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Energy requirement for alkali assisted microwave and high pressure reactor pretreatments of cotton plant residue and its hydrolysis for fermentable sugar production for biofuel application

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ABSTRACT

In the present work, alkali assisted microwave pretreatment (AAMP) of cotton plant residue (CPR) with high pressure reactor pretreatment was compared. Further, modeling of AAMP was attempted. AAMP, followed by enzymatic saccharification was evaluated and the critical parameters were identified to be exposure time, particle size and enzyme loading. The levels of these parameters were optimized using response surface methodology (RSM) to enhance sugar yield. AAMP of CPR (1 mm average size) for 6 min at 300 W yielded solid fractions that on hydrolysis resulted in maximum reducing sugar yield of 0.495 g/g. The energy required for AAMP at 300 W for 6 min was 108 kJ whereas high pressure pretreatment (180 °C, 100 rpm for 45 min) requires 5 times more energy i.e., 540 kJ. Physiochemical characterization of native and pretreated feedstock revealed differences between high pressure pretreatment and AAMP.

1. Introduction

There is a growing global interest in the development of economically viable bioenergy production systems that can at least partly displace the use of fossil fuels. Increased energy security, reduction in greenhouse gas emissions, use of renewable resources and establishing a carbohydrate-based chemical process industry are the main benefits of using lignocellulosic materials as a stable energy source (Klinke et al., 2004; Kashaninejad and Tabil, 2011).

Second generation biofuels are better than first generation biofuels in terms of energy balances, greenhouse gas emission reduction, land requirement and competition for land, food, fiber and water. The main reason they have not yet been taken up for commercialization, despite their potential advantages, is that the involved production technologies are not technically proven at a commercial scale and their costs are estimated to be significantly higher than petroleum products and most first generation biofuels. Therefore, there is still much work to be done for the improvement of the existing processes and for the development of new efficient technologies (Perego and Bianchi, 2010).

The performance of lignocellulosic biomass-to-ethanol processes greatly depends upon the characteristics of feedstocks and conversion technology including pretreatment, enzymatic saccharification and fermentation (Guo et al., 2009). Lignocellulosic biomass resists enzymatic hydrolysis in bioconversion processes (Shao et al., 2009). This is because native cellulose is in a highly crystalline structure and embedded deep into lignin and hemicelluloses. Hence attachment of cellulase to cellulose is extremely difficult. Pretreatment processes separate lignin from polysaccharides and reduce cellulose crystallinity making the glycoside bonds more accessible to the hydrolytic enzymes (Feng et al., 2011). Operational integration of pretreatment and enzymatic saccharification is a crucial issue, where pretreatment efficiency can be assessed by estimating the sugar released by enzymatic hydrolysis. It is necessary to have optimum conditions both for pretreatment and enzymatic hydrolysis, so that the overall efficiency of fermentable sugar production enhances.

India is the first among cotton growing countries of the world with respect to area - 9.4 million hectares in 2009–10, which is about 35% of world cotton area (ISAAA, 2009). After harvesting the cotton balls, the entire plant, consisting of stalk and leaves, is a residue which remains in the field and the farmers usually destroy it by burning. It has been estimated that 11.4 MMT cotton plant residue (CPR) is available as feedstock for commercial bio-based processes (Pandey et al., 2009). In this scenario, utilization

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of the cotton plant residues for bioethanol production seems to be very promising.

Pretreatment is one of the most expensive and least technologically developed steps in the process of converting biomass to fermentable sugars (Binod et al., 2012a). Costs are due to the use of steam and chemicals and the need for expensive corrosion resistant reactors (Rabelo et al., 2009). Most of these conventional pretreatment methods produce compounds that might inhibit subsequent fermentation. Among different pretreatment methods, acid pretreatment separates pentoses and hexoses; while alkali pretreatment separates lignin from lignocellulosic biomass. Efficiency of pretreatment can be linked to the increase in cellulose available for hydrolysis which would increase the yield from fermentation (Krishna and Chowdhry, 2000; Chapple et al., 2007).

Microwave irradiation is an alternative approach to conduction heating and has proved to be a highly effective heating source in chemical reactions. Irradiation uses direct interaction between the object to be heated and an applied electromagnetic field to generate heat. This heating mechanism can accelerate the reaction rate, provide better yields, uniform and selective heating and achieve greater reproducibility of reactions (Oliver, 2005). Also, it can reduce process energy requirements and has the ability to instantaneously start and stop the process (Gabriel et al., 1998; Datta, 2001). Combination of microwave treatment with either acid or alkali or combined acid/alkali might be an alternative for pretreatment of lignocellulosic materials.

