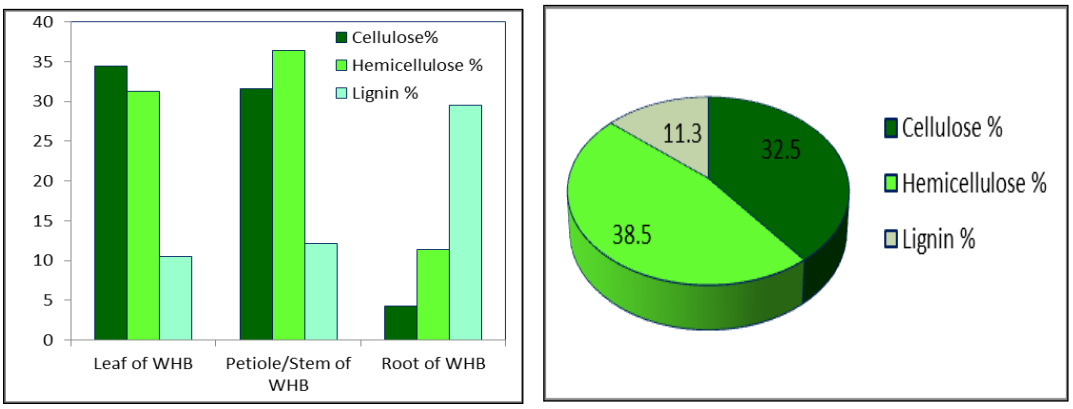

(a) **(b)**

Fig. 2. (a) Cellulose, hemicellulose and lignin content (%) in Leaf, Stem and Root of water hyacinth biomass; (b) Cellulose, hemicellulose and lignin content (%) in uniform mixer of leaf and petiole from water hyacinth biomass

3.2. FTIR Analysis

FTIR analysis of untreated and dilute acid treated WHB was done, which indicates variation in their transmittance of spectral bands as shown in Fig 3. Sharp peak at 812 cm^{-1} indicates presence of C1 group of β- glycosidic linkage between xylose units in hemicellulosic fraction of the biomass. It also shows higher content of hemicellulose. Peak at 1512 cm^{-1} in untreated biomass shows C-O aromatic ring vibration, whereas the peak is reduced to 1489 cm^{-1} in treated biomass which reduction delignification. Also peak at 1864 cm^{-1} is observed due to acetyl and uronic ester linkage of carboxylic group of lignin polymer. Peaks ranging from 812 cm-1 to 920 cm^{-1} indicates presence of C-O and C-H bonds which corresponds to higher content of hemicellulose.

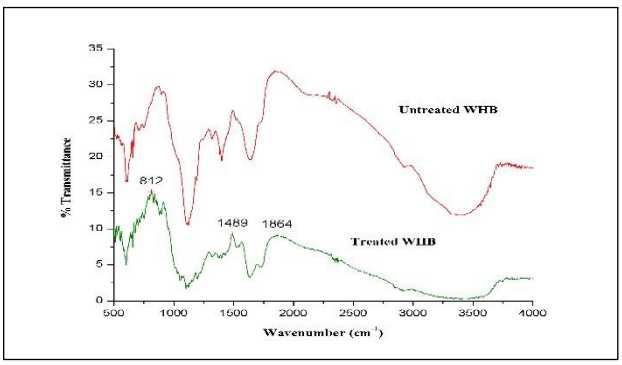

Fig 3. FTIR spectra of untreated and dilute acid treated water hyacinth biomass (WHB)

3.3. Effects of Driving Parameters on Xylose Yield by Dilute Acid Hydrolysis of WHB

Acid hydrolysis of water hyacinth biomass was performed using different parametric conditions as mentioned in Table 1 which includes soaking time (1, 2, 3, 4 h), soaking temperature (30 ºC – 60 ºC/ 303K-333K), agitation speed (130, 160, 190 rpm), acid concentration (1 %, 3%, 5%) and boiling time

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(5, 10, 15, 20, 25, 30 min.) at 100 ^{o}C . Depolymerisation of hemicellulose occurred during dilute sulphuric acid hydrolysis where the effects of different parameters were studied (Fig 4a -4e) to find out the best set of parameters which gives major product as xylose (65%) along with mixture of monomeric sugars, its derivatives as phenolics and furan products and organic acids during the catalytic reaction. The xylose yield % in the hydrolysate, measured by phologlucinol assay is calculated as

