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Investigations on the effect of driving parameters for xylitol production from water hyacinth biomass

Anamica Bhattacharya[1], Amit Ganguly[2], Anup Kumar Sadhukhan[1] and Pradip Kumar Chatterjee[2]*

[1]Department of Chemical Engineering, National Institute of Technology Durgapur, Durgapur 713209, India
[2]Thermal Engineering, CSIR-Central Mechanical Engineering Research Institute, Durgapur 713209, India

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Acid hydrolysis of fresh water hyacinth biomass has been performed under different conditions; soaking time (1 - 4 h), temperature (30°C, 40°C, 50°C and 60°C), agitation speed (130, 160 and 190 rpm), dilute acid solution (1%, 3% and 5% of H_2SO_4) and treatment time (5 - 30 min) for obtaining the optimum xylose yield with further fermentation towards xylitol production in the present study. The parametric conditions were optimized by using MATLAB and Design Expert software. The maximum xylose yield of 211 mg/g was obtained at the optimal condition of soaking time of 2 h at 50°C temperature with agitation speed of 160 rpm with 3% of dilute acid solution and 20 min of treatment time. The xylose obtained was fermented to get 0.63 g and 0.61 g of xylitol using *Pichia stipitis* and *Candida shehatae* respectively after 48 h of fermentation at 30°C from 1 g of xylose.

Keywords: Water hyacinth biomass, xylitol, acid hydrolysis, detoxification, fermentation

Introduction

Xylitol is a 5-carbon sugar poly-alcohol, a sugar substitute for diabetic patients and a non-toxic, harmless food with many beneficial effects for human organism. Water hyacinth (*Eichhornia crassipes* (Mart.) is considered to be the world's worst aquatic weed, however, it is a potential resource characterised by a high hemicellulose content (38%) in comparison to other available lignocellulosic materials. So far, water hyacinth is an economic and ecological burden to many tropical and sub-tropical regions of the world, causing problems for millions of users of water resources. Bioconversion of lignocellulosic biomass to value added product can be considered a beneficial aspect of research given the increasing concern about environmental issues and better human nutrition. Lignocellulosic biomass is the most abundantly available, renewable and inexpensive material on earth which can be efficiently used to generate xylitol, which is a highly valuable product with huge importance in food and pharmaceutical industries. Water hyacinth biomass (WHB) is sufficiently rich in cellulose and hemicellulose to produce monomeric sugars such as hexoses and pentose, from which several value added products can be obtained. Xylitol ($C_5H_{12}O_5$) is a value added product which has attracted worldwide interest due to its properties. It is a five-carbon sugar polyalcohol, which is also called wood or birch sugar[1] due to its high sweetening capacity. It is found in significant quantities as a natural constituent of various fruits and vegetables[2]. Traditional production of xylitol involves direct chemical hydrogenation of the hemicellulosic hydrolysate over a Raney-Nickel catalyst followed by extensive purification from non-specific reduction products[1]. Alternative methods of xylitol production are chemical or microbial reduction of D - xylose or hydrolysate of xylan rich hemicelluloses material. Microbial xylitol production is more favorable for industrial applications due to mild fermentation conditions like atmospheric pressure and ambient temperature. Yeasts are considered to be the best xylitol producers. A number of yeasts and moulds can produce xylitol from xylose because they possess the enzyme xylose reductase. *Candida boidinii, Candida guilliermondii, Candida tropicalis, Candida magnolia, Debaryomyces hansenii* and *Pichia stipitis*[3] are some of the yeasts with xylitol production capability. Xylose fermenting yeasts reduce xylose to xylitol by the NAD(P) H-dependent xylose reductase (XR). Recently considerable attention has been drawn to the bioconversion of xylose into xylitol from lignocellulosic hydrolysate. Dilute acid hydrolysis refers to the hydrolysis of hemicellulosic material by

*Author for correspondence:
Tel: +91-343-6510236; Fax: +91-343-2546745
pradipcmeri@gmail.com

acids (typically sulphuric, hydrochloric or phosphoric acid) at concentrations of 1 - 10% using a moderate temperature (100 - 150°C). Dilute acid hydrolysis is mostly used because it can effectively solubilize hemicelluloses to xylose and other sugar monomers. However, it proves less effective in the formation of hexoses under these relatively moderate operating conditions. Dilute acid pretreatment process, is inexpensive, avoids corrosion and is easy to perform[4]. Detoxification is a major step for removing or decreasing the concentration of inhibitors. Some of the methods to overcome these inhibitors are the 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[5].

The kinetics of hemicellulose hydrolysis is very important for understanding the complex reactions, process development and sugar recovery. Reactions using dilute acid are very complex, mainly because the substrate is in a solid phase while the catalyst is in a liquid phase. 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 intricate reactions[6-8]. Hemicellulose hydrolysis model was proposed to follow a first order dependence on reactant concentration of Seamen[9]. The kinetic models based on dilute sulphuric acid pre-treatment have to be studied extensively in order to maximize xylose yield, while avoiding the degradation reaction.

The present study was intended to obtain an optimum xylose yield from dilute acid hydrolysis of water hyacinth biomass (WHB) by including driving parameters as soaking time, soaking temperature, agitation speed, treatment time and acid concentration. Kinetic modeling of hemicellulose hydrolysis by dilute acids has been included to study the reaction kinetics of hydrolysis. MATLAB 7.10.0 (The Math works Inc. Ver. 7.10.0. US) was used to optimize xylose yield by Reed-Solomon (RS) encoder tool, surface fitting tool and non-linear multivariable regression analysis. The respective error was comparable to find the best fitting model and the analytical yield was compared with the predicted yield and the parameters were optimized.

