Supporting information for

Enzyme mediated biomass pretreatment and hydrolysis: a biotechnological venture towards bioethanol production

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SUPPLEMENTARY TABLES AND FIGURES

(A) Fig. S1 One variable at a time approach (OVAT) for enzymatic delignification/pretreatment

(B) Fig. S2 One variable at a time approach (OVAT) for enzymatic hydrolysis

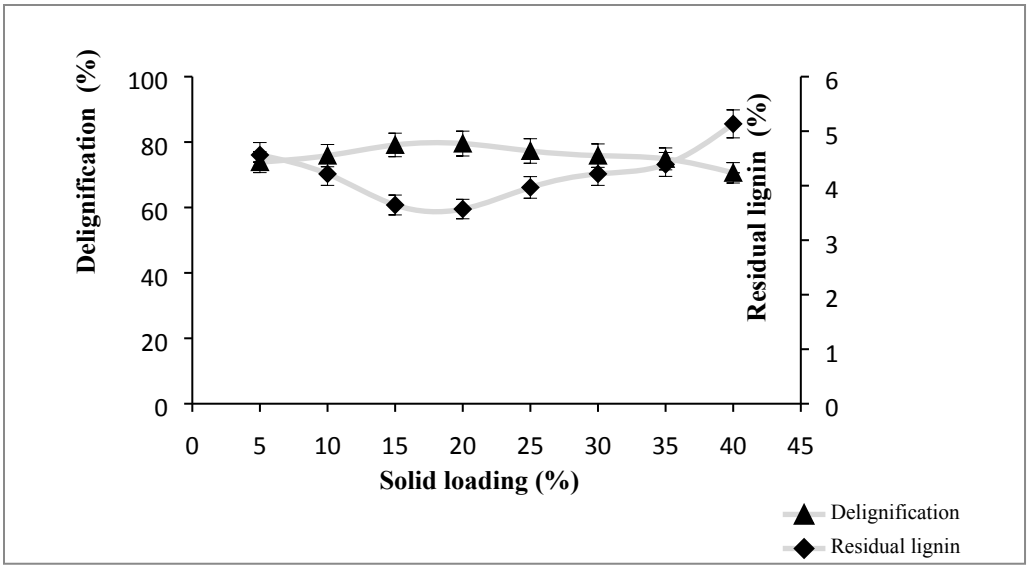

