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A Novel Surfactant-assisted Transition Metal Pretreatment of Chili Post Harvest Residue for the Production of Bioethanol by Separate Hydrolysis and Fermentation

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ABSTRACT

In this work, a novel surfactant assisted transition metal pretreatment strategy was evaluated for the production of bioethanol from chili post harvest residue. Among the various transition metals screened surfactant assisted ferrous sulphate pretreatment was found to be more effective in terms of reducing sugar yield. Various process parameters affecting pretreatment were optimized by adopting a Taguchi design. The optimum conditions of pretreatment were (% w/w) surfactant concentration of 1, FeSO₄ concentration of 2, biomass loading of 5 and pretreatment time of 20 min. Under optimized conditions 0.245g/g of reducing sugar per g of dry biomass (g/g) was obtained. The hydrolyzate is devoid of major fermentation inhibitors such as furfural, 5-hydroxymethylfurfural and organic acids such as citric acid, propionic acid and succinic acid. Fermentation of the non-detoxified hydrolyzate yielded 1.84%v/v of ethanol with a fermentation efficiency of 79%.

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1. Introduction

Increase in energy demand and depletion of fossil fuel reserves leads to search for alternative strategies of energy. Lignocellulosic biomass serves as a potential source for the production of bioethanol. It is composed of cellulose, hemicelluloses and lignin as the major component. Cellulosic bioethanol is considered as the most promising renewable biofuel in transportation sector in the coming few decades [Galbe and Zacchi, 2007]. For the production of ethanol from lignocellulosic biomass, it requires three major unit operations like pretreatment, hydrolysis and fermentation. Pretreatment is one of the key steps for bioethanol production. Pretreatment is the most expensive and energy intensive step accounting for about 20% of total cost [Yang and Wyman, 2008]. During the last decades, several pretreatment strategies have been developed either by employing totally new strategies or by modification of existing conventional strategies of pretreatment like acid or alkali with addition of catalysts for effective hemicelluloses and lignin removal.

India is one of the chief producers of chili in the world. The residue which is left out after harvesting of chili represents chili-post harvest residue (CPHR). It is an underexploited biomass and is usually disposed by direct burning in the field. This will create many societal and environmental issues. Hence utilization of this residue for bioethanol production seems promising.

Few reports are available on pretreatment of CPHR. This includes acid and alkali pretreatment [Preeti et al., 2012], crude glycerol-assisted surfactant pretreatment [Sindhu et al., 2015], sono-assisted acid pretreatment [Sindhu et al., 2016], microwave-assisted surfactant pretreatment [Sindhu et al., 2016] and ultrasound-assisted alkali pretreatment [Sindhu et al., in press]. Surfactants are known to play an important role in delignification and transition metals are known for hemicelluloses removal [Liu et al., 2009; Zhao et al., 2011]. For the exploitation of delignification and hemicelluloses removal properties of surfactant and transition metal are exploited in this study. The exact mechanism is not fully understood.

The objective of the present study was to select the best transition metal for surfactant assisted transition metal pretreatment of chili post-harvest residue (CPHR) and to optimize various process parameters affecting surfactant-assisted transition metal pretreatment of CPHR (SATMP CPHR) and utilization of the hydrolyzate obtained after enzymatic saccharification for the production of bioethanol.

2. Materials and methods

2.1. Feed stock

Chili post-harvest residue (CPHR) received from Virudhanagar, Tamil

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Nadu, India was used in this study. The samples were dried and milled using a knife mill. Mixed particle size samples were used throughout the experiments. Compositional analysis of native and pretreated samples was carried out by adopting NREL protocol [Sluiter et al., 2008].

2.2. Screening of various transition metals for surfactant assisted transition metal pretreatment of chili post-harvest residue (SATMP CPHR)

Screening experiments were carried out with four different transition metals – $MnSO_4$, $FeSO_4$, $CuSO_4$ and $ZnSO_4$ at an initial concentration of 1% (w/w), biomass (solid) loading of 10% (w/w), surfactant concentration of 3% w/w and pretreatment was carried out in a laboratory autoclave at 121°C for 60 min. After pretreatment, the samples were washed with tap water and air dried.

2.3. Optimization of various process parameters affecting surfactant assisted transition metal pretreatment of chili post-harvest residue (SATMP CPHR)

Optimization of various process parameters affecting surfactant assisted transition metal pretreatment of CPHR was carried out by adopting a Taguchi design. The experiment consisted of a total of 16 runs. The details are presented in Table 1. The parameters selected were biomass loading, surfactant concentration, $FeSO_4$ concentration and pretreatment time. All the parameters were selected at four levels.

