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Prospects of utilizing seawater as a reaction medium for pretreatment and saccharification of rice straw

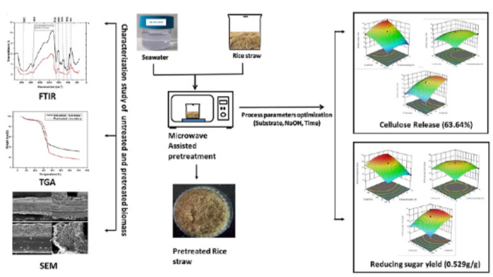
Binita Dev [a], Arindam Bakshi [b], Balasubramanian Paramasivan [c,*]

[a] Department of Life Science, National Institute of Technology Rourkela, Odisha, 769008, India
[b] Department of Food Science and Human Nutrition, Iowa State University, Iowa, 50011, USA
[c] Department of Biotechnology & Medical Engineering, National Institute of Technology Rourkela, 769008, Odisha, India

HIGHLIGHTS

- Pretreatment and saccharification of rice straw was performed in seawater medium.
- Box Behnken design used for optimization of microwave-NaOH pretreatment parameters.
- Optimized pretreatment generated 65.43% cellulose and 0.554 g/g reducing sugar yield.
- SEM, FTIR, BET and TGA confirmed the pretreatment mediated changes in biomass.

GRAPHICAL ABSTRACT



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ABSTRACT

The transition towards a bio-based economy has led to an unprecedented surge in fresh water consumption that renders biofuel a high water footprint product. The depleting fresh water resources have exacerbated the situation which necessitates the exploration of non-potable water for biorefinery purposes. In the current study, seawater is used as a plausible alternative reaction medium for pretreatment and saccharification of rice straw. Response Surface Methodology (RSM) based on Box-Behnken Design (BBD) was employed to model, predict and validate cellulose release and reducing sugar yield from rice straw subjected to microwave-NaOH pretreatment. The optimized pretreatment conditions were determined to be 8.54% substrate loading, 1.94% NaOH and 4.09 min which resulted in the maximum cellulose release of 65.43% and reducing sugar yield of 0.554 g/g. Several physico-chemical studies of the raw and pretreated biomass were carried out using bomb calorimetry, scanning electron microscopy (SEM), Fourier transform infrared (FTIR) spectroscopy, Brunauer–Emmett–Teller (BET) analysis and thermal gravimetric analysis (TGA) to examine the efficacy of pretreatment. Evidences of an apparent delignification was substantiated by the increase in surface area from 7.719 to 44.188 $m^2\ g^{-1}$ and pore volume from 0.039 to 0.071 mlg^{-1} which was consistent with the decrease in energy density and distorted surface morphology of the pretreated biomass. Further, the FTIR revealed a reduced peak in the absorption spectral bands at 1636 cm^{-1} which confirmed the pretreatment mediated degradation of lignin and hemicellulose. This finding provides evidence on the prospects of utilizing abundantly available seawater resource as a reaction medium for sustainable biofuel production.

* Corresponding author.
E-mail address: biobala@nitrkl.ac.in (B. Paramasivan).

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1. Introduction

The overgrowing population and increasing demand of transportation fuel has caused depletion of non-renewable fossil based energy resources (Tsegaye et al., 2019). The dawn of the 21st century entails us to self-introspect so as to mitigate the global energy crisis by using greener, sustainable and renewable energy source. This has driven the research of achieving zero emissions by exploring lignocellulosic based biofuels (Morone et al., 2018; Alagumalai et al., 2020; Rai and Sahoo, 2021). Currently, lignocellulosic biomass is regarded as the most preferred substrate for bioenergy production owing to its plenitude of supply, economic viability in addition to being non-competitive and sustainable in nature (Ahmed et al., 2017; Sorn et al., 2019). Bioethanol derived from lignocellulosic biomass is considered to be an eminent non-petroleum fuel due to its higher combustion efficiency of hydrocarbons with reduced greenhouse gas emissions by 86% (Ahmed et al., 2017; Karimi et al., 2019, 2021). Among the lignocellulosics, rice straw is regarded as the most preferred biomass for bioethanol production because of its wide availability with a reported gross annual production of 650–975 million tons globally (Momayez et al., 2017). The low lignin (3–30%), predominant cellulose (30–56%) and hemicellulose content (10–27%) also makes it a suitable candidate for bioethanol production (Abraham et al., 2016; Prasad et al., 2020). However, the recalcitrant property and complex lignocellulosic structure hinders the utilization of rice straw for bioconversion processes. In order to circumvent this structural inhibition, an appropriate pretreatment is necessary to alter the recalcitrant properties of the biomass (Kumari et al., 2021). Pretreatment leads to changes in terms of depolymerization, porosity, accessible surface area and amorphous cellulose, which consequently improves the yield of reducing sugar and ethanol during saccharification and fermentation respectively (Kaur and Phutela, 2016; Jin et al., 2020). Several physical, chemical and biological pretreatment have been evaluated widely for compositional and structural alteration of biomass matrix (Ren et al., 2020; Wang et al., 2020). Among the various modes of chemical pretreatment, alkaline method have been shown to be feasible in selective degradation of lignin, lack of furan formation unlike acidic pretreatment, low sugar loss, reduced cellulose crystallinity and enhanced surface area for saccharification (Wu et al., 2018; Molaverdi et al., 2019).

Microwave based pretreatment have been extensively used over conventional slower heating methods for lignocellulosic conversion due to its higher heating efficiency and swift operation time (Hoang et al., 2021). The microwave induced molecular collisions caused by dielectric polarization breaks down the supramolecular lignocellulosics into simpler molecules through swelling of the fiber structure and fragmentation resulting in enhanced enzymatic saccharification (Sorn et al., 2019; Prasad et al., 2020). Further, appropriate combinations of two or more pretreatment approaches have been recognized for preserving the maximum cellulose from the pretreated biomass (Akhtar et al., 2016b).

