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Studies on the Optimization of Phenolics during Production of Xylitol from Water Hyacinth

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Abstract
The study investigates the production of xylose by acid hydrolysis from water hyacinth (*Eichhornia crassipes*) and its subsequent bioconversion into xylitol. Lignin derived phenolic compound is inhibitory to the xylitol production process. Hence pretreatment of water hyacinth was first performed with sodium hydroxide to remove phenolics derived from lignin. Response surface methodology (RSM) was incorporated to evaluate the combined effect of independent factors affecting the pre-treatment process to remove lignin. The factors affecting the process are concentration of sodium hydroxide solution (2%, 3% and 4% w/v), soaking time (2-4 h), agitation speed (100 rpm, 130 rpm, 160 rpm), treatment time (7-15 min) and operating treatment temperature (30-70 ^{o}C) respectively. Acid hydrolysis was performed by autoclaving at 121 ^{o}C for 15 min with the pre-treated water hyacinth biomass, which released maximum phenolics (3524.16 mg/l) in the pre- treatment hydrolysate. 34.22 g/l of xylitol was produced by *Pichia stipitis* at 48 h of fermentation, with a yield of 0.645g xylitol/g xylose.

Keywords: Water hyacinth, Phenolics, Acid hydrolysis, Fermentation, Xylitol

1. Introduction
Lignocellulosic biomass from forests, agriculture and agro-industry residues are considered as abundant and inexpensive source of polysaccharides. The useful exploitation of this resource depends on the degradation of these polymers to sugars of which hemicellulose is important in the overall conversion process. Water hyacinth (*Eichhornia crassipes*) represents hemicellulosic rich (38%) noxious biomass that could be utilized for a variety of value added products such as xylitol. It is an exceptionally fast growing plant with very high productivity and abundant availability in major parts of the world, making it a suitable feed stock for distributed xylitol production. Production of Xylitol from biomass involves hydrolysis, detoxification and fermentation process. Optimization of the factors affecting pretreatment are necessary for achieving higher amount of desired yields. This process aims to remove lignin and various uronic acid substitutions in hemicellulose which lowers the enzyme accessibility to carbohydrate polymers. Sodium hydroxide (NaOH) pretreatment is the most commonly studied alkaline pretreatment process as it causes swelling of lignocellulosic materials which allow the separation of structural linkages between lignin and carbohydrate polymers, decreases cellulose crystallinity and cause lignin disruption which might lead to an increase in internal surface area[1,2,3].

Dilute acid hydrolysis is a simple and rapid method and is widely used for hydrolysis of biomass. Unfortunately the sugar liquors obtained from acid hydrolysis contains several microbial inhibitors such as furans, aliphatic acids and lignin-derived phenolics, with low molecular weight[4,5]. A sequential two step process pretreatment followed by hydrolysis of the pre-treated biomass) usually generate an inhibitor free hydrolysates. Hydrolysate fermentability and removal of inhibitors can be done via, several detoxification treatments like chemical, physical and biological methods. Since detoxification increases the cost of the process[6] (Sivers *et al*. 1994) it is essential either to overcome detoxification steps or to develop cheap and efficient methods. However, the needs for detoxification must be assessed in each case, since it depends on the chemical composition of the hydrolysate and is strain specific. Xylitol ($C_5H_{12}O_5$), due to its unique properties finds applications in pharmaceutical, healthcare, and food industries. Fruits and vegetables, which naturally contain xylitol, are not used for xylitol extraction because of their low content (less than 9 mg/g), thereby making manufacturing process expensive. Xylitol is currently produced using catalytic hydrogenation of commercial xylose but the process is expensive and has low yields (60%), due to the separation of xylitol from the chemical compounds formed during manufacturing.

Biotechnology provides an alternative pathway, which utilizes microorganisms such as bacteria, molds and yeasts that can convert xylose into xylitol, a highly specific and economic process, since 80% of the sugars are transformed into sugar alcohol. The biotechnological alternative is all the more appealing when using low cost raw matter such as cellulose from agricultural residues[7,8]. The fermentation organism must be able to ferment all monosaccharide present and in addition, withstand potential inhibitors in the hydrolysate. Yeasts are reckoned as the best xylitol producers among the microorganisms investigated. In the present work, NaOH pretreatment was performed and the oligosaccharides containing liquors were hydrolyzed using dilute acid.

Materials and Methods

2.1 Preparation and treatment of water hyacinth biomass

Fresh water hyacinth with long stem was collected from a natural pond inside the premises of CSIR-CMERI, (CSIR LAB), Durgapur, India. Water hyacinth was thoroughly washed several times with tap water to remove adhering dirt, chopped into small pieces of size 1-2 cm (approx), and ground to make a paste of water hyacinth biomass. A sample of moist water hyacinth (8 g) was mixed with 2%, 3% and 4% (v/v) of sodium hydroxide to a final volume of 30 ml. The mixtures were soaked for 1, 2 and 4 h respectively. The reactions were carried out in an incubator shaker in the temperature range of 30 °C, 50 °C and 70 °C with agitation speed of 100 rpm, 130 rpm and 160 rpm respectively. The hydrolysate was filtered using Whatman paper no.1 to remove the unhydrolysed material. The filtrate was collected and subjected to analysis of the phenolics content.