The objective of the present work is to compare the energy required for pretreatment of cotton plant residue using a high pressure reactor and a microwave treatment. Also, the best conditions for pretreatment and hydrolysis for cotton plant residues were evaluated using a single design matrix. The physio–chemical characteristics of the native and pretreated biomass were also compared.

2. Methods

Cotton plant residues (CPR) were obtained from Maharashtra, India. It contained mainly dried stalks. It was milled in a knife mill to reduce the particle size in the range of 0.2–1.5 mm. The milled materials were stored in an air tight container. The compositional analysis of native and pretreated residue was carried out by two stage acid hydrolysis protocol developed by National Renewable Energy Laboratory (Sluiter et al., 2008).

2.1. Optimization of parameters for pretreatment and enzymatic hydrolysis

Optimization of parameters for pretreatment and saccharification of biomass was performed in two stages. Initially, 11 variables (including a dummy variable) were screened using a Plackett–Burman design to identify the significant parameters that affected the pretreatment and enzymatic saccharification of CPR by alkali assisted microwave pretreatment (AAMP). In the second stage, the levels of these parameters were optimized using a response surface design.

2.1.1. Screening of parameters affecting pretreatment and hydrolysis by Plackett–Burman design

Plackett–Burman design is an efficient mathematical approach to determine the most significant variables in any process. Plackett–Burman design offers fast screening procedure and mathematically computes the significance of a large number of factors in one experiment, which is time saving and gives the effect of change in more than one factor in a single experiment (Reddy et al., 2008; Singh et al., 2011).

In this study, biomass loading (BL), alkali concentration (AC), microwave power (MW), microwave exposure time (ET), particle size (PS), soak time in alkali (ST), substrate concentration for hydrolysis (SC), enzyme loading (EL), incubation time (IT), tween 80 concentration (T80) and antibiotic concentration (ABC) were selected as the independent variables. These variables were investigated and 12 experiments were carried out. Each variable was set at two levels, a high level and a low level. The experimental design is given in Table 1a. The significance of regression coefficient was tested by analysis of variance (ANOVA).

2.1.2. Optimization of significant parameters by response surface methodology

The effect of three independent variables (ET, PS and EL) on the response (reducing sugar yield) was studied using a factorial Central Composite Design (CCD) of response surface methodology. In this study, a polynomial quadratic equation, as shown in Eq. (1) was employed to evaluate the effect of the variables on the response.

$$Y = \beta_0 + \beta_1 A + \beta_2 B + \beta_3 C + \beta_{11} A^2 + \beta_{22} B^2 + \beta_{33} C^2 + \beta_{12} AB + \beta_{13} AC + \beta_{23} BC \quad (1)$$

where Y is the predicted response (reducing sugar yield); $ß_0$ is a constant; $ß_1$, $ß_2$, $ß_3$ are the linear coefficients; $ß_{12}$, $ß_{13}$, $ß_{23}$ are the cross-coefficients; $ß_{11}$, $ß_{22}$, $ß_{33}$ are the quadratic coefficients (Ferreira et al., 2009; Tan et al., 2011).

Each of the variables were investigated at five coded levels ((-1)- α, -1, 0, 1, (1)+ α) and the complete experimental design matrix for this study is shown in Table 2a. A total of twenty experiments, including eight for factorial design, six for axial points and six repetitions at the central point were performed. The responses were analyzed using Design Expert (Stat-Ease Inc.) and Minitab (Minitab Inc.) software. Their significance was checked by ANOVA.

Table 1a
Plackett–Burman design for identification of significant parameters in alkali assisted microwave pretreatment and hydrolysis of cotton plant residue.