In addition, the growing scarcity of Earth's freshwater resources is an aftermath of the escalating consumption and population explosion which further heightens the magnitude of environmental pollution. Interestingly, biofuels have been portrayed as a high water footprint product due to the estimated requirement of almost 1900–5900 L of water per liter of biofuel production. The approximate quantity of water needed for 1 L of bioethanol varies between 1388 L and 9812 L (Fang et al., 2015; Ren et al., 2016; Mathimani et al., 2018; Pei and Jiang, 2018). It has been estimated that the fresh water requirement for biofuel production would exceed the available supply by 5.5% in 2030, which would aggravate the existing burden on fresh water reservoirs (Gerbens-Leenes et al., 2012). Therefore, the unrestrained use of fresh water in biorefineries risks the future depletion of limited water reservoirs and hence, is not feasible. Addressing the present and future fresh water scarcity, the search for non-potable water resources is paramount for large scale bioprocesses (Domínguez de María, 2013). In this context, seawater is an infinite resource which can be utilized as a suitable alternative to fresh water usage in biorefineries (Dev et al., 2019a; Zaky et al., 2020). In contrast to the low availability of fresh water at about 2.5%, boundless reserves of seawater encompasses our planet by approximately 96.5% covering an estimated 71% area of the Earth's surface (Dev et al., 2019b). In addition, the presence of a large quantity of essential minerals in seawater limits the necessity of external addition of such minerals (Zaky et al., 2018). A number of coherent studies have provided conclusive evidence on the utilization of seawater as a reaction media for various pretreatment, enzymatic, fermentative and chemical transformations (Indira et al., 2016; Ren et al., 2016; Indira and Jayabalan, 2020; Zaky et al., 2021). However, the precise implications of using seawater in optimization studies of delignification of rice straw remain elusive.

The objective of the present study is to understand the outcome of using seawater based reaction media for delignification and saccharification of rice straw. Response surface methodology (RSM) based on Box Behnken Design (BBD) was used to obtain optimum microwave-NaOH assisted pretreatment conditions in seawater for studying cellulose release and reducing sugar production. Furthermore, in order to examine the pretreatment induced structural, functional and microscopic changes in the biomass, scanning electron microscopy (SEM), attenuated total reflection Fourier transform infrared (ATR-FTIR), Brunauer–Emmett–Teller (BET) analysis and thermal gravimetric analysis (TGA) were performed. The findings of the present study could provide insights on the utilization of seawater as an alternative to fresh water usage in biorefineries.

2. Materials and methods

2.1. Seawater (SW) preparation

The seawater (SW) used in experiments was collected from Digha, West Bengal (21.6222° N, 87.5060° E) situated along the coast of Bay of Bengal, India. The seawater with salinity of 35 ppt was filtered using glass microfiber filters (Whatmann pore size 1.2 μm) and autoclaved at 121 °C for 15 min and then stored at 4 °C until further use.

2.2. Substrate and reagents

Rice straw was procured from a local market of Rourkela, Odisha, India. The rice straw was thoroughly rinsed for removal of debris, dried at 60 °C and initially chopped into 2–3 cm with paper knife. 500 gm of the dried rice straw was then homogenized in a hammer mill to 60 mesh and stored in polythene bag at room temperature. The commercial cellulase enzyme blend Cellic CTec2 was purchased from Sigma-Aldrich.

2.3. Proximate analysis and extractive determination of rice straw

The moisture and ash content of native rice straw was determined by conventional oven method and NREL/TP-510-42,622 respectively (Sluiter et al., 2008a). For extractives determination, extraction of 2 g m of native rice straw was carried out using Soxhlet apparatus as per NREL/TP- 510–42619 (Sluiter et al., 2008c). As per the protocol, two consecutive steps of extraction were performed independently using ethanol and distilled water respectively.

2.4. Biochemical composition of rice straw

For lignin determination, 300 mg of native and pretreated rice straw was hydrolyzed using 72% (w/w) H_2SO_4in water bath at 30 °C for 1 h. Upon completion, the acid was diluted to 4% and autoclaved at 121 °C, 15 l b/inch^2 for 1hr. The reaction mixture containing acid insoluble lignin (AIL) was cooled and subsequently filtered, dried at 105 °C and burned at 500 °C for ash quantitation. The acid soluble lignin (ASL) in the filtrate was measured spectrophotometrically at 205 nm (Sluiter et al., 2008b). Anthrone method was used for hemicellulose estimation of native and pretreated dried biomass (Marlett and Lee, 1980)."Semi-micro determination of cellulose" method was used for the estimation of cellulose content of raw and pretreated biomass (Updegraff, 1969).

2.5. Preliminary screening

2.5.1. Steam acid/alkali pretreatment

Pretreatment were carried out in seawater with 10% solid loading, 1% (w/v) of different acids (HCl, H_2SO_4 and H_3PO_4) and 2% (w/v) of different alkali (NaOH and Ca $(OH)_2$) at 121 °C for 60 min. The pretreated samples were then washed to neutral pH with deionized water and dried at 60 °C overnight prior to enzymatic hydrolysis. The preliminary screening was performed to compare the efficiency in terms of reducing sugar yield for each acid and alkali pretreatment respectively. Thereafter, based on higher reducing sugar yield, the selected acid and alkali was chosen for ultrasonication and microwave based pretreatment.

2.5.2. Ultrasonication acid/alkali pretreatment

Ultrasonication aided pretreatment was carried out with 10% solid loading, 1% (w/v) H_2SO_4 and 2% (w/v) NaOH prepared in seawater for 30 min at an amplitude of 60%. After sonication, the pretreated samples were washed with deionized water to neutral pH, dried overnight at 60 °C and stored for enzymatic hydrolysis.

2.5.3. Microwave acid/alkali pretreatment

Microwave assisted pretreatment was performed with 10% solid loading, 1% (w/v) $H_2SO_4$and 2% (w/v) NaOH prepared in seawater using standard microwave oven (2.4 GHz). The pretreated samples were washed to neutral pH, dried overnight at 60 °C and stored for enzymatic hydrolysis.

2.6. Influence of various process parameters on microwave assisted pretreatment of rice straw

Microwave based pretreatment was selected on the basis of preliminary screening and one variable at a time (OVAT) approach was employed for further analysis. The range of the chosen process parameters were as follows: solid loading (2.5–25%), NaOH (0.25–5%), microwave power (90–500 W) and time (1–10 min).

2.7. Process optimization for microwave assisted NaOH pretreatment using response surface methodology (RSM)

Response surface methodology (RSM) based on Box-Behnken design (BBD) was used to obtain optimal pretreatment conditions of rice straw. Two response variables were chosen for optimization namely, cellulose release (CR) and reducing sugar yield (RSY). Design Expert software (Version 11) was specifically dedicated to performing design of experimental (DOE). All the pretreatment experiments were executed in triplicates with a microwave power of 160 W.

2.8. Enzymatic saccharification

Enzymatic saccharification of rice straw was conducted at 50°Cfor 48 h with 2.5% of biomass, an enzyme load of 20FPU/gm and 0.02% sodium azide in citrate buffer (pH 4.8, 0.05 M) prepared in seawater. The reducing sugar concentration in the hydrolysate was determined by 3,5-Dinitrosalicylic acid (DNS) method using glucose as standard (Miller, 1959).

