Process Optimization for Recovery of Reducing Sugar from Coconut Pith Using Sequential Hydrothermal Pretreatment and Enzymatic Saccharification

Marttin Paulraj Gundupalli, Nishanth Senthilkumar, and Debraj Bhattacharyya

***Abstract*—In this study, pith was hydrothermally pretreated using high pressure batch reactor under different conditions of time and temperature a) case I (2-10 min: 160-220^{o}C); b) case II (10-60 min: 100-160^{o}C). The enzymes used for this study were cellulase (97 FPU/ml) and β – glucosidase (5.1 CBU/mg). The hydrothermal pretreatment process was optimized using Response Surface Methodology (RSM). The solid residues collected after pretreatment were studied for morphological changes using XRD and SEM image evaluation. During the pretreatment step, the reducing sugar recovered was 14% for case I and 4% for case II. During the saccharification process, 43% and 16% of reducing sugar yield was observed for case I and case II, respectively. Total reducing sugar recovery (pretreatment and saccharification) of 57% (13.68 mg/g of dry pith biomass) was observed for pith pretreated under optimum condition for case I, which was higher the case II (20%).**

***Index Terms*—Pretreatment, response surface methodology reducing sugar, saccharification.**

I. Introduction

Environmental pollution, climate change and renewable energy, are the primary areas of research nowadays. Increase in consumption of energy in the form of fuel has led to consequent depletion of fossil fuel reserve [1]. It is essential to find a suitable replacement for fossil fuels. Lignocellulosic biomass with abundant sugars (cellulose and hemicellulose) has the potential to serve as the alternative renewable energy source. However, fuel production from lignocellulosic biomass is still under development [2]. This study was carried out by using coconut pith (lignocellulosic biomass) as the source for recovery of reducing sugar. The annual production of coconut pith in India is 7.5 million tons of which not all are utilized as per the reports [3]. The other motive for this study was to reduce the management and accumulation of coconut pith in coconut industries. It is believed that coconut pith causes two major problems (a) occupation of land space and (b) contamination of groundwater table with phenolic compounds due to leaching [4].

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The above challenge is overcome by converting pith with a value-added by product, for example, bioethanol. To achieve this, pretreatment has to be carried out to break the linkage bonds between lignin and increase cellulose accessibility during saccharification process. This work emphasizes on understanding the effect of time and temperature on coconut pith during hydrothermal pretreatment. Though there are different pretreatment methods, hot water pretreatment is an eco-friendly method and has the advantage of lower sugar degradation to other byproducts [5]. Other pretreatment methods, at higher temperature and pressure tend sugar to degrade to furfural and hydroxymethylfurfural [6]. These furan compounds inhibit the yeast metabolism during the fermentation process [7]. This work was carried out for the following two cases, a) lower reaction time versus high temperature, and b) higher reaction time versus lower temperature.

For the case I, the reaction time and temperature were varied from 2-10 min & 160-220^{o}C similar to condition reported [8], [9]. For the second case (Y_{caseII}), the reaction time and temperature were changed from 10-60 min: 100-160^{o}C. Based on Response surface methodology (RSM), an optimal point of time and temperature for both the cases were determined. RSM is a collection of statistical techniques that are used for developing and optimizing the process parameters [10]. After the pretreatment process, enzymatic saccharification was done for the pretreated solid using cellulase and β – glucosidase. The saccharification yield, (%) was determined for the pretreated solids with high recovery of reducing sugar during pretreatment.

II. Materials and Methods

A. Collection and Sample Preparation

The coconut pith was collected from a coconut processing industry (Anu coconut Industries Private Limited) located at the region of Nagole, Hyderabad, Telangana. The obtained sample was analyzed for moisture content by drying in the hot air oven at 105^{o}C for 24h [11]. The sample was mechanically reduced to a particle size (< 1 mm) using the food processor. The reduced sample was stored in airtight containers until further use.