Fig. S1 (a) Effect of solid loading on enzymatic delignification

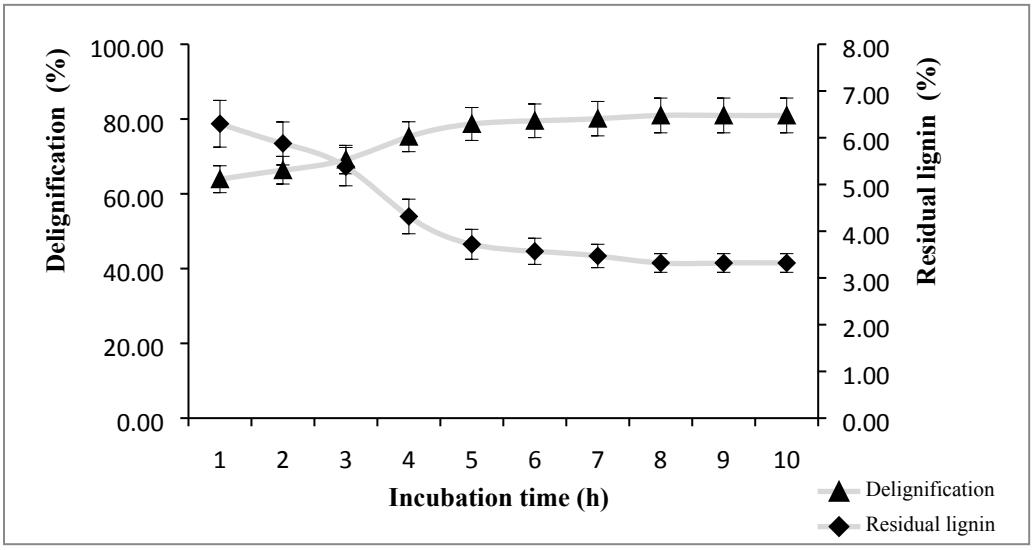

Fig. S1 (b) Effect of incubation time on enzymatic delignification

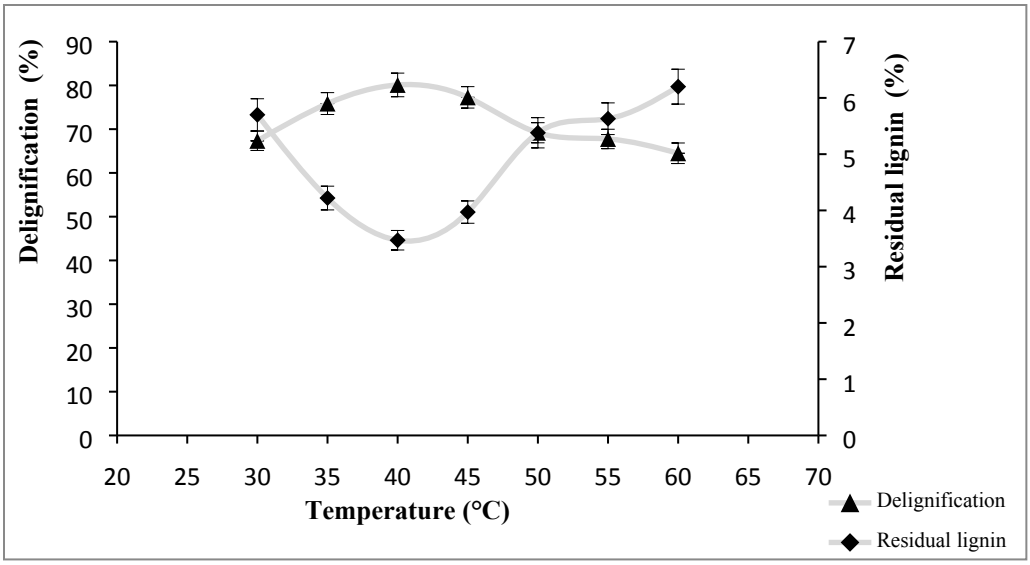

Fig. S1 (c) Effect of temperature on enzymatic delignification

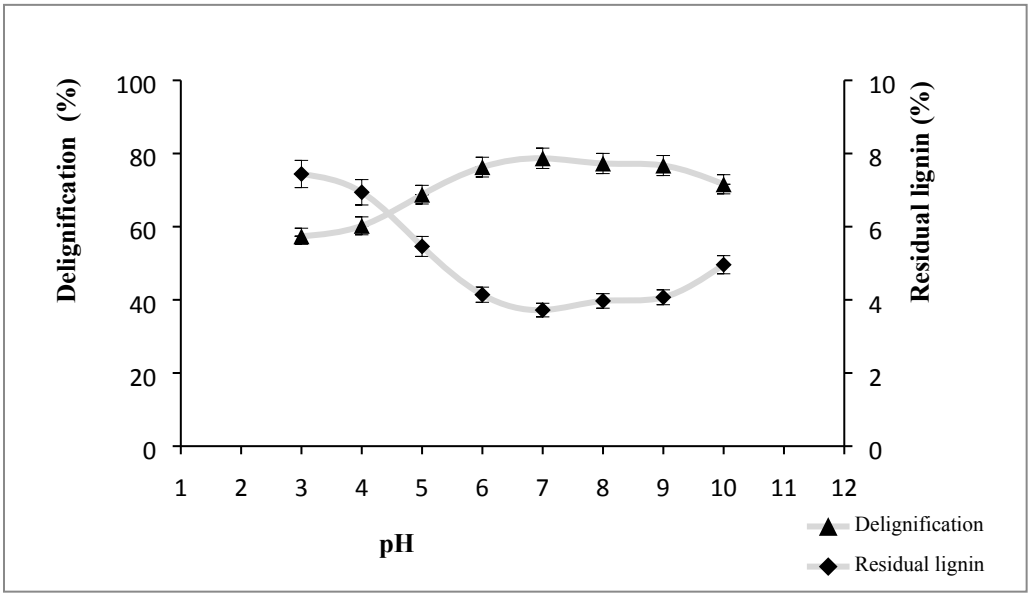

Fig. S1 (d) Effect of pH on enzymatic delignification