Xylose yield % = (Xylose yield /Amount of dried biomass used) x 100 …..(8)

Table 1. Experimental range with different parametric condition and levels of independent process variables

Factor	Parameters	Low	High
A	Soaking Temperature, ^{o}C/K	30/303	60/333
B	Soaking time (h)	1	4
C	Agitation speed (rpm)	130	190
D	Acid Concentration (%)	1	5
E	Boiling Time (min)	5	30

Fresh and dried water hyacinth biomass were compared which concluded fresh biomass paste gives a more xylose yield (Fig 4a). Simultaneously the effect of agitation was included in respect to soaking time along with a soaking temperature and it was concluded that agitation has a pronounced effect on dilute acid hydrolysis. It may be due to the enhanced level of interaction of the biomass with an acid solution, which is more while agitation during soaking than only soaking without agitation (Fig 4b).

The effect of different acid concentration on xylose yield was studied and it was observed that the xylose yield is increasing with an increase in acid concentration upto 3 % and after that the yield is reduced. The reason of the decrease is may be due to the enhancement in formation of degradation product at higher acid concentration (Fig 4c). There is an increasing trend of xylose yield upto 160 rpm when the agitation speed is increased during soaking time of 3h, at soaking temperature of 50 ^{o}C (323K), using 3% dilute sulphuric acid, but the xylose yield starts reducing at higher agitation speed (Fig 4d). The reason behind the reduction is may be due to production of more inhibitory product like furfural, which may degrade the xylose formed. It is also observed that while increasing the soaking temperature from 30 ^{o}C to 60 ^{o}C (303K to 333K), keeping other parameters fixed at 3 h soaking, using 3% acid solution, at 160 rpm agitation speed, the xylose yield increases with boiling time up to 20 min and then reduces (Fig 4e). A maximum xylose yield of 77.4 % was obtained at operating temperature of 50 ^{o}C/323 K, with 3 h soaking time at 160 rpm agitation speed using 3 % acid solution and boiling time of 20 min.

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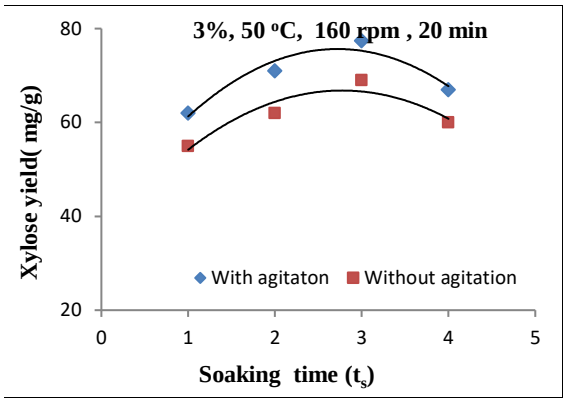

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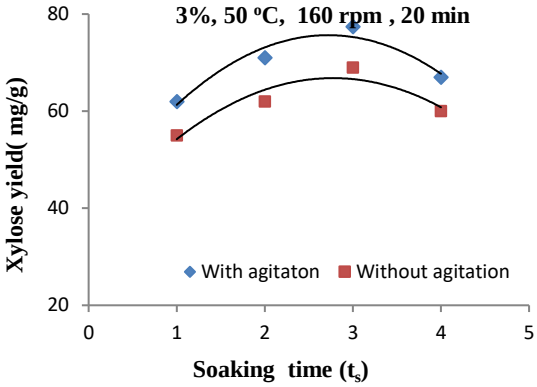

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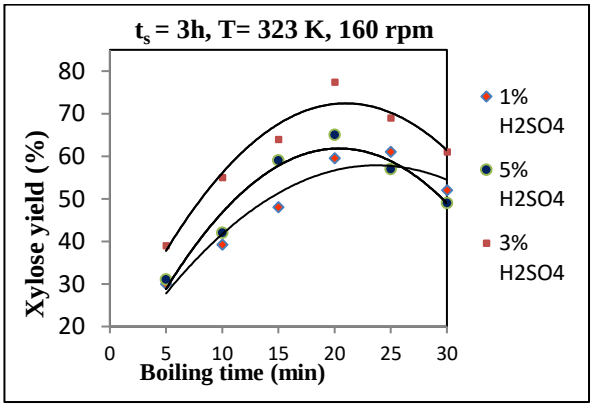

