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A novel microwave assisted surfactant pretreatment of chili post-harvest residue for the production of bioethanol and biopolymer

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ABSTRACT

A novel microwave assisted surfactant pretreatment strategy was evaluated for the production of bioethanol and biopolymer from chili post-harvest residue. Among the various surfactants screened microwave assisted Tween - 20 pretreatment was found to be more effective. Various process parameters affecting pretreatment were optimized by adopting a Taguchi design. The optimum conditions of pretreatment were surfactant concentration of 4% w/w, MW pretreatment time for 1 min, MW power of 550W, biomass loading of 5% w/w and pretreatment time for 45 min. Under optimized conditions 0.316g/g of reducing sugar per g of dry biomass (g/g) was observed. The hydrolyzate is devoid of major fermentation inhibitors like furfural, 5-hydroxymethylfurfural and organic acids like citric acid, propionic acid, succinic acid and formic acid. Fermentation of the non-detoxified hydrolyzate yielded 1.66% of ethanol and 67.85% of PHB.

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1. Introduction

Depletion of fossil fuels and increase of energy consumption leads to search for alternative fuels. Bioethanol serves as an important alternative for fossil fuels [Balat et al., 2008]. Lignocellulosic biomass serves as a promising feed stock for the production of biofuels. Conversion of lignocellulosic biomass to bioethanol involves three major unit operations-pretreatment of biomass, enzymatic saccharification of the pretreated biomass and fermentation of sugars into ethanol. The major challenge for the enzymatic saccharification of lignocellulosic biomass is hemicelluloses and lignin content. Recalcitrance of lignocellulosic biomass to hydrolysis requires some kind of pretreatment for the conversion of biomass to biofuels and other value added products. Pretreatment is the important and costly unit operation in lignocellulosic biorefinery. The most common pretreatments currently in practice includes acid, alkali, organosolvents, organic acid, ammonia or other chemicals. Most of the pretreatments are energy intensive and economically non-viable.

An ideal pretreatment condition yields maximum sugar and minimum degradation products. Since the composition of biomass is different for each feed stock, optimization of the pretreatment conditions to be carried out to get maximum sugar yield. Pretreatment in a cost effective manner is a major challenge in cellulose to ethanol technology.

Microwave radiation is a conduction heating and has proved to be a highly effective heating source in chemical reactions [Vani et al., 2012]. Advantages of microwave pretreatment include less energy requirements, selective processing and precise control, change the ultra-structure of

cellulose and degrade lignin and hemicelluloses [Xiong et al., 2000; Azuma et al., 1984]. The heating by microwave irradiation is volumetric and rapid since the heat is generated by rapid interaction between the electromagnetic field and components of the heated material. Microwave irradiation is a promising strategy for pretreatment of lignocellulosic biomass since it utilizes thermal and non-thermal effects generated by microwave in aqueous environments [Keshwani and Cheng, 2010]. The thermal and non-thermal effects of microwave cause fragmentation and swelling leading to degradation of hemicelluloses and lignin in the lignocellulosic biomass [Verma et al., 2011].

Several reports were available on microwave assisted pretreatment. This includes microwave assisted alkali pretreatment of cotton plant residue [Vani et al., 2012], microwave assisted acid pretreatment of sugarcane bagasse [Binod et al., 2012], microwave assisted H₂O₂ pretreatment of rice straw [Singh et al., 2013], microwave assisted ionic liquid pretreatment of kenaf core fiber [Ninomiya et al., 2014], microwave assisted dilute ammonia pretreatment of sorghum [Chen et al., 2012], microwave assisted FeCl₃ pretreatment of rice straw [Lu and Zhou, 2011a] and microwave assisted lime pretreatment of ramie decortications waste [Kurniasari et al., 2016].

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

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2. Materials and methods

2.1. Feed stock

Chili post-harvest residue (CPHR) received from Virudhanagar, Tamil Nadu, India was used in this study. The samples were dried and milled using a knife mill. Compositional analysis of native and pretreated samples was carried out by adopting NREL protocol [Sluiter et al., 2008].