Materials & Methods

Biomass Preparation

Fresh water hyacinth (*Eichhornia crassipes*) was collected from a natural pond inside CSIR-CMERI, Durgapur (23.5500° N, 87.3200° E) campus, India 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 were also chopped into small pieces, dried at 80°C for 8 h and ground to fine powder for biomass characterisation. The cellulose, lignin and hemicellulose fraction of water hyacinth were analyzed using standard detergent (ADF & NDF) extraction method[10].

Preparation of Hemicellulose Acid Hydrolysate

A sample (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 soaking for a period of 1, 2, 3, 4 h, agitated at 130, 160, 190 rpm at different temperatures ranging from 30 - 60°C. The samples were boiled for 5, 10, 15, 20, 25 and 30 min at 100°C in heating mantle after soaking. 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.

Scanning Electron Microscopy (SEM)

The treated and untreated biomass was examined under scanning electron microscope (SEM), HITACHI S3000N, which uses a focused beam of high energy electrons to generate a variety of signals at the surface of solid specimens. The signals that were generated from the electron sample interactions gave information about the sample including external morphology (texture), chemical composition, and crystalline structure and orientation of materials present in the biomass to clearly distinguish between treated and untreated biomass. Areas ranging from approximately 1 cm to 5 microns in width can be imaged in a scanning mode using conventional SEM techniques (magnification ranging from 20X to approximately 30,000X, spatial resolution of 50 to 100 nm). Scanning electron micrographs (SEM) were taken for untreated and treated water hyacinth biomass.

Concentration and Detoxification of Acid Hydrolysate

Concentration of the hydrolysate was carried out to increase the amount of sugars in hydrolysate of water

hyacinth by rotary evaporator (Yamato Rotary Evaporator, RE-300). Calcium oxide was added with stirring to the concentrated hydrolysate, until the pH of the hydrolysate reached 10. It was then incubated for half an hour followed by centrifugation (3000 rpm, 20 min) and filtration. Concentrated sulphuric acid was added to make the pH back to 6. Activated charcoal (3.5% w/v) was added to the hydrolysate after over liming 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.

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 medium used for inoculums preparation contained (g/L): yeast extract 10, peptone 20 and 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 rpm for 10 minutes to obtain the pellets. The cell pellets were washed and suspended in sterile distilled water.

Fermentation

The detoxified hydrolysate was supplemented with (g/l) NH_4Cl 0.5, KH_2PO_4 2.0, $MgSO_4.7H_2O$ 0.5, yeast extract 1.5, $CaCl_2.\ 2H_2O$ 0.1, $FeCl_3.2H_2O$ 0.1, $ZnSO_4.7H_2O$ 0.001 and was inoculated with 10% (v/v) inoculums of *P. stipitis*, at pH 5.0 and incubated at 30°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.

Analytical Methods

The total reducing sugars, released after acid hydrolysis were estimated by the dinitro salicyclic acid (DNS) method[11] and xylose sugar was determined using the phloroglucinol assay[12]. The fermentation inhibitors (furans and phenolics) were analyzed by spectroscopic analysis. Phenolics estimation was carried out by Folin-Ciocalteu method[13].

Xylitol Estimation

Xylitol and the sugars present in the fermented broth were estimated by high performance liquid chromatography (HPLC) using instrument, Cecil Adept system 9 fitted with Hichrome Cosmosil Sugar D column (4.6 mm I.D. x 250 mm) with refractive index (RI) detector (CE 4700) using acetonitrile-water (75 : 25) as mobile phase at a flow rate of 1 mL/min at 30°C.

Kinetics of Acid Hydrolysis of Hemicellulose

The kinetics of hemicellulose hydrolysis is extremely important for understanding the complex reactions, process development 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 compositional structure of hemicellulose makes the hydrolysis reaction very complex. The reaction rate of hydrolysis depends on a number of variables like temperature, acid concentration, time, substrate concentration and substrate composition. 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 carbon cation 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[8]. 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 below:

$$(C_5H_8O_4)_n + nH_2O \longrightarrow nC_5H_{10}O_5$$

Where, $(C_5H_{10}O_5)_n$ 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. In correspondence to Seaman's previous work on dilute sulphuric acid catalysed cellulose hydrolysis[9], the hemi cellulose hydrolysis reactions were also modelled 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 hemi cellulose fibres a more networked model was proposed by Kobayshi and Sakai (1956)[14].

Hemicellulose Hydrolysis Models

As proposed by Saeman[9] in 1945 the simplest model describing a monophasic hemicellulose hydrolysis based on a two step first-order is shown in Figure 1. According to this approach, hemicellulose is hydrolyzed to xylose, which in turn breaks down to degradation products in a second reaction:

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

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

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

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

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

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

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

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

Owing to the structural complexities of the hemi-cellulose fibres a more networked model was prepared by Kobayshi and Sakai (1956)[14]. Based upon their experimental observations, they proposed the existence of a bi-phasic pattern during the hemi cellulose hydrolysis. This pattern shows that one part (easy-to-hydrolyse hemi cellulose) of the total hemi cellulose complex can hydrolyse more easily than the other fraction (hard-to-hydrolyse hemi cellulose).

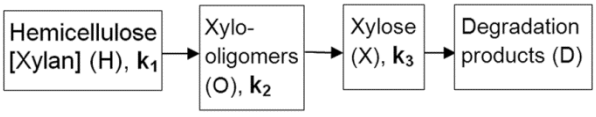

Fig. 1 — Schematic diagram of monophasic hydrolysis model of hemi cellulose.