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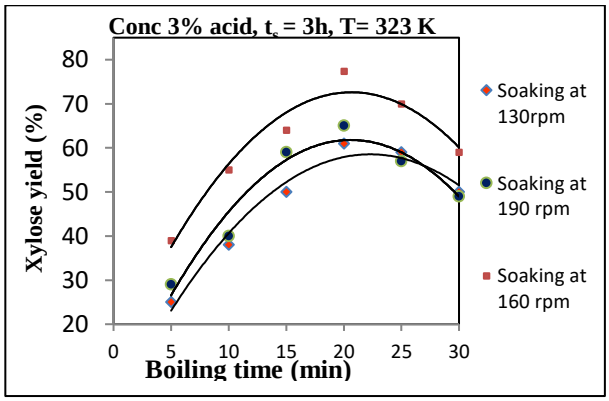

(d)

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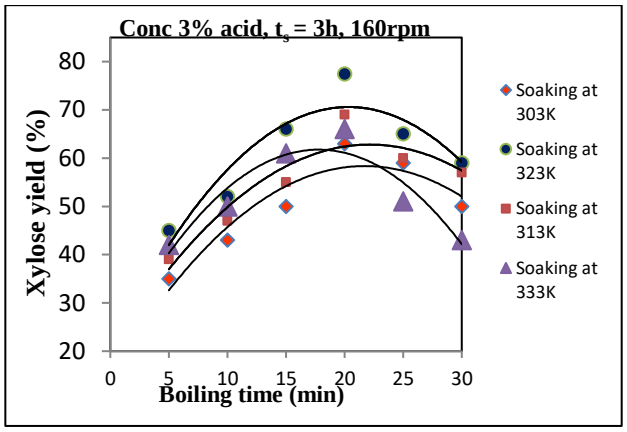

(e)

Fig 4. (a) Xylose yield from dry and powdered WHB and fresh WHB paste; (b) Effect of agitation on xylose yield in respect to soaking time (t_s) at 50 ^oC/323 K, 160 rpm, 15 min. boiling; (c) Effect of different acid concentration on xylose yield in respect to boiling time at soaking time ($t_{s)}$ = 3 h at 50 ^oC/ 323 K, 160 rpm; (d) Effect of agitation on xylose yield in respect to boiling time during soaking with 3% acid, 3 h soaking time; (e) Effect of different operating temperature on xylose yield in respect to boiling time xylose yield in respect to boiling time during 3 h soaking at 50 ^oC with 3% acid.

3.5. Reaction Kinetics and Estimation of Reaction Constants

The dilute acid hydrolysis reactions are in general very complex as the substrate and catalyst are in different phase. As discussed above the reaction follows formation of the products and simultaneously degradation of the products by forming derivatives like furfurals, furans etc. which is explained in the following equation:

$$\text{Hemicellulose (Xylan)} \xrightarrow{k_1} \text{Xylo-oligomers} \xrightarrow{k_2} \text{Xylose} \xrightarrow{k_3} \text{Degradation products} \quad (9)$$

Using the differential equations discussed above, the value of kinetics constant are estimated. Also the effect of all the operating parameters on the kinetic constant and xylose were studied (Fig 5a–5e). The change in rate constants at different operating temperature using 3% acid solution is shown in Fig 5a. There is a gradual increase in the kinetic rate constant with the increase in temperature from 303 K to 333 K. As the temperatures rise, the collision increases and the reaction rate is increased. The variation of rate constants at different acid concentration at 323 K is shown in Fig 5b.

After the estimation of kinetic constant at different operating conditions, some other important kinetic information has been derived using equation (10). The straight line in the plots shows that dilute acid catalyzed hydrolysis follows Arrhenius law which follows the following equation as-

$$\mathbf{k_2 = Ae^{(-Ea/RT)}} \quad (10)$$

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Here 'k_2' denotes the kinetic rate constant (min^{-1}) mainly the rate constant for xylose production, 'A' denotes the frequency factor (min^{-1}), the frequency at which the molecules collide during chemical reaction, also known as pre-exponential factor, 'E_a' is the activation energy ($KJmol^{-1}$), 'R' is the universal gas constant(8.314 $KJmol^{-1}K^{-1}$), 'T' is the absolute temperature in K. Here pre-exponential factor build a link between acid concentration and temperature. It is observed that with the increase in operating temperature the reaction constant increases exponentially. Acid concentration has an extreme effect in changing the rate constants (Gamez et al., 2004) which may be explained through the regression analysis as explained by the equation 11.