2.2. Screening of various surfactants for microwave assisted surfactant pretreatment of chili post-harvest residue (MWASP CPHR)

Initial screening experiments were carried out with three different surfactants (Tween 20, Polyethylene Glycol (PEG) and Triton X 100 at an initial concentration of 1% w/w, biomass (solid) loading of 10% w/w, Microwave power of 500 W, Microwave incubation time of 2 min followed by pretreatment carried out in a laboratory autoclave at 121°C for 60 min. After pretreatment the samples were used for hydrolysis after washing and drying.

2.3. Optimization of various process parameters affecting microwave assisted surfactant pretreatment of chili post-harvest residue

Optimization of various process parameters affecting microwave assisted surfactant pretreatment of CPHR was carried out by adopting a Taguchi design. The experiment consists of a total of 16 runs. The details were presented in Table 1. The parameters selected were biomass (solid) loading, surfactant concentration, microwave power, microwave incubation time and pretreatment time. Parameters like biomass loading, surfactant concentration, microwave incubation time and pretreatment time were selected at four levels, while the microwave power was selected at two levels (400W and 550 W).

Table 1 Taguchi design for optimization of various process parameters affecting Microwave assisted surfactant pretreatment of chili post-harvest residue

Run No:	Surfactant Conc. (% w/w)	MW time (min)	Residence Time (min)	Biomass Loading (% w/w)	MW power (W)	Reducing Sugar Yield (g/g)
1	1	0	15	5	0	0.235
2	1	1	30	10	400	0.243
3	1	2	45	15	550	0.259
4	1	3	60	20	550	0.251
5	2	0	30	15	0	0.277
6	2	1	15	20	550	0.251
7	2	2	60	5	400	0.273
8	2	3	45	10	400	0.266
9	3	0	45	20	0	0.27
10	3	1	60	15	400	0.276
11	3	2	15	10	550	0.29
12	3	3	30	5	550	0.305
13	4	0	60	10	0	0.270
14	4	1	45	5	550	0.316
15	4	2	30	20	400	0.175
16	4	3	15	15	400	0.189

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 (Zytek India Ltd, Mumbai, India). The enzyme loading was 30 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 48 hours. After incubation the samples were centrifuged to remove the un-

hydrolyzed biomass. Reducing sugar analysis was carried out by 3, 5-dinitrosalicylic acid method [Miller, 1959].

2.6. Scanning electron microscopy

Scanning electron microscopic images (JEOL JSM-5600) of native and pretreated biomass were taken to evaluate the morphological differences between the native and pretreated biomass. The biomass samples were mounted on a double sided conductive tape on precut brass stubs and sputter coated with gold palladium using a JEOL JFC-1200 fine coater. The images were taken with a 10-15 kV accelerating voltage and magnification 500X.

2.7. Inhibitor analysis of the hydrolyzate

The hydrolyzate obtained after enzymatic saccharification of MWASP CPHR was centrifuged to remove the unhydrolyzed biomass 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.

2.8. Fermentation

2.8.1. For the production of bioethanol

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. It was inoculated with seed culture (2% v/v) of 18 hrs old *Saccharomyces cerevisiae* and incubated at 30°C for 72 hrs. 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).

2.8.2. For the production of biopolymer

Fermentation of non-detoxified hydrolyzate obtained after MWASP CPHR was used for biopolymer (poly-3-hydroxybutyrate) production using *Bacillus firmus* NII 0830. Fermentation was carried out with the hydrolyzate as such after adjusting to pH 7.0. The media was inoculated with 1% (v/v) of seed culture (3×10^6 CFU/ml) and incubated at 30°C for 72 hrs. After fermentation the samples were centrifuged and the biomass pellets were lyophilized. Poly-3-hydroxybutyrate (PHB) assay was carried out by the method of Law and Slepecky (1961).

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 contains 39.95% cellulose, 17.85% hemicelluloses and 25.32% lignin. Control 1 (water alone) contains 41.05% of cellulose, 16.79% of hemicelluloses and 24.11% of lignin. Control 2 (Microwave alone) contains 42.11% of cellulose, 16.41% of hemicelluloses and 23.15% of lignin. MWASP CPHR contains 45.63% of cellulose, 9.11% of hemicelluloses and 4.32% of lignin.