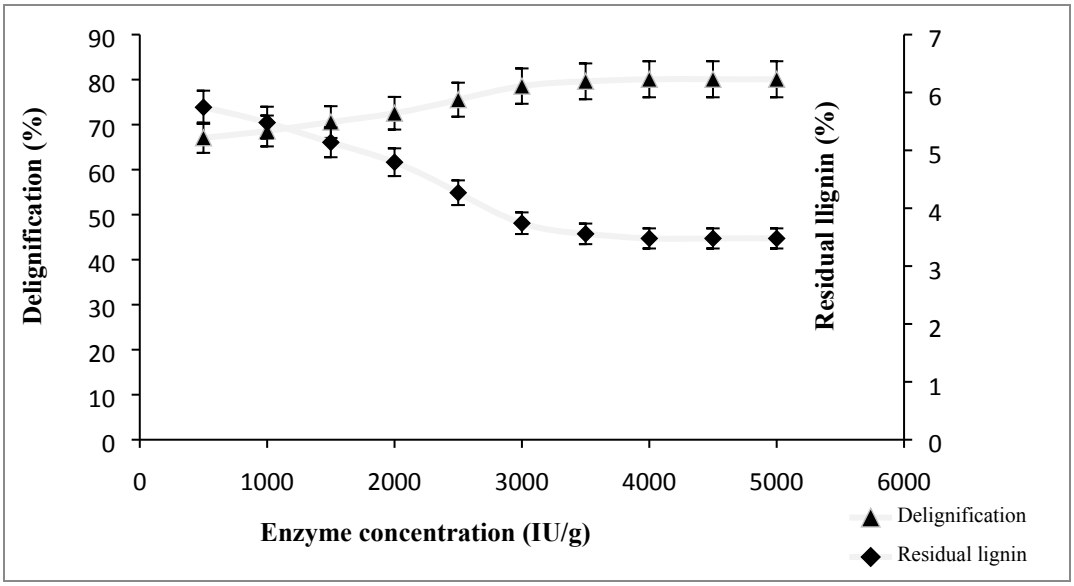

Fig. S1 (e) Effect of enzyme concentration on enzymatic delignification

(B) One variable at a time approach (OVAT) for enzymatic hydrolysis

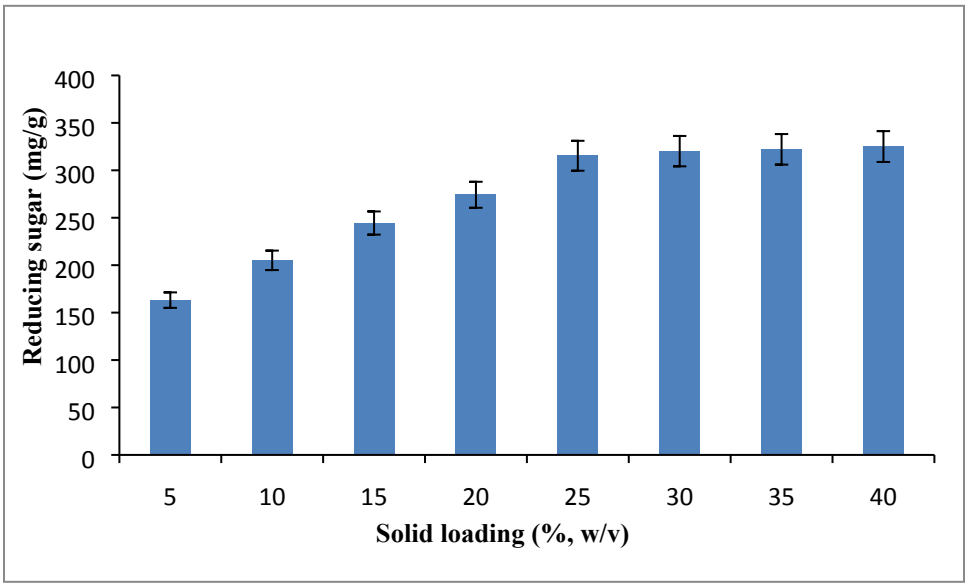

Fig. S2 (a) Effect of solid loading on enzymatic hydrolysis

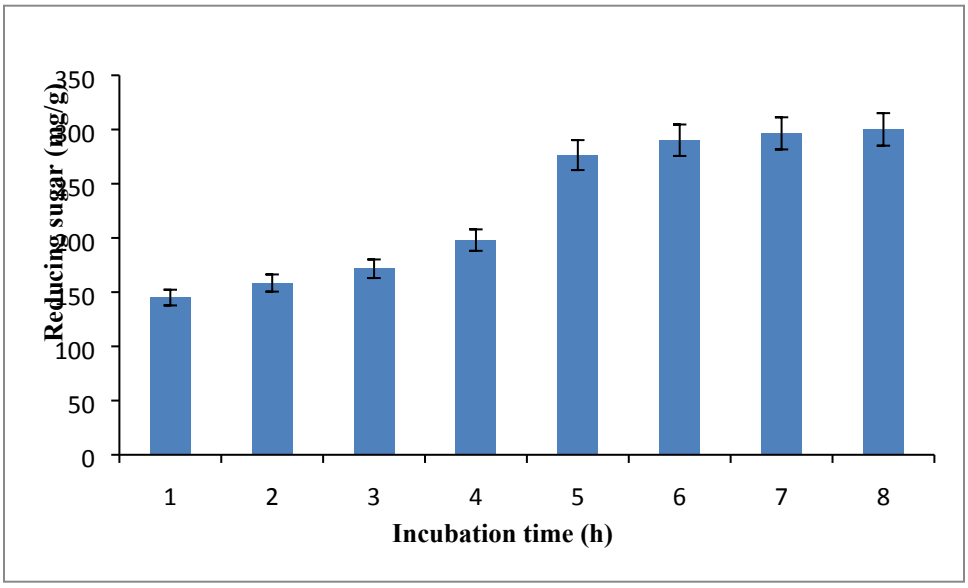

Fig. S2 (b) Effect of incubation time on enzymatic hydrolysis

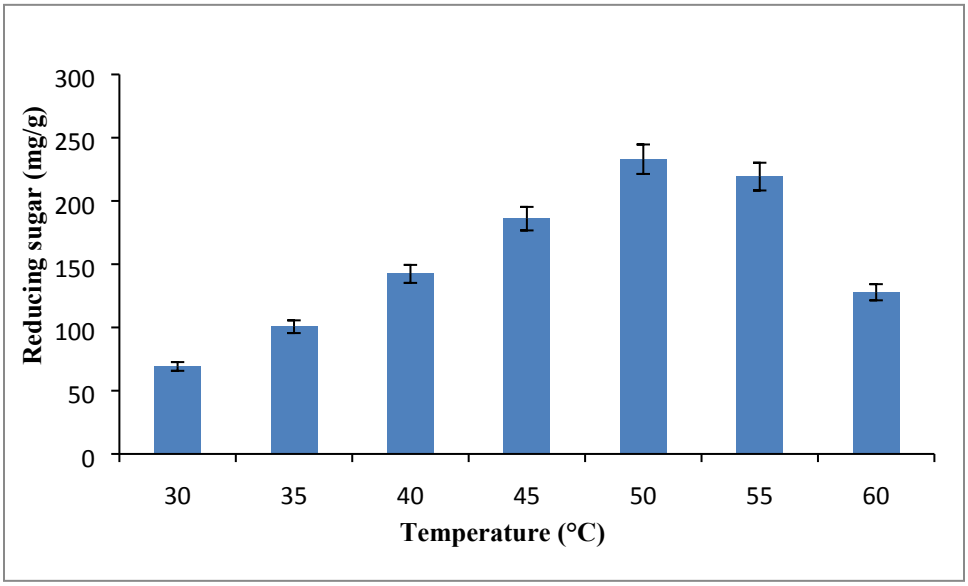