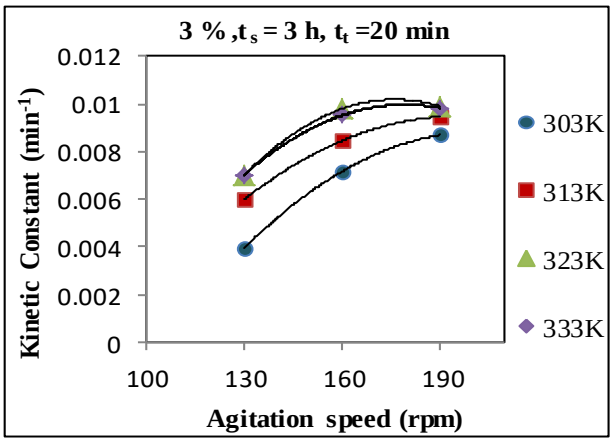
$$\mathbf{A=a[A_c]^n} \qquad (11)$$

Here, A_c is the acid concentration, a, n are the regression parameters, A is the frequency factor. So from equation (10), k_2 is first calculated, based on which all other reaction constants can be determined. From equation (10) and (11), we can have the equations as follows –

$$\mathbf{\ln k_2 = (-E_a/R)\,(1/T) + \ln A} \qquad (12)$$

(- E_a/R) denotes the slope of the straight line. And from equation (11) we can have equation (13) as follows :

$$\mathbf{\ln A = n \ln[Ac] + \ln a} \qquad (13)$$



(a)

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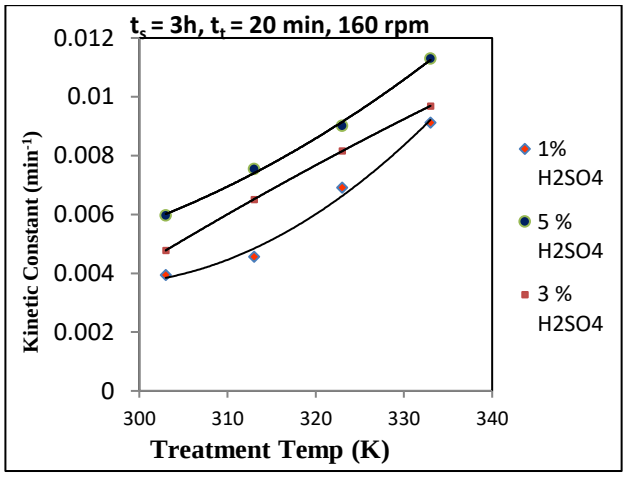

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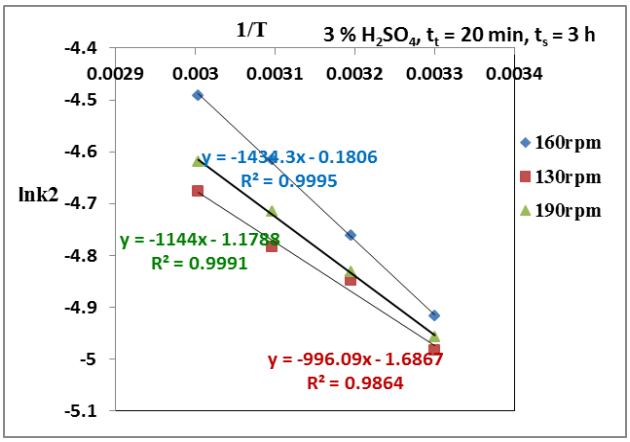