Mass balance analysis revealed a 33% loss of biomass during the pretreatment process. MWASP was found to be effective in removing hemicelluloses and lignin.

3.2. Screening profile of various surfactants for microwave assisted surfactant pretreatment of chili post-harvest residue

Three different surfactants –Tween 20, Triton X100 and PEG at 1% w/w were used for initial screening to select the best surfactant for MWASP of CPHR. The results were presented in Fig. 1. Control experiments were carried out with water alone, microwave alone and surfactants alone. Initial screening was carried out with 10% (w/w) of biomass (solid) loading, Microwave power of 550W, Microwave time of 2 min, surfactant concentration of 1% (w/w) and pretreatment time of 60 min in a laboratory autoclave at 121°C. Control samples were the pretreatment carried with water alone gave a reducing sugar yield of 0.05 g/g, with microwave alone gave a reducing sugar yield of 0.09 g/g. Control samples were the pretreatment was carried out with surfactants alone- PEG, Tween 20 and Triton X100 gave a reducing sugar yield of 0.11, 0.18 and 0.10 g/g respectively. MW assisted Tween 20, MW assisted PEG and MW assisted Triton X100 gave a reducing sugar yield of 0.27, 0.24 and 0.21 g/g, respectively. MWASP CPHR gave a better reducing sugar yield when compared to microwave pretreated alone or surfactant pretreated alone

samples. Since MW assisted Tween 20 gave higher reducing sugar yield it was selected for further optimization of different process parameters

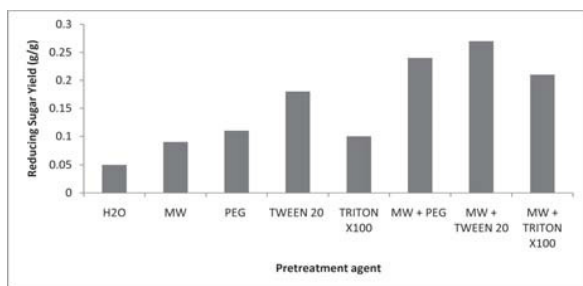


Fig.1. Screening profile of various surfactants on MWASP CPHR

affecting MWASP of CPHR by adopting a Taguchi design. The positive effects of Tween on pretreatment have been earlier reported for pretreatment of corn stover and sugarcane tops [Qing et al., 2010; Sindhu et al., 2013a]. Surfactant pretreatment improves lignin solubility and also prevents unproductive binding of enzyme on biomass surface [Sindhu et al., 2013a].

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

The results were presented in Table 1. Maximum reducing sugar yield (0.316 g/g) was observed in Run No: 14 where the conditions of pretreatment were surfactant concentration of 4% (w/w), MW pretreatment time for 1 min, MW power of 550W, biomass loading of 5% (w/w) and pretreatment time for 45 min. Contour plots showing interactions between various process parameters affecting MWASP of CPHR were depicted in Figs. 2A-H.