2.9. Characterization of native and pretreated rice straw

2.9.1. Brunauer–Emmett–Teller (BET) and Barrett-Joyner-Halenda (BJH) analysis

Dried native and pretreated sample were initially degassed for 3 h at 200 °C and the nitrogen adsorption-desorption isotherms were recorded at −196 °C using Quantochrome BET surface area and porosity analyzer. The surface area, pore size and pore volume of the native and pretreated rice straw were measured using BET region of the nitrogen adsorption isotherm and BJH region of the nitrogen desorption isotherm respectively.

2.9.2. Energy density measurement

Raw and delignified biomass was subjected to energy density measurement using standard bomb calorimeter (Parr 6100 Calorimeter). The samples were made moisture free using oven drying overnight at 60 °C and then cast into pellet. The energy density of the samples was measured as per Eq. (1).

$$Energy\ density\left(\frac{kJ}{g}\right) = \frac{W\ X(T_2 - T_1)}{M} \tag{1}$$

where, W is the water equivalent of calorimeter (2330 cal°C^{-1}), M is the sample mass and (T_2-T_1) is the rise in temperature.

2.9.3. SEM, FTIR and TGA analysis

The physico-chemical properties of both native and pretreated rice straw were analyzed by scanning electron microscopy (SEM), attenuated total reflection Fourier transform infrared (ATR-FTIR) and thermal gravimetric analysis (TGA). Surface morphology was observed using scanning electron microscope (JEOL JSM- 6480LV) at 250X and 500X magnification. The effect of pretreatment on the functional group of the constituents of rice straw was examined using Fourier Transform Infrared Spectrophotometer (Thermofisher, Nikolet iS-10) within the wave number range of 500 — 4000 $cm^{-1}$by recording the average of 25 scans per sample. The thermal behaviour of native and pretreated biomass was assayed on a thermal gravimetric analyzer (PerkinElmer, USA) from 30 to 800 °C at a rate of 10 °C/min under Nitrogen conditions.

3. Results and discussion

3.1. Proximate analysis and extractive determination in native rice straw

The ash and moisture content of native rice straw were 3.45% and 6.29% respectively. Similar results were reported for ash (1.3–9.8%) and moisture (6–7.9%) content of native wheat straw and barley straw (Naik et al., 2010; Akhtar et al., 2017). Further, the ethanol and water extracts were calculated to be 2.96% and 9.11% respectively which corroborates with the earlier reports of ethanol (2.1–8%) and water extract (8.5–10.3%) of wheat straw and barley straw respectively (Naik et al., 2010; Akhtar et al., 2017).

3.2. Preliminary screening

In order to examine the effect of different acids and bases during steam assisted pretreatment of rice straw, experiments were carried out in seawater based reaction medium and reducing sugar yield was determined. In addition, pretreatment was also performed using fresh water based reaction medium and used as a control for comparative analysis. The results suggested that 2% NaOH generated the maximum reducing sugar yield of 0.462 g/g in seawater medium (Fig. 1a). Interestingly, the reducing sugar yield was found to be in the similar range in the freshwater based pretreatment (Fig. 1b). These observations further validated the rationale of using seawater as a reaction medium for the pretreatment of rice straw. Also, reducing sugar yield of 0.450 g/g and

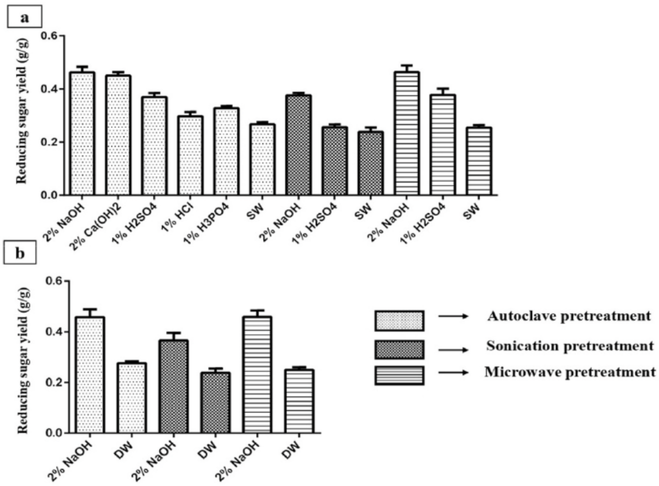

Fig. 1. Preliminary screening of reducing sugar yield using rice straw upon different modes of pretreatment (Autoclave, Sonication, Microwave).

0.369 g/g was detected for pretreatment using $Ca(OH)_2$and 1% H_2SO_4 respectively in seawater. Since the highest reducing sugar yield was detected for 2% NaOH and 1% H_2SO_4, these reagents were selected for further screening using other modes of pretreatment. As expected, sonication and microwave pretreatments also revealed 2% NaOH to be the most efficient reagent yielding 0.463 g/g and 0.375 g/g reducing sugar respectively which was also found to be comparable with the freshwater control (Fig. 1b). Previous studies have reported that NaOH pretreatment leads to higher solubilization of the substrate and hence, it is the widely preferred choice over other bases for delignification (Kang et al., 2013; Kim et al., 2016). Furthermore, microwave-assisted alkali pretreatment leads to fragmentation of the biomass and subsequent removal of lignin and hemicellulose to a greater extent along with the enhanced release of reducing sugars upon enzymatic hydrolysis (Cao et al., 2018; Sewsynker-Sukai and Kana, 2018). Although, the reducing sugar yield of steam and microwave assisted pretreatment were comparable in the current study, the latter was chosen for further experiments due to its lower duration and lesser energy consumption (Sombatpraiwan et al., 2019).

In order to determine the range of the different pretreatment parameters of microwave assisted pretreatment of rice straw, OVAT experiments were conducted in seawater based reaction medium with the four parameters – solid loading (%), NaOH (%), Microwave power (W) and time (min). Each of these parameters was varied one at a time while keeping the rest constant (Fig. 2 a-d).