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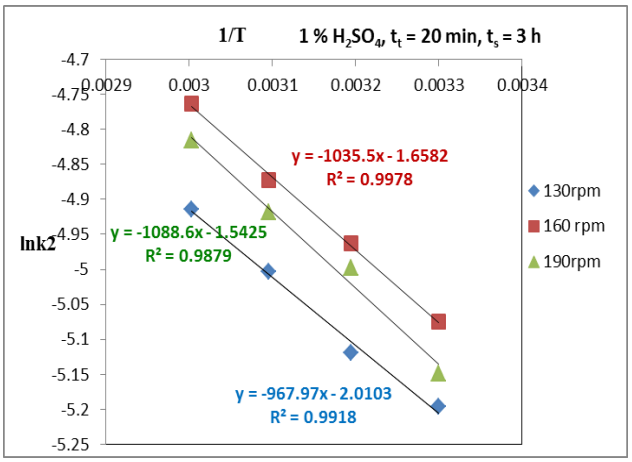

(d)

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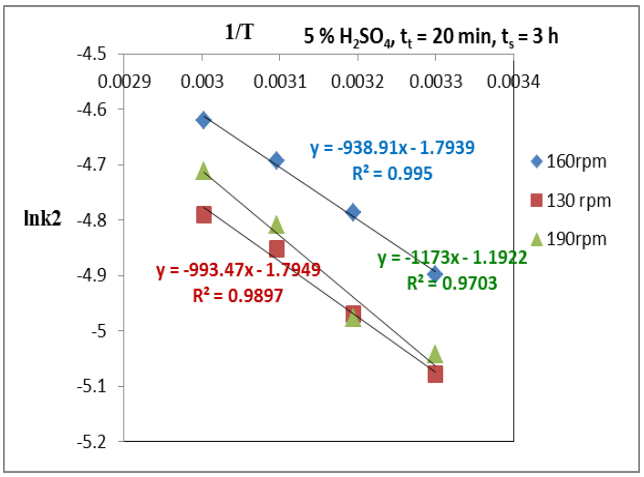

(e)

Fig 5. (a) Graph with variation of Kinetic constant (k_2) at different agitation to study the effect of hydrolysis at different operating temperature using 3 % acid solution with 3 h soaking time (t_s), and 20 min boiling time(t_t) of water hyacinth biomass; (b) Graph with variation of Kinetic constant (k_2) at different temperature to study the effect of hydrolysis of water hyacinth biomass at different acid concentration (%) at 3 h soaking time at 160 rpm with 20 min boiling time; (c) Arrhenius plot for hydrolysis of water hyacinth biomass at different agitation using 3 % acid solution with 3 h soaking time, and 20 min boiling time; (d) Arrhenius plot for hydrolysis of water hyacinth biomass at different agitation using 1 % acid solution with 3 h soaking time, and 20 min boiling time; (e) Arrhenius plot for hydrolysis of water hyacinth biomass at different agitation using 5 % acid solution with 3 h soaking time, and 20 min boiling time

Here, Fig 5a shows with an increase in the operating temperature during soaking, the rate of reaction also increases. Also the effect of change in the kinetic constant while using different acid concentration at different operating temperature is shown in Fig 5b. It can be clearly observed that the rate constant gradually increases with the increase in operating temperature. Fig 5c shows that with increase in treatment temperature, the value of lnk gradually decreases. Moreover, from the graph it is evident that as agitation speed differs the slope of the line also changes accordingly, which is higher in case of 160 rpm agitation. From the slope of each straight line activation energy and frequency factor is estimated and tabulated in Table 2.

Here higher R^2 correlation value, more than 0.95, shows good agreement with the experimental value which follows the equation. A maximum of 77.4% of xylose yield was obtained at an agitation speed of 160 rpm with 3 h soaking time at soaking temperature of 323 K using 3% acid and 20 min of boiling time.

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Table 2. Estimation of Kinetic parameters of water hyacinth biomass hydrolysis at 3h soaking time with 20 min boiling time.

Agitation speed (rpm) & Acid concentration (%)	**Kinetic constants, k_2(min $^{-1}$)** Temperature (K)				**Activation Energy, E_a (kJ mol^{-1})**	**R^2**
130 rpm	**303**	**313**	**323**	**333**		
1%	0.00554	0.00598	0.00672	0.00734	8.05	0.99
3%	0.00685	0.00784	0.00837	0.00931	8.28	0.99
5%	0..00623	0.00694	0.00781	0.00831	8.25	0.98
160 rpm						
1%	0.00625	0.00699	0.00765	0.00854	8.60	0.99
3%	0.00732	0.00856	0.00989	0.00931	11.92	0.99
5%	0.00745	0.00812	0.00889	0.00923	7.80	0.97
190 rpm						
1%	0.00581	0.00675	0.00731	0.0081	9.04	0.98
3%	0.00703	0.00798	0.00895	0.00987	9.51	0.98
5%	0.00645	0.00689	0.00816	0.00899	9.75	0.97