Table 1 Taguchi design for optimization of various process parameters affecting SATMP

Run No:	Surfactant Conc. (% w/w)	$FeSO_4$ Conc. (% w/w)	Pretreatment time (min)	Biomass Loading (% w/w)	Reducing Sugar Yield (g/g)
1	1	1	15	5	0.155
2	1	2	20	5	0.245
3	1	3	25	5	0.156
4	1	4	30	5	0.127
5	2	2	25	10	0.174
6	2	1	30	10	0.194
7	2	4	15	10	0.142
8	2	3	20	10	0.144
9	3	3	30	15	0.168
10	3	4	25	15	0.135
11	3	1	20	15	0.155
12	3	2	15	15	0.144
13	4	4	20	20	0.139
14	4	3	15	20	0.173
15	4	2	30	20	0.176
16	4	1	25	20	0.158

2.4. Validations of optimized conditions of pretreatment

For the validation of the model, three confirmation experiments were carried out within the range defined previously and correlation analysis was performed based on the experimental and the predicted responses.

2.5. Enzymatic saccharification

Enzymatic saccharification of the pretreated biomass was carried out in 150 ml stoppered hydrolysis flasks by incubating 10% w/w of pretreated biomass with commercial cellulase (Zytext India Ltd, Mumbai, India). The enzyme loading was 20 FPU per g of pretreated dry biomass, 0.1% (w/w) of Tween 80 was used as surfactant, 200µl of antibiotic solution (Penicillin- Streptomycin cocktail, Hi-media, India) were added and the total reaction volume was made up to 30 ml with 0.1 M citrate buffer (pH 4.8). The samples were incubated in a shaking water bath at 50°C for 36 hours. After incubation the samples were centrifuged at 4°C, 10,000 rpm for 10 min to remove the un-hydrolyzed biomass. Reducing sugar analysis was carried out by 3, 5-dinitrosalicylic acid method [Miller, 1959].

2.6. Inhibitor analysis of the hydrolyzate

The hydrolyzate obtained after enzymatic saccharification of SATMP CPHR was centrifuged to remove the unhydrolyzed residue and filtered through 0.2µm PES membrane filters (Pall, USA) and the filtrate was evaluated for inhibitors such as furfural, 5-hydroxymethylfurfural, citric acid, succinic acid, propionic acid, acetic acid and formic acid by HPLC. The inhibitors were analyzed using a photodiode array detector kept at 55°C. Rezex ROA columns (Phenomenex) were used with an injection

volume of 10µl and flow rate was maintained at 0.6 ml/min. The concentrations of inhibitors were analyzed using the standard curve.

2.7. Fermentation

The hydrolyzate obtained after enzymatic saccharification was centrifuged at 10,000 rpm, 4°C for 10 min to remove the solids. Fermentation was carried out in stoppered bottles containing non-detoxified hydrolyzate and was inoculated with seed culture (2% v/v) of 18 hrs old *Saccharomyces cerevisiae* and incubated at 30°C for 72 h. After fermentation, the samples were centrifuged and filtered through 0.2µm filters (Pall, USA). The ethanol was analyzed by Gas Chromatography (Sindhu et al., 2011).

3. Results and discussion

3.1. Compositional analysis of native and pretreated chili post-harvest residue

Compositional analysis of the biomass revealed that the native biomass contained 39.95% cellulose, 17.85% hemicelluloses and 25.32% lignin. SATMP CPHR contained 42.11% of cellulose, 11.23% of hemicelluloses and 12.13% of lignin. Mass balance analysis revealed a 30% loss of biomass during the pretreatment process. This shows that SATMP was effective in removing hemicelluloses and lignin from the biomass.

3.2. Screening profile of various transition metals for surfactant assisted transition metal pretreatment of chili post-harvest residue