Run	BL % (A)	AC % (B)	MW W (C)	ET min (D)	PS mm (E)	ST h (F)	SC g (G)	EL FPU/g (H)	IT h (J)	T80 % (K)	ABC % (L)	Reducing sugar (g/g biomass)
1	30	0.5	600	7	1	12	0.5	10	48	0	2	0.012 ± 0.001
2	30	5	100	1	0.6	24	0.5	60	48	0	2	0.166 ± 0.029
3	30	0.5	100	1	1	12	5	60	12	1	2	0.016 ± 0.001
4	5	5	100	7	1	12	5	60	48	0	0	0.171 ± 0.013
5	30	5	100	7	1	24	0.5	10	12	1	0	0.057 ± 0.001
6	5	0.5	100	7	0.6	24	5	10	48	1	2	0.060 ± 0.001
7	5	5	600	7	0.6	12	0.5	60	12	1	2	0.292 ± 0.01
8	30	0.5	600	7	0.6	24	5	60	12	0	0	0.375 ± 0.014
9	5	0.5	600	1	1	24	0.5	60	48	1	0	0.217 ± 0.012
10	5	0.5	100	1	0.6	12	0.5	10	12	0	0	0.081 ± 0.006
11	5	5	600	1	1	24	5	10	12	0	2	0.037 ± 0.002
12	30	5	600	1	0.6	12	5	10	48	1	0	0.007 ± 0.00

Table 1b
ANOVA for Plackett–Burman model based on alkali concentration as error.

Source	Sum of squares	DF	Residual	*F* value	*P* value Prob > F
Model	0.160	10	0.016	192.563	0.049
A-Biomass loading	0.004	1	0.004	51.170	0.088
C-Microwave power	0.013	1	0.013	151.575	0.052
D-Exposure time	0.016	1	0.016	196.935	0.045
E-Particle size	0.018	1	0.018	222.034	0.043
F-Soak time	0.009	1	0.009	112.028	0.060
G-Substrate concentration	0.002	1	0.002	24.689	0.126
H-Enzyme loading	0.081	1	0.081	973.417	0.020
J-Incubation time	0.004	1	0.004	50.631	0.089
K-Tween 80	0.003	1	0.003	38.320	0.102
L-Antibiotic concentration	0.009	1	0.009	104.833	0.062
Residual	8.289E-05	1	8.289E-05		
Cor total	0.160	11			

Table 2a
RSM design matrix for optimization of significant parameters for pretreatment and hydrolysis of cotton plant residue.

Run	Factor 1; exposure time (min)	Factor 2; particle size (mm)	Factor 3; enzyme loading (FPU/g)	Reducing sugar (g/g biomass) observed	Reducing sugar (g/g biomass) predicted
1	4	0.8	30	0.355 ± 0.03	0.363
2	2	1	45	0.418 ± 0.004	0.401
3	2	0.6	15	0.207 ± 0.004	0.229
4	6	1	45	0.495 ± 0.001	0.468
5	6	1	15	0.325 ± 0.00	0.333
6	0.64	0.8	30	0.274 ± 0.010	0.293
7	2	1	15	0.304 ± 0.010	0.277
8	4	0.8	30	0.358 ± 0.011	0.363
9	4	0.8	30	0.372 ± 0.011	0.363
10	4	0.8	30	0.365 ± 0.008	0.363
11	4	0.8	30	0.340 ± 0.038	0.363
12	7.36	0.8	30	0.392 ± 0.026	0.380
13	4	0.46	30	0.370 ± 0.022	0.341
14	4	0.8	55.23	0.474 ± 0.010	0.491
15	2	0.6	45	0.418 ± 0.017	0.406
16	6	0.6	45	0.430 ± 0.017	0.453
17	4	0.8	30	0.389 ± 0.019	0.363
18	6	0.6	15	0.253 ± 0.023	0.267
19	4	0.8	4.77	0.240 ± 0.025	0.229
20	4	1.14	30	0.358 ± 0.004	0.394

Table 2b
ANOVA for response surface quadratic model.

Source	Sum of squares	DF	Mean square	*F* value	*P* value Prob > *F*
Model	0.098	9	0.010	14.624	0.0001 – Significant
A-Exposure time	0.009	1	0.009	12.167	0.006
B-Particle size	0.003	1	0.003	4.451	0.061
C-Enzyme loading	0.083	1	0.083	111.027	<0.0001
AB	0.0002	1	0.0002	0.260	0.621
AC	5.942E-05	1	5.942E-05	0.079	0.784
BC	0.001	1	0.001	1.794	0.210
A^2	0.001	1	0.001	1.713	0.220
B^2	3.639–05	1	3.639E-05	0.049	0.830
C^2	1.596E-05	1	1.596E-05	0.021	0.887
Residual	0.007	10	0.0007		
Lack of fit	0.006	5	0.001	4.501	0.062 – Not significant
Pure error	0.001	5	0.0003		
Cor total	0.106	19			

Three-dimensional plots were drawn to illustrate the effects of independent variables on the response. Three additional experiments were conducted to verify the validity of the predicted optimum values by the program.