2.2 Analysis of chemical composition

Cellulose lignin and hemicellulose fraction of water hyacinth (Eichhornia crassipes) was analyzed [9]. Lignocellulosic compositions obtained from water hyacinth are shown in Table 1.

Table 1: Experimental range and levels of independent process variables

Name	Low	High
Soaking time(h)	2	4
Conc. Of NaOH%(v/v)	2	4
Agitation speed(rpm)	100	160
Treatment time(min)	7	15
Treatment temp(oC)	30	70

Scanning Electron Microscopy

Scanning Electron Microscope (SEM) (JEOLJSM-5600) analysis was carried out to understand the change in structural morphology of the alkali followed by acid treated, only acid treated and untreated water hyacinth biomass.

2.4 Fourier Transform Infrared Spectroscopy (FTIR)

Fourier Transform Infrared Spectroscopy (FTIR) spectroscopy was used to investigate and quantify chemical changes in pretreated and treated sample. IR spectra were studied using Shimadzu spectrometer (Japan). In brief, samples were prepared by mixing 2 mg of biomass and 198 mg of spectroscopic grade potassium bromide. After grinding, the mixture was pressed to form disks. The spectra were generated with an average scan of 16 scans with a resolution of 4 cm^{-1} within a range of 500-4500 cm^{-1}.

2.5 Fermentation inhibitors

Fermentation inhibitors (i.e. phenolics) were analyzed by spectroscopic analysis. Phenolics estimation was carried out by Folin ciocalteus method[10].

2.6 Preparation of hemicellulose acid hydrolysate

1 g of dried water hyacinth biomass was mixed with 3% (v/v) sulphuric acid to make a final volume of 10 ml. The mixture was autoclaved at 121 ^oC for 15 min. The hydrolysate was filtered using Whatman Paper no. 1 to remove the unhydrolysed material. The filtrate was gathered and subjected to analyze the xylose content.

2.7 Determination of xylose content by Phloroglucinol assay

Xylose content was identified using the Phloroglucinol assay with the hydrolysate obtained from acid hydrolysis. The colouring reagent mixture was heated in a water bath and rapidly cooled to room temperature before measuring in a THERMO UV1 100 Double beam scanning Spectrophotometer at 554 nm. The treated hydrolysate was then used for the fermentation studies.

2.8 Microorganism and maintenance

Pichia stipitis NCIM 3500 were procured commercially from NCIM, Pune. 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 ^oC.

2.9 Inoculum preparation

A loopful of cells was transferred in 250 ml Erlenmeyer flasks containing 50 ml of medium having the following media components (g/l): D-Xylose(50); Glucose(5); yeast extract(3); malt extract(3); peptone(5); the cells were then incubated in incubator shaker at 125 rpm, 30^o C for 20 h, and the broth was centrifuged at 10,000 rpm for 10 min to obtain cell pellets and it was washed and suspended in sterile distilled water.

2.10 Fermentation condition

Batch fermentation of the detoxified hydrolysate (50 ml) was carried out by supplementing with the following nutrients (gl^{-1}); yeast extract 1.5; KH_2PO_4 2; $MgSO_4.7H_2O$ 0.5 and $(NH_4)_2SO_4$ 0.5, $CaCl_2.2H_2O$ 0.1, $FeCl_3.2H_2O$ 0.1, $ZnSO_4.7H_2O$ 0.001 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 followed by centrifugation or 10 min. The supernatant was being filtered using 0.02 micron filter and the xylitol and residual sugar concentration was analyzed using HPLC.

2.11 Analytical Procedure

Concentration of remaining xylose and xylitol were determined using High Performance Liquid Chromatography (HPLC) with Hypersil APS-2 column (250 mm x 4.6 mm) and RI detector (CECIL 4200) using acetonitrile-water (82:18) as mobile phase at 1.23 ml/min flow rate at room temperature.

2.12 Design of experiments

Response Surface Methodology (RSM)

RSM is an empirical based statistical approach, which helps

us to construct a proper experimental design and simultaneously use it to solve multivariate equations. On the basis of design of experiment; we have to perform certain experiments to obtain the experimental responses quantitatively. In RSM, quantitative experimental responses are used to perform multiple regression analysis thus generating a mathematical model. In the present study, RSM was used to develop an experimental design and evaluate the optimum condition for alkaline hydrolysis and maximize the amount of phenolics content (mg/l).Central composite rotatable design (CCRD) was implemented to investigate the interactive effects of the five independent process variables. Independent variables selected for our process are: soaking time, concentration of NaOH, agitation speed, treatment time and treatment temperature. The input variables are scaled to coded levels based on the following equation:

$$x_i = \frac{X_i - X_{cp}}{\Delta X_i}, \qquad i = 1,2,3....k \tag{1}$$

where 'x_i' is a dimensionless parameter of the independent process variable, 'X_i' indicates the real value of the independent variable, 'X_{cp}' implies the real value of an independent variable at the centre point and 'ΔX_i', represents the step change in the real value of the variable 'i' upon an unit change in the dimensionless value of the variable 'i'. All experiments were performed in triplicates to observe the variability in measurements.

$$Y = \beta_o + \sum_{i=1}^{k} \beta_i X_i + \sum_{i}^{k} \beta_{ii} X_i X_i + \sum_{i=1}^{k-1} \sum_{j=i+1}^{k} \beta_{ij} X_i X_j \tag{2}$$

Where "Y" is predicted response, β_o is constant, β_i is linear co-efficient, β_{ii} is coefficients of squared terms, β_{ij} isco-efficient of cross termed products and k is no. of independent factors.

