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Bifurcation of lignocellulosic biomass (*Areca catechu*) using alkaline pretreatment: An efficient method.

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AGENDA:-

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- Introduction
- Methodology
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MOTIVATION

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- Lignocellulosic residues : major environmental liabilities in the agricultural sector.
- Conversion of agro-residues to bioenergy or value added products
- Recalcitrant nature of the biomass should be reduced.
- Pretreatment finds a way of its applications to reduce the recalcitrance.
- Lignin forms the main group causing the hindrance.
- Disposed on open lands causing nuisances by spreading diseases and pest growth due to their slow deterioration

Introduction

- ❖ Agriwastes - immense biomass potential
- ❖ “Lignocellulosic biomass”
- ❖ Second generation biofuels (SGB)
- ❖ Value- added products

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Biomass resource categorization

Biomass can be categorized broadly as follows.

- Woody biomass
  - ➢ Consists of forests, agro-industrial plantations and trees
  - ➢ Wood, bark, branches, leaves, stalk and twigs of Acacia, Eucalyptus, Shisham, Teak, Neem, Conifers.
  - ➢ Have high lignin content.
- Non-woody biomass
  - ➢ comprises crop residues like stalk, straw, husk, pod, cobs, shell and leaves of various crops like wheat, cotton, rice, coconut, arecanut, etc.
  - ➢ Processing residues like saw dust, bagasse and domestic wastes
  - ➢ Have moderate lignin content.

Arecanut husk (*Areca catechu*)