2.2. Alkali assisted microwave pretreatment

Microwave pretreatment was carried out using a commercial microwave oven (Samsung, CE2877 N, Korea) with an operating frequency of 2.450 GHz. The mixtures were placed in a sealed conical flask and treated by microwave–alkali according to the experimental design. After pretreatment, the samples were neutralized to pH 6.0 using 1 N H_2SO_4. The pretreated material was filtered; air dried (30 °C) and was subjected to hydrolysis. All experiments were performed in triplicates and mean values reported.

2.3. Enzymatic hydrolysis

Enzymatic saccharification of pretreated CPR was carried out using commercial cellulase from Zytex (Zytex India Private

Limited, Mumbai, India). To the pretreated biomass, enzyme was added and incubated at 50 °C and 120 rpm. Hydrolysis conditions were as given by the experimental design. After incubation, the samples were centrifuged to remove the un-hydrolyzed residue. The supernatant was estimated for reducing sugar by 3,5 dinitrosalicylic acid method (Miller, 1959). Results are expressed as g of reducing sugar/g pretreated biomass.

2.4. Characterization of native and pretreated biomass

2.4.1. XRD analysis

The X-ray diffractogram which indicates the crystallinity of CPR before and after pretreatment was analyzed in a PANalytical (Netherlands), X- pert pro diffractometer set at 40 kV, 30 mA; radiation was Cu Kα (λ = 1.54 Å) and grade range between 10 and 30° with a step size of 0.03°.

2.4.2. FT-IR analysis

Fourier Transform Infrared (FT-IR) spectroscopic analysis was carried out to detect changes in functional groups present on the biomass due to pretreatment. FT-IR spectrum was recorded between 4000–400 cm^{-1} using a Shimadzu Spectrometer with detector at 4 cm^{-1} resolution and 25 scans per sample. Disks were prepared by mixing 3 mg of dried sample with 300 mg of KBr (Spectroscopic grade) in an agate mortar. The resulting mixture was pressed at 10 MPa for 3 min.

2.4.3. SEM analysis

Physical changes in the native and pretreated CPR were observed by scanning electron microscope (SEM). Images of the native and pretreated CPR were taken at magnification of 1500× using a JEOL JSM-5600 SEM. The specimens to be coated were mounted on a conductive tape and coated with gold palladium using a JEOL JFC -1200 fine coater and observed using a voltage of 10–15 kV.

2.5. Comparison of energy consumption in high pressure reactor and microwave oven

Pretreatment was carried out in a high pressure reactor (Amar Equipments, Mumbai, India) with maximum temperature and pressure rating of 250 °C and 100 bar respectively, equipped with a pitch blade turbine stirrer with rpm range 50–1450, and heated through a ceramic mantle.

The power consumption in a stirred vessel depends upon various geometrical parameters, rotational speed and fluid properties. Power input to the pretreatment mixture is due to heating by the mantle and agitation by the impeller. Heat transfer by the mantle is given by

$$Q_{heating} = m * C_p * \Delta T \quad (2)$$

where:

m = mass of the batch liquid being heated or cooled, expressed as kg.

C_p = heat capacity of the liquid batch, expressed as J/kg°C.

ΔT = the temperature of the batch liquid at any time, expressed as °C.

To calculate the Mechanical Power (P_i) being transmitted to the fluid by a turbine/impeller of a given design, the following equation needs to be used:

$$P_0 = P_i/\rho N^3 D^5 \quad (3)$$

where P_o is power number, N is impeller rotational speed, D is impeller diameter (D) and ρ is liquid density.

To calculate the total energy input to the broth, the impeller power input P_i and the mixing time for the agitated system needs to be known. P_i can be calculated from P_o. P_o depends on the geometry of the system and this dependence can be expressed by the equation

$$P_o = 1.507(T/D)^{-0.365}(h/D)^{-0.165}(H/T)^{0.140} n_B^{0.171} (sin\alpha)^{2.077} \quad (4)$$

This equation is valid for values of the *Reynolds number* greater than 10^4 (Medek, 1980; Fořt et al., 2001). It consists of n_B –number of impeller blades, α –pitch angle of the impeller blades, h –impeller off-bottom clearance, T – diameter of tank, D = diameter of impeller, H = height of tank.