Design of Experiments

In order to estimate the combined effect of the five independent factors affecting the hemicellulose hydrolysis of WHB to obtain a maximum xylose yield and optimize the process parameters for maximum xylose yield, a set of 864 data of xylose yield were obtained from experimental analysis in laboratory. All data were analysed to obtain an optimized xylose yield from regression analysis through RS tool and surface fitting tool (3D) from MATLAB (The Math Works Inc. Ver. 7.10.0). The RS tool opens a graphical user interface for interactively investigating one-dimensional contours of multi-dimensional response surface models for calculating the root mean square error (RMSE) and residuals. The best fit regression equation was compared by plotting the graph and calculating the error for different models as linear. pure quadratic, interactions and full quadratic models to find out the best model fitted for our experiment. Also a central composite design of the full quadratic model of central composite design of 87 experiments was designed based on the independent parameters using Design Expert version 7.0.0 to perform the regression analysis and statistically analyze the results for optimization and to obtain the coefficients. The statistical significance of the model was established by F-test analysis of variance (ANOVA). The significance of each coefficient of the model was evaluated by F values and p values.

Results and Discussion

Characterisation of Water Hyacinth Biomass

The composition of water hyacinth as analysed in the laboratory is shown in Table 1. The moisture content of the WHB paste (uniform paste leaf and

Table 1 — Comparative analysis of lignocellulosic characterization of WHB

Plant material (Expressed in %)	Present study uniform mix of leaf and stem (petiole)
Cellulose	32.5
Hemicellulose	38.1
Lignin	11.3
Ash	1.86
Crude protein	14.2

petiole) was measured to be 95.2%. Simultaneously lignocellulosic content in the root, leaf and stem (petiole) of the plant is also analyzed separately. It was found that the hemicellulose content in leaf and stem (petiole) of the plant is higher than the root, so uniform mixture (1 g of leaf and 1 g of the stem were mixed) of leaf and stem were analyzed which is given in Table 2.

Scanning Electron Microscopy (SEM)

The processed and untreated biomass was further analyzed by scanning electron microscopy (SEM). Figure 2a shows that the untreated biomass is generally homogenous, rigid and characterised by a highly ordered structure with smaller pore sizes. Meanwhile, Figure 2b shows that the treated biomass has more pores exhibiting highly distorted structures. The heterogeneous distribution of the pores and rough texture on the surface with more distorted structures present in the treated biomass confirms the pronounced effect of hydrolysis increasing the surface area of water hyacinth biomass.

Influence of Different Parameters on Acid Hydrolysis of Water Hyacinth Biomass (WHB)

Acid hydrolysis of water hyacinth biomass was performed using different parameters which include soaking time (1, 2, 3, 4 h), soaking temperature (30°C - 60°C), agitation speed (130, 160, 190 rpm), acid concentration (1%, 3%, 5%) and boiling time (5, 10, 15, 20, 25 and 30 min.). The effects of different parameters were studied to find out the best set which gives a maximum xylose yield. Fresh and dried water hyacinth biomass were compared which concluded that the fresh biomass paste gives a higher xylose yield as shown in Figure 3a. Furthermore, the effect of agitation during soaking at different temperatures was studied and it was observed that agitation has a pronounced effect on dilute acid hydrolysis, which may be due to the enhanced level of interaction of the biomass with the acid solution. The xylose yield was higher with agitation during soaking than only soaking without agitation as shown in Figure 3b. After soaking, the sample was boiled at 100°C so that the inhibitory products formed during hydrolysis may be evaporated and maximum xylose sugar can be recovered for fermentation. There was an increase in xylose yield with the increase in soaking time, agitation speed and treatment time using 3% dilute sulphuric acid. However, the xylose yield started decreasing after 3 h of soaking at 60°C with an increase in agitation speed as shown in Figure 3c. The reason behind the reduction is may be the production of more inhibitory product like furfural which may degrade the xylose formed. It has been observed that when the boiling time was increased other parameters being fixed (2 h soaking, using 1, 3 and 5% acid solution, 160 rpm agitation), the xylose yield increased with treatment time up to 20 min and then decreased as shown in Figure 3d. However, at 3% acid solution, 2 h soaking time, the kinetic constant decreased with the increase in soaking temperature as shown in Figure 3e.

The Arrhenius plot showing the variation of the logarithm of kinetic constant (lnk) with 1/T for various acid concentrations, agitation speed and soaking time is represented in Figure 4a, 4b and 4c. Figure 4a shows that with increase in treatment temperature, the value of lnk decreases. Figure 4a shows that the higher is the acid concentration, the more pronounced is the change in slope of the straight line. Similar pattern is noticed in Figure 4b, where with the increase in treatment temperature (T) there is a decrease in the value of lnk. Moreover, from the graph it is clear that as agitation speed increases the slope of the line also increases. Figure 4c shows that with an increase in treatment temperature T, the value of lnk decreases with increasing soaking time. Therefore, the longer the soaking time, the steeper is the slope of the line. The straight line in the plot indicates that the hydrolysis reaction follows the Arrhenius law as in the following equation:

$$k = Ae^{(-E/RT)} \quad \ldots (8)$$

Table 2 — Lignocellulosic characterization in leaf, stem (petiole) and root of WHB

Component	Leaf of WHB (%)	Stem of WHB (%)	Root of WHB (%)
Cellulose	33.4	29.6	9
Hemicellulose	29.1	36.2	20
Lignin	12.4	14.1	31.5

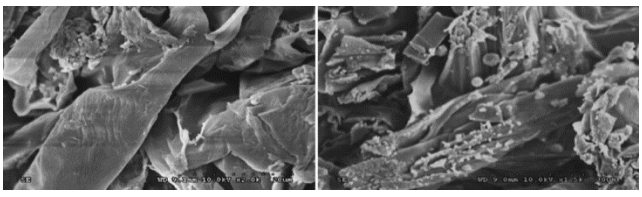

Fig. 2 — Scanning electron micrograph (a) Untreated biomass, (b) Treated with 3% sulphuric acid, 160 rpm, 2 h soaking at 50°C and boiled for 20 min.