B. Compositional Characterization of Coconut Pith

Coconut pith sample was characterized for sugars and lignin by following the NREL method [12]. The solid residue was analyzed for insoluble lignin and the liquid collected after vacuum filtration was analyzed for soluble lignin and total carbohydrates. The total monosaccharides in liquid

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hydrolysate were analyzed following the phenol-sulphuric acid method [13]. The contents of hemicellulose, cellulose, and lignin were determined to be 0.6 %, 24.2%, and 45 % respectively. The liquid hydrolysate obtained after pretreatment was analyzed for reducing the sugar by following the dinitrosalicylic acid method [14]. All analytical measurements were carried out in a UV Visible spectrophotometer (Make: Labindia, model: 3000 plus).

C. Design of Experiments

For the optimization of time and temperature in both the cases, Central Composite Design (CCD) was applied during the hydrothermal pretreatment [15]. Though the full factorial design is preferred for two factors, CCD was done to generate a second order model for the hydrothermal pretreatment that includes a full factorial run, central runs and axial runs (±α). The levels for independent variables were represented as uncoded and coded values which are summarized in Table. I. These values were derived from the expression as follows

$$X_{coded} = \frac{X_{uncoded} - X_0}{\frac{X_{+1} - X_{-1}}{2}} X_{uncoded} \qquad (1)$$

where, X_{coded} represents the coded value of the independent variable; $X_{uncoded}$ represents the real value of the independent variable; X_0 is the real value at the center level; X_{-1} is the real value at the lower level, and X_{+1} is the real value at the higher level [10]. All statistical analyses were done using Statistica 7.0. A second order polynomial model expressing yield as a function of the independent variables was used which is described below

$$Y = \beta_o + \sum_{j=1}^{k} \beta_j x_j + \sum_{j=1}^{k} \beta_{jj} x_j^2 + \sum\sum_{i<j=2}^{k} \beta_{ij} x_i x_j \quad (2)$$

where Y is the dependent variable, x_i and x_j are the independent variables, and β_0, β_i, β_{ii}, and β_{ij} are the model coefficients that were obtained using the least-squares method [16]. In this study, for hydrothermal pretreatment, two independent variables, temperature, (X_1, °C) and time, (X_2, min) were considered. Recovery of reducing sugar, (%)(Y_{caseI} & Y_{caseII}) in % was selected as the response for both the cases. Each run was carried out based on the CCD matrix summarized in Table II. For each individual run, the reducing sugar was determined and recorded.

The model significance test was checked by determining the coefficient of determination R^2 and adjusted R^2 [10], [17]. The adjusted R^2 is defined for the principal effect terms, and multiple R^2 is determined for main, interaction and quadratic terms. For a proper fit model, the coefficient of determination (R^2) should have a minimum value of 0.80 [18], and adjusted R^2 should be at least 0.70. Further, to check the adequacy of the model, the insignificance of lack of fit must be obtained for the second order model.

TABLE I: CODED AND UNCODED VALUES FOR CCD DESIGN

Independent variable		**Coded values**				
		-α	-1	0	+1	+α
Case I						
Temperature °C	X_1	160	172	200	228	240
Time, min	X_2	2	3.2	6	8.8	10
Case II						
Temperature °C	X_1	100	109	130	152	160
Time, min	X_2	10	17	35	53	60

TABLE II: DESIGN OF CCD FOR THE INDEPENDENT VARIABLES ON RECOVERY OF REDUCING SUGAR

Run	Experimental factors					
	X_1, °C	X_2, min	$Y_{caseI,\%}$	X_1, °C	X_2, min	$Y_{caseII,\%}$
1	172	3	1	109	17	2
2	172	9	11	109	53	3
3	228	3	4	151	17	3
4	228	9	5	151	53	3
5	160	6	7	100	35	2
6	240	6	2	160	35	3
7	200	2	2	130	10	2
8	200	10	14	130	60	4
9	200	6	13	130	35	6
10	200	6	14	130	35	4
11	200	6	13	130	35	4
12	200	6	14	130	35	5
13	200	6	10	130	35	4
14	200	6	13	130	35	5

D. Hydrothermal Pretreatment of Coconut Pith

The hydrothermal pretreatment of coconut pith was carried out by using a high-pressure batch reactor (HPBR) as shown in Fig. 1. A coconut pith sample of 1:10 w/w was added to the reactor and operated at a different time and temperature as summarized in Table II. The HPBR (jacketed vessel - SS 316) is equipped with pressure gauge, safety values, and chiller. The liquid sample is collected from the dip tube provided internally to the HPBR. After pretreatment, on the release of pressure, the liquid hydrolysate passes through the dip tube leaving the solid residue within the reactor. The collected liquid is subjected to determination of reducing sugar, and the solid residue was dried in a hot air oven.