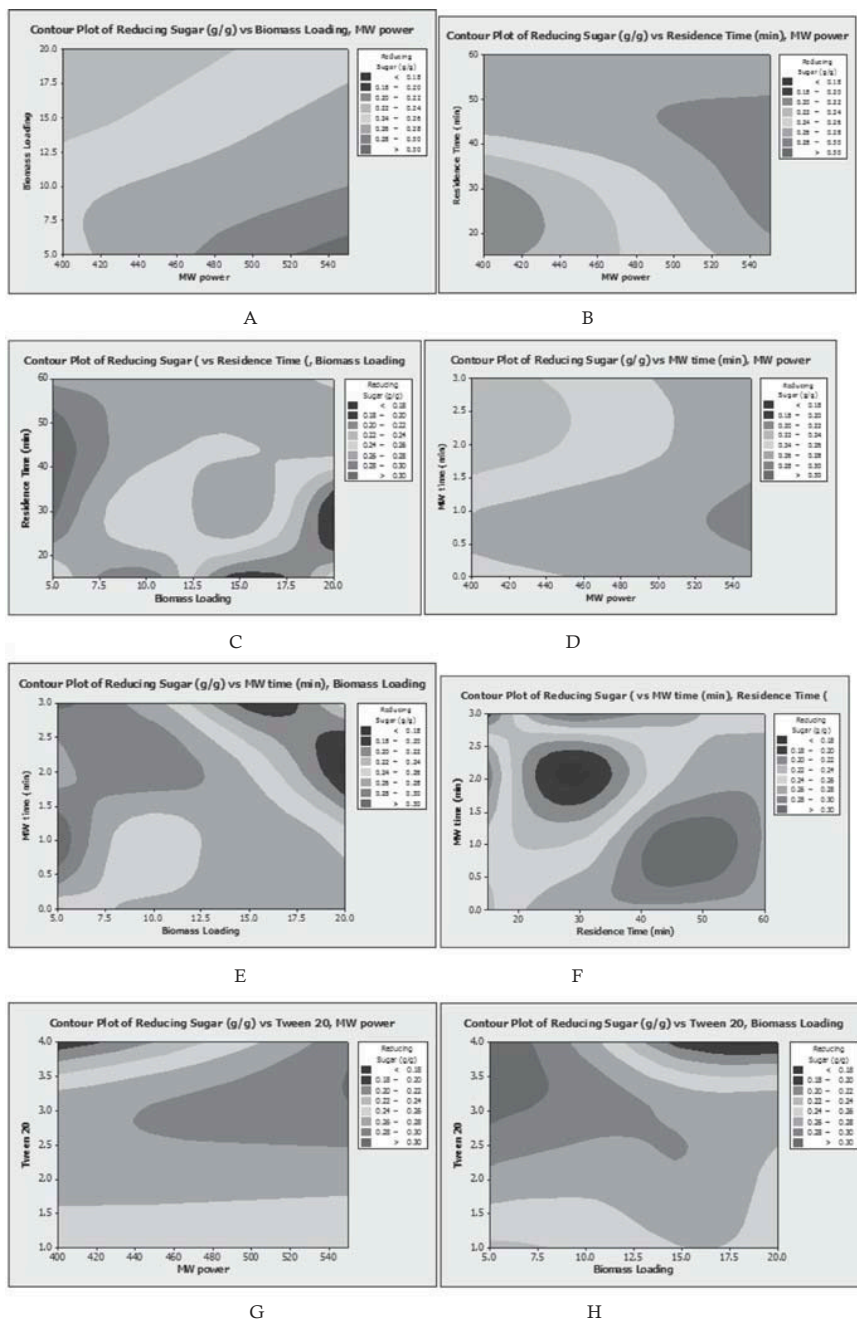


Fig. 2 A-F Contour plots showing interactions of various process parameters affecting MWASP CPHR (A) interactions between biomass loading and MW power (B) interactions between residence time and MW power (C) interactions between residence time and biomass loading (D) interactions between MW time and MW power (E) interactions between MW time and biomass loading (F) interactions between MW time and residence time (G) interactions between surfactant concentration and MW power and (H) interactions between surfactant concentration and biomass loading

An interaction between biomass loading and MW power is depicted in Fig. 2A. At low levels of biomass loading (5.0 – 7.5% w/w) the reducing sugar yield is high (0.30 g/g). It decreases with increase of biomass loading (10 – 20 % w/w). At low levels of MW power (400 – 460 W) the reducing sugar yield is low; it increases with increase of MW power. Maximum reducing sugar yield (0.30 g/g) was observed with low levels of biomass loading (5.0 – 7.5% w/w) and high levels of MW power (550W). Contrary observations were earlier reported by Vani et al., 2012 for alkali assisted MW pretreatment of cotton plant waste, where maximum reducing sugar yield was observed at 300 W for 6 min. An identical observation was earlier reported by Binod et al., 2012 for MW assisted alkali pretreatment of sugarcane bagasse where maximum reducing sugar yield was observed at high levels of MW power.