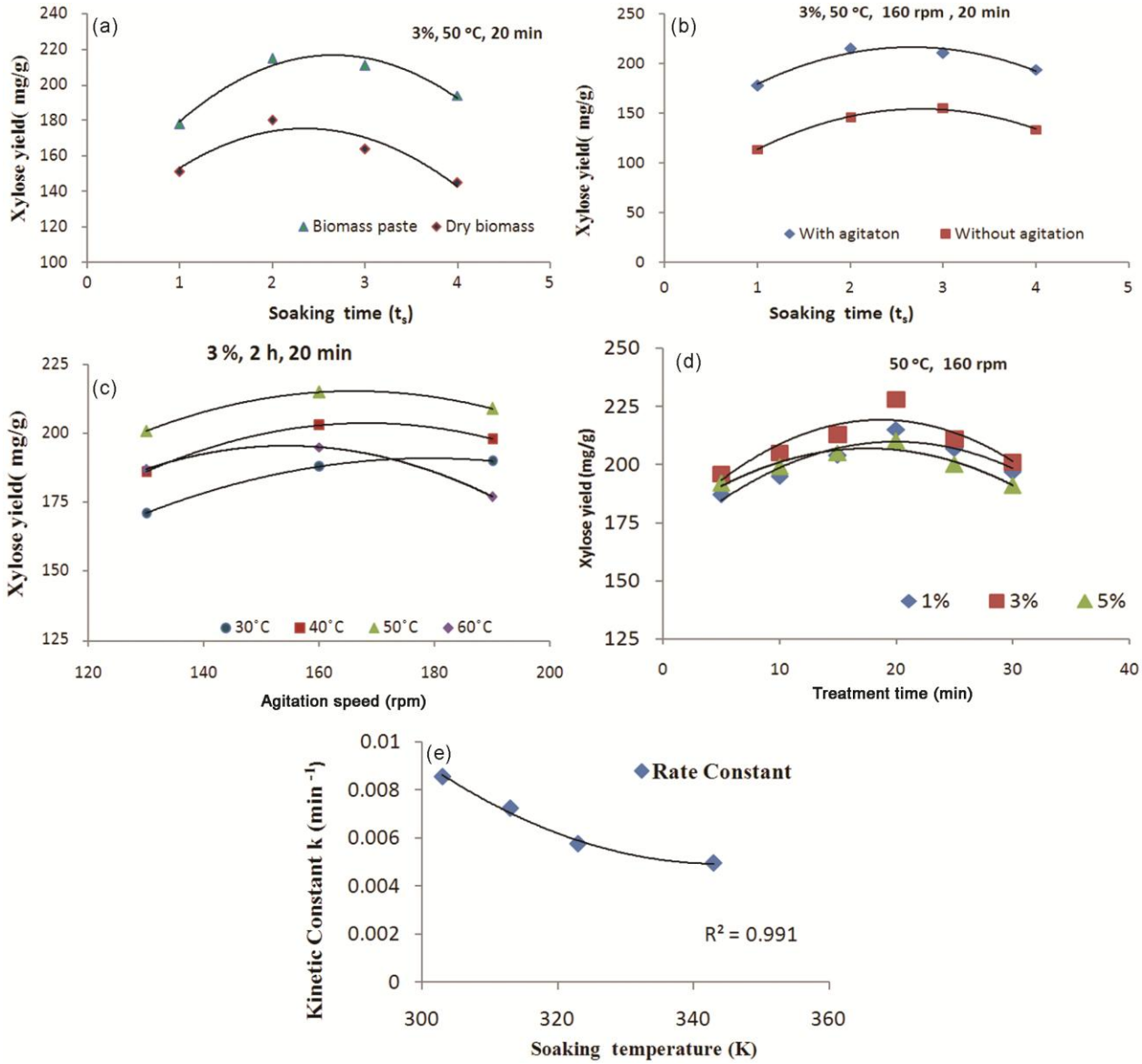

Fig. 3 — (a) Xylose yield from dry WHB and fresh WHB paste, (b) Effect of agitation on xylose yield in respect to soaking time (t_s) at 50°C, 160 rpm, 15 min. boiling; (c) Effect of temperature on xylose yield in respect to agitation during soaking with 3% acid, 2 h soaking time, 15 min. boiling time (d) Effect of acid concentration on xylose yield in respect to boiling time during 2 h soaking at 50°C with 3% acid, (e) Graph with variation of Kinetic constant (k) at different treatment temperature, (K) at 3% acid solution, 2 h soaking time.

Where 'A' is defined as the pre-exponential factor or the frequency factor and it depends on the concentration of the reacting medium used. 'E' represents activation energy and T is the treatment temperature in degrees Kelvin. 'R' represents the molar gas constant. 'E' and 'A' can be easily found out from the slope and the intercept of the curves respectively.