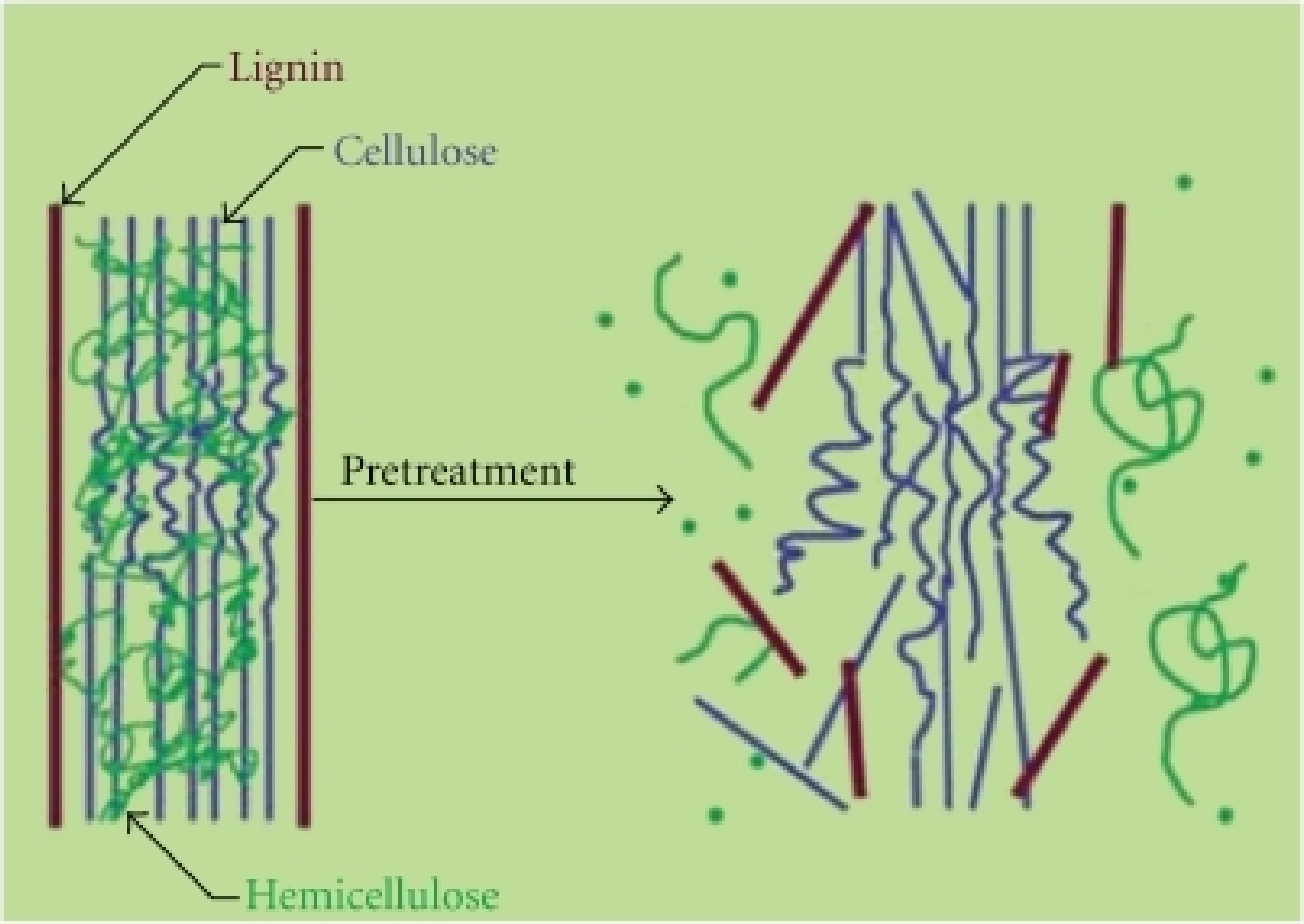
Arecanut and its husk

- India has a large leading production of arecanut husk, AH (*Areca catechu*) (40%- 50%) and China comes the next (Singh et al., 2017)

- Botanical nomenclature
  - Class: *Liliopsida*
  - Family: *Arecaceae*
  - Genus: *Areca*
  - Scientific name: *Areca catechu*
- During the extraction of arecanut from the arecanut crop, it was observed and measured that 100 kg of arecanut yields 70kg of residue (arecanut husk).
- Areca husk left unnoticed in the plantation causes bad odour and other decay related issues
- Creates environmental problems - burning, fire, termite attack, leaching phenols from heaped leaf wastes and proliferation of pests and diseases.
- At present majority of arecanut waste is disposed of by burning which resulted into a loss of potential source of organic matter and valuable plant nutrients. (Nagaraja et al., 2014)

Pretreatment of lignocellulosic biomass

- Pretreatment such as physical, chemical, biological, enzymatic, thermal and their combinations on various lignocellulosic biomass - to overcome the recalcitrance through structural and chemical changes during hydrolysis.
- Physical pretreatment: mechanical (milling and grinding), hydrothermal (liquid or gaseous), irradiation and extrusion
- Chemical pretreatment: alkaline, dilute acid, organosolv, oxidizing agents, etc.
- Biological pretreatment - bacterial and fungal action to rupture the rigid lignocellulosic cell wall.
- Biological pretreatment: low cost, inhibition free and environmental friendly but time consuming process.
- Now more researches are interested towards the combination of various pretreatments, i.e., physicochemical, thermochemical, etc.



Schematic diagram biomass composition of agricultural waste (Ramakrishnana et al. 2013)

Materials & methods

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- The dehusked arecanut husk (Central Plantation Crops Research Institute (CPCRI) Kasargod, Kerala, India): washed, cleaned, separated the fibres and size reduced to 0.2-0.5 cm.
- Alkaline pretreatment :Sodium hydroxide (NaOH- 97%) Sigma-Aldrich product was used.
- Batch pretreatment studies
  - Extractive free arecanut husk samples were considered
  - Check the delignification and the total reduced sugars (TRS) which helps to bifurcate into lignin and lignin free biomass
  - Parameters considered: dosage of alkali used (%), solids loading and soaking time (hrs).
  - To 1 g of arecanut husk, targeted concentrations range from 2- 10% (w/v) of alkaline solution (sodium hydroxide) were added. The solids loading were also varied as 1:25- 1: 100 and the mixture is incubated at 35^{0}C for soaking periods (12hr-48 hr) at 150 rpm.
- A sequence of batch studies was performed to find the efficacy of the pretreatment process with respect to two responses includes delignification and TRS using response surface methodology (RSM).
- In this research, a set of 17 experiments were executed as per the layout is given by three variable Box-Behnken Design (BBD) approach.