Four different transition metalsalts- $MnSO_4$, $FeSO_4$, $CuSO_4$ and $ZnSO_4$ at an initial concentration of 1% (w/w) were used for initial screening to select the best transition metal for SATMP of CPHR. The results are presented in Figure 1. Control experiments were carried out with water alone. Initial screening was carried out with 10% (w/w) of biomass loading, surfactant concentration of 3% (w/w), transition metal concentration of 1% (w/w) and pretreatment time of 60 min in a laboratory autoclave at 121°C. Control samples pretreated with water alone gave a reducing sugar yield of 0.059 g/g. Surfactant assisted- $MnSO_4$, surfactant assisted- $ZnSO_4$, surfactant assisted - $FeSO_4$ and surfactant assisted - $CuSO_4$ pretreatment gave a reducing sugar yield of 0.076, 0.090, 0.160 and 0.122g/g respectively. Since surfactant assisted $FeSO_4$ samples gave highest reducing sugar yield, it was selected for further studies. Optimization of various process parameters affecting SATMP of CPHR was carried out by adopting a Taguchi design. The positive effects of ferrous ion on pretreatment have been earlier reported for pretreatment of sugarcane trash [Raghavi et al., 2016]. Transition metals play an important role in hemicelluloses removal [Liu et al., 2009]. The role of surfactant for effective delignification has been earlier reported [Sindhu et al., 2012; Sindhu et al., 2013].

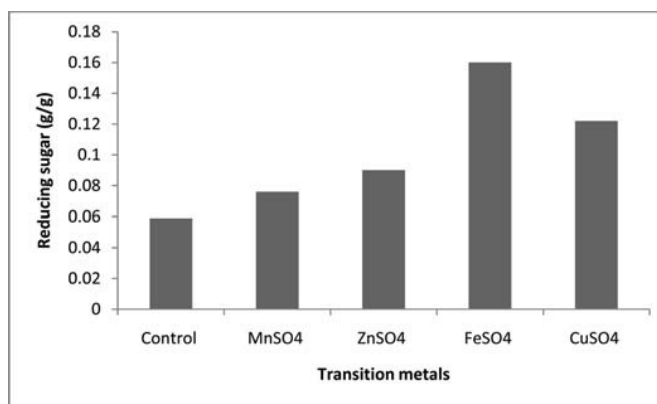


Fig.1. Screening profile of various transition metals for SATMP CPHR

3.3. Effect of different process parameters on surfactant assisted transition metal pretreatment of chili post-harvest residue

The results are presented in Table 1. Maximum reducing sugar yield (0.245 g/g) was observed in Run No: 2 where the conditions of pretreatment were surfactant concentration of 1% (w/w), $FeSO_4$ concentration of 2% (w/w), biomass loading of 5% (w/w), and pretreatment time of 20 min. Contour plots showing interactions between various process parameters affecting SATMP of CPHR are depicted in Figures 2A-2D.

An interaction between FeSO₄ concentration and pretreatment time is depicted in Figure 2A. At low levels of FeSO₄ concentration (1.0 – 1.5% w/w), the reducing sugar yield is low (0.18 g/g). It increased with increase of FeSO₄ concentration (1.6 - 2.4 % w/w). At low levels of pretreatment time (15-17 min), the reducing sugar yield was low; it increased with increase of pretreatment time. Maximum reducing sugar yield (0.24 g/g) was observed with middle levels of pretreatment time (18 – 21 min) and middle levels of FeSO₄ concentration (1.6 - 2.4 % w/w). An identical observation was reported by Raghavi et al., (2016) for sequential pretreatment of sugarcane trash. The role of ferrous ion co-catalyst for enhancing dilute acid pretreatment has been reported by Wei et al., (2011).

An interaction between surfactant concentration and pretreatment time is depicted in Figure 2B. At low levels of pretreatment time (15 – 17 min) the reducing sugar yield was low (0.18 g/g), which increased with increase of residence time (17 – 22 min). At low levels of surfactant concentration (1.0 – 1.5% w/w) the reducing sugar yield is low (0.18 g/g); it increases with increase of surfactant concentration. Maximum reducing sugar yield (0.24 g/g) was observed at middle levels of pretreatment time (17–22 min) and middle levels of surfactant concentration (1.7-2.2%, w/w). Role of surfactants for effective delignification has been earlier reported by Qing et al., 2010. The study revealed that surfactants during biomass pre treatment captured the lignin released into the liquid phase by forming emulsions, thereby reducing re-deposition of lignin to biomass.

An interaction between surfactant concentration and FeSO₄ concentration is depicted in Figure 2C. At low levels of surfactant concentration, the reducing sugar yield was low (0.18 g/g); it increased with increase of surfactant concentration (1.5 -2.2% w/w). At low levels

of FeSO₄ concentration (1.0 – 1.5%, w/w), the reducing sugar yield was low (0.18 g/g), which increased with increase of FeSO₄ concentration (1.5 – 2.2 % w/w). Maximum reducing sugar yield (0.24 g/g) was observed at middle levels of surfactant concentration (1.5-2.2%, w/w) and middle levels of FeSO₄ concentration (1.5 – 2.2 % w/w).