3.6. Detoxification of Acid Hydrolysate

Over liming and activated charcoal treatments were used to reduce the effect of inhibitors produced during acid hydrolysis after concentration of hydrolysate in vacuum rotator, which improved the bioconversion of the sugars into xylitol. After detoxification the total phenolics were reduced from 975 mg/l to 223 mg/l (75.2% removal) and xylose yield was increased to 79% after detoxification.

3.7. Fermentation

Batch fermentation was performed from the concentrated and detoxified water hyacinth hemicellulosic hydrolysate, using 10%(v/v) *Pichia stipitis* (NCIM 3500) and *Candida shehatae* (NCIM 3497). 33.5 g/l of xylitol was produced using *Pichia stipitis* with a yield of 0.63 g xylitol/g of xylose at 48 h and 31.1g/l of xylitol was produced usiting *Candida shehatae* with a yield of 0.61g xylitol /g of xylose at 48h as shown in Fig 6a. The HPLC plot for xylitol production by *Candida shehatae* and *Pichia stipites* is given in Fig 6b and Fig 6c. Our results are in accordance with of 26 g/l xylitol produced from corn husk by *Pichia stipitis* CBS 5773 as reported earlier (Chaudhary et al., 2013) and 13.02 g/l xylitol

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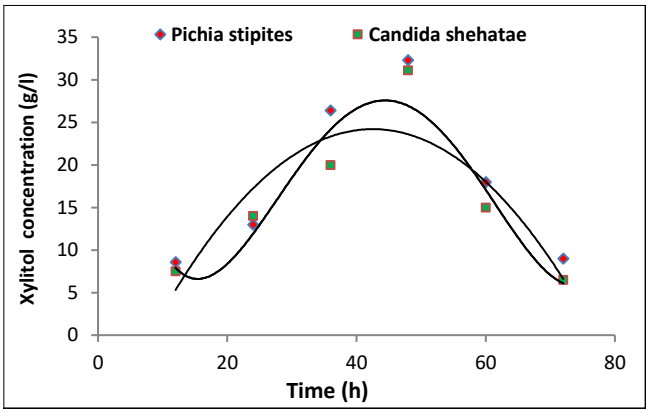
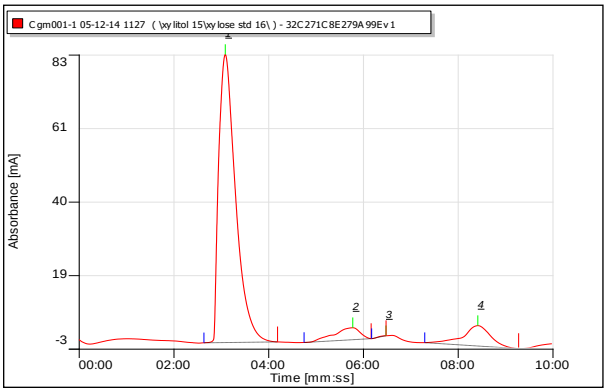
(Zhang et al., 2014) from cotton stock by *Candida tropicalis*. 32.5 g/l of xylitol (Kalhornia et al., 2013) was obtained by *Candida tropicalis*Y-27405 from WHB.



(a)



(b)

Report Layout for *Candida shehatae*

No.	Peak Name	Ret. Time [mm:ss]	Start Time [mm:ss]	End Time [mm:ss]	Area [mAs]	Height [mA]	Quantity
001	Solvent	03:05.7	02:38.6	04:11.8	2065.9	83.7	N/A
002	Xylitol	05:47.1	04:45.4	06:10.4	134.4	3.5	N/A
003	Xylose	06:29.2	06:11.0	06:29.3	1.1	0.0	N/A
004	Furfuryl	08:25.5	07:18.2	09:17.2	238.8	5.9	N/A

