Chapter

Enzymatic Saccharification of Canola Straw and Oat Hull Subjected to Microwave-Assisted Alkali Pretreatment

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Abstract

Pretreatment of lignocellulosic biomass is a critical step in removing substrate-specific barriers to the cellulolytic enzyme attack. The study compared the effectiveness of microwave-assisted alkali pretreatment and alkali treatment in the enzymatic saccharification of canola straw and oat hull. Microwave pretreatments were employed by immersing the biomass in dilute alkali solutions (NaOH and KOH) at concentrations of 0, 0.75, and 1.5% (w/v) for microwave-assisted heating times of 6, 12, and 18 min. Alkali treatments were carried out using the same procedure but by soaking and without microwave heating. The highest glucose yields after enzymatic saccharification of both canola straw and oat hull were obtained when these feedstocks were ground using 1.6 mm hammer mill screen size and subjected to microwave-assisted alkali pretreatment using 1.5% and 0.75% NaOH for 18 min, respectively. SEM analysis indicated a more significant modification in the structure of biomass samples subjected to microwave-assisted alkali pretreatment compared to untreated and alkali-treated biomass samples. Results indicated that microwave-assisted alkali pretreatment with short residence time is effective in improving the glucose yield of canola straw and oat hull during enzymatic saccharification.

Keywords: microwave, lignocellulosic biomass, pretreatment, glucose yield

1. Introduction

Lignocellulosic biomass is widely available, abundant at low cost, and a possible source of energy that is estimated to contribute up to 10% to 14% of the global energy supply [1, 2]. Sustainable biofuel and biomass-based transport fuel produced from cellulosic biomass is an energy-dense fuel characterized by lower carbon emissions compared to fossil-based petroleum [3]. Research reports indicated that global biofuels supply since 2000 has increased by a factor of 8% to equal 4% of the transport fuels in 2015 [4, 5]. Furthermore, technologies aimed at converting agricultural biomass into bioethanol and bioproducts are being developed using different techniques [6].

The production of bioethanol from lignocellulosic biomass utilizes biotechnological techniques to convert carbohydrate polymers in biomass into fermentable sugars, which are subsequently used for the production of ethanol and other bioproducts [7]. According to a review by Sarkar et al. [8], there is a transitional shift of energy source by many countries from fossil fuels to renewable sources due to environmental challenges associated with fossil fuels. Liu et al. [1] reported that bioenergy production has the potential to minimize the emission of greenhouse gases (GHG), and governments have used mandates to encourage biofuel production. The total biomass production in Canada was estimated to be 37.3 million metric tons (MT) and is dominated by agricultural and forestry residues. Research on the use of cellulosic biomass from the Canadian agricultural sector to produce energy, including bioethanol, is on-going [1]. However, the economic and environmental sustainability of bioethanol conversion from biomass may be affected by pretreatment efficiency, cost, and enzyme preparation [9, 10].

The production of biofuels is carried out using various feedstocks through thermochemical and biochemical conversion. Pre-processing and biochemical pretreatment increase the accessibility of cellulase enzymes that convert cellulose into glucose [11]. The extent of glucose production is dependent on the type of feedstock used. Several research groups have already examined pretreatment using microwave heating on various feedstocks and have reported suitable glucose yields [12]. However, there is no information on the use of microwave-assisted alkali pretreatment and enzymatic saccharification of oat hull or canola straw.

Lignocellulosic biomass must be pretreated to increase the accessibility of the carbohydrate fraction and modify or partially remove lignin prior to converting the components into final market products such as bioethanol, biopower, and bioproducts [13]. Pretreatment can be carried out in the form of physical, chemical or physico-chemical processes (mechanical, extrusion, alkali, microwave-assisted, organic solvent, and lime treatments) and biological pretreatment methods. Also, combinations of pretreatments have been reported, and many studies are still ongoing [12, 14]. According to Alvira et al. [14], microwave pretreatment technology shows highly increased accessibility of the surface area, cellulose decrystallization, lignin removal, and structural alteration. The method also features low hemicellulose solubilization and minimal production of toxic compounds compared to other pretreatment methods. Microwave-assisted alkali pretreatment penetrates the biomass, thereby causing direct vibration of the molecules and fast heating and break-down of the lignocellulosic cell wall structure [15, 16]. Microwave heating combined with chemical treatment showed high carbohydrate recovery. The yield of carbohydrates upon alkaline pretreatment is dependent on the feedstock used [17, 18]. The study aimed to investigate the effects of microwave-assisted alkali (sodium and potassium hydroxide) pretreatment and alkali treatment alone (no heating) on glucose yield during enzymatic saccharification of canola straw and oat hull. The morphology and structural changes of canola straw and oat hull biomass after pretreatment were analyzed using scanning electron microscopy (SEM).