Fig. 2a demonstrates the effect of solid loading (2.5–25%) on microwave pretreatment with a maximum reducing sugar yield of 0.471 g/g obtained at 5%. An increase in solid loading beyond 10% resulted in a decrease of reducing sugar yield which could be due to the reduced accessibility of the alkaline reagent (NaOH) to the effective surface area of the substrate. Fig. 2b illustrates the pretreatment of rice straw at different concentrations of NaOH (0.25–5%) where 1.5% resulted in maximum reducing sugar yield of 0.511 g/g. Fig. 2c and d shows the reducing sugar yield at different microwave power (90–500 W) and time duration (1–15 min) respectively. The results of the OVAT experiment indicated a maximum reducing sugar yield of 0.509 g/g and 0.511 g/g at 7.5 min pretreatment time and 160 W microwave power respectively. Therefore, further experiments of optimization of the process parameters was carried out with microwave power kept constant at 160 W.

3.3. Process optimization of seawater based pretreatment of rice straw

The key parameters that influence the outcome of pretreatment conditions in terms of cellulose release and reducing sugar yield were examined by Response Surface Methodology (RSM) using Box-Behnken design. The different combinations of the three independent variables for pretreatment, namely solid loading (*A*), alkali concentration (*B*) and time of pretreatment (*C*) generated 17 experimental runs and 5 center points as summarized in Table 1. In the present study, rice straw was chosen as the lignocellulosic biomass and the compositional analysis

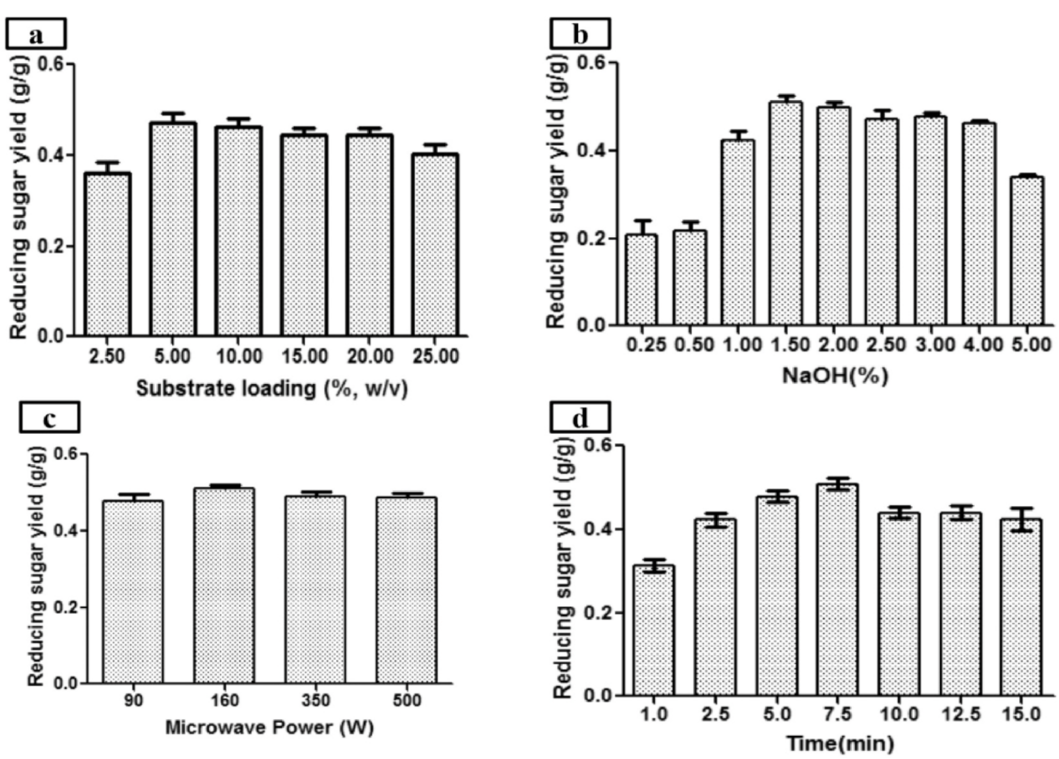

Fig. 2. Comparative study of reducing sugar yield by one variable at a time approach (OVAT) for microwave assisted pretreatment of rice straw.

Table 1
Box-Behnken Design (BBD) for optimization of process parameters affecting cellulose release (CR) and reducing sugar yield (RSY) during microwave assisted pretreatment of rice straw in seawater medium.

Run	Substrate loading –Rice straw (%) (A)	NaOH (%) (B)	Time, (min) (C)	Cellulose release (CR) (%)		Reducing sugar yield (RSY) (g/g)	
				Predicted	Observed	Predicted	Observed
1	5	1.125	10	47.83	47.21	0.387	0.387
2	5	2	5.5	54.21	54.43	0.454	0.450
3	10	1.125	10	50.03	49.57	0.398	0.400
4	5	0.25	5.5	44.70	44.64	0.313	0.320
5	7.5	0.25	1	43.51	43.11	0.287	0.283
6	7.5	1.125	5.5	57.18	56.66	0.483	0.489
7	10	1.125	1	54.78	55.40	0.397	0.398
8	10	2	5.5	63.58	63.64	0.535	0.529
9	7.5	1.125	5.5	57.18	56.99	0.483	0.483
10	7.5	2	1	61.16	60.48	0.482	0.488
11	7.5	1.125	5.5	57.18	57.67	0.483	0.485
12	7.5	1.125	5.5	57.18	56.83	0.483	0.493
13	5	1.125	1	48.60	49.06	0.342	0.341
14	7.5	2	10	55.45	55.85	0.500	0.505
15	10	0.25	5.5	43.72	43.50	0.297	0.301
16	7.5	1.125	5.5	57.18	57.73	0.483	0.466
17	7.5	0.25	10	43.72	44.40	0.315	0.310

revealed the presence of 42.56% cellulose, 23.95% hemicellulose and 17.45% lignin. Extensive studies on different lignocellulosic biomasses indicated significant changes in composition upon pretreatment (Singh et al., 2015; Zhang et al., 2021). As expected, microwave assisted alkali pretreatment altered the composition of the biomass in terms of cellulose, hemicellulose and lignin content which was examined to be 63.64%, 13.58% and 10.70% respectively. The maximum cellulose release and reducing sugar yield were obtained at 10% solid loading, 2% NaOH and pretreatment time of 5.5 min. At this pretreatment condition, 63.64% cellulose and 0.529 g/g reducing sugar was obtained. Reports suggest that the microwave pretreatment of lignocellulosic biomass leads to an apparent disruption in structure and physico-chemical properties that confers enhanced accessibility towards enzymatic hydrolysis (Tsegaye et al., 2019; Hoang et al., 2021). Therefore, it is likely that the pretreatment conditions would have led to significant structural and morphological changes in rice straw resulting in enhanced saccharification (Hartati et al., 2021).