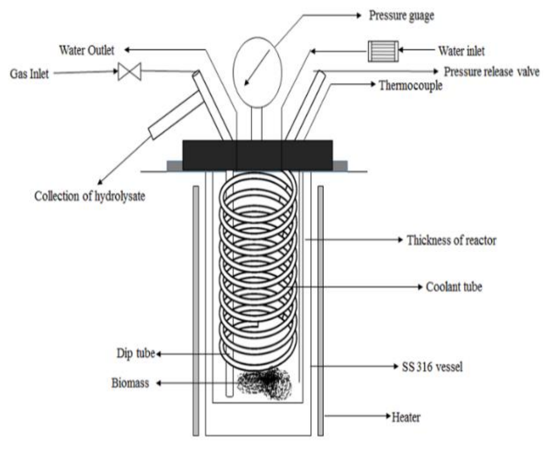


Fig. 1. High pressure Batch Reactor (HPBR).

E. Structural Characterization of Untreated and Treated Pith

The crystallinity index was determined from the XRD pattern (model:Xpert Pro, PW3040/60. make: PANlytical, Germany). The XRD equipment was equipped with a sealed tube Cu Kα source, diffracted beam PreFIX carrier, and line detector. Scans were collected from 2θ = 5 – 80° with the step size of 0.0167° at 4s per step. The percent of crystallinity was estimated for the untreated and treated pith from the expression.

$$CrI = \frac{I_{cryz} - I_{am}}{I_{cryz}} \times 100 \quad (3)$$

where I_{cryz} is the total scattered intensity of crystalline cellulose; I_{am} is the scattered intensity due to amorphous cellulose [19]. The crystallinity index of untreated and treated pith is compared and later studied. For SEM image evaluation, the samples were dried and coated with gold-palladium alloy using a sputtering machine (Model: SC7620 Mini sputter coater; Make: Quorum Technologies, UK). Sample imaging was carried out using scanning electron microscope (Model: ZEISS EVO18; Make: ZEISS, USA).

F. Enzymatic Saccharification of Treated Pith

Enzymatic saccharification studies for untreated and treated pith were performed using commercially available cellulase, 97 (FPU/ml) and β – glucosidase, (5.1 CBU/mg) enzyme. To a 100 ml Erlenmeyer conical flask, 100 mg/ml of pith was added. The solid and liquid ratio was maintained as 1:10 (w/v). The enzymatic mixture of cellulase and β – glucosidase ratio was 2:1.

To the conical flask 20 FPU/ml of cellulase and 10 CBU/mg of β – glucosidase was added with 0.05 M citrate buffer (pH 4.8) [20]. To the mixture 0.3 % sodium azide and 0.2 % Tween 80 was added. Biomass and enzyme mixture was incubated at 50°C with continuous mixing at 150 RPM. The samples were collected at different intervals of time (24h, 48h, 72h, 96h, & 120h) to determine the saccharification yield %. The yield, (%) was determined from the following expression

$$Yield, \% = \frac{Reducing\ sugar, mg/ml\ \times 0.9\ \times\ 100}{Carbohydrate\ in\ biomass, mg/ml}$$