To calculate the total energy transferred to the broth by the impeller, Eq. (5) is used.

$$Q_{impeller} = P_{impeller} * \text{exposure time} \quad (5)$$

For pretreatment in a microwave oven, energy transferred to the broth is only due to heat given by the microwaves. Assuming no loss of energy in the closed system, heat transferred to the broth is given by Eq. (6).

$$Q_{microwave} = \text{Power input} * \text{exposure time} \quad (6)$$

3. Results and discussion

3.1. Plackett–Burman design

Preliminary experiments were performed to determine the significant factors by Plackett–Burman design. The effects of different factors (BL, AC, MW, ET, PS, ST, SC, EL, IT, T80 and ABC) were evaluated on the basis of reducing sugar released after the pretreatment and hydrolysis of CPR (Table 1a). Among the variables ET, PS and EL were identified as the most significant (Fig. 1) with a range of 0.64–7.34 min, 0.46–1.14 mm and 4.77–55.23 FPU/g respectively. To test the significance of the developed model, ANOVA was performed and the results are presented in Table 1b. A model is considered significant if its p-value (also known as the 'Prob > F' value) is lower than 0.05. In this case this value was found to be lower than 0.05 and hence the model is significant. The coefficient of determination (R^2) of the model was 0.99, implying a high correlation between the observed and predicted values.

3.2. RSM results

The results of RSM experiments for studying the effect of the three independent variables (optimized by Plackett–Burman Design) are presented along with the mean predicted and observed responses in Table 2a. The levels of the non – significant parameters were maintained at the average of low and high value. The experimental data shown in Table 2a was used to determine the regression coefficients of the second-order polynomial equation using Design-Expert software and the following model that describes the reducing sugar yield was obtained.

$$\begin{aligned}\text{Reducing Sugar(g/g biomass)} = {} & 0.0231 + 0.0192ET + 0.0947PS \\ & + 0.0086EL + 0.0123ET * PS \\ & + 9.084e^{-5} * ET * EL - 0.0043PS \\ & * EL - 0.0024 * ET^2 + 0.0397 \\ & * PS^2 - 4.677E - 06 * EL^2\end{aligned}$$

To test the significance of the developed model, ANOVA was performed and the results are presented in Table 2b. As the p value for the model is very low (0.0001), the model equation adequately

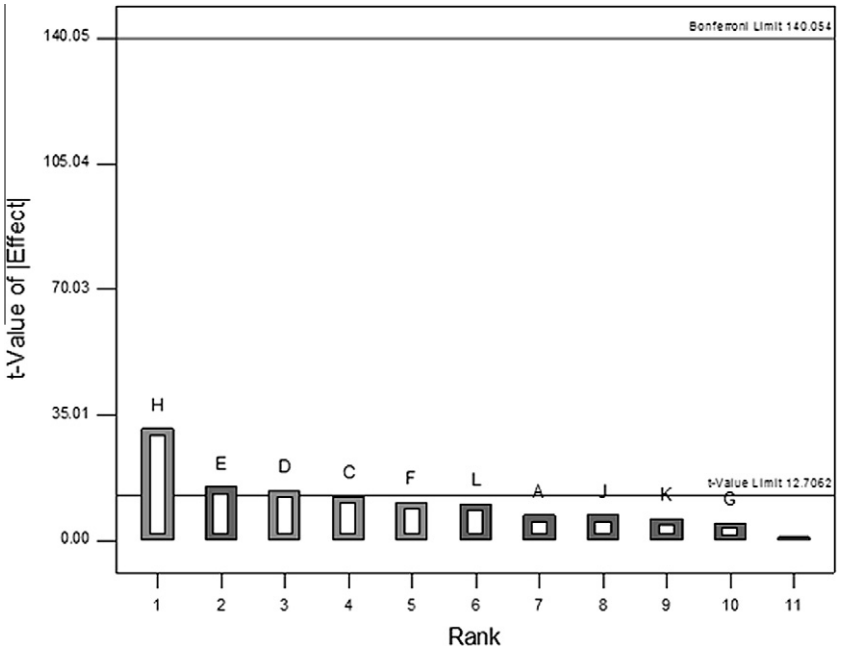

Fig. 1. Pareto chart showing the significant parameters from Plackett–Burman design.

describes the response and the model is significant. Also, $p < 0.01$ values for model terms suggest that ET, PS and EL have significant effects on the sugar yield. The lack of fit test used to determine the adequacy of the model, indicates an insignificant lack of fit with an *F*-value of 4.501. The R^2 of the model was 0.929, implying a high correlation between the observed and predicted values. All of these statistical tests showed that the developed model was suitable for representing the data and provided a good description of the relationship between the process variables and response.