Fig. S2 (c) Effect of temperature on enzymatic hydrolysis

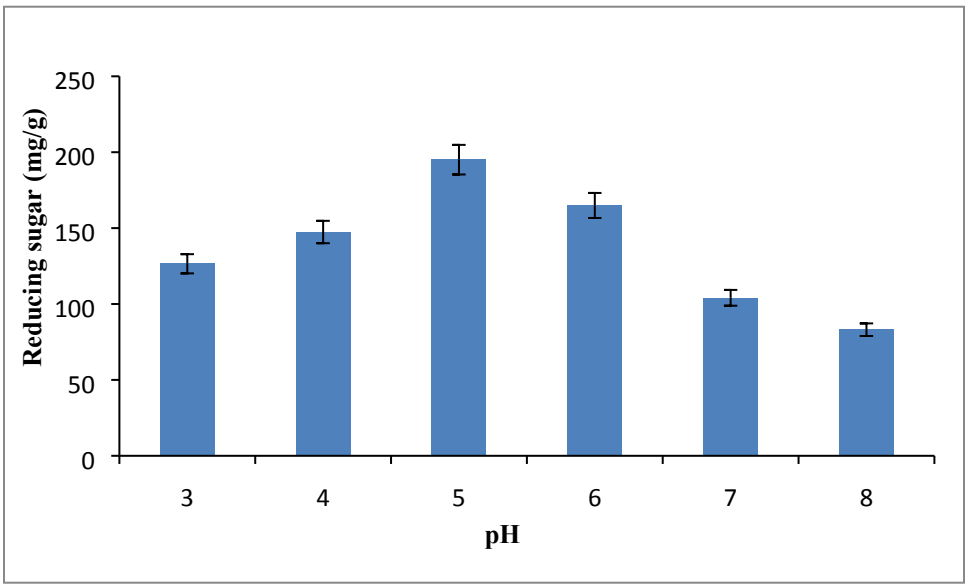

Fig. S2 (d) Effect of pH on enzymatic hydrolysis

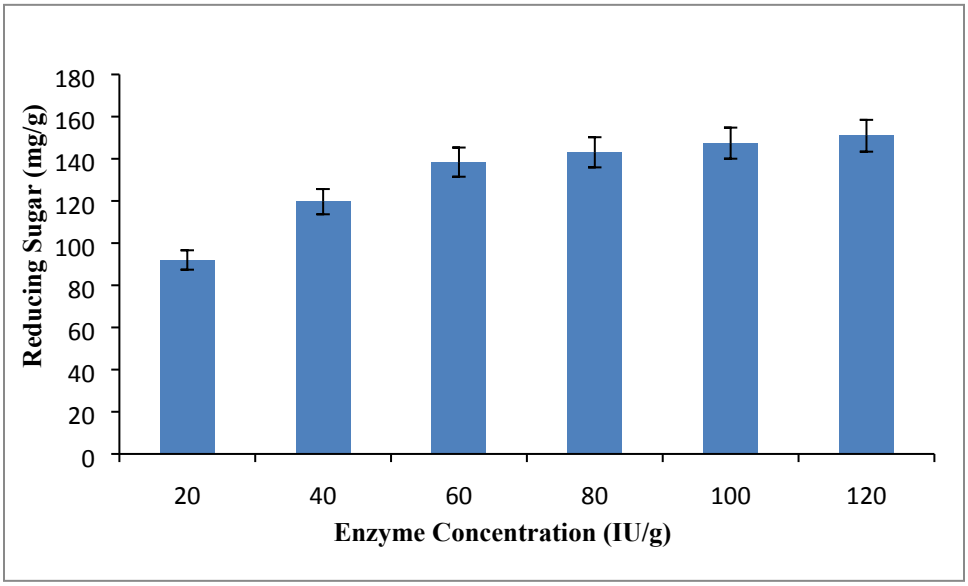

Fig. S2 (E) Effect of enzyme concentration on enzymatic hydrolysis

Table S1 Central composite design based experimental designs (variables and responses) for enzymatic pretreatment of Kans grass in terms of coded level of variables