The second-order-polynomial co-efficient were calculated using Design Expert Version 9.0.3. this proved to be effective in finding out the optimization efficiency and also to estimate the responses of the dependent variable. The range of the independent parameters for the alkaline hydrolysis and the experimental design setup of the RSM along with experimental responses is defined in Table 1 and 2 respectively.

Table: 2 RSM model, for finding out average values using phenolics as a response under different conditions

		Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Response 1
Std	**Run**	**A:Soaking time**	**B:Concentration**	**C:Agitation**	**D:Treatment Time**	**E:Treatment Temperature**	**phenolics**
		h	%	Rpm	min	°C	mg/l
13	1	2	2	160	15	30	927.49
25	2	2	2	100	15	70	1885.98
26	3	4	2	100	15	70	2112.04
48	*4*	*3*	*3*	*130*	*11*	*50*	*1104.02*
34	5	5	3	130	11	50	1376
44	6	3	3	130	11	50	1245.52
4	7	4	4	100	7	30	881.81
20	8	4	4	100	7	70	2074.87
37	*9*	*3*	*3*	*59*	*11*	*50*	*1176.06*
32	10	4	4	160	15	70	2433.99
38	11	3	3	201	11	50	1453.12
24	12	4	4	160	7	70	2230.79
21	13	2	2	160	7	70	1836.89
41	*14*	*3*	*3*	*130*	*11*	*2.5*	*752.6*
12	15	4	4	100	15	30	897.8
35	16	3	0.6	130	11	50	1338.01
15	17	2	4	160	15	30	921.09
10	18	4	2	100	15	30	895.95
23	19	2	4	160	7	70	2077.67
27	*20*	*2*	*4*	*100*	*15*	*70*	*2110.15*
3	*21*	*2*	*4*	*100*	*7*	*30*	*835.38*
22	22	4	2	160	7	70	1991.41
7	23	2	4	160	7	30	951.42
40	24	3	3	130	21	50	1571.69
45	25	3	3	130	11	50	1104.02
29	26	2	2	160	15	70	2089.49
36	27	3	5	130	11	50	1480.48
30	28	4	2	160	15	70	2261.82
11	29	2	4	100	15	30	807.585
17	30	2	2	100	7	70	1639.19
5	31	2	2	160	7	30	915.8
50	32	3	3	130	11	50	1104.02
14	33	4	2	160	15	30	1016.88
47	34	3	3	130	11	50	1104.02
8	35	4	4	160	7	30	954.18
42	*36*	*3*	*3*	*130*	*11*	*98*	*3524.16*

19	37	2	4	100	7	70	1970.93
28	38	4	4	100	15	70	2358.88
43	39	3	3	130	11	50	1104.02
1	40	2	2	100	7	30	885.62
6	41	4	2	160	7	30	914.31
16	42	4	4	160	15	30	931.44
33	43	0.6	3	130	11	50	1202.89
31	44	2	4	160	15	70	2247.84
2	45	4	2	100	7	30	912.702
39	46	3	3	130	1.5	50	1157.6
18	47	4	2	100	7	70	1810.53
46	48	3	3	130	11	50	1104.02
9	49	2	2	100	15	30	948.48
49	50	3	3	130	11	50	1104.02

3. Results and Discussion

3.1 Chemical compositions of water hyacinth

Chemical composition of the water hyacinth for root, leaf and stem was determined individually. The result suggests that the composition of the water hyacinth varies in each plant body. It was noted that the stem contains relatively more hemicellulose compared to leaf and root. Also it was estimated that root has high lignin content, therefore it might cause inhibition in the hydrolysis step, and hence root was excluded further from our study. The chemical composition of root, stem and leaf is shown in Fig 1. Stem biomass of water hyacinth was selected for pretreatment as it contained highest hemicellulose concentration, which can be converted into xylan monomers.