An interaction between biomass loading and FeSO₄ concentration is depicted in Figure 2 D. At low levels of biomass loading (5% w/w) the reducing sugar yield was high (0.24 g/g); it decreased with increase of biomass loading (7.5 – 20%, w/w). At low levels of FeSO₄ concentration (1.0 – 1.5%, w/w), the reducing sugar yield was low (0.18 g/g); it increased with increase of FeSO₄ concentration (1.5 – 2.0 % w/w). Maximum reducing sugar yield (0.24 g/g) was observed at low levels of biomass loading (5%, w/w) and middle levels of FeSO₄ concentration (1.5 – 2.0 % w/w).

The regression coefficient for reducing sugar yield was best with pretreatment time. The p value verifies the significance of each of the coefficients and identifies the pattern of interactions between the selected variables. In this model pretreatment time, FeSO₄ concentration and surfactant concentration were the significant factors. Other factors such as biomass loading was insignificant since the p value was greater than 0.05 (p value less than 0.05 is significant). The R² value explains the variability in the reducing sugar yield. The coefficient of determination (R²) was calculated as 87.09, indicating that the statistical model could explain 87.09% variability in response. The details are presented in Table 2.

For the validation of the model, three confirmation experiments were carried out within the range defined previously. The results are presented in Table 3. Correlation analyses were performed based on the predicted