It has been reported that microwave irradiation causes a physical explosion effect among the microfibers, causing the disintegration of the recalcitrant structures of the biomass. Also, the electromagnetic field used in microwaves produces physio-chemical effects that accelerate the breakdown of the crystalline regions (Hu and Wen, 2008). The performance advantage of microwaves is because of the non-thermal effects associated with it (Ooshima, 1984; Huang et al., 2009). These might be the reasons that an increase in ET and EL was found to increase the reducing sugar yield as is evident from Fig. 2a. The higher reducing sugar yields upon higher microwave exposure can be attributed to the direct interaction of microwaves with the cellulose and more efficient heating due to small particle size (Fig. 2b).

Higher microwave power and exposure time leads to high localized overheating and results in sugar degradation (Kappe, 2005). The first reported use of microwave pretreatment of lignocellulose was by Ooshima, 1984. They showed the benefit of microwave-assisted water pretreatment of rice straw and bagasse relative to untreated biomass. Microwave-assisted stepwise alkali/acid/peroxide pretreatment of rice and wheat straw has also been investigated (Zhu, 2006).

Fig. 2c shows that a higher enzyme loading and smaller particle size enhances enzyme digestibility. The particle size is critical only above a certain size range. Lignocellulosic biomass must be milled to less than 10 mm in size for highest observed conversion. Finer size is necessary to maximize the surface area for enzymatic digestion (Jannasch et al., 2001).

The optimum pretreatment and hydrolysis condition was found to be 6 min ET, 1 mm PS and 45 FPU/g EL. This yielded a reducing sugar amount of 0.495 g/ g pretreated biomass. As is evident from the three response surface plots, an increase in EL and ET can lead to an increase in reducing sugar yield. But, increase in ET leads to charring of the biomass and considering the cost constraints, it is always better to maintain the enzyme loading at as low a value as possible.

3.2.1. Validation of the model

The adequacy of the model equation was validated by performing three verification experiments within the experiment range as given in Table 3. The data of the validation runs were statistically analyzed to find the correlation between observed and predicted values. The R^2 between experimental and predicted values was found to be 0.955 indicating that the experimental values were in good agreement with those of the predicted, demonstrating the accuracy of the model.

3.3. Biomass composition and physio–chemical characterization

3.3.1. Biomass composition

The chemical composition of the native and pretreated biomass is given in Table 4. It is evident that there is a difference in the composition of native, high pressure reactor treated and AAMP biomass. It has been reported that microwave irradiation facilitates lignin and hemicelluloses dissolution in alkali solutions. The primary role of NaOH pretreatment is the disruption of ester bonds between lignin and carbohydrates in the biomass that results in solubility of lignin (Zhu et al., 2005; Kumar et al., 2009). The cellulose and hemicelluloses content of AAMP samples is lower than high pressure reactor treated samples.

3.3.2. XRD studies

The X- ray diffraction profile of native, high pressure reactor treated and AAMP CPR is shown in Supplementary Fig. S1. The crystallinity of the native biomass is 45.32% and that of high pressure reactor pretreated biomass is 57.87%. Maximum crystallinity for AAMP biomass was calculated to be 60%. This may be due to the removal of lignin from the biomass.