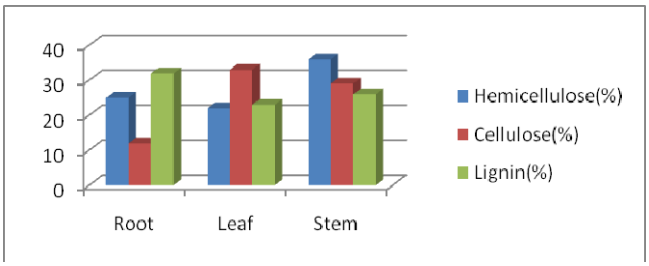


Fig: 1 Chemical composition of water hyacinth

3.2 Characterization of treated and untreated biomass

Scanning electron microscope SEM and Fourier transform infrared spectroscopy FTIR based instrumental analysis were performed to better support the observations in Table 2. SEM of WHB is shown in Fig 2(A-C).The untreated WHB samples showed a firm, and highly ordered structure, while the treated samples exhibited dispersed and distorted structures. Acid hydrolyzed WHB underwent greater degree of disruption than alkali pre-treated WHB. The FTIR spectra of the WHB are represented in Fig 3, indicating changes in the shape, location and transmittance of the FTIR spectral bands. Absorption peaks at 875 cm^{-1} normally indicates a C-O-C strectching present in the β-(1-4)- glycosidic linkage in cellulose and hemicellulose. The absorption peak at 1035 cm^{-1} can be attributed to the vibrational modes of CH_2OH groups which is normally coupled with C-O bending of the C-OH functional groups of the carbohydrates. This spectral absorption normally indicates the decrease in xylose content of the biomass due to solubilisation of hemicellulose. A characteristic band at 1325 cm^{-1} can be observed due to C-O linkages of syringyl ring of lignin. The absorption peak at 1500 cm^{-1}can be attributed due to aromatic ring vibration of lignin. This absorption peak is present distinctly in the untreated sample. But there is significant reduction of this peak intensity for the alkali pre-treated and the acid hydrolysed sample implying lignin depolymerisation by pre-treatment. The peak observed at 1735 cm^{-1} can be either acetyl or uronic ether linkages of carboxylic group in the ferulic and p-coumeric acids. Ferulic and p-coumeric acids are important constituents in lignin biopolymer. Disappearance of the 1620 cm^{-1} peak from the alkali pre-treated WHB sample indicates an effective de-lignification. A characteristic band at 1383 cm^{-1} corresponds to the C=C linkages, which is present in the guaiacyl ring of the lignin. Absorption peaks at 2924 corresponds to C-H stretching of lignin which is enhanced after alkali pretreatment followed by acid hydrolysis. Finally band widening at 3500 cm^{-1} can be correlated to stretching of H- bonded hydroxyl (OH) functional groups of lignin.

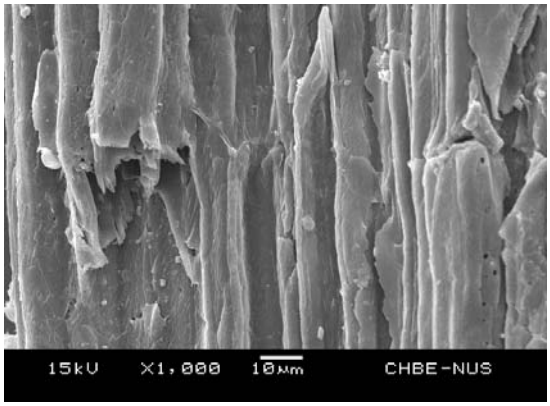


Fig 2(A)

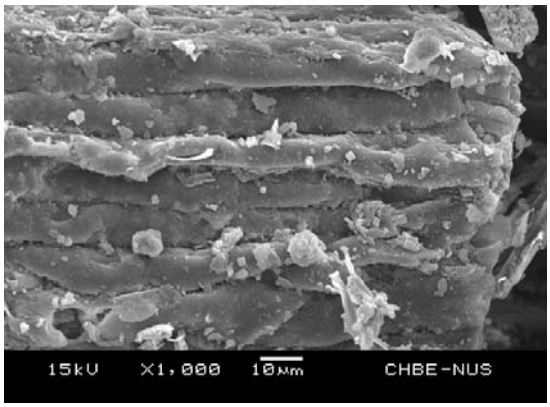


Fig 2(B)

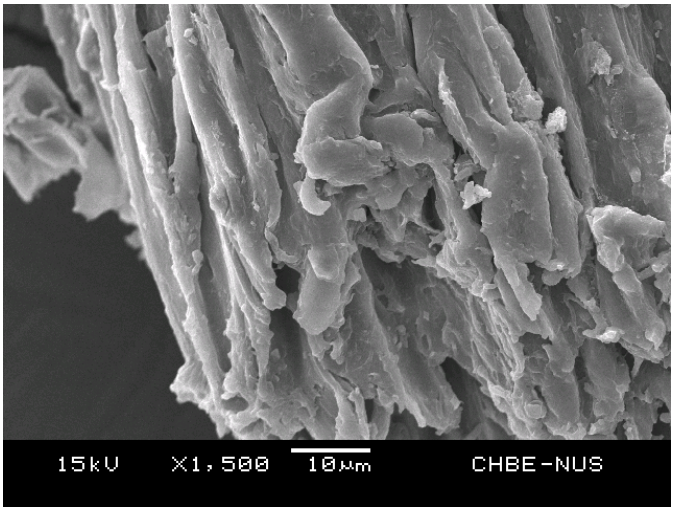


Fig 2(C)