III. Results and Discussion

G. Response Surface Methodology – Model development

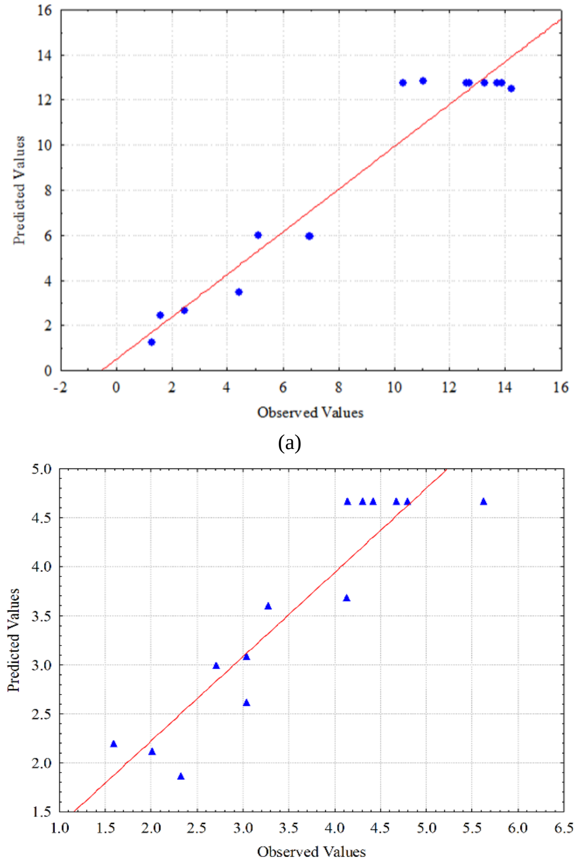
According to the results based on Table II for both cases, the relationship between the response (Y) and independent variables were:

$$Y_{CASE_I} = -243.607 + 2.240X_1 - 0.00527X_1^2 + 10.869X_2 - 0.329X_2^2 - 0.028X_1X_2 \quad (4)$$

$$Y_{CASE_{II}} = -41.07 + 0.616X_1 - 0.0022X_1^2 + 0.235X_2 - 0.0027X_2^2 - 0.0004X_1X_2 \quad (5)$$

In the significance test, with p-value < 0.05, the model for both the cases is considered significant. To validate the model coefficient of determination, the predicted and the observed values were plotted and shown in Fig. 2 (a) and Fig. 2 (b).

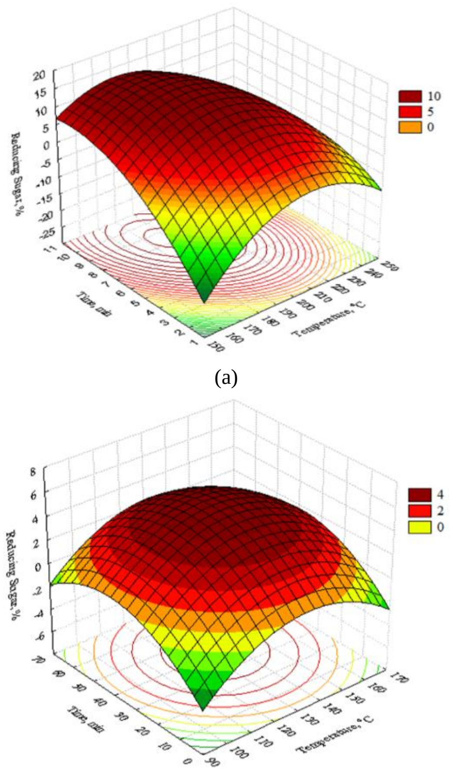
The multi-regression analysis for both the cases with response Y_{caseI} and Y_{caseII} revealed that the R^2 and adjusted R^2 were 0.83, 0.72 and 0.86, 0.77 respectively. Further, the p-value > 0.05 for the lack of fit for both models signifies that the model is fit and the errors are normally distributed [21]. The model obtained can be used to predict the reducing sugar from pith for hydrothermal pretreatment. To understand the effect of independent variables on recovery of reducing sugar, the three dimensional contour plots were developed. The three dimensional plots for Y_{caseI} and Y_{caseII} are shown in Fig. 3.



(a)

(b)

Fig. 2. Predicted versus observed values (a – Y_{caseI}: b – Y_{caseII}) for hydrothermal pretreatment of coconut pith.



(a)

(b)

Fig. 3. Surface and contour plots for responses Y_{caseI} (a) and Y_{caseII} (b).

Considering the case I, in the Fig. 3 (a), the influence of time and temperature on recovery of reducing sugar is seen. The reducing sugar increased as the temperature increased initially predicting a maximum recovery of 10 %. However as the temperature increases, further the recovery was observed to decrease with time.