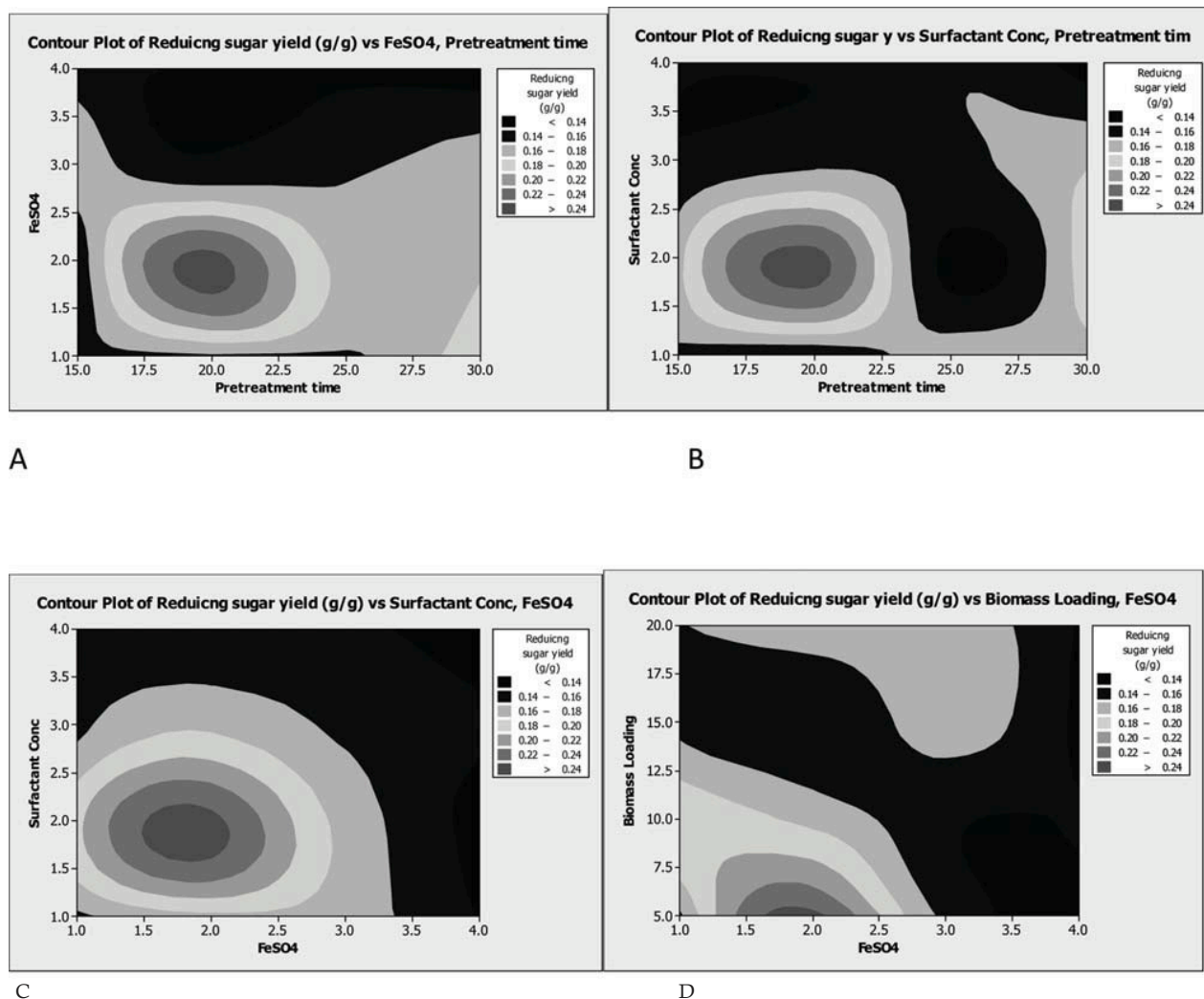


Fig. 2 A-D Contour plots showing interactions of various process parameters affecting SATMP CPHR (A) interactions between pretreatment time and FeSO₄ concentration (B) interactions between pretreatment time and surfactant concentration (C) interactions between surfactant concentration and FeSO₄ concentration (D) interactions between biomass loading and FeSO₄ concentration.

Table 2: Analysis of variance

Source	DF	Seq SS	Adj SS	Adj MS	F	P
BL	3	0.0008422	0.0008422	0.0002807	0.54	0.087
Surfactant	3	0.0039797	0.0039797	0.0013266	2.55	0.031
FeSO ₄	3	0.0048847	0.0048847	0.0016282	3.13	0.017
Pretreatment time	3	0.0008207	0.0008207	0.0002736	0.53	0.005
Error	3	0.0015607	0.0015607	0.0005202		
Total	15	0.0120879				

S = 0.0228085

R-Sq = 87.09% R-Sq(adj) = 35.44%

Table 3: Validations of optimized conditions for surfactant assisted transition metal pretreatment of chili post-harvest residue

Surfactant Conc. (% w/w)	FeSO ₄ Conc. (%w/w)	Pretreatment time (min)	Biomass loading (%w/w)	Reducing sugar (g/g)	
				Predicted	Experimental
2	2	20	5	0.213	0.245
3	2	30	20	0.133	0.176
3	4	15	10	0.119	0.142

Table 4: Inhibitor profile of hydrolyzate of surfactant assisted transition metal pretreated CPHR

Inhibitor	Surfactant assisted transition metal pretreated CPHR
Furfural	ND
5-hydroxymethyl furfural	ND
Formic acid	0.013
Acetic acid	0.019
Citric acid	ND
Succinic acid	ND
Propionic acid	ND

ND - Not detected

results and the experimental values. Correlation coefficient was 0.981, indicating that the model developed was accurate.

3.4. Inhibitor profile of the hydrolyzate obtained after SATMP CPHR

Inhibitor profile of hydrolyzate obtained after enzymatic saccharification of SATMP CPHR is presented in Table 4. In an ideal pretreatment strategy, there will be minimal generation of inhibitors. Major fermentation inhibitors such as furfural, 5-hydroxymethylfurfural and organic acids such as citric acid, succinic acid and propionic acid were absent in the hydrolyzate obtained after enzymatic saccharification of SATMP CPHR. Acetic acid and formic acid were present at low concentration in SATMP CPHR hydrolyzate. However since the concentrations of acetic and formic acid was low these did not inhibit fermentation by *Saccharomyces cerevisiae*. An identical observation was earlier reported by Sindhu et al., (2015) for crude glycerol assisted surfactant pretreatment of chili post-harvest residue.

3.5. Fermentation for the production of bioethanol

Fermentation of the non-detoxified hydrolyzate obtained after enzymatic saccharification of SATMP CPHR with *S. cerevisiae* yielded 1.84% of ethanol with a fermentation efficiency of 79% based on the theoretical ethanol yield from glucose. Since this yield was without any optimization of various process variables of fermentation, it could be expected that this yield could be improved by fine tuning.

4. Conclusions

Compositional analysis of SATMP CPHR revealed that there was effective removal of hemicelluloses and lignin from the pretreated biomass when compared to native samples. The hydrolyzate obtained after SATMP CPHR were devoid of major fermentation inhibitors such as furfural, 5-hydroxymethylfurfural and organic acids such as citric acid, succinic acid and propionic acid. To the best of our knowledge, this is the first report on SATMP of CPHR. Absence of inhibitors in hydrolyzate will effectively eliminate the detoxification step, offering economic advantages. Fermentation of the non-detoxified hydrolyzate yielded 1.84% v/v of ethanol with a fermentation efficiency of 79%.

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