Run Order	Solid Loading (%)	Incubation Time (h)	Temperature (° C)	pH	Enzyme Concentration (IU/g)	Delignification (%)	
						Predicted	Experimental
1	20	6	40	7	3000	75.01	79.80
2	20	6	35	6	3500	72.23	72.83
3	20	5	40	7	3000	73.80	73.59
4	15	6	40	7	3000	72.20	72.83
5	20	6	40	7	3000	75.01	79.80
6	15	5	35	8	3000	71.75	72.83
7	20	6	40	7	3000	75.01	79.80
8	25	7	35	7	3000	72.04	70.48
9	20	6	40	8	3500	59.34	59.28
10	15	7	45	7	3000	80.09	80.02
11	25	6	40	6	3500	59.98	60.24
12	25	5	35	7	3000	71.04	70.18
13	15	7	45	8	2500	70.46	70.26
14	25	5	35	6	2500	73.02	73.10
15	15	5	35	8	3000	63.03	63.18
16	20	7	40	7	3500	73.65	72.18
17	20	6	40	8	3500	79.50	79.28
18	25	7	35	8	3500	71.86	71.98
19	20	6	40	8	2500	74.62	74.58
20	15	7	35	8	2500	63.55	63.18

21	15	5	45	7	3000	75.01	73.46
22	20	6	40	7	3000	75.01	79.80
23	20	6	45	6	3500	73.28	73.72
24	20	6	40	6	2500	62.14	62.42
25	20	6	40	6	3500	75.85	76.10
26	25	7	45	6	3000	74.27	72.26
27	25	5	45	6	3000	77.71	77.62
28	15	7	35	7	3000	75.01	73.68
29	20	6	40	6	2500	79.93	80.16
30	15	5	45	8	2500	71.67	71.26
31	25	5	45	7	2500	74.88	75.42
32	25	7	45	7	2500	77.41	76.68

Table S2 Central composite design based experimental designs (variables and responses) for enzymatic hydrolysis of Kans grass in terms of coded level of variables

Run Order	Solid Loading (%)	Incubation Time (h)	Temperature (° C)	pH	Enzyme Concentration (IU/g)	Reducing Sugar (mg/g of substrate)	
						Predicted	Experimental
1	25	5	50	5	60	461.44	479.89
2	30	4	55	6	40	187.63	190.48
3	30	4	45	6	80	152.54	155.87
4	25	6	50	5	60	380.55	377.60
5	20	6	55	6	40	104.56	105.83
6	20	6	45	6	80	97.279	103.02
7	25	5	50	5	40	280.53	278.10
8	25	5	50	5	60	461.44	479.89
9	20	4	45	4	80	353.26	358.10
10	20	4	55	6	80	396.84	401.68
11	30	6	45	4	80	97.52	101.25
12	25	5	50	5	80	341.08	336.85
13	25	5	50	5	60	461.44	479.89
14	20	4	45	6	40	103.94	108.81
15	20	5	50	5	60	322.72	320.32
16	30	6	55	4	40	296.58	299.83
17	30	4	55	4	80	109.20	112.02
18	25	5	50	6	60	416.57	412.23
19	30	5	50	5	60	316.71	310.45
20	25	5	50	5	60	461.44	479.896
21	25	5	55	5	60	334.74	332.42
22	25	5	50	5	60	461.44	479.896

23	20	4	55	4	40	151.26	155.62
24	30	6	55	6	80	407.16	410.89
25	20	6	55	4	80	432.94	438.18
26	25	5	45	5	60	265.33	260.99
27	25	5	50	5	60	461.44	479.896
28	30	6	45	6	40	202.62	206.37
29	25	4	50	5	60	360.90	359.18
30	30	4	45	4	40	255.27	258.12
31	25	5	50	4	60	455.56	453.24
32	20	6	45	4	40	264.48	269.75

Table S3 The DSC-thermoporometry temperature program and the corresponding pore diameters.

Step (i)	Temperature (°C)	Pore diameter (nm)
0	-30	Estimating C_0
1	-15	2.6
2	-13	3.04
3	-7	5.65
4	-6	4.84
5	-5.19	12.85
6	-2	19.8
7	-1.5	26.4
8	-1.3	30.4
9	-0.8	49.5
10	-0.2	198
11	-0.1	396

Table S4 ANOVA analysis for regression coefficients and corresponding *F*- and *P*- values for enzymatic pretreatment

Source	DF[a]	Seq SS[b]	Adj SS[b]	Adj MS[c]	*F*	*p*
Regression	20	894.844	894.844	44.742	10.04	< 0.001
Linear	5	462.17	22.387	4.4774	1.00	0.459
Square	5	146.30	146.30	29.261	6.56	< 0.005
Interaction	10	286.36	286.36	28.636	6.42	0.002
Residual error	11	49.038	49.038	4.4580		
Lack-of-fit	6	15.471	15.471	2.5785	0.38	0.862
Pure error	5	33.567	33.567	6.7134		
Total	31	943.882				

R^2 = 94.80 %, $R^2_{(adj)}$ = 85.36 %

a Degrees of Freedom.
b Sum of Squares.
c Mean Squares.