The decrease of the reducing sugar is probably due to the degradation of sugars at higher temperature [22]. In case II, Fig. 3 (b), the effect of reaction time and temperature was clearer. A maximum of 4 % reducing sugar recovery was predicted due to the interaction of time and temperature. The ANOVA for Y_{caseI} and Y_{caseII} is shown in Table III and Table IV

TABLE III: ANOVA AND MODEL SIGNIFICANCE FOR Y_{CASEI}

Source	SS	df	MS	F-value	P - value
X_1	10.5	1	10.5	6.3	0.054
X_{11}	131	1	131.6	78.3	0.000
X_2	100.4	1	100.4	59.7	0.001
X_{22}	51.2	1	51.2	30.4	0.003
X_{12}	20.6	1	20.6	12.2	0.017
Lack of fit	9.8	3	3.3	1.9	0.241
Pure error	8.4	5	1.7		
Total SS	320.8	13			

SS - sum of squares; MS – mean sum of squares; df - degrees of freedom

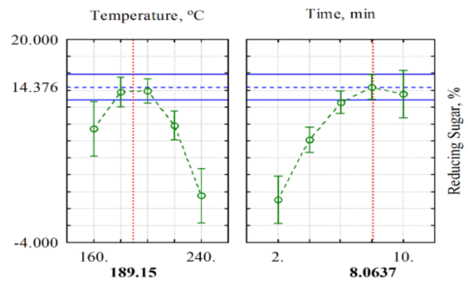
TABLE IV: ANOVA AND MODEL SIGNIFICANCE FOR Y_{CASEII}

Source	SS	df	MS	F-value	P - value
X_1	0.94	1	0.936	3.323	0.128
X_{11}	7.88	1	7.877	27.96	0.003
X_2	2.22	1	2.218	7.873	0.038
X_{22}	5.53	1	5.529	19.62	0.007
X_{12}	0.005	1	0.005	0.017	0.900
Lack of fit	1.15	3	0.384	1.363	0.355
Pure error	1.41	5	0.282		
Total SS	18.18	13			

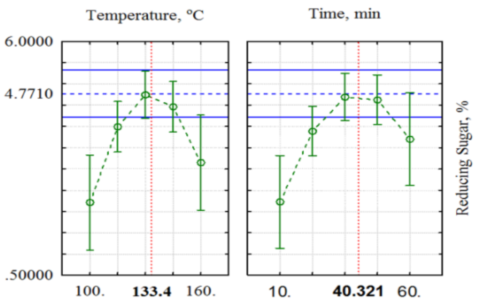
SS - sum of squares; MS – mean sum of squares; df - degrees of freedom

H. Optimization and Validation of the Model

The second order model for the responses were validated. The optimal points for Y_{caseI} and Y_{caseII} using Response Surface Methodology was determined and shown in Fig. 4.



(a)



(b)

Fig. 4. Optimal points validated using Response surface Methodology for Y_{caseI} (a) and Y_{caseII} (b).

These optimal points were experimentally verified in triplicates. The values obtained for both the cases were similar to the predicted values. From the result, it is concluded that case I is highly preferred due to its maximum reducing sugar recovery of 14.2 %. However, further study of enzymatic saccharification was done for both the cases to observed maximum saccharification yield.

Though reducing sugar recovery was less for case II, pretreatment can change the morphology of pith, thereby, enhancing the yield of sugar during saccharification. The morphological changes were examined for the residue collected after pretreatment. Considering the XRD pattern as shown in Fig. 5, the difference in crystallinity was observed for the samples.

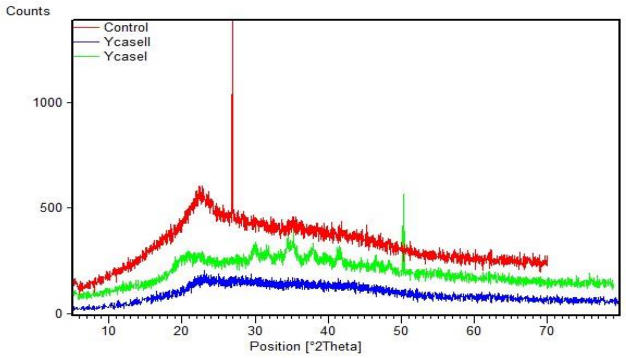


Fig. 5. XRD pattern studied for control, Case I and Case II to determine the changes in crystalline property after hydrothermal pretreatment.