3.3.3. FT-IR analysis

The removal of lignin was also evident from FT-IR spectrum of untreated and pretreated CPR (Supplementary Fig. S2). In the

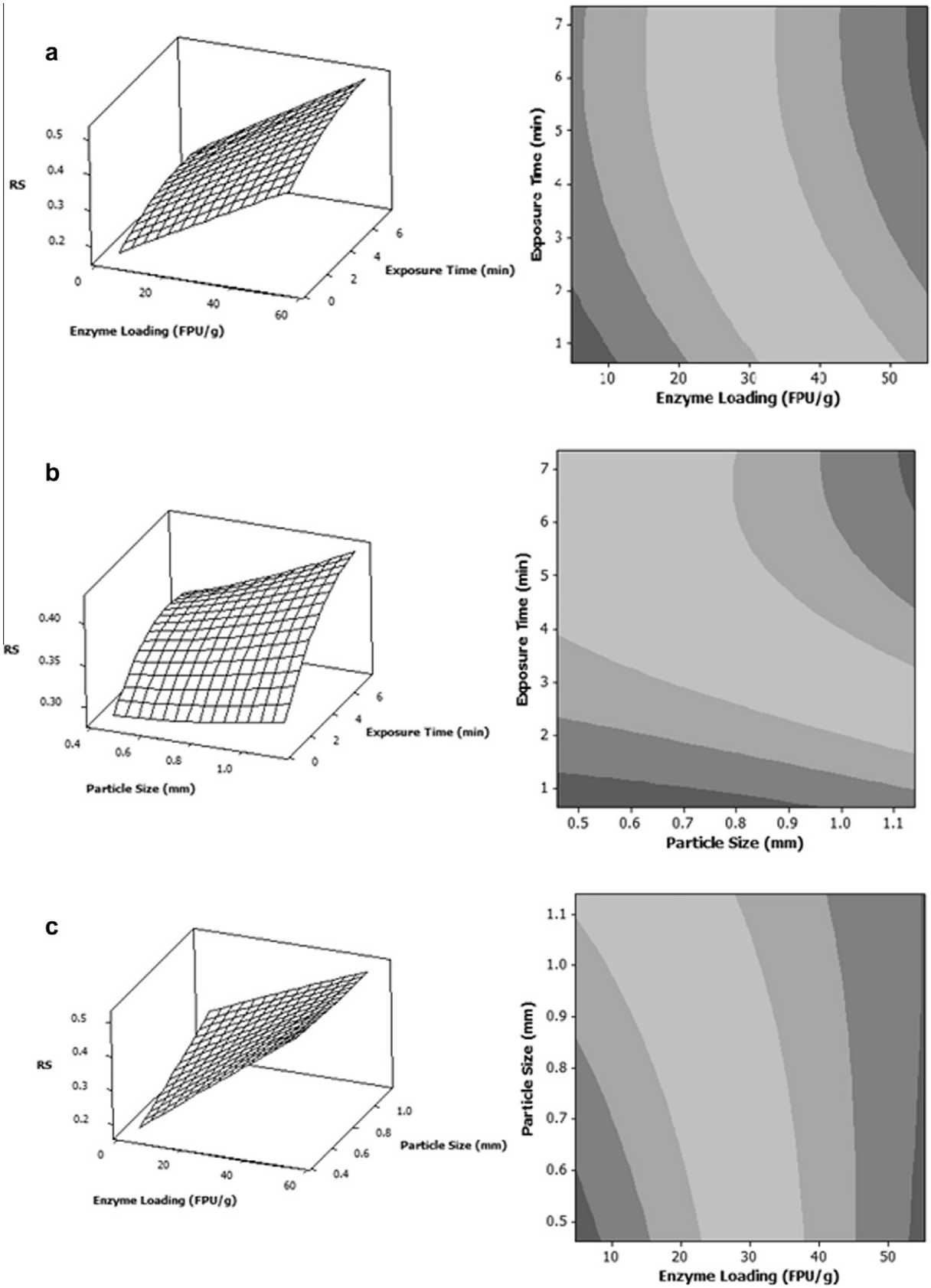

Fig. 2. Surface and contour plot for the optimization of pretreatment parameters (a) effect of enzyme loading and microwave exposure time on reducing sugar yield; (b) effect of particle size and microwave exposure time on reducing sugar yield; (c) effect of enzyme loading and particle size on reducing sugar yield.

Table 3
Validation of RSM Model.

Run	Factor 1; exposure time (min)	Factor 2; particle size (mm)	Factor 3; enzyme loading (FPU/g)	Reducing sugar (g/g biomass) observed	Reducing sugar (g/g biomass) predicted
1	3	0.6	25	0.310 ± 0.013	0.306 ± 0.027
2	4.5	0.8	35	0.390 ± 0.014	0.395 ± 0.027
3	5.5	1.0	20	0.331 ± 0.016	0.353 ± 0.027

Table 4
Composition of native and pretreated cotton plant residue.