Optimization of Parameters for Hemicellulose Hydrolysis

A set of experiments were performed in different parametric conditions to study the effect on sugar yield and to optimize the parameters to obtain maximum bioconversion to xylose sugar for xylitol production. A set of 864 data of xylose yield was obtained from experimental analysis in the laboratory and all the data were analysed to obtain an optimized xylose yield from regression analysis through RS tool and surface fitting tool (3D) from MATLAB (The Math Works Inc. ver. 7.10.0), where RS tool opens a graphical user interface for interactively investigating one-dimensional contours of multi-dimensional response surface models. The best fit regression equation was obtained by plotting the graph and calculating the error for different models as

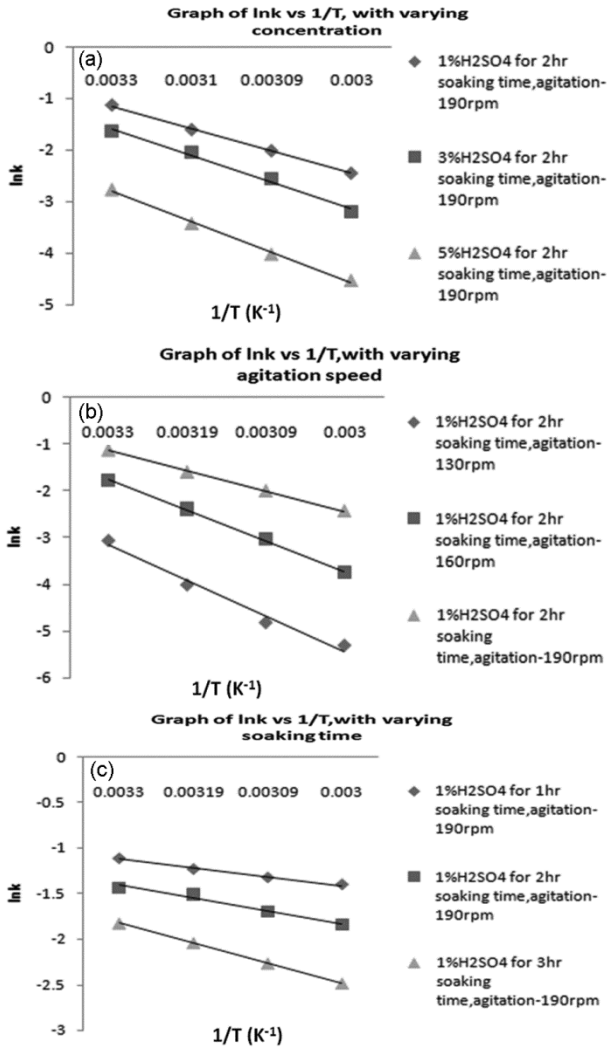

Fig. 4 — Changes in lnk with 1/T for varying (a) Concentration, (b) Agitation speed and (c) Soaking time.

linear. Pure quadratic interactions and full quadratic models were studied to find out the best model fitted for our experiment. The details of the parameters considered are shown in Table 3. Apart from the optimization tool in MATLAB, point prediction was also done using Design Expert 7.0.0 to discover the predicted response values with confidence intervals. Predicted xylose yield was then compared with the present experimental xylose yield as showed in Table 4.

From RS tool in MATLAB, all the experimental data were fed to obtain the best set of parameters with maximum yield and the best fit model from comparison of the calculated RMSE and residuals. After fitting all the data in the pure quadratic model, it was found that the predicted value of xylose yield is 212.05 mg/g of WHB at soaking time 2.54 h, soaking temperature 47.16^{o}C, agitation speed of 166.33 rpm, acid concentration of 3.20% and boiling time of 19.90 min with RMSE of 15.22. However, when the data were simultaneously fed into the full quadratic model, the predicted value of xylose yield was 211.92 mg/g of WHB with parametric conditions as soaking time of 2.46 h, soaking temperature of 46.58^{o}C, agitation speed of 165.75 rpm, acid concentration of 3.45% and boiling time of 19.06 min with RMSE value of 13.87 which proves the data show the best agreement with the full quadratic model with a lower error value of 13.87 in comparison with the pure quadratic model with RMSE of 15.22. The prediction is close to the xylose yield of 215 mg/g of WHB obtained from the experiments. The graphical presentation of the contour plot is shown in Figure 5.

Table 3 — Experimental range and levels of independent process variables

Factor	Name	Low	High
A	Soaking temperature, ^{o}C	30	60
B	Soaking time (h)	1	4
C	Agitation speed (rpm)	130	190
D	Acid Concentration (%)	1	5
E	Treatment time (min)	5	30

Calculation of Coefficients from Design Expert

Final equation in terms of coded factors:

Xylose yield = +208.44 +24.40 *A +0.24 *B +2.00 *C +5.37 *D+3.61 *E-2.90 *A *B -0.025 *A *C -0.069 *A *D -3.10 *A *E -1.28 *B *C-0.069 *B *D -0.35 *B * E -0.13 * C * D +0.27 *C * E +0.069 *D *E -2.58 * A^2 -6.56 *B^2+1.91 * C^2-22.18 * D^2-22.18 * E^2

The interaction effect of the process parameters which had the maximum effect on the total yield of xylose for hemicellulose hydrolysis of WHB was studied by plotting the three dimensional surface plots and fitting the data using surface fitting tool in MATLAB as shown in Figure 6 & 7. It indicates xylose yield as a function of the variables soaking time, soaking temperature and agitation. At the lower level of agitation and soaking time at low temperature, a lesser yield is achieved. The yield of xylose was raised with the increase in temperature and agitation. A slight reduction in the yield of xylose sugar was observed at the highest level of the soaking time and soaking temperature which may be due to feedback inhibition of the system due to the presence of various inhibitory factors.