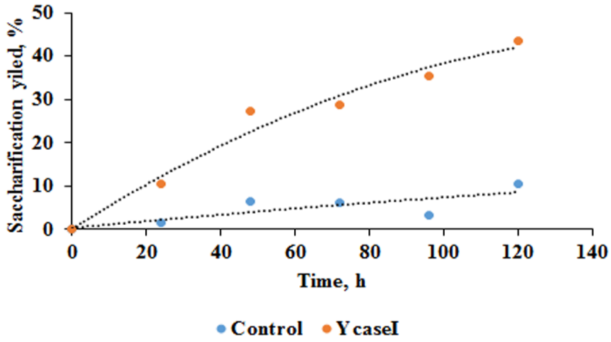
The crystallinity indices for all three samples has been shown in Table V. For case I there is a 20% reduction in crystallinity index whereas, for case II, only a 4% reduction in crystallinity index was observed. The crystallinity index for case I is lower compared to control and case II. The possibility of reducing sugar yield during enzymatic saccharification can be higher for case I compared to case II. The crystallinity index for control and case II were almost similar, thereby showing that case II conditions are having a lower effect on pith. Therefore the modification of structure was observed more for case I compare to case II. The solid residue for both the states was subjected to enzymatic saccharification.

TABLE V: CRYSTALLINITY INDEX FOR UNTREATED AND TREATED PITH (CASE I AND CASE II)

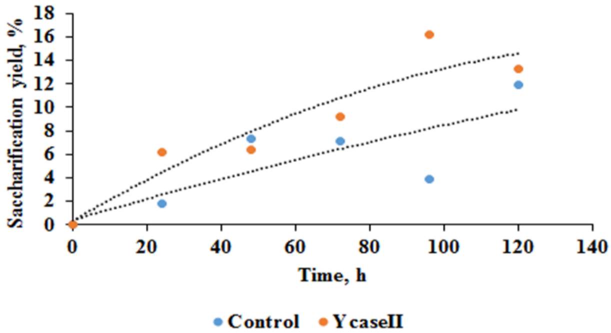
S. No	Sample	CrI
1	Control	36.6
2	Case I	29.5
3	Case II	35.1

I. Enzymatic Saccharification

Enzymatic saccharification was done for the residue acquired after the pretreatment step for both the cases. By using commercial enzymes of cellulase and β – glucosidase, the saccharification was done. The saccharification step was carried out for both untreated (control) and treated pith (case I and case II). The yield for both the cases from 24h – 120h was plotted in Fig. 6. The reducing sugar varied from 10 – 43 % for case I and 6 – 16 % for case II. For untreated pith (control), the yield of reducing sugar was upto 11 %. From the results, it is inferred that the reducing sugar yield after saccharification was higher in case I compared to case II.



(a)



(b)

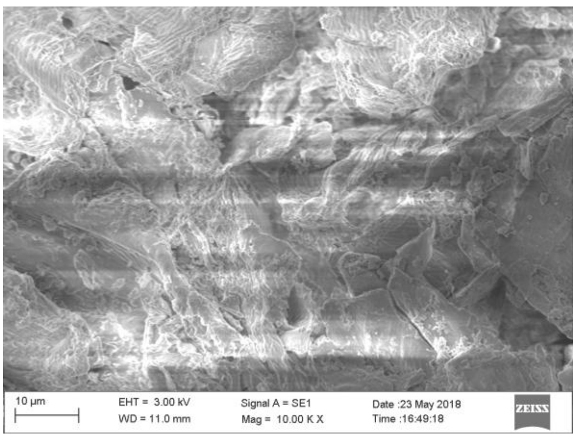
Fig. 6. Enzymatic saccharification yield for Case I (a) and Case II (b).

It is observed for both cases, that the yield increases as the time interval increases. However, the maximum yield of 43 % was attained for solid residue (case I) compared to the solid residue (case II) with yield <15%.