3.4. Influence of process parameters on cellulose release

In order to demonstrate the interactive effects of solid loading, NaOH and time on the cellulose release, 3D response surface plots were generated by feeding the experimental data into design expert software version 11. These plots represent the interaction between two factors while the third factor is kept constant at the center value (Fig. 3 a-c). The shapes of the 3D response plots indicate the extent and nature of

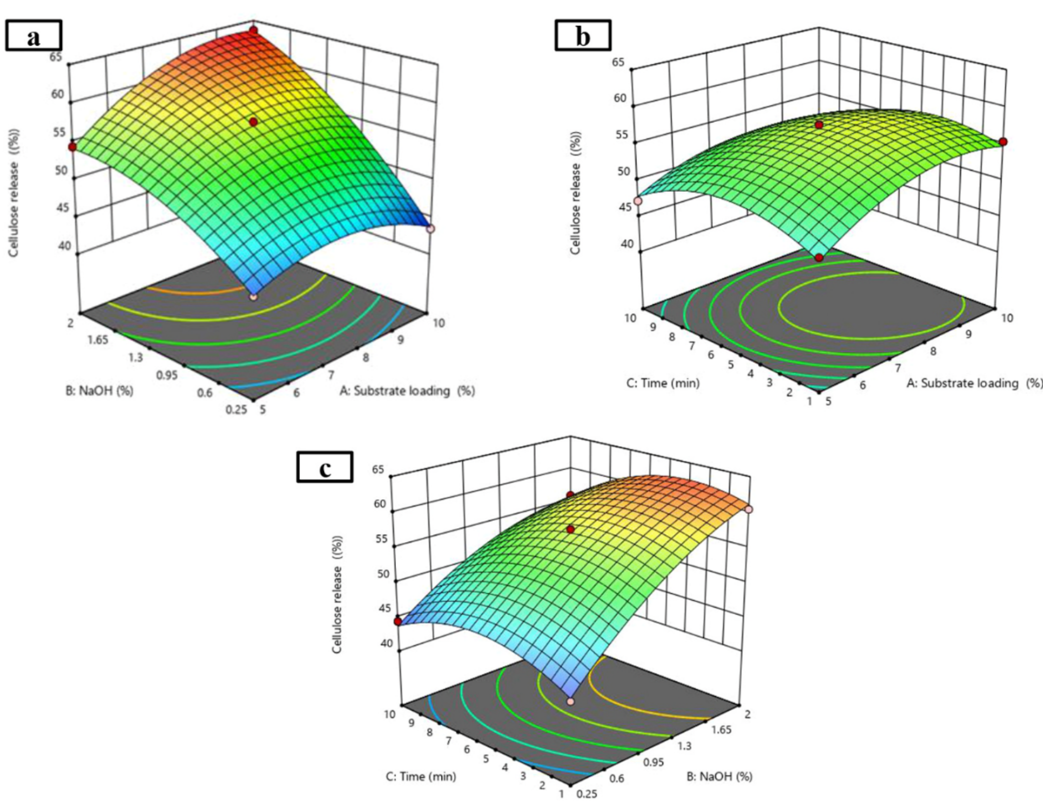

Fig. 3. Influence of process parameters on cellulose release during microwave assisted NaOH pretreatment of rice straw.

Table 2
One-way ANOVA analysis for Cellulose release of microwave assisted NaOH pretreatment of rice straw in seawater medium.

Source	Sum of squares	Df	Mean square	F-value	p- value	
Model	661.59	9	73.51	145.79	<0.0001	significant
A-Substrate loading	35.15	1	35.15	69.72	<0.0001	
B– NaOH	431.45	1	431.45	855.66	<0.0001	
C- Time	15.18	1	15.18	30.11	0.0009	
AB	26.78	1	26.78	53.11	0.0002	
AC	3.96	1	3.96	7.85	0.0264	
BC	8.76	1	8.76	17.38	0.0042	
A^2	41.43	1	41.43	82.16	<0.0001	
B^2	26.04	1	26.04	51.64	0.0002	
C^2	58.56	1	58.56	116.13	<0.0001	
Residual	3.53	7	0.5042			
Lack of fit	2.56	3	0.8527	3.51	0.1283	not significant
Pure Error	0.9715	4	0.2429			
Cor Total	665.12	16				

Adj R-squared - 0.9879.
R-squared -0.9947.

interaction between the process variables. A stronger interaction between the variables leads to the elliptical nature of the response plot whereas a circular nature indicates weak or negligible interaction (Tsegaye et al., 2019). On the other hand, *F*-values correspond to the parameter significance on the magnitude of generated response. A closer examination of 3D plots in the current study indicates the robust effect of NaOH concentration and substrate loading in generating higher response variable (Fig. 3a). The concentration of NaOH was found to have a strong influence on cellulose release as reflected by the *F*-values (855.56). Further, the effect of the interactive (AB, AC and BC) and quadratic (A^2, B^2 and C^2) terms were examined from their respective *F*-values (Table 2). The results indicated that substrate-NaOH (*AB*) and the square factor of time (C^2) exhibited a strong influence on the cellulose release which was evident from the higher *F* values.

The regression coefficient of the quadratic equation describing the cellulose release (CR) has been represented by Eq. (2).

$$CR = 9.2228 + 7.5224\boldsymbol{A} + 8.8967\boldsymbol{B} + 2.8058\boldsymbol{C} + 1.1828\boldsymbol{AB} - 0.0884\boldsymbol{AC} - 0.3758\boldsymbol{BC} - 0.5018\boldsymbol{A}^2 - 3.2480\boldsymbol{B}^2 - 0.1841\boldsymbol{C}^2 \quad (2)$$

The statistical significance of the model for the cellulose release was tested by one-way analysis of variance (ANOVA) (Table 2). The model was found to be significant as evident by the *p*-value of 0.0001 (<0.05). Further, the significantly high values of *R*-square (0.9947) and adjusted *R*-square (0.9879) validated the adequacy of the model.

The cellulose release from the experimental runs of different combination of the process parameters was found to range within 43.5%–63.64%. It was previously reported that the increase in cellulose content of *Catalpa* sawdust up to 55.78% and 56.28% when subjected to microwave assisted NaOH and $Ca(OH)_2$ pretreatment respectively (Jin et al., 2016). Therefore, the findings of the present study have conclusively established the role of NaOH and substrate loading in generating higher cellulose release in microwave pretreated rice straw.