Characteristics of arecanut husk

Table 1 Arecanut husk characteristics

Sl. No.	Parameter	Method of analysis
1.	➢ Proximate analysis ▪ TS (%) ▪ Moisture content (%) ▪ VS (% of TS) ▪ Fixed content (% of TS)	APHA standard method (1999) • Take known quantity of sample as initial weight. • TS & moisture content: Oven dry method at 105 °C for 12 hrs. • VS & fixed: Ignite in muffle furnace at 550°C for 2 hrs. • Difference in initial and final weights of sample.
2.	➢ Ultimate analysis ▪ Carbon, Hydrogen, Nitrogen, Sulphur, Oxygen (CHNSO)	• Elemental analyser
3.	➢ Cellulose ➢ Hemicellulose ➢ Lignin	• Cellulose and hemicellulose by Tappi method • NREL procedure for acid soluble and acid insoluble lignin
4.	Total organic carbon	• Loss of ignition method (LOI)
5.	Reduced sugar	• DNS method

Optimization of parameters using BBD

- To optimize the selected factors such as pretreatment dosage, solids loading and soaking time for maximizing the delignification efficiency and TRS content in the residues after pretreatment.
- This design is best suited for the generation of the polynomial model of second degree through quadratic response surfaces.
- A Box-Behnken Design (BBD) developed by Design Expert 10.0.3 with three level and three factors
- The levels of each factor and their range were based on the preliminary experiments, and it includes three levels as shown in Table 1 given below

Table 2 Levels of input parameters considered for BBD

Name	Units	Type	Low (-1)	Central (0)	High (+1)
Pretreatment dosage	%	Factor	2	6	10
Solids loading		Factor	1:25	1:40	1:100
Soaking time	hrs	Factor	12	30	48

- The significance of the independent variable interactions can be studied from the ANOVA (Analysis of Variance) table (Kumar and Phanikumar, 2013).
- The experimental data was allowed to fit one among the various models such as linear, 2FI, Quadratic and Cubic.
- The model fitness was based on the highest score gained in the sum of squares.
- The significance of the model was determined by the larger F-value (Fischer) and smaller p-value (< 0.0001).
- The correlation coefficient (R^2) value give the fitness of the model.
- Surface plots of both 2-D and 3-D are drawn which shows trends in response surface with the input process parameters (M Manohar, Jomy Joseph, 2013).

Results and discussion

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Characterization of AH

- The TS, VS, moisture content and ash content in AH was found to be 88.09%, 97.22%, 11.91% and 2.78% respectively
- Due to the variation in the water content, a slight change in the values can be observed for the dry, ripe and raw husk (Julie Chandra et al., 2016; Nagaraja et al., 2014; Sadasivuni et al., 2016).

Table 3 Ultimate analysis of Arecanut husk

Chemical component	Value
C	45.52±0.13%
H	6.31±0.10%
N	0.36±0.16%
S	0.00
O	47.81±0.07%

Table 4 TOC and Biomass compositional characteristics of the AH

Parameter	Value
TOC	54.64%
Total extractives	2.156%
Cellulose	45.02%
Hemicellulose	28.25%
Lignin	22.47%
Ash content	2.1%