Components (%)	Native cotton plant waste	High pressure reactor pretreated	Microwave–alkali pretreated
Cellulose	38.26	48.77	42.37
Hemicellulose	13.70	8	2.81
Total lignin	29.95	0	2.12

FT-IR spectra, the range of 1850–500 cm^{-1} contains lignin related information (Raiskila et al., 2007). The dip in the spectra of pretreated samples in the range 1150–1350 cm^{-1} shows an increase in cellulose content. The FT-IR spectral analysis of untreated CPR also showed absorption bands in 1720, 1670, 1590, 1500, 1463, 1423, 1360, 1330, 1270, 1215, 1140, 1125, 1087, 1031, 970, 855 and 815 cm^{-1} regions. For high pressure reactor treated and AAMP samples, the corresponding bands in these regions were absent. This shows significant removal of lignin during pretreatment.

3.3.4. SEM analysis

The native sample appears rigid and contains a hard surface (Supplementary Fig. S3A). The high pressure reactor treated sample does not show a rigid surface and appears porous (Supplementary Fig. S3B). The AAMP CPR appears to be even more open than the conventional-pretreated samples, with fiber ruptured in many different directions (Supplementary Fig. S3C). The increased disintegration within the structure may be due to the non-thermal effects caused by microwave pretreatment (Ooshima, 1984; Hu and Wen, 2008).

3.4. Comparison of energy consumption in high pressure reactor treatment and AAMP.

In a high pressure reactor, for treating 300 ml at 180 °C, 100 rpm and 45 min (Binod et al., 2012b),

N = 100 rpm; D = 0.035 m; T = 7 cm; H = 17.7 cm; h = 0.1 cm; α = 60°; m = 296.7 g (300 ml slurry); ρ = 989 kg/m^3; C_p = 12.1335 J/K/g; ΔT = (453 K-303 K)

From Eq. (4),

P_o = 2.25212
$P_i = P_o * \rho N^3 D^5$ = 0.001083 J/s
$Q_{heating}$ = 540001.4175 J (from Eq. (2))
$Q_{impeller} = P_i * 45$ min = 2.9241 J (from Eq. (5))
$Q_{total} = Q_{heating} + Q_{impeller}$ = 540004.342 J

In the case of microwave treatment, the energy input will be

$Q_{microwave}$ = 108000 J (for 6 min operation at 300 W) (from Eq. (6))

Microwave treatment of pulverized CPR (average 1 mm particle size) for 6 min at 300 W, yielded solid fraction that on hydrolysis resulted in a maximum reducing sugar yield of 0.495 g/g biomass. The energy required for microwave pretreatment at 300 W for 6 min was calculated as 108 kJ. Compared to this, the energy requirement for high pressure pretreatment of CPR (180 °C, 100 rpm for 45 min) was 540 kJ and the pretreated biomass on subsequent hydrolysis under similar conditions gave 0.79 g sugar/g biomass (Binod et al., 2012a).

The biomass loading in high pressure reactor treatment is 15 g/300 ml alkali (5% w/v) whereas for AAMP, biomass loading was 17.5 g/100 ml alkali (17.5% w/v). So when considering biomass loading also, AAMP is more economical. To improve the economics of pretreatment and the overall economics of the process, biomass loading must be at least 20% (w/v). A higher solid loading is required to achieve at least break-even economics by taking advantage of higher throughput and increased energy usage relative to lower biomass loading (Martin, 2009).

The microwave pretreatment process has a higher investment, lower operating cost, and higher operating income, relative to the conventional pretreatment process. The outlook and scale-up potential for microwave pretreatment is still in its infancy. Commercial outlook is best realized through the scale up of a continuous microwave reactor system. The scalability of the microwave technology has been limited.

4. Conclusions

The potential of utilizing AAMP for energy efficient pretreatment of lignocellulosic feed stock for bioethanol production has been demonstrated. The energy requirement for pretreating unit quantity of CPR by AAMP was calculated to be 5 times lesser than high pressure reactor treatment. AAMP yielded 0.495 g/g of reducing sugar after 6 min of treatment whereas high pressure reactor treatment yielded 0.79 g/g reducing sugar after 45 min of treatment. Hence AAMP is also faster. Further studies need to be carried out to determine the overall process efficiencies and practicality of the process.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.biortech.2012.02.076.

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