3.5. Influence of process parameters on reducing sugar yield

The 3D response plots generated for the effects of NaOH concentration, substrate loading and time on reducing sugar yield was calculated. The elliptical nature of the response plots indicated a stronger interaction between NaOH and substrate loading parameters compared to the substrate-time and time-NaOH interactions (Fig. 4 a-c). Furthermore, the concentration of NaOH was found to be the prominent factor in yielding higher reducing sugar which was reflected by the high *F*-value (757.37). All the quadratic terms have a relatively stronger influence on reducing sugar yield while the interactive term NaOH-time (BC) have weaker effect as indicated by the *F*-value (0.2636) (Table 3).

The regression coefficient of the quadratic equation describing the reducing sugar yield (**RSY**) has been represented by Eq. (3).

$$\mathbf{RSY} = -0.2280 + 0.1171\boldsymbol{A} + 0.1279\boldsymbol{B} + 0.0391\boldsymbol{C} + 0.0112\boldsymbol{AB} - 0.0009\boldsymbol{AC} - 0.0006\boldsymbol{BC} - 0.0078\boldsymbol{A}^2 - 0.0445\boldsymbol{B}^2 - 0.0025\boldsymbol{C}^2 \quad (3)$$

The significance of the model for reducing sugar yield was tested by ANOVA analysis which revealed a *p*-value <0.0001 (Table 3). The high *R*-square and adjusted *R*-square values of 0.9947 and 0.9879 validated the high correlation between the predicted and the observed values. These results demonstrated that the model is best fitted and is able to bring a good estimate of the influence of variables as well as levels of reducing sugar release.

The total reducing sugar yield during microwave pretreatment was found to range between 0.301 g/g and 0.529 g/g. These findings emphasized on the efficiency of microwave assisted pretreatment used in the current study which facilitated the enzymatic hydrolysis and subsequent release of reducing sugars. In addition, optimum levels of NaOH (2%) and substrate loading (10%) were found to be necessary in penetrating the lignin barrier of the biomass. Similar observations were previously reported in sugarcane bagasse where microwave alkali pretreatment resulted in reducing sugar yield of 0.665 g/g and lignin removal of about 90% (Binod et al., 2012).

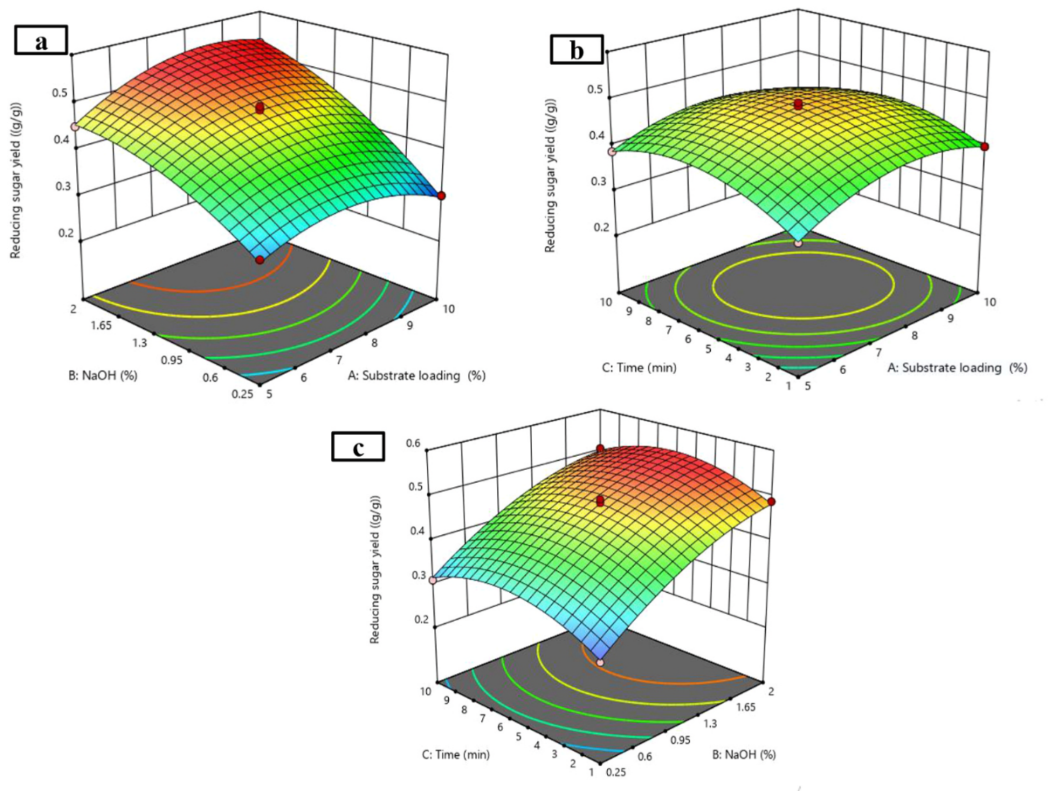

Fig. 4. Influence of process parameters on reducing sugar yield during microwave assisted NaOH pretreatment of rice straw.

Table 3
One-Way ANOVA analysis for reducing sugar yield of microwave assisted NaOH pretreatment of rice straw in seawater medium.

Source	Sum of squares	Df	Mean square	F-value	p- value	
Model	0.1076	9	0.0120	126.08	<0.0001	significant
A-Substrate loading	0.0021	1	0.0021	22.28	0.0022	
B– NaOH	0.0718	1	0.0718	757.37	<0.0001	
C- Time	0.0011	1	0.0011	11.16	0.0124	
AB	0.0024	1	0.0024	25.32	0.0015	
AC	0.0005	1	0.0005	5.10	0.0584	
BC	0.0000	1	0.0000	0.2636	0.6234	
A^2	0.0102	1	0.0102	107.04	<0.0001	
B^2	0.0049	1	0.0049	51.63	0.0002	
C^2	0.0116	1	0.0116	122.85	<0.0001	
Residual	0.0007	7	0.0001			
Lack of fit	0.0002	3	0.0001	0.7307	0.5853	not significant
Pure Error	0.0004	4	0.0001			
Cor Total	0.1083	16				

Adj R-squared- 0.9860.
R-squared –0.9939.

Table 4
Validation of the optimized pretreatment condition obtained for rice straw on different substrates.