The effects of all the process parameters as soaking time, soaking temperature, agitation speed, acid

Table 4 — Comparison between predicted xylose yields and experimental data

a) Design summary through central composite design by Design Expert 7.0.0

Study type: response surface initial design: Central Composite design model: quadratic — Runs: 86 blocks: no blocks

Factor	Name	Level	Low level	High level	Std. dev.	Coding
A	Soaking temp	56.42	30.00	60.00	0.00	Actual
B	Soaking time	2.51	1.00	4.00	0.00	Actual
C	Agitation	160.00	130.00	190.00	0.00	Actual
D	Acid conc	3.31	1.00	5.00	0.00	Actual
E	Treat. Time	17.99	5.00	30.00	0.00	Actual

b) Point prediction using Design Expert 7.0.0 with predicted response values (xylose yield) and confidence intervals

Response	Prediction	SE Mean	95% CI low	95% CI high	SE pred	95% PI low	95% PI high
Xylose yield	225	5.03	214.99	235.01	20.75	183.70	266.30

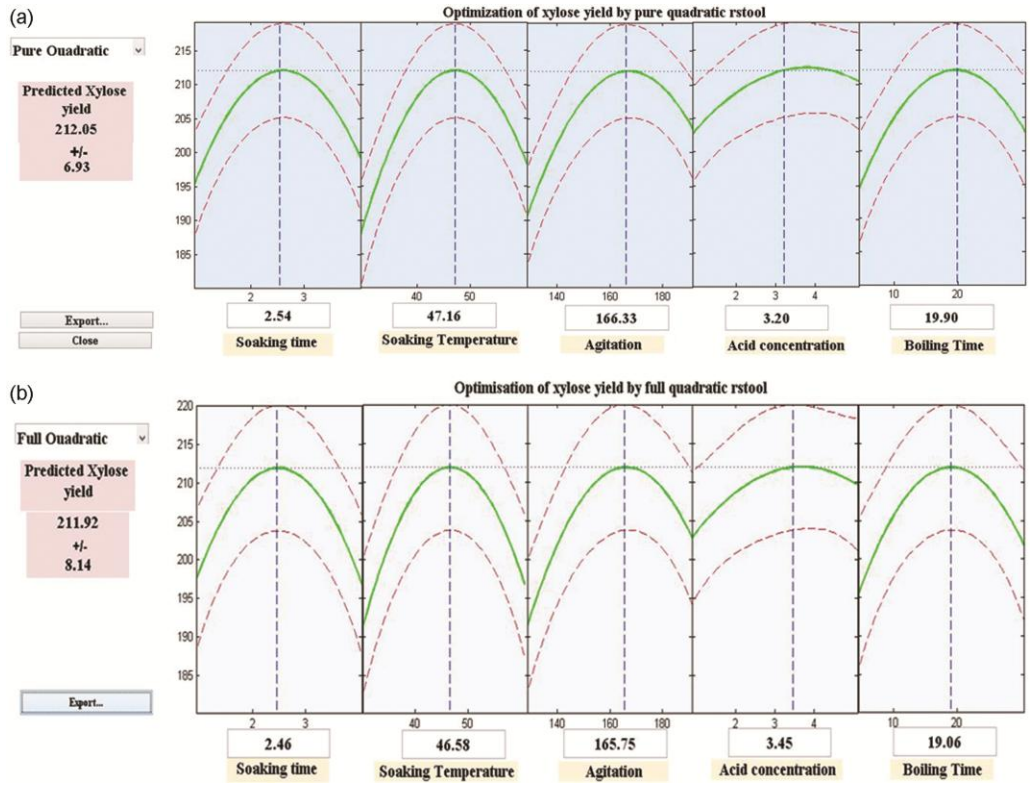

Fig. 5 — (a) Graphical presentation of pure quadratic model through RS tool of MATLAB (RMSE of pure quadratic = 15.22) and (b) Graphical presentation of full quadratic model through RS tool of MATLAB (RMSE of full quadratic = 13.87).

concentration and treatment time were collectively studied for optimization by plotting graphs in 3D surface by Design Expert software as shown in Figure 7 (a - j). The interaction effect of soaking time and soaking temperature on xylose yield is represented in Figure 7a. The xylose yield was very low at the lower temperature and short soaking time but it increases with increase in soaking time and soaking temperature. Interaction between agitation and soaking temperature is shown in Figure 7b. It has been observed that the yield of xylose sugar increases as moved from the lower levels of the factors to the middle levels and then there was a reduction in the yield. This can be explained by the presence of inhibitory factors causing sugar degradation. The interaction between acid concentration and soaking