Table 5 BBD for the pretreatment variable and their responses

	Factor 1	Factor 2	Factor 3	Response 1	Response 2
Run	A: Pretreatment dosage	B: Solids loading	C: Soaking time	Delignification efficiency	Total Reduced Sugars
	%		hrs	%	mg/mL
1	6	1:40	30	64.36	20.23
2	2	1:40	12	26.78	8.78
3	10	1:40	48	38.11	11.67
4	2	1:100	30	46.44	9.77
5	10	1:100	30	69.07	10.14
6	2	1:25	30	59.55	9.34
7	6	1:25	12	65.08	18.54
8	10	1:25	30	61.46	9.25
9	6	1:40	30	60.12	21.07
10	6	1:25	48	55.41	16.57
11	2	0.025	48	42.63	5.84
12	6	0.025	30	57.34	20.92
13	6	0.01	12	57.39	20.58
14	6	0.025	30	58.33	21.71
15	6	0.025	30	61.28	20.84
16	10	0.025	12	57.07	12.5
17	6	0.01	48	44.85	19.52

Table 6 Analysis of delignification efficiency & TRS

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	Delignification efficiency						TRS					
Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F		Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	1851.15	9	205.68	14.96	0.0009	significant	487.02	9	54.11	27.82	0.0001	significant
A-Pretreatment dosage	316.39	1	316.39	23.02	0.0020		12.08	1	12.08	6.21	0.0415	
B-Solids loading	70.51	1	70.51	5.13	0.0579		4.98	1	4.98	2.56	0.1537	
C-Soaking time	80.14	1	80.14	5.83	0.0465		5.78	1	5.78	2.97	0.1284	
AB	107.33	1	107.33	7.81	0.0267		0.053	1	0.053	0.027	0.8737	
AC	302.93	1	302.93	22.04	0.0022		1.11	1	1.11	0.57	0.4741	
BC	2.06	1	2.06	0.15	0.7102		0.21	1	0.21	0.11	0.7538	
A^2	259.17	1	259.17	18.85	0.0034		439.52	1	439.52	225.94	< 0.0001	
B^2	188.42	1	188.42	13.71	0.0076		5.21	1	5.21	2.68	0.1459	
C^2	536.98	1	536.98	39.06	0.0004		4.55	1	4.55	2.34	0.1700	
Residual	96.23	7	13.75				13.62	7	1.95			
Lack of Fit	66.11	3	22.04	2.93	0.1632	not significant	12.49	3	4.16	14.83	0.0124	not significant
Pure Error	30.12	4	7.53				1.12	4	0.28			
Cor Total	1947.38	16					500.64	16				

- The coefficient of determination (R^2) of the model was 0.95 and the adj-R^2 value was 0.88.
- . The coefficient of variation (C.V.) obtained was 6.81%.
- Adequate precision value (13.47) measures the signal to- noise ratio and a ratio greater than 4 is generally desirable.
- The final equation for delignification efficiency in terms of actual factors was given in Eq.1 shown below.

Delignification efficiency = -11.97 + (13.24 x Pretreatment dosage) + (2.57 x Soaking time) – (86.33 x Pretreatment dosage x Solids loading) – (0.12 x Pretreatment dosage x Soaking time) – (0.49 x Pretreatment dosage^2) + (29731.11 x Solids loading^2) – (0.03 x Soaking time^2) (1)

- The coefficient of determination (R^2) of the model was 0.97 and the adj-R^2 value was 0.93.
- The coefficient of variation (C.V.) obtained was 9.22%.
- Adequate precision value (12.96) measures the signal to- noise ratio

The final equation for TRS in terms of actual factors was given in Eq.2 shown below.