Fig: 2 SEM photograph of untreated and treated biomass

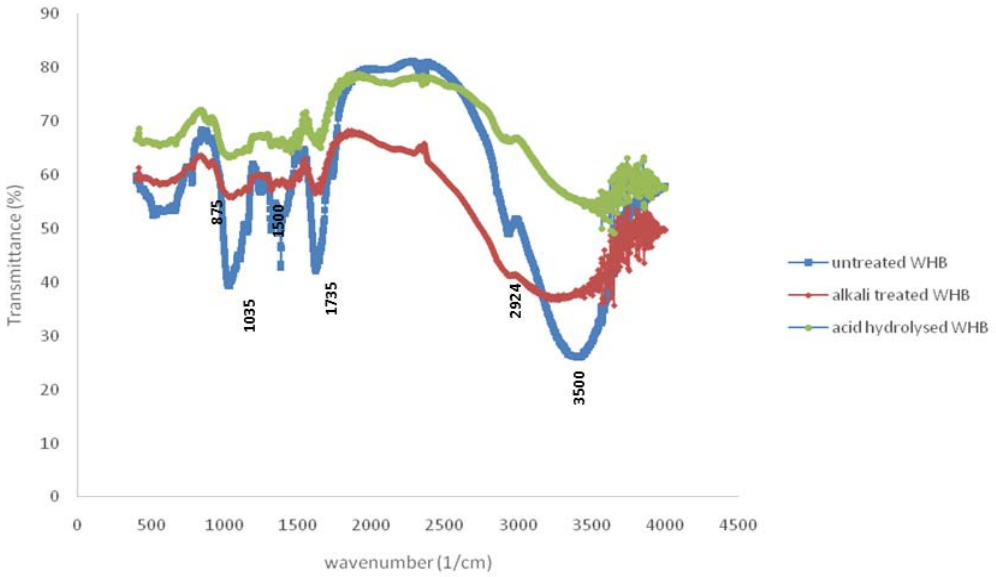


Fig 3: FTIR of untreated, alkali treated and alkali +acid treated Water hyacinth biomass

3.3 Experimental designs and fitting of a quadratic model

From experimental studies on phenolics concentration by alkali pretreatment, it has been established that soaking temperature, concentration of sodium hydroxide, treatment time, agitation speed and soaking time are the most important factors. Therefore, regression model equation has been developed considering the above factors. In order to estimate the combined effect, 50 experiments were constructed of the independent factors: soaking time, concentration of NaOH, agitation speed, treatment time and treatment temperature affecting the amount of phenolics. Design Expert Version 9.0.3 (Stat Ease, USA) was used to perform the regression analysis and obtain the co-efficient of the second-order-polynomial involving the neutral parameters. Table 3 summarizes results obtained for the ANOVA study of the second order response surface model. The statistical significance of the model was established by F-test ANOVA.

Table 3: Analysis of Variance of the quadratic model for phenolics content of alkali pre-treated WH B

ANOVA for Response Surface Quadratic model						
Analysis of variance table [Partial sum of squares - Type III]						
	Sum of		Mean	F	p-value	
Source	Squares	df	Square	Value	Prob > F	
Model	1.736E+007	20	8.682E+005	566.93	< 0.0001	significant
A-Soaking time	1.113E+005	1	1.113E+005	72.69	< 0.0001	
B-Concentration	1.154E+005	1	1.154E+005	75.38	< 0.0001	

C-Agitation	1.257E+005	1	1.257E+005	82.08	< 0.0001	
D-Treatment Time	1.924E+005	1	1.924E+005	125.60	< 0.0001	
E-Treatment Temperature	1.452E+007	1	1.452E+007	9478.23	< 0.0001	
AB	94.51	1	94.51	0.062	0.8056	
AC	276.93	1	276.93	0.18	0.6738	
AD	3061.39	1	3061.39	2.00	0.1680	
AE	45299.22	1	45299.22	29.58	< 0.0001	
BC	86.41	1	86.41	0.056	0.8139	
BD	7811.03	1	7811.03	5.10	0.0316	
BE	1.397E+005	1	1.397E+005	91.22	< 0.0001	
CD	72.79	1	72.79	0.048	0.8289	
CE	17114.67	1	17114.67	11.18	0.0023	
DE	98170.69	1	98170.69	64.10	< 0.0001	
A^2	68971.56	1	68971.56	45.04	< 0.0001	
B^2	1.805E+005	1	1.805E+005	117.89	< 0.0001	
C^2	74947.53	1	74947.53	48.94	< 0.0001	
D^2	1.115E+005	1	1.115E+005	72.79	< 0.0001	
E^2	1.830E+006	1	1.830E+006	1194.69	< 0.0001	
Residual	44412.39	29	1531.46			
Lack of Fit	26893.67	22	1222.44	0.49	0.9058	not significant
Pure Error	17518.73	7	2502.68			
Cor Total	1.741E+007	49				

The results in Table 3 suggest that the objective parameters viz. Soaking temperature, soaking time, agitation speed and treatment time, have a significant influence on the amount of total phenolics content during alkaline hydrolysis. For understanding the interaction effect between the process parameters ANOVA study was very helpful. The developed a model for RSM proved to be highly significant due to its high Fisher's F-value (566.93) with a low probability value ($p<0.0001$). Regression co-efficient was determined to additionally understand the goodness of fit. Regression co-efficient was $r^2 = 0.9974$, implying that 99.74% of the total variance was attributed to this RSM model. The predicted R-squared (0.9928) and the adjusted R-squared (0.9957) were in reasonable argument with each other indicating the model is efficient. The residual variation is measured utilizing co-efficient of variance (CV) relative to the size of the mean. A low value of CV is desirable to have sufficient confidence in the experimental results. A very low (2.73%) value of CV implies a sufficient precision and reliability on the experimental results. The model shows the values of standard deviation and means as 39.13 and 1434.73 respectively.