Table S5 ANOVA analysis for regression coefficients and corresponding *F*- and *P*- values for enzymatic hydrolysis

Source	DF[a]	Seq SS[b]	Adj SS[b]	Adj MS[c]	*F*	*p*
Regression	20	476212	476212.6	23810.6	11.20	<0.001
Linear	5	50128	50104	10025.6	4.72	0.015
Square	5	251949	241946	50389.7	23.71	<0.001
Interaction	10	174136	174139	17413.6	8.19	0.001
Residual error	11	23378	23378.1	2125.3		
Lack-of-fit	6	13378	13378.1	3896.3		0.031
Pure error	5					
Total	31	499590				

R^2 = 95.32 %, $R^2_{(adj)}$ = 86.81 %

a Degrees of Freedom.
b Sum of Squares.
c Mean Squares.

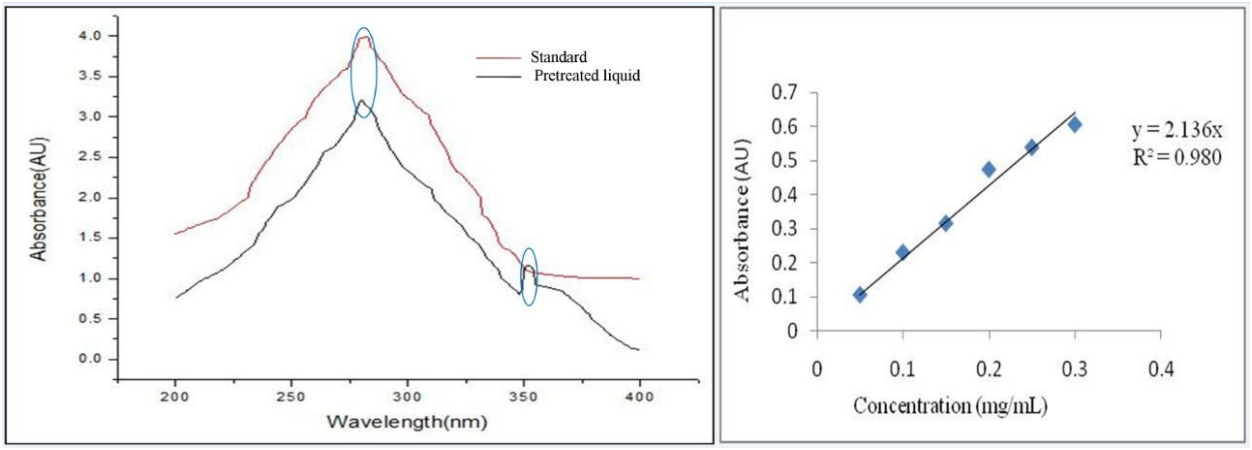

Fig. S3 (a) UV-spectra of pretreated liquid and standard at various concentrations (b) shows the standard Kraft lignin concentrations against absorbance 280 nm.