TRS = -6.72321 + 7.79806 * Pretreatment dosage + (-0.638562 x Pretreatment dosage$^{2)}$ (2)

- Optimization of the three variables can be checked using the contour plot and their 3D responses for delignification efficiency

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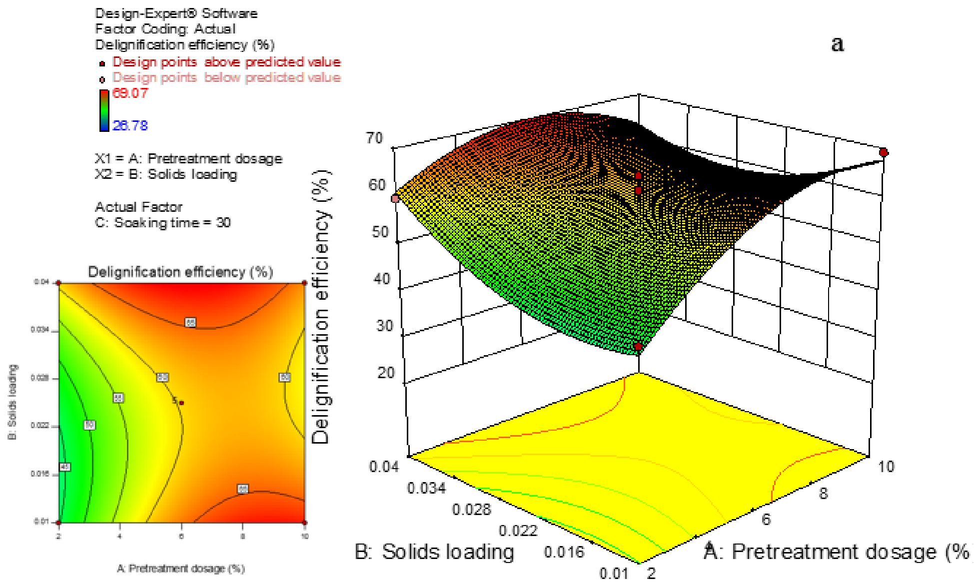
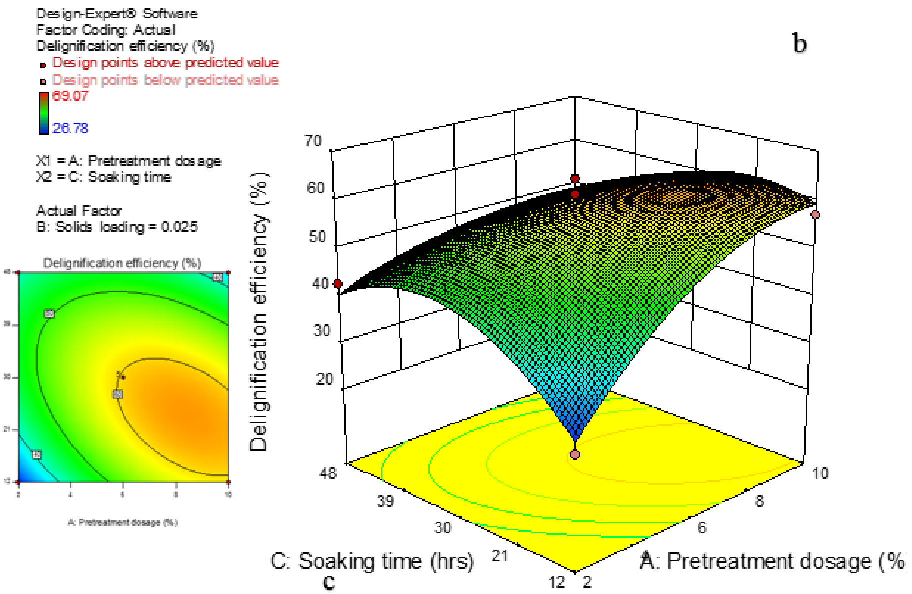
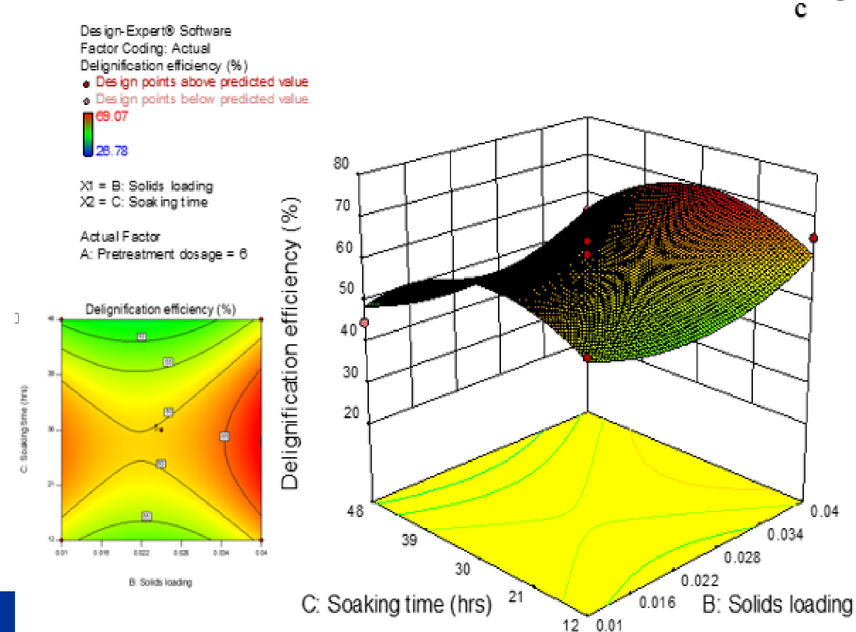
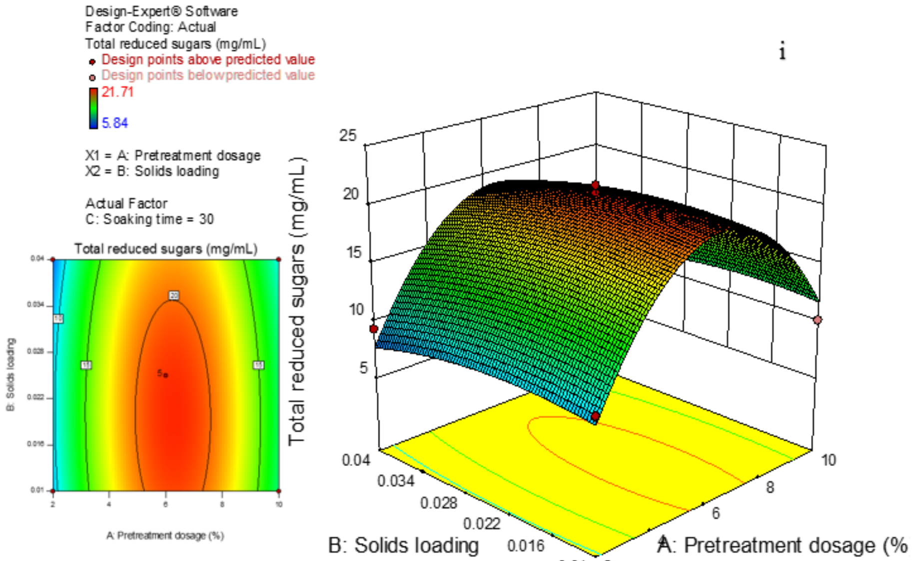
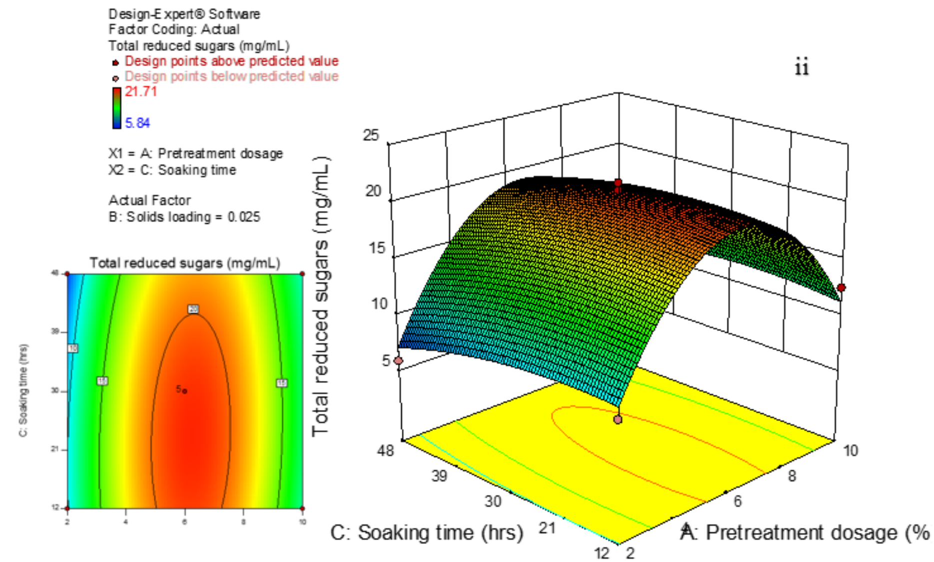