An interaction between MW power and residence time is depicted in Fig. 2B. At low levels of residence time (10 – 40 min) the reducing sugar yield is low (0.22 g/g); it increases with increase of residence time (40 – 60 min). At low levels of MW power (400 – 460 W) the reducing sugar yield is low (0.22 g/g); it increases with increase of MW power. Maximum reducing sugar yield (0.30 g/g) was observed at middle to high levels of incubation time (25 – 50 min) and high levels of MW power (550 W).

An interaction between residence time and biomass loading is depicted in Fig. 2C. At low levels of biomass loading the reducing sugar yield is high; it decreases with increase of biomass loading. At low levels of residence time (10 – 30 min) the reducing sugar yield is low (0.24 g/g), it increases with increase of residence time (30 -50 min). Maximum reducing sugar yield (0.3 g/g) was observed at low levels of biomass loading (7.5 – 10 % w/w) and middle to high levels of residence time (30 – 50 min). Adequate moisture in biomass is a key factor for successful pretreatment using microwaves. At high biomass loading charring occurs. Role of moisture for successful pretreatment of biomass was earlier reported by Puligunda et al., 2016.

An interaction between MW power and MW time is depicted in Fig. 2D. At low to middle levels of MW power (400 – 500 W) the reducing sugar yield is low (0.24 g/g); it increases with increase of MW power (520 – 540 W). At low levels of MW time (0.5 to 1.5 min) the reducing sugar yield is high (0.3 g/g), it decreased with increase of MW time (1.5 – 3.0 min). Maximum reducing sugar yield (0.3 g/g) was observed at high levels of MW power (520 – 540 W) and low levels of MW time (0.5 – 1.5 min). Microwave irradiation produces high power densities which improves saccharification efficiency. An identical observation was reported by Xu et al., 2011 where the microwave power positively affected glucose recovery from microwave assisted alkali pretreated wheat straw. Contrary observation was reported by Binod et al., 2012 for microwave assisted acid, microwave assisted alkali and microwave assisted acid-alkali pretreatment of sugarcane bagasse where an inverse relationship was observed between microwave power and reducing sugar yield.

An interaction between biomass loading and MW time is depicted in Fig. 2E. At low levels of biomass loading (5.0 % w/w) the reducing sugar yield is high (0.3 g/g); it decreases with increase of biomass loading (7.5 – 20 % w/w). At low to middle levels of MW time (0.5 – 1.5 min), the reducing sugar yield is high (0.3 g/g); it decreases with increase of MW time (1.5 – 3.0 min). Maximum reducing sugar yield (0.3 g/g) was observed with low levels of biomass loading (5% w/w) and low to middle levels of MW time (0.5 – 1.5 min). Biomass loading is an important factor affecting microwave pretreatment efficiency. Increase of substrate concentration will decrease the saccharification rate. An identical observation was earlier reported by Yang et al., 2004 for microwave pretreatment of rice straw where samples with high biomass loading showed lower straw digestibility. At high biomass loading the samples will have low water holding and will receive less energy adsorbed by the water due to oscillation of water molecules. Microwave irradiation time is one of the significant factors which affect the pretreatment severity. Kabel et al., 2007 reported that increase in MW pretreatment time can cause high temperature within the sample and may lead to decomposition of reducing sugar.

An interaction between MW time and residence time is depicted in Fig. 2F. At low to middle levels of residence time (10 – 35 min), the reducing sugar yield is low (0.24 g/g); it increases with increase of residence time (40 – 60 min). At low levels of MW time (0.5 – 1.5 min) the reducing sugar yield is high (0.3 g/g), it decreases with increase of MW time (1.5 -3.0 min). Maximum reducing sugar yield (0.3 g/g) was observed with high levels of residence time (40 – 60 min) and low levels of MW time (0.5 – 1.5 min). Contrary observations were earlier reported by Keshwani et al., (2007) for MW pretreatment of switch grass where maximum reducing sugar yield was observed at high levels of MW time (10 min).