Phenolics = +3313.85614 -298.61925 * Soaking time -451.17181* Concentration
-10.19128* Agitation -70.62804* Treatment Time -44.12504*Treatment
Temperature + 1.71853 * Soaking time * Concentration -0.098059*
Soaking time * Agitation +2.44526 * Soaking time * treatment time
+1.88122*Soaking time*Treatment Temperature -0.054774 * Concentration
* Agitation -3.90588*Concentration*Treatment Time+
3.30359*Concentration * Treatment Temperature -0.012568 * Agitation * Treatment Time +0.038544 * Agitation * Treatment Temperature +0.69235 * Treatment Time * Treatment Temperature +39.54891*Soaking time^2 +63.98620 * Concentration^2 +0.040995 * Agitation^2 +2.66317* Treatment time^2 + 0.44861 * Treatment Temperature^2

The Fig. 4 gives clear evidence that model predicted results are very close to agreement with the experimental ones. As most experimental results lie on the 45 degree line implying the model had fitted the experimental data with an excellent accuracy resulting in lesser deviation from the predicted values.

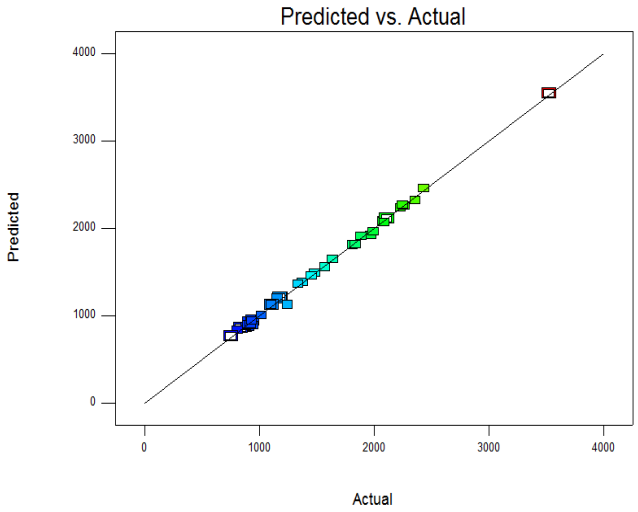
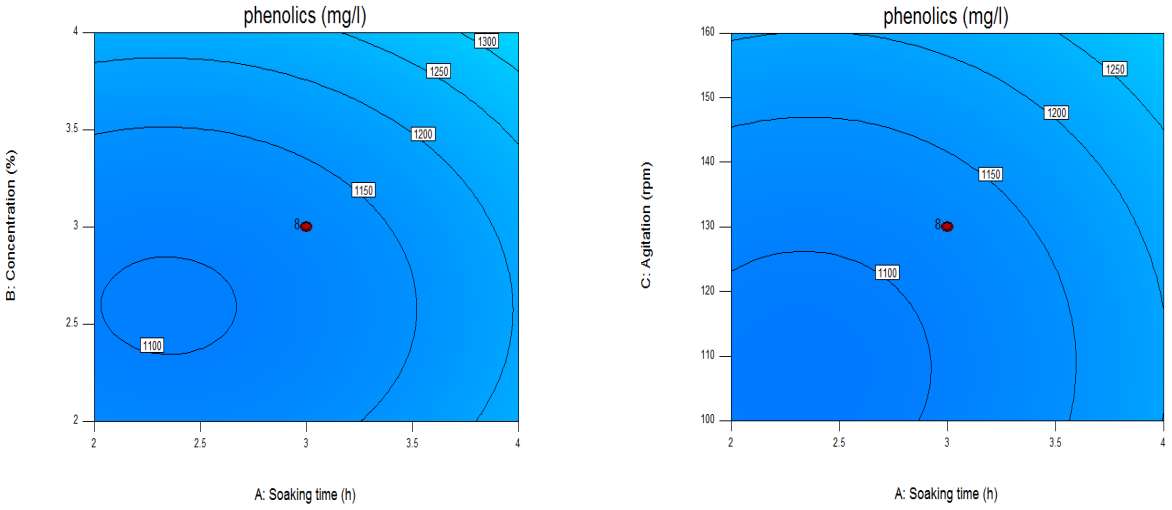


Fig: 4 Experimental data plotted against RSM model predicted data for alkaline hydrolysis of WHB using phenolics as a response

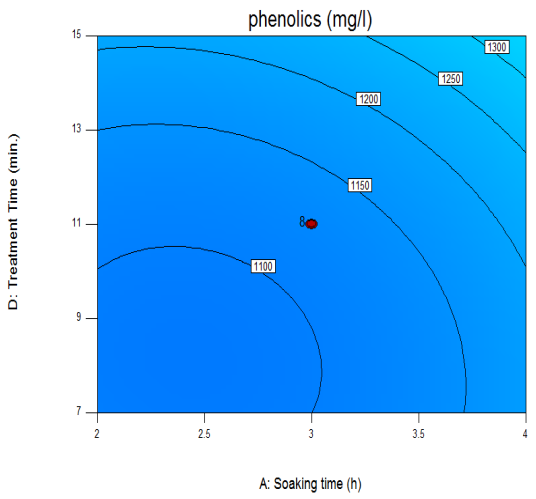
3.4 Contour Plots

The contour plot determines the interaction of the components and optimum level of each component for maximum response. These plots were obtained from the pair-wise combination of independent factors, while keeping other factors at its center point level. The elliptical contour plot shown in Fig. 5(A–F), it clearly indicates that the mutual interaction is prominent among factors.