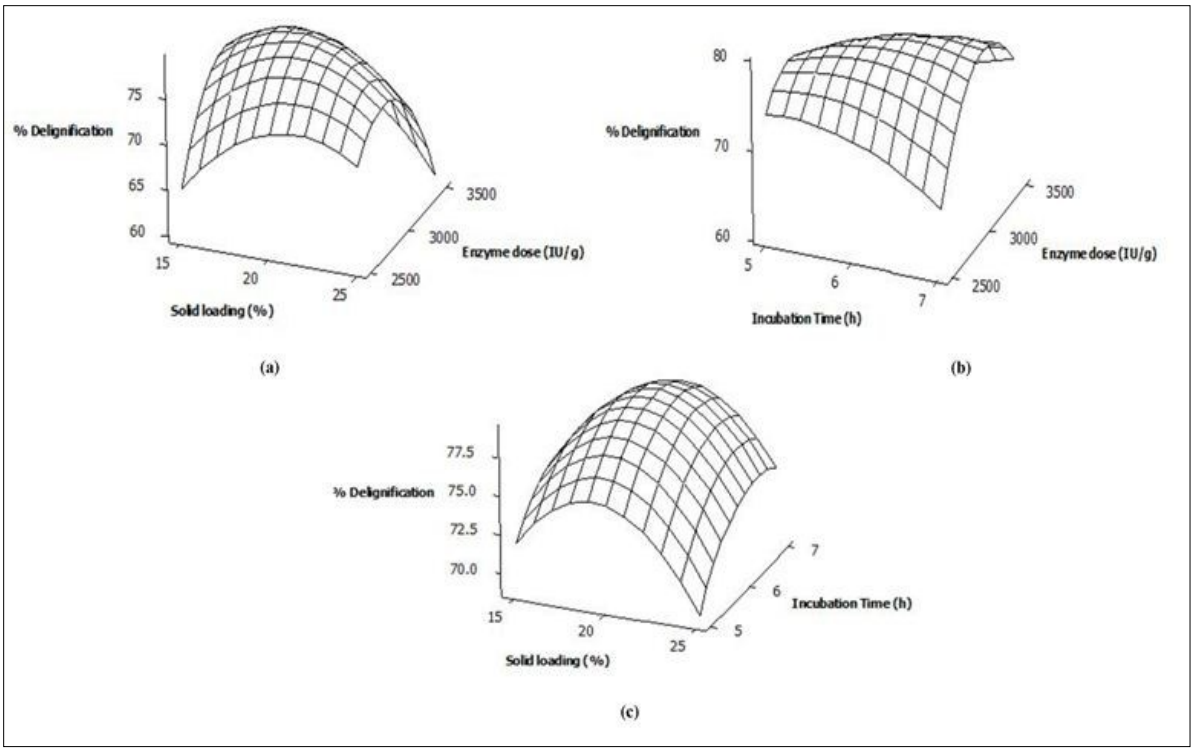

Fig. S4 Response surface plots for (a) solid loading and enzyme dose (b) incubation time and enzyme dose (c) solid loading and incubation time. % delignification represents amount of lignin degraded and horizontal axis represents different process variables.

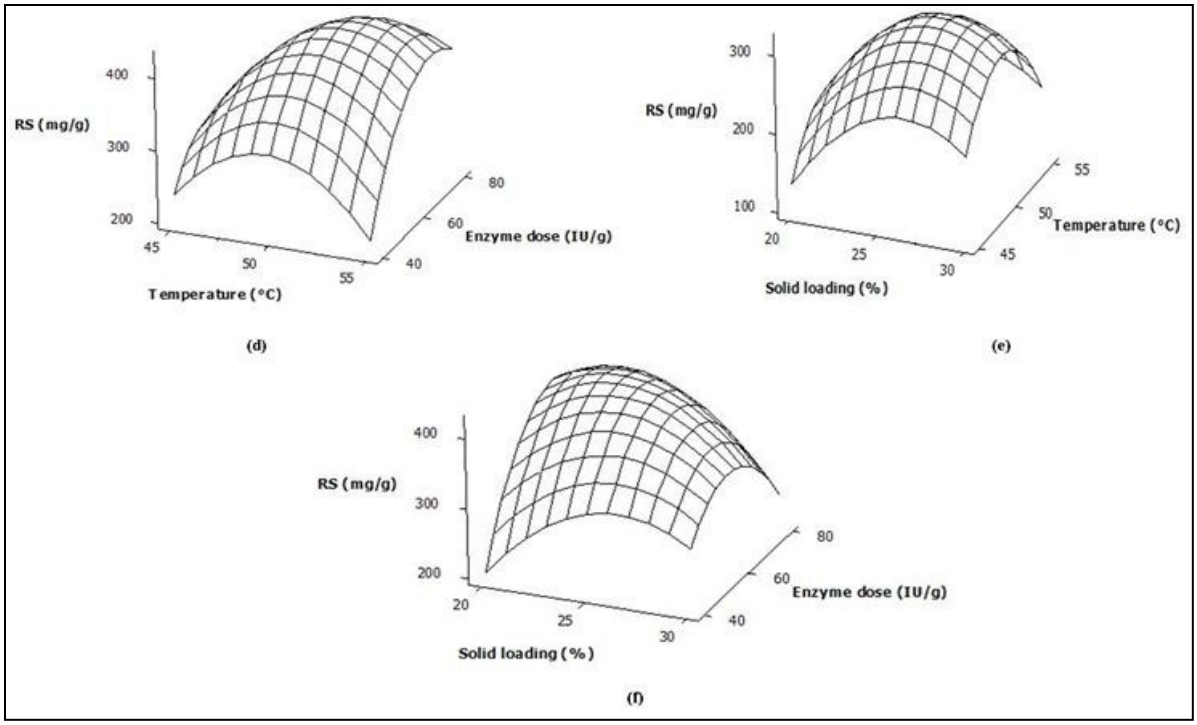

Fig. S5 Response surface plots for (d) temperature and enzyme dose (e) solid loading and temperature (f) solid loading and enzyme dose. RS represents reducing sugar concentration and horizontal axis represents different process variables.

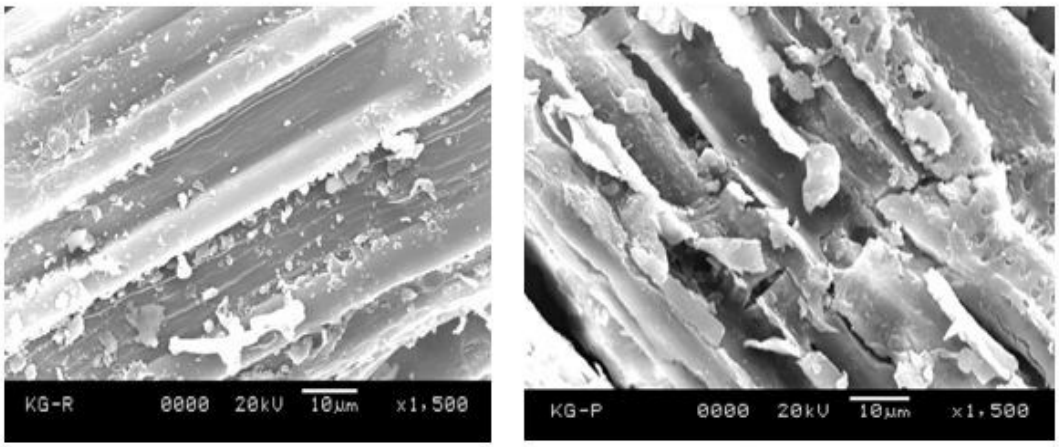

Fig. S6 SEM images for (a) raw substrate (b) pretreated substrate.