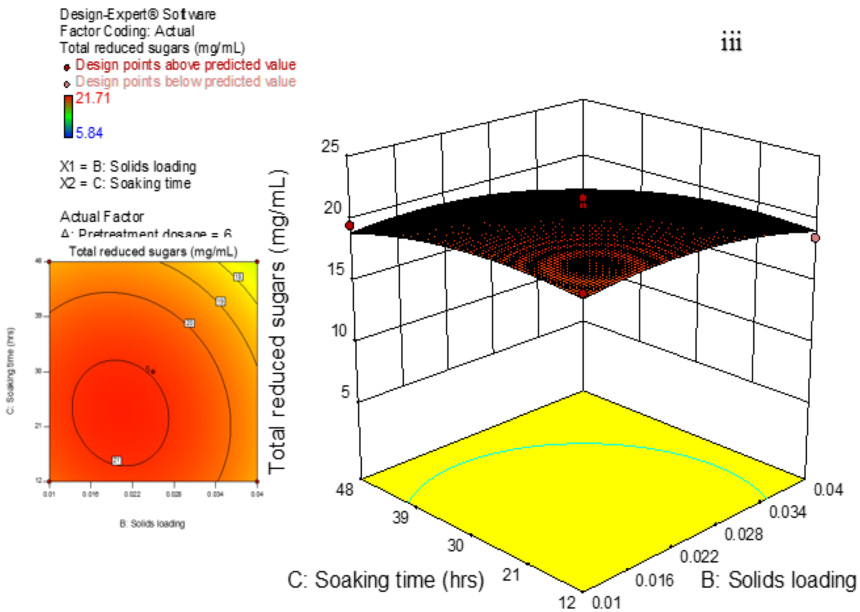




- Optimization of the three variables can be checked using the contour plot and their 3D responses for TRS