Substrate	Pretreatment strategy	Cellulose release (%)	Reducing sugar yield (g/g)
Rice straw	8.54% substrate loading, 1.940% NaOH, 4.09 min, 160 W	65.43 ± 2.10	0.554 ± 0.06
Sugarcane bagasse		68.40 ± 1.71	0.640 ± 0.06
Kans grass		56.44 ± 2.37	0.487 ± 0.04
Teawaste		32.56 ± 0.86	0.267 ± 0.01

3.6. Validation of the developed model

The validity of the RSM-BBD model was experimentally tested using the optimized pretreatment parameters. The optimal pretreatment condition derived from the model was 8.54% substrate loading, 1.94% NaOH and 4.09 min duration resulting in a predicted cellulose release of 63.81% and reducing sugar yield of 0.543 g/g. Based on the experimental results, the optimized pretreatment condition was found to generate a cellulose release of 65.43% and reducing sugar yield of 0.554 g/g which validated the developed model (Table 4).

3.7. Assessment of the optimized pretreatment parameter on other lignocellulosic residues

The feasibility of the optimized microwave-alkali pretreatment strategy was tested on different lignocellulosic residues such as Kans grass, sugarcane bagasse and spent tea waste using seawater as reaction medium. The highest sugar yield and cellulose release was found in sugarcane bagasse followed by Kans grass and tea waste respectively (Table 4). These results conclusively establish the effectiveness of seawater mediated pretreatment which can be employed to reduce the consumption of fresh water in bioprocesses.

3.8. Physico-chemical changes in the pretreated biomass

3.8.1. BET analysis

Extensive studies have shown that the changes in the biomass surface area upon pretreatment leads to enhanced enzymatic hydrolysis. Therefore, it was of interest to examine the alterations in the surface area, pore size and pore volume of the untreated and pretreated rice straw by studying the nitrogen adsorption/desorption isotherms using BET-N_2 surface analyzer. The untreated rice straw exhibited the surface area of 7.719 m^2 g^{-1} with pore volume of 0.039 mlg^{-1} and pore diameter of 3.96 nm. Due to the pretreatment, there was a noticeable increase in

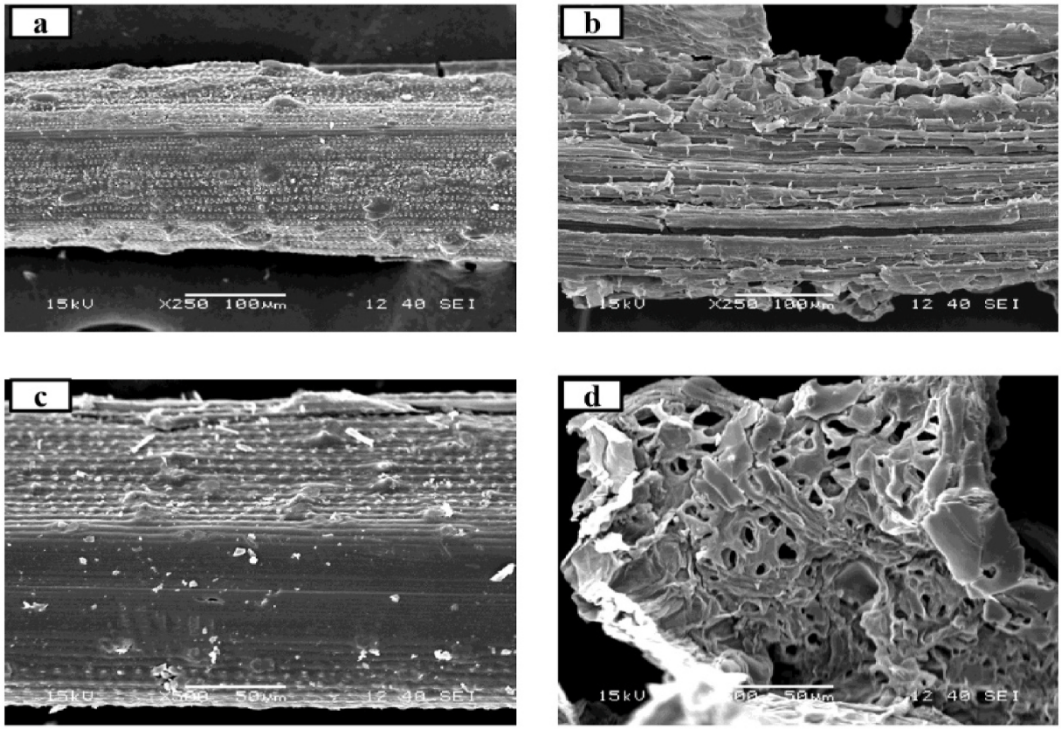

Fig. 5. Scanning electron microscopy of untreated (a–b) and microwave assisted NaOH pretreated rice straw (c–d).

rice straw surface area to 44.188 m^2 g^{-1} and pore volume to 0.071 mlg^{-1}, possibly due to the degradation of lignin. These surface changes in the biomass had a significant impact on saccharification which was evident from the increased reducing sugar yields. Interestingly, several earlier reports have also found positive correlation between enhanced surface area and enzymatic saccharification of different pretreated biomasses (Gaur et al., 2015; Suriyachai et al., 2020; Thoresen et al., 2021).

3.8.2. *Energy density measurement*

One of the major factors in determining the energy and cost balance during biofuel production is the estimation of change in energy density value upon pretreatment. Most of the agricultural residues have high energy density with heating values in the range of 15–17 kJ/g (Kargbo et al., 2010). The heating value corresponds to the amount of lignin present in the biomass due to its polyphenolic nature (Mendu et al., 2012). In order to understand the efficacy of the current pretreatment strategy, it was of interest to examine the energy density values of the pretreated and untreated biomass using bomb calorimeter. The results indicated that the energy density of rice straw markedly decreased from 16.67 kJ/g to 14.244 kJ/g upon pretreatment. This implied an apparent breakdown of the lignin component of the biomass which is reflected by the reduction in the energy density values. Similar findings were previously reported in switchgrass subjected to ionic liquid pretreatment where it was shown that the decrease in lignin content was in accordance with the lesser energy density values of the pretreated biomass (16.62 kJ/g) compared to its untreated form (18.37 kJ/g) (Li et al., 2013).