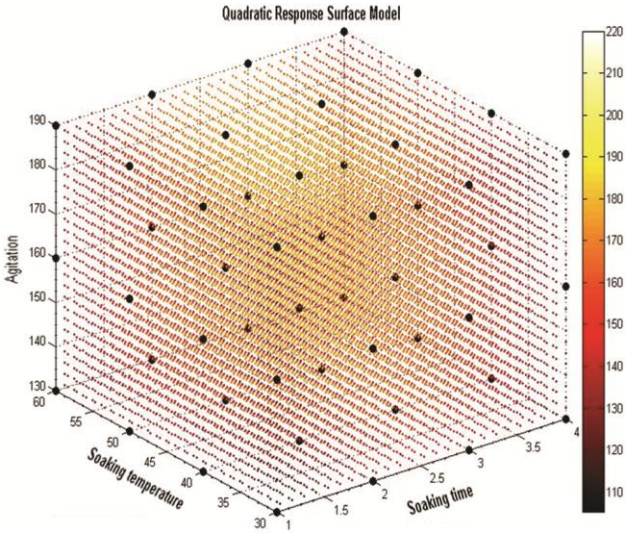

Fig. 6 — 3D quadratic response surface model of experimental data by surface fitting tool in MATLAB.

temperature is shown in Figure 7c, where it can be observed that with the increase in acid concentration and soaking temperature the xylose yield increases. Interaction between treatment time and soaking temperature is shown in Figure 7d, where it can be observed that xylose yield was increasing as it moved from the lower levels of the parameters to the middle levels and then there was a reduction in the yield. Figure 7e shows the interaction between agitation and soaking time, which suggests the increase in the levels of the yield. Figure 7f shows the interaction between acid concentration and soaking time where it appears that with increase in acid concentration and soaking time the yield increases to the middle level and then decreases. This may be due the formation of inhibitory products during the hydrolysis process. Interaction between treatment time and soaking time is presented in Figure 7g, which shows a similar trend as found in Figure 7d. Figure 7h shows the effect of treatment time and acid concentration. It was found that the yield increases with the increase in acid concentration and treatment time. The effect of agitation and acid concentration is shown in Figure 7i, where the yield initially increased with increase in acid concentration and agitation but at a higher level the yield did decrease. The interaction between the treatment time and agitation is shown in Figure 7j, where it was found that the xylose sugar yield increased significantly with the increase in treatment time from the lower level of factors to middle level and then decreased at higher levels due to the formation of degrading products.

Optimization of Results

The summary of the optimized parametric condition for hemicellulose hydrolysis of WHB using RSM of Design Expert software and RS tool and surface fitting tool of MATLAB is shown in Table 5. The optimum conditions predicted by RS tool of MATLAB and RSM were very similar except agitation. RS tool of full quadratic model predicted the xylose yield to be 211.92 mg/g with an error of 13.87 as RMSE and pure quadratic model predicted the xylose yield 212.05 mg/g with an error of 15.22 as RMSE which demonstrates that the full quadratic model has a better fit with the experimental data. From experimentally validating the optimized results, xylose yield of 215 mg/g of WHB was obtained. Similarly, when using RSM, the predicted xylose yield was 209 mg/g, whereas the experimental xylose yield was 212.5 mg/g under optimal conditions.

Detoxification of Acid Hydrolysate

Over liming and activated charcoal treatments were used to reduce the effect of inhibitors produced during acid hydrolysis 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 concentration was increased to 255 mg/g from the initial xylose yield of 215 mg/g (Table 6). The results of the present investigations are in accordance with the earlier findings[19]. The authors of this study obtained total phenolics of 200 ± 10 mg/L (80% removal) from 1000 mg/L with an increase in sugar concentration of 52.5 ± 0.25 g/L from 28 ± 0.34 g/L extracted from water hyacinth biomass after detoxification. It was also reported that the yield increased to 52 g/L xylose from 32 g/L of xylose after detoxification in corn cob husk and to 35 g/L from 19 g/L xylose after detoxification using $Ca(OH)_2$ and charcoal powder[16]. Decrease in the concentration of total phenolics has also been reported by Deuk *et al*[17].

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). An amount of 32.3 g/L of xylitol was produced from *Pichia stipitis* with a yield of 0.63 g xylitol/g of xylose at 48 h and 31.1 g/L of xylitol were produced from *Candida shehatae* with a yield of 0.61g xylitol/g of xylose at 48 h as shown in Figure 8. The results are in accordance with the work of Singleton and Rossi[13]