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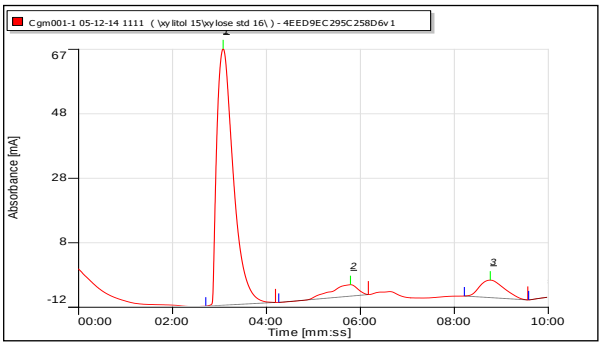

(c)

Report Layout for *Pichia stipites*

No.	Peak Name	Ret. Time [mm:ss]	Start Time [mm:ss]	End Time [mm:ss]	Area [mAs]	Height [mA]	Quantity
001	Solvent	03:05.4	02:43.1	04:12.2	1912.2	78.6	N/A
002	Xylitol	05:48.0	04:16.4	06:10.8	142.5	3.5	N/A
003	Furfuryl	08:46.6	08:13.5	09:34.5	195.3	5.4	N/A

Fig 6. (a)Xylitol production from hemicellulose hydrolysate of WHB by *Pichia stipitis* (NCIM 3500) and *Candida shehatae* (NCIM 3497); (b) HPLC graph for Xylitol production by *Candida shehatae* (NCIM 3497), with report layout; (c) HPLC graph for Xylitol production by *Pichia stipites*(NCIM 3500) with report layout

4. Discussion

With the increase in worldwide interest for Xylitol production and consumption for the benefit and better accessibility to the mankind, lignocellulosic material can prove to be an efficient source of hemicellulose from where higher xylose containing hydrolysate can be produced by performing hydrolysis with dilute sulphuric acid with proper selection of the parametric conditions like (soaking time (1-4 h), temperature (30 ^{o}C, 40°C, 50 ^{o}C and 60 ^{o}C), agitation (130, 160 and 190 rpm), dilute acid solution (1%, 3% and 5% of H_2SO_4), and boiling time (5-30 min) for obtaining the maximum xylose yield in the present study. A comprehensive study of acid catalyzed hemicellulose hydrolysis reaction kinetics shows that there is a distinct relationship of agitation, soaking time, soaking temperature acid concentration and boiling time during hydrolysis of water hyacinth biomass. Arrhenius equation was used to study the relation of the activation energy, frequency factor and kinetic rate constants. With the increase in temperature from 303 K to 323 K and agitation from 130 rpm to 160 rpm, the activation energy increases with higher xylose yield. Maximum xylose yield of 77.4% was obtained at an operating

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temperature of 323 K with soaking time of 3 h at an agitation speed of 160 rpm and boiling time of 20 min. After microbial fermentation of WHB hydrolysate, 32.3g/l of xylitol was produced from *Pichia stipitis* with an yield of 0.63 g xylitol/g of xylose at 48 h and 31.1g/l of xylitol was produced from *Candida shehatae* with a yield of 0.61g xylitol /g of xylose at 48h. 26 g/l xylitol produced from corn husk by *Pichia stipitis* CBS 5773 as reported earlier (Chaudhary et al., 2013) and 13.02 g/l xylitol (Zhang et al., 2014) from cotton stock by *Candida tropicalis.* 32.5 g/l of xylitol (Kalhornia et al., 2013) was obtained by *Candida tropicalis* Y-27405 from WHB. WHB hydrolysate gave higher xylitol yield when compared to the production of xylitol from other lignocellulosic substrates.

5. Conclusion

Xylitol is an extremely important constituent in food and pharmaceutical industries. It is a sugar substitute for diabetic patients and a non-toxic, harmless food with many beneficial effects for human organism and finds wide application in pharmaceutical industries owing to its suitability for diabetic patients, non-cariogenic properties and chemically inert nature. Both extraction from natural resources and industrial process being very expensive, alternatively microbial reduction of hemicellulosic fraction of lignocellulosic biomass is considered as a sustainable and eco-friendly process. Water hyacinth is a better competent and promising lignocellulosic substrate which can be effectively used as a novel substrate for the bioconversion to highly value added product. However, the effective and economic production of xylitol needs further emphatic research in order to make it more economically abundant at the industrial level. The present study concludes that water hyacinth can be effectively used as a novel substrate for the bioconversion of lignocelluloses to highly value added product xylitol, which opens the prospective of further studies on the optimization of xylitol yield.

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