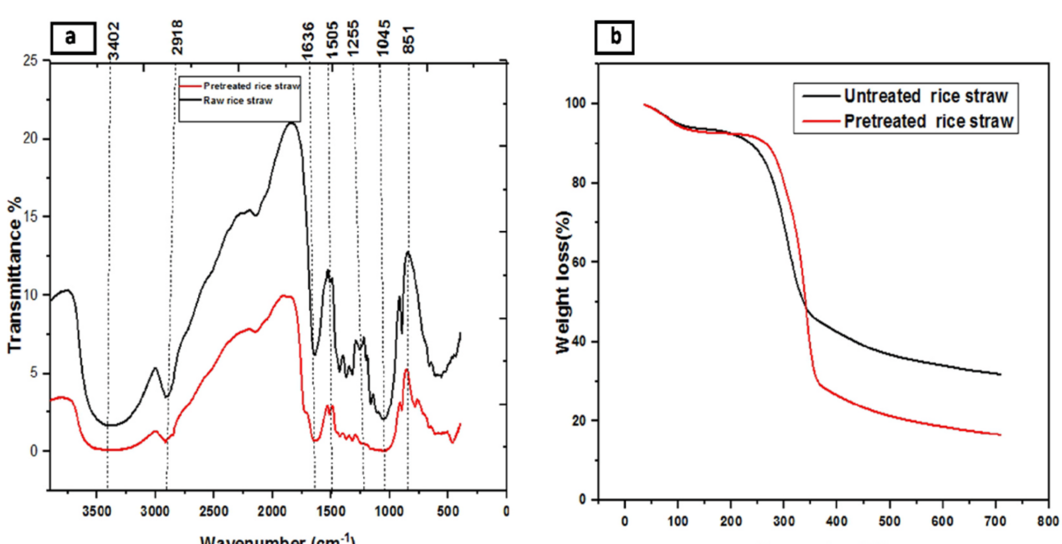

Fig. 6. (a) Fourier transform infrared analysis and **(b)** Thermal gravimetric analysis of untreated and microwave assisted NaOH pretreated rice straw.

3.8.3. SEM

The effects of pretreatment on the changes in surface morphology and structural integrity of rice straw was studied using SEM. The micrographs of the raw/untreated rice straw revealed a smooth surface with intact morphology (Fig. 5). However, the pretreated rice straw showed structural alterations along with the formation of numerous pores, clearly indicating the pretreatment induced disruption of the lignocellulosic matrix. Extensive studies have previously shown that pretreatment results in distortion of surface morphology of biomass leading to enhanced enzymatic hydrolysis. Also, such structural changes are reported for other microwave pretreated lignocellulosic biomasses where delignification has led to the formation of rough intrusions over the biomass surface (Binod et al., 2012; Akhtar et al., 2017; Moodley and Kana, 2017; Tsegaye et al., 2019).

3.8.4. FTIR analysis

The effects of pretreatment on rice straw was studied by understanding the changes in the absorption spectral bands of various functional groups attributed to cellulose, hemicellulose and lignin using FTIR analysis. The most prominent transmittance was recorded at 3402, 2918, 1636, 1505, 1255, 1045 and 851 for native and pretreated rice straw as shown in the FTIR spectra (Fig. 6a). The transmittance bands were assigned to specific constituents of the biomass based on the data gathered from earlier reports (Meng et al., 2012; Sasmal et al., 2012; Zhang et al., 2013; Wang et al., 2015). The broad transmittance band at 3600-3000 $cm^{-1}$is a manifestation of the stretching vibration imposed by the O–H group present in cellulose (Mandal and Chakrabarty, 2011). In addition, the transmittance observed at 3402 cm^{-1} indicated the result of stretching vibration of hydrogen bond with the hydroxyl groups possibly from β,1–4 glycosidic linkages of cellulose or phenolic and alcoholic group of lignin (Akhtar et al., 2017). Also, the peak at 2918 cm^{-1}represent the C–H stretching, which suggests that it undergoes apparent breakdown of methyl and methylene groups of cellulose (Xiao et al., 2011). The reduced peak at 1636 cm^{-1} indicates C=O stretching of hemicellulose acetyl groups and/or vibrations of aromatic rings which confirmed the pretreatment induced degradation of lignin and hemicellulose (Sindhu et al., 2013). The peak at 1255 cm^{-1} in untreated rice straw is attributed to C–O plane stretching vibration of aryl group in lignin which expectedly was absent in the spectra of pretreated rice straw (Mandal and Chakrabarty, 2011). The characteristic band at 1045 cm^{-1} is designated to C–O vibration in cellulose and hemicellulose (Akhtar et al., 2016a). These results coherently establish the pretreatment induced alterations in FTIR spectra which were consistent with the findings of enhanced release of reducing sugar (Fig. 6a).

3.8.5. TGA

Thermogravimetric analysis (TGA) was performed to understand the physico-chemical changes in microwave pretreated rice straw. A comparative analysis of pretreated and raw biomass was carried out by plotting the change in weight of the biomass against increasing temperature as shown in the Fig. 6b. The results indicated an initial weight reduction at a temperature below 100 °C, which correspond to the evaporation of the adsorbed moisture. Subsequently, a second weight reduction was detected at the temperature range of 250–365 °C which could be attributed to the disintegration of hemicellulose and cellulose (Sasmal et al., 2012). However, the occurrence of a third weight reduction between 300 and 700 °C is assigned to the degradation of lignin. It was found that the weight reduction was significantly higher in the pretreated biomass when compared to the raw untreated rice straw. This suggests that microwave based pretreatment had disrupted the lignocellulosic biomass significantly and resulted in change in its physico-chemical properties. The evidence of increased weight reduction confirms the impact of the pretreatment which concurs with previous reports for alkaline treated agave bagasse (Ávila-Lara et al., 2015) and sugarcane bagasse treated with dilute acid (Chen et al., 2012).

4. Conclusion

The findings of the present study demonstrated the effect of microwave assisted alkali pretreatment using seawater based reaction medium. The pretreatment conditions were designed using response surface methodology and the coefficient of determination ($R^2 > 0.99$) for the predicted models of both cellulose release and reducing sugar yield implied that the model could account for 99% variation in the observed data. The optimized pretreatment conditions resulted in a cellulose release of 65.43% and reducing sugar yield of 0.554 g/g. Evidences of structural and morphological changes in the biomass confirmed the success of pretreatment which was evident from the increased surface area of the biomass accompanied by the enhanced release of reducing sugars. Further, SEM analysis revealed alterations in surface morphology of the pretreated rice straw and formation of pores on the surface which led to enhanced enzymatic hydrolysis. The feasibility of the optimized model of pretreatment was also tested among other substrates which validated the effectiveness of seawater mediated pretreatment, thereby suggesting a plausible solution to the plight of fresh water usage in biorefineries.

Credit author statement

BD: Conceptualization, Experimentation, Data Formal analysis, Funding acquisition, Writing – original draft preparation. AB: Investigation, Supervising, Writing – original draft preparation; Writing-Reviewing and Editing. BP: Conceptualization, Investigation, Funding acquisition, Writing- Reviewing and Editing, Final approval.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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