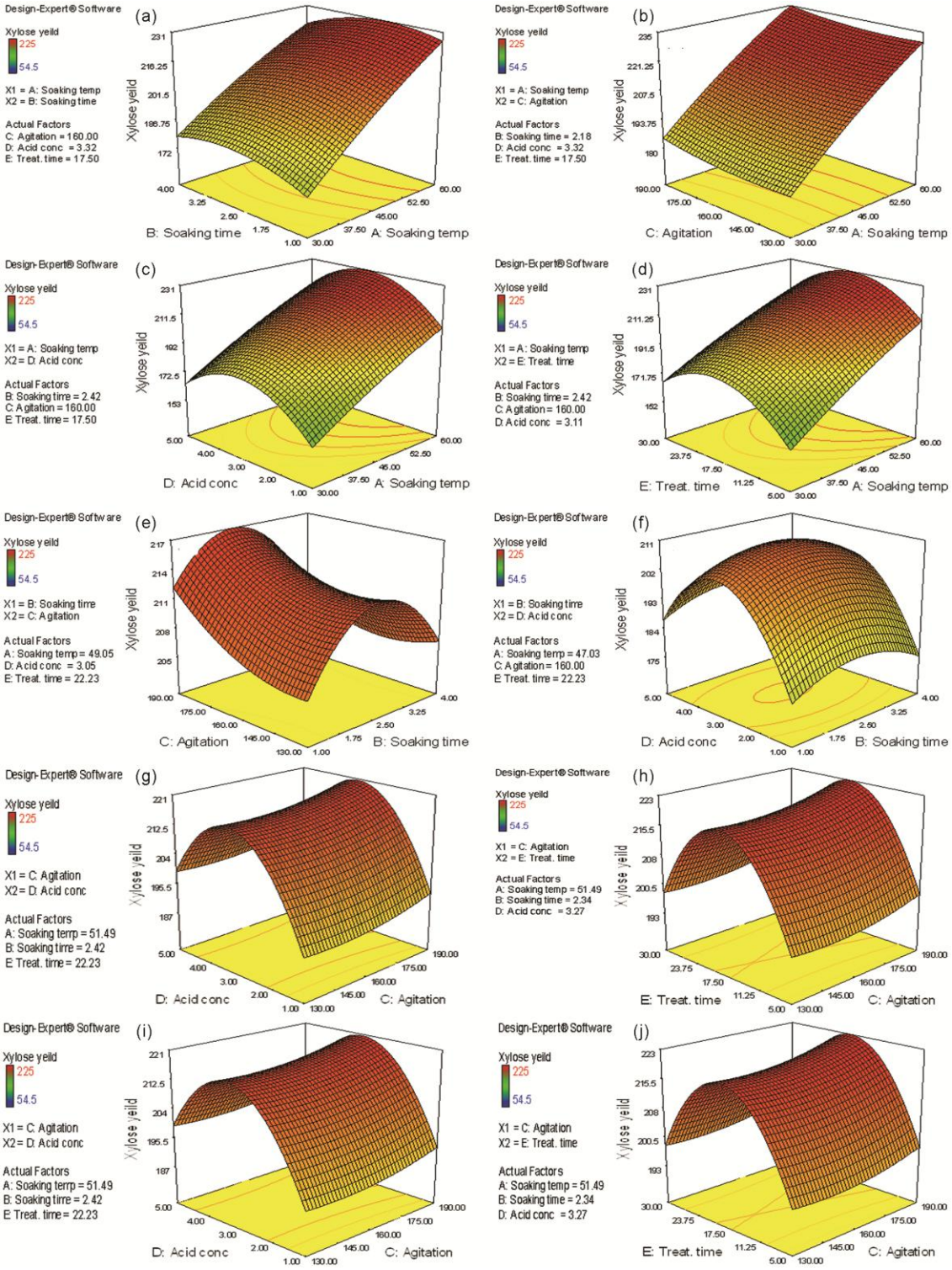

Fig. 7 — Effect of operating parameters on the hemicellulose hydrolysis of WHB for xylose sugar yield: (a) Effect of soaking temperature and soaking time on xylose yield, (b) Effect of soaking temperature and agitation on xylose yield, (c) Effect of soaking temperature and acid concentration on xylose yield, (d) Effect of soaking temperature and treatment time on xylose yield, (e) Effect of soaking time and agitation on xylose yield, (f) Effect of soaking time and acid concentration on xylose yield, (g) Effect of soaking time and treatment time on xylose yield, (h) Effect of treatment time acid concentration on xylose yield, (i) Effect of acid concentration and agitation on xylose yield and (j) Effect of agitation and treatment time on xylose yield.

Table 6 — Effect of detoxification

Component	Before detoxification	After detoxification
Xylose (mg/g)	215	255
Reducing sugar (mg/g)	137.5	167.5
Total phenolics (g/l)	0.975	0.223

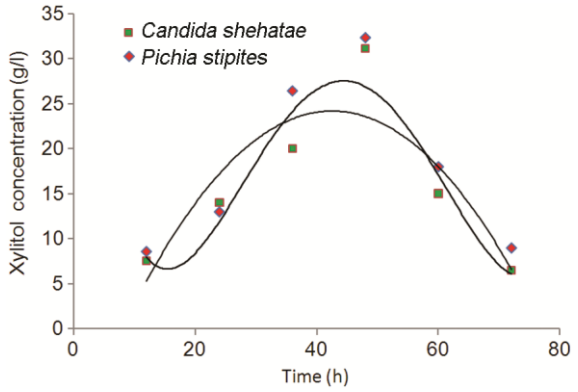

Fig. 8 — Xylitol production from hemicellulose hydrolysate of WHB with time by *Pichia stipitis* (NCIM 3500) and *Candida shehatae* (NCIM 3497).

where 26 g/L xylitol were produced from corn husk by *Pichia stipitis* CBS 5773, Zhang *et al*[18] where 13.02 g/L xylitol were produced from cotton stock by *Candida tropicalis* and Kalhorinia *et al*[19] where 32.5 g/L of xylitol were obtained by *Candida tropicalis* Y-27405 from WHB.

Conclusion

Lignocellulosic material can prove to be an efficient source of hemicellulose from which higher xylose containing hydrolysate can be produced by performing hydrolysis with dilute sulphuric acid with proper selection of the parametric conditions like, soaking time, temperature, agitation, dilute acid solution and treatment time for further fermentation to produce xylitol by using microorganisms. It has been observed that the agitation speed during soaking plays an important role in the hemicellulose-xylose conversion. Various process parameters were optimized using MATLAB and Design Expert software and the effects of the interaction between different parameters were studied. It has been observed that a maximum xylose yield of 211.9 mg/g of water hyacinth biomass (WHB) was obtained under optimal condition, which increases to a value of 255 mg/g of WHB after detoxification. Fermentation of the above xylose produces 0.63 g of xylitol using *Pichia stipitis* and 0.61 g xylitol using *Candida shehatae* after 48 h of fermentation at 30^{o}C from 1 g of xylose. 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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