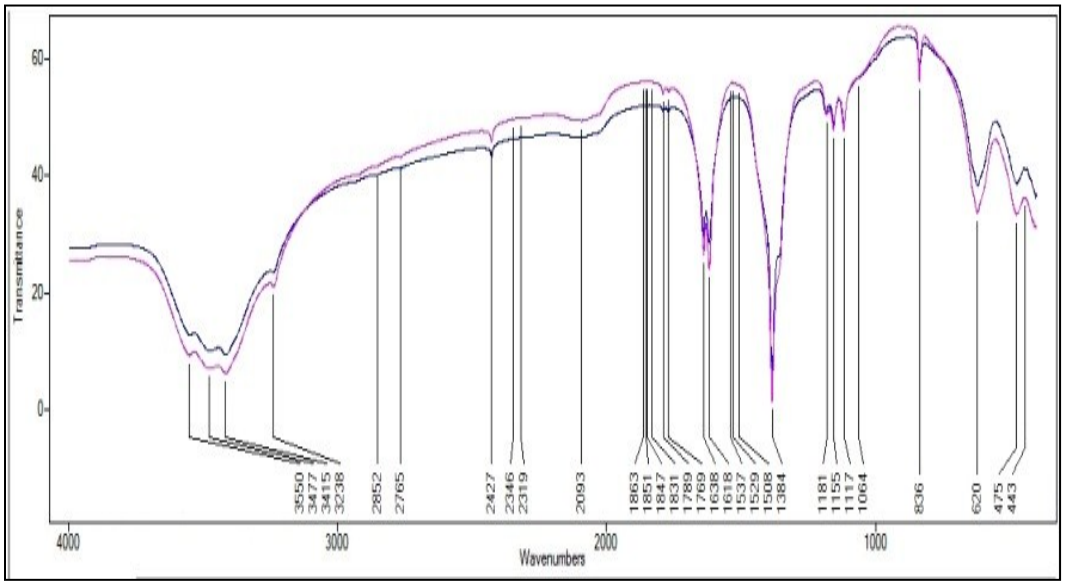

Fig. S6 (c) FT-IR spectra for raw and pretreated substrate.

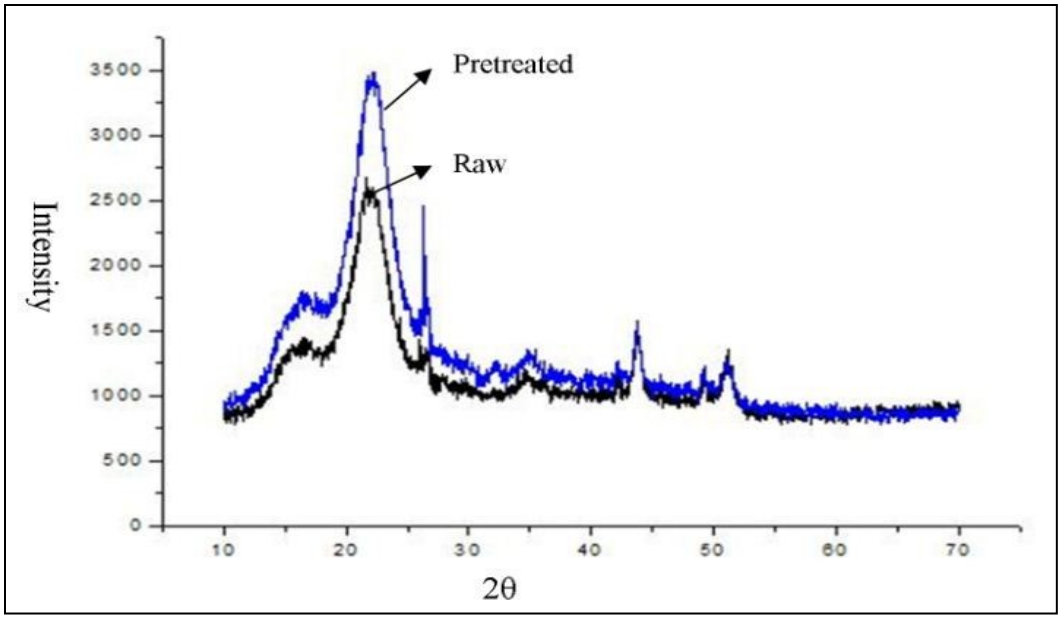

Fig. S7 XRD for raw and pretreated substrate.