(A)

(B)



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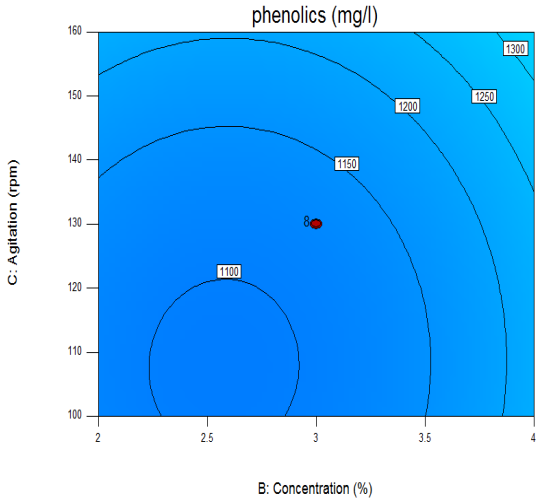

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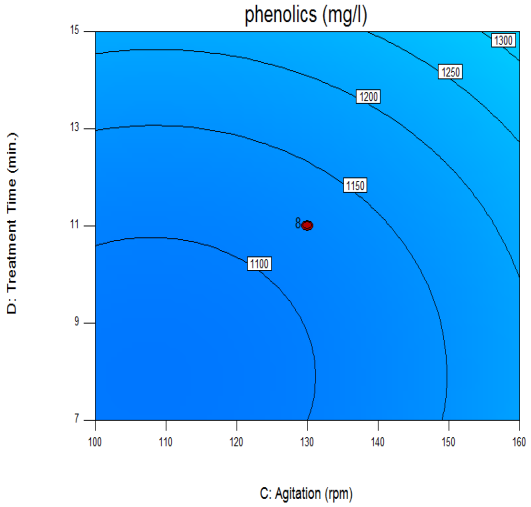

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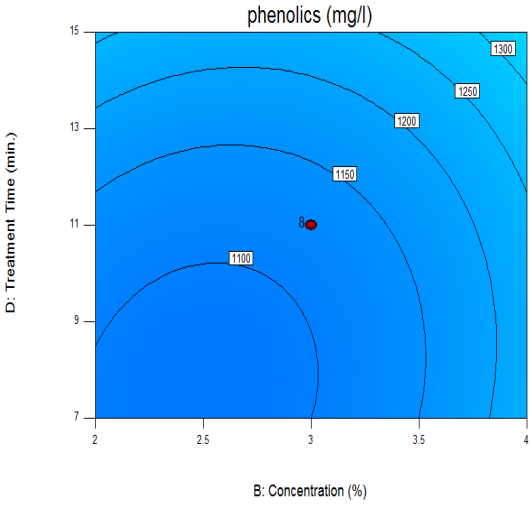

(F)

Fig: 5 Elliptical Counter Plots showing mutual interaction between the factors (A) Effect of concentration of NaOH and soaking time on phenolics, (B) Effect of agitation and soaking time on phenolics, (C) Effect of treatment time and soaking time on phenolics, (D) Effect of agitation speed and concentration of NaOH on phenolics, (E) Effect of treatment time and agitation speed on phenolics, (F) Effect of treatment time and concentration of NaOH on phenolics.

3.5 Response Surface Plots

Design Expert 9.0.3 was also used for plotting response surface plots. The surface plots were beneficial to determine the interaction between process parameters on the total phenolics content for the alkaline hydrolysis of water hyacinth biomass. The Response Surface plots shown in Fig. 6(A–F), it clearly indicates that the mutual interaction is prominent among the different process parameters.

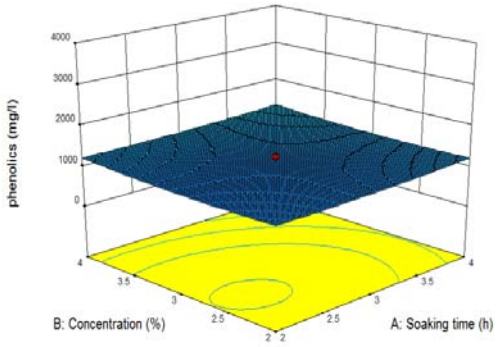

Fig (A)

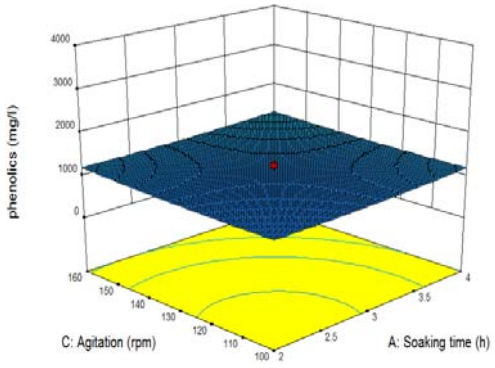

Fig (B)