An interaction between MW power and surfactant concentration (Tween 20) is depicted in Fig. 2G. At low to middle levels of MW power (400 – 440 W) the reducing sugar yield is low (0.24 g/g); it increases with increase of MW power (440 – 540 W). At low to middle levels of surfactant concentration (1.0 – 2.5% w/w) the reducing sugar yield is low (0.24 g/g), it increases with increase of surfactant concentration (2.5 – 3.5% w/w). An identical observation was earlier reported by Sindhu et al., 2013a for surfactant assisted ultrasound pretreatment of sugarcane tops where maximum reducing sugar yield was observed at high levels of surfactant concentration (5.0 – 6.0% w/w). Maximum reducing sugar yield (0.3 g/g) was observed at middle to high levels of MW power (440 – 540 W) and high levels of surfactant concentration (2.5 – 3.5% w/w).

An interaction between surfactant concentration (Tween 20) and biomass loading is depicted in Fig. 2H. At low levels of biomass loading (5.0 – 7.5 % w/w) the reducing sugar yield is high (0.3 g/g), it decreases with increase of biomass loading (7.5 – 20% w/w). At low levels of surfactant concentration (1.0 – 2.0% w/w) the reducing sugar yield is low (0.24 g/g); it increases with increase of surfactant concentration (2.5 – 4.0 % w/w). Maximum reducing sugar yield (0.3 g/g) was observed at high levels of surfactant concentration (2.5 – 4.0% w/w) and low levels of biomass loading (5.0 – 7.5% w/w).

The regression coefficient for reducing sugar yield was found to be best with MW power. The p value verifies the significance of each of the coefficients and identifies the pattern of interactions between the selected variables. In this model MW power is the significant factors. Other factor like surfactant concentration, MW time, residence time, biomass loading were found to be insignificant since the p value was greater than 0.06. p value less than 0.05 is found to be significant. The R² value explains the variability in the reducing sugar yield. The coefficient of determination (R²) was calculated as 98.20, indicating that the statistical model can explain 98.20% variability in response. The details were presented in Table 2.

Table 2: Analysis of variance

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Tween 20	3	0.0054213	0.0054213	0.0018071	9.87	0.093
MW time	3	0.0012253	0.0012253	0.0004084	2.23	0.324
Residence time	3	0.0032793	0.0032793	0.0010931	5.97	0.147
Biomass loading	3	0.0047208	0.0047207	0.0015736	8.59	0.106
MW power	1	0.0053290	0.0053290	0.0053290	29.10	0.033
Error	2	0.0003662	0.0003662	0.0001831		
Total	15	0.0203418				

S = 0.0135324

R-Sq = 98.20% R-Sq(adj) = 86.50%

For the validation of the model, three confirmation experiments were carried out within the range defined previously. The results were presented in Table 3. Correlation analyses were performed based on the predicted results and the experimental values. Correlation coefficient was found to be 0.962, indicating that the model developed is accurate.

3.4. Scanning electron microscopy

Scanning electron micrographs (SEM) of native and MWASP CPHR is presented in Fig. 3A-B. Morphological changes of native and pretreated biomass were evaluated by SEM. Native biomass showed a smooth, well ordered and compact structure while the MWASP CPHR showed an irregular and highly distorted structure. An identical observation was earlier reported by Sindhu et al., 2016 for sono-assisted acid pretreated CPHR.

3.5. Inhibitor profile of the hydrolyzate obtained after MWASP CPHR

Inhibitor profile of hydrolyzate obtained after enzymatic saccharification of control samples (microwave pretreated alone and surfactant pretreated alone) and MWASP CPHR were presented in Table 4. Major fermentation inhibitors like furfural, 5-hydroxymethylfurfural and organic acids like formic acid, citric acid, succinic acid and propionic acid were absent in control and pretreated samples. Acetic acid was present in control and MWASP CPHR hydrolyzate. Lu et al., 2011b reported that for microwave assisted pretreatment of biomass the inhibitor generation is minimum and is the most promising pretreatment method to change the native structure of cellulose with lignin and hemicelluloses degradation and thereby increasing enzymatic hydrolysis efficiency. An identical observation was reported by Pang et al., 2012 for steam explosion-microwave pretreatment of corn stover where acetic acid is present.