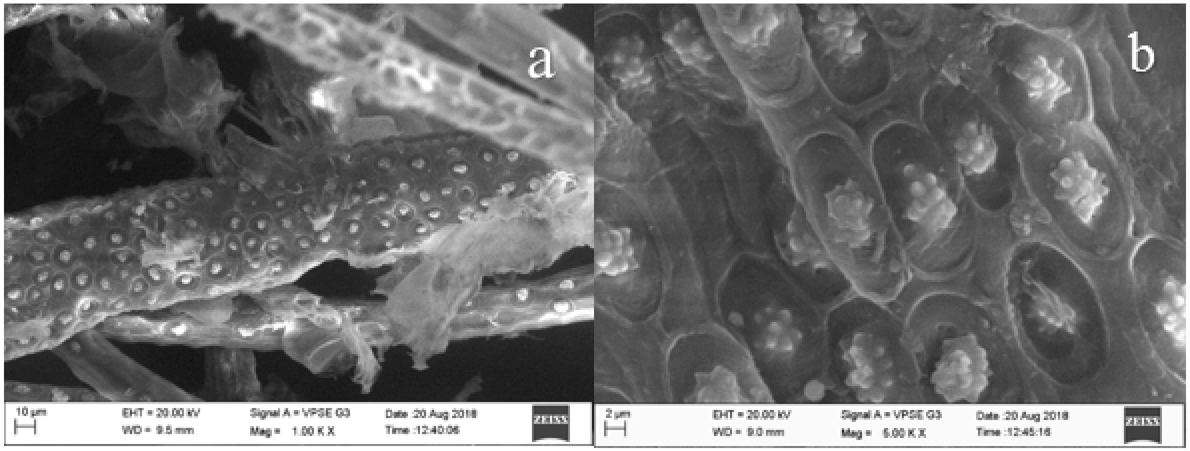




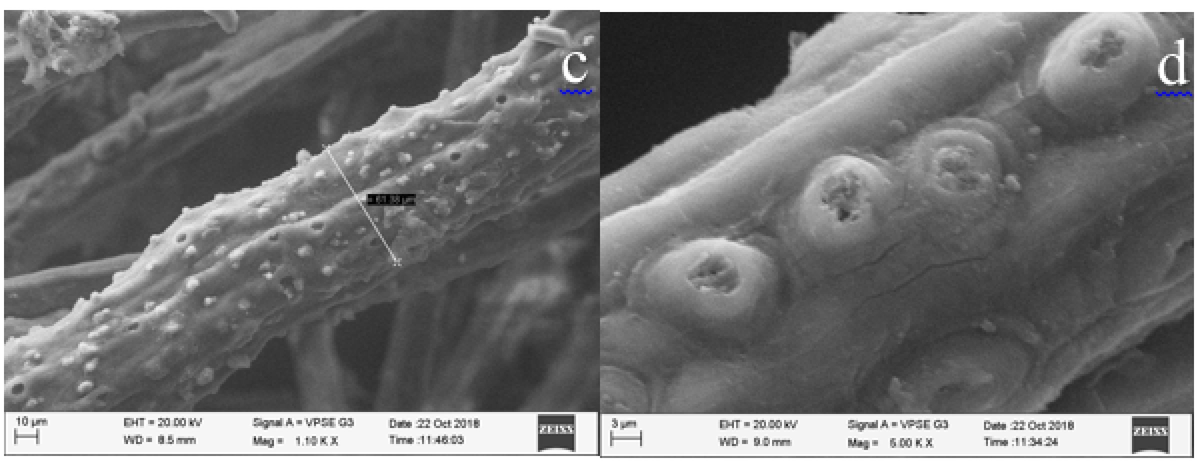


Morphological analysis

➢ SEM for the morphological changes



SEM images for (a) Raw AH at 1000X (b) Raw AH at 5000X



SEM images for (c) Raw AH at 1100X (d) Raw AH at 5000X

- Raw AH surface images show a compact arrangement of hemicellulose and cellulose in the lignin matrix.
- Raw arecanut husk showed highly ordered fibrils with acute edges due to the trimming process and exhibited a rigid structure.
- The thorn-like structure on the surface depicts the phytoliths (silica storing bodies) which form an intact epidermis.
- On alkali pretreatment, this intact epidermis form broke and thereby reduces the recalcitrance.

Conclusion

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- Alkaline pretreatment - best methods can be employed for the fractionalisation of the arecanut husk.
- Helps in the removal of lignin and increases the accessibility to the cellulose.
- Facilitates the bifurcation of its biomass composition into lignin and recalcitrant free residues of AH.
- Using BBD, the optimal values obtained for the maximum delignification efficiency and TRS :
  - pretreatment dosage of 4.78%,
  - solids loading of 1:25 and
  - soaking time of 24.52 hours.
- The desirability of the optimization was found to be 0.868.
- The achieved delignification efficiency and TRS were of 68.64% and 17.92mg/mL respectively.
- The recovered lignin can be utilised for the various applications and can also be used for the synthesis of various chemicals and value-added products.

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Thank You