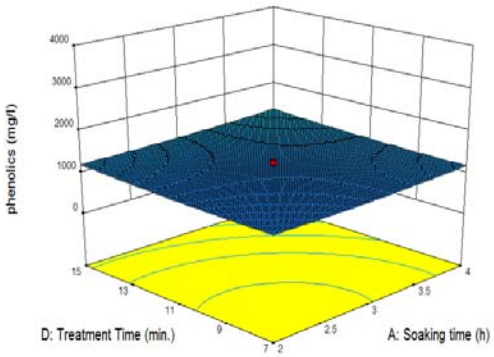

Fig (C)

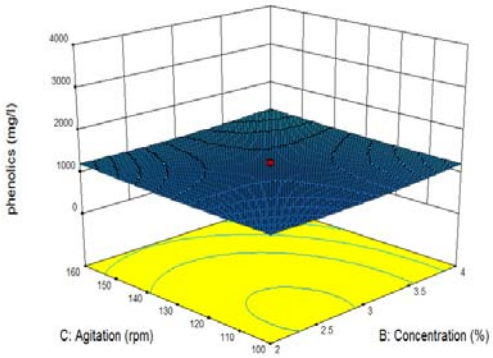

Fig (D)

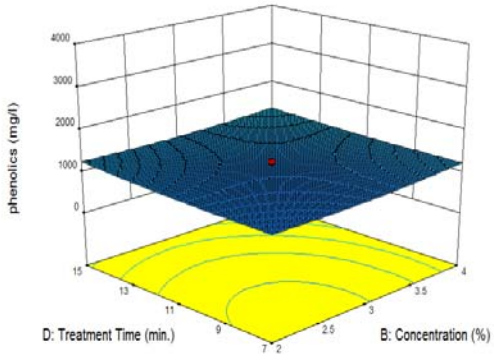

Fig (E)

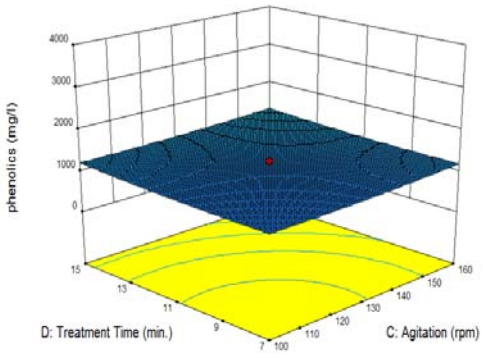

Fig (F)

Fig: 6 Response Surfaces showing interactions for phenolics response between (A) concentration of NaOH and Soaking Time, (B) agitation speed and soaking time, (C) treatment time and soaking time, (D) treatment temperature and soaking time, (E)agitation speed and concentration of NaOH, (F) treatment time and agitation speed**.**

3.6 Analysis of phenolics in Compound

Solubilization of lignin can lead to the production of phenolic degradation products, like vanillin and guaiacol, as well as organic acids, like acetic and formic[11,12]. Highest concentration of phenolics was 3524.16 mg/l obtained at the soaking temperature of 3 h, 3% concentration, agitation speed of 130 rpm, treatment time of 11 min and soaking temperature of 98 ºC.

3.7 Concentration of acid hydrolysate

Water hyacinth biomass (WHB) hydrolysate contained 27.23 g/l of fermentable monosaccharide after post-hydrolysis. Xylitol bio production is favoured at high xylose concentrations therefore; WHB hydrolysates were previously concentrated for an efficient xylitol production. After detoxification the total phenolics were reduced and xylose concentration was increased to 51.49 g/L from the initial 27.20 g/L of xylose.

3.8 Fermentation

Batch fermentation was performed from the concentrated water hyacinth hemi cellulosic hydrolysate, using 10% (v/v) *Pichia stipitis* (NCIM 3500). 34.22 g/l of xylitol was produced from *Pichia stipitis* at 48 h fermentation. Our results are in accordance with 32.5 g/l of xylitol[13] (Kalhorinia *et al*. 2013) obtained by *Candida tropicalis*Y-27405 from WHB. With the hike in worldwide interest for Xylitol production, lignocellulosic material can prove to be a better alternative. Water hyacinth biomass can act as an efficient source for the analysis of phenolics by NaOH Pretreatment. The process parameters were optimized using software package Design Expert Version 9.0.3.which released a maximum phenolics of 3524.16 mg/l at soaking time 3 h, concentration of NaOH 3%, agitation speed 130 rpm, treatment time 11min, treatment temperature 98 ^{o}C. Then acid hydrolysis was carried out with the dried pretreated biomass by autoclaving at 121 ^{o}C for 15 min which yielded 272.26 mg of xylose/g of dry biomass. The concentrated hydrolysate after 48h fermentation yielded 0.645 g of xylitol/g of xylose using *Pichia stipitis*. In this study, for the production of xylitol from water hyacinth, the results obtained were encouraging with higher yield of xylitol. Therefore, it can be concluded that water hyacinth can be effectively used as a novel alternative for the bioconversion of lignocelluloses to highly value added product Xylitol, which opens the prospective for further studies on biodegradation kinetics of lignin and optimization of xylitol yield.

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