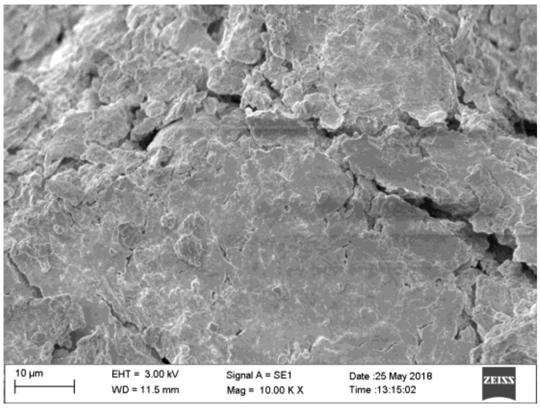
J. SEM Image Evaluation

For control and case I, the SEM image was studied based on the results previously concluded. The SEM image evaluation was done based on the structural alteration of the pith before and after pretreatment.

The SEM images are shown in 10X magnification for better observance and understanding. It is evident from the Fig. 7 that, the morphology of pith is altered compared to that of control sample. For control pith, the surface appears to be smooth without any cracks. On the other hand, for pith pretreated under case I conditions, cracks were noticed on the surface of the pith. This explains, the cause for higher yield of reducing sugar during enzymatic saccharification compared to the control sample.



(a)



(b)

Fig. 7. SEM image evaluation: (a) – Control; (b) - Case I Pith.

IV. Conclusion

Hydrothermal pretreatment under two different condition was studied using High-Pressure Batch Reactor. From this study it is concluded

- A maximum reducing sugar of 14% was recovered for pith pretreated under conditions case I.
- The morphological characteristics were studied for both the solid residues through XRD pattern and SEM image evaluation.
- The XRD pattern showed the crystallinity index reduced by around 20% for case I compared to only a 4% reduction in case II.
- After pretreatment step, the solid residues were subjected to enzymatic hydrolysis using commercial enzymes.
- During the enzymatic saccharification process, the maximum reducing sugar yield of 43 % was observed for case I.
- In this study, it is concluded that pretreatment of pith using case I conditions is considered effective compared to case II conditions.

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Nishanth is having work experience in bioenergy field and wastewater treatment. His Internships program include 1. Summer Research Intern at Indian Institute of Technology, Hyderabad. 2. Intern student at URC Constructions Pvt. Limited, 3. Engineering Intern at IITB-KILA summer school for Canal Rejuvenation in Alappuzha. Current research interests includes solid waste management, bioenergy and biomass conversion and wastewater treatment. Mr. Nishanth Senthilkumar has been an active participant in workshops and research.

Debraj Bhattacharyya was born in Kolkata, India, on 3rd July 1976. He did his schooling from Kolkata. He completed his bachelor of engineering in civil engineering from Bengal Engineering College (presently Indian Institute of Engineering Science and Technology Shibpur), West Bengal, India, in the year 1999. He obtained master of technology in civil engineering, with specialization in environmental engineering, from Indian Institute of Technology Roorkee, India, in the year 2002. He received doctor of philosophy from the University of New Brunswick, Canada, in 2009. Dr. Debraj's Ph.D research was on wastewater treatment.

Dr. Debraj worked as a postdoctoral research fellow in the Department of Civil Engineering at the University of New Brunswick, Canada, after completion of his Ph.D. He joined as an assistant professor in the Department of Civil Engineering at the National Institute of Technology Calicut, Kerala, India, in 2010. He moved to Indian Institute of Technology Hyderabad, India, in 2012. Currently he is serving as an associate professor in the Department of Civil Engineering at Indian Institute of Technology Hyderabad. Dr. Debraj has presented several papers in peer reviewed conferences and published papers in several peer reviewed journals in the area of wastewater treatment and renewable energy.

His earlier research was on biological wastewater treatment using aerobic and anaerobic bacteria. Nowadays, he is focusing on electrocoagulation treatment of industrial wastewater, domestic wastewater treatment using microalgae, and pretreatment of lignocellulosic biomass for subsequent production of biofuel (ethanol and butanol).

Dr. Bhattacharyya coordinates the examination for postgraduate (M. Tech.) admission in his institute. He is also a member of several committees which look after the administrative duties of the institute. He has also served as a task force member of the state pollution control board in India. He is a regular reviewer of papers of reputed journals in the field of Environmental Engineering.