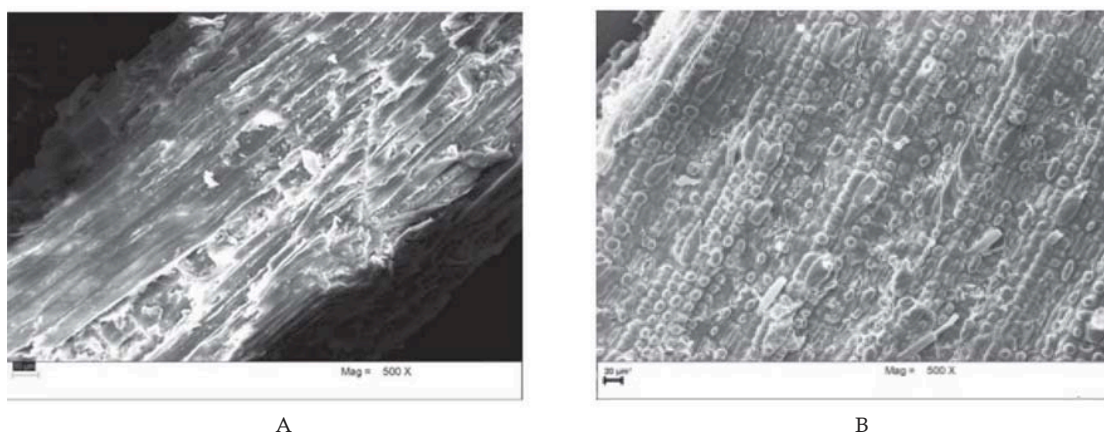


Fig. 3. Scanning electron micrographs of (A) native and (B) pretreated chili post-harvest residue

Table4: Inhibitor profile of hydrolyzate of control samples and microwave assisted surfactant pretreated CPHR

Inhibitor	Control 1 (Microwave alone)	Control 2 (Surfactant alone)	Microwave assisted surfactant pretreated CPHR
Furfural	ND	ND	ND
5-hydroxymethyl furfural	ND	ND	ND
Formic acid	ND	ND	ND
Acetic acid	0.115	0.019	0.211
Citric acid	ND	ND	ND
Succinic acid	ND	ND	ND
Propionic acid	ND	ND	ND

ND- Not detected

3.6. Fermentation for the production of bioethanol and biopolymer

Fermentation of the non-detoxified hydrolyzate obtained after enzymatic saccharification of MWASP CPHR with *Saccharomyces cerevisiae* yielded 1.66% of ethanol with a fermentation efficiency of 81.23% based on the theoretical ethanol yield from glucose. The yield can be improved by fine tuning of various process parameters affecting fermentation. An improved ethanol yield (2.14%) was observed by Sindhu et al., 2015 for fermentation of the sono-assisted acid pretreated hydrolyzate of CPHR with *Saccharomyces cerevisiae*.

Fermentation of the non-detoxified hydrolyzate obtained after MWASP CPHR was evaluated for biopolymer (poly-3-hydroxybutyrate) production by *Bacillus firmus* NII 0830. The strain produced 67.85% of poly-3-hydroxybutyrate (PHB). The results indicate that the hydrolyzate obtained after MWASP CPHR is suitable for the production of PHB. Till date only one report is available for PHB production from biomass hydrolyzate using *Bacillus firmus* NII 0830 [Sindhu et al., 2013]. The strain produced 89% of PHB from pentose rich hydrolyzate obtained after acid pretreatment of rice straw.

4. Conclusions

Compositional analysis data revealed that hemicelluloses and lignin were removed during MWASP of CPHR. The major fermentation inhibitors like furfural, 5-hydroxymethylfurfural and organic acids like citric acid, succinic acid, formic acid and propionic acid were absent. Absence of inhibitors will eliminate the detoxification step. To the best of our knowledge, this is the first report on MWASP of CPHR. Fermentation of the non-detoxified hydrolyzate yielded 1.66% of ethanol and 67.85% of PHB. Fine tuning of various process parameters will make the process economically viable.

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