2. Materials and methods

The experiments were performed in triplicates, and the data reported were expressed in mean values and standard deviation. The lignocellulose composition of the canola straw and oat hull, including their hydrolyzed residues, is expressed on a dry weight basis.

2.1 Feedstock collection and conditioning

Dried canola straw was collected from the black soil zone in Maymont, SK and oat hull was sourced from Richardson Milling Ltd., Martensville, SK and stored at room temperature. The biomass samples ground using a hammer mill (Glen Mills Inc., Clifton, NJ) with screen sizes of 1.6 and 3.2 mm. The physical properties of canola straw and oat hull were reported in Agu et al. [19].

2.2 Alkali pretreatment

Samples of ground canola straw or oat hull (20 g) were mixed with 180 g of NaOH or KOH solutions at concentrations of 0, 0.75, and 1.5% (w/v). Each mixed sample was placed in a 600 ml beaker covered with aluminum foil and incubated at room temperature for soaking times of 6, 12, and 18 min. The process was replicated three times. The moisture of the pretreated samples was determined using ASAE S358.2 [20]. The alkali-treated samples were dried and conditioned, as reported in Agu et al. [19], followed by lignocellulose analysis using the National Renewable Energy Laboratory (NREL) standard [21] and subsequent enzymatic saccharification (see below).

2.3 Microwave-assisted alkali pretreatment

Microwave-assisted alkali pretreatment was carried out on the ground canola straw and oat hull using a microwave oven (Model NNC980W 2450 MHz, Panasonic Canada Ltd., Mississauga, ON). Microwave power was set at 713 W based upon previous experiments [19]. Twenty grams of sample (ground canola straw or oat hull) were mixed in 180 g of NaOH or KOH solutions at concentrations of 0, 0.75, and 1.5% (w/v). The mixture was heated in the microwave oven with constant rotation for heating times of 6, 12, and 18 min. The pretreatment process was replicated three times. After the pretreatment, the sample was dried to 12% w.b. using an air oven at 42 °C; after cooling, the sample was stored in a sealed plastic bag as reported in Iroba et al. [22]. The sample was subjected to composition analysis followed by enzymatic saccharification and glucose yield analysis.

2.4 Enzymatic saccharification

Enzymatic saccharification of microwave pretreated and alkali-treated samples was conducted using cellulase (C2730-50 ml, cellulase from *Trichoderma reesei* ATTC 26921, Sigma-Aldrich Co., St. Louis, MO) and β-glucosidase (C6105-50 ml, Novozyme 188, Sigma-Aldrich Co., St. Louis, MO). Cellulase activity is measured in filter paper units (FPU Eq. (1)) [23]. The products of the enzymatic saccharification were quantified using the total reducing sugar, as reported by Wood et al. [24].

$$\text{FPU / ml} = \left(\frac{A_{540\,Sample}}{A_{540}\ /\ mgstandard} \right) \left(5.55\ \mu mole\ /\ mg \right) \text{x} \left(\frac{1}{60\ \text{min}} \right) \left(\frac{1}{\text{Xml}} \right) \quad (1)$$

where FPU/ml is the determined cellulase activity; A_{540} sample is the absorbance at 540 nm observed for a 1 mg glucose standard; 5.55 μmole/mg equates to the number of μmole of glucose in 1 mg, and X ml (0.02 ml) the volume of cellulase used. According to the NREL protocol [25], the reactions were done and included a 2% sodium azide solution to prevent microbial growth during incubation [26]. A 20 μl aliquot of the reaction products was collected and prepared for the DNS assay in a microplate format [23, 24]. Three replicates of each sample were analyzed.

2.5 Chemical composition analysis

The NREL standard was used to determine the chemical composition of microwave-alkali pretreated and alkali-treated biomass samples [21], and the sample selection was based on canola straw or oat hull pellet quality [19]. The lignin determination process and the calculation of acid-insoluble lignin content of the samples were done using the methods reported in Iroba and Tabil [27] and Sluiter et al. [21]. Briefly, 0.3 g of dried biomass sample was slurried in 72% H_2SO_4 and 4% H_2SO_4, autoclaved, and solids separated by filtration. The solid material was then dried in the air over at 105 °C for 24 h. Acetone was evaporated at room temperature for 3–4 h, and the solids left oven-dried at 105 °C for 24 h. The data was used to calculate acid-insoluble lignin content in the samples [21].

Glucose, xylose, galactose, mannose, and fucose were quantified using the Waters Acquity UPLC – MS system (Acquity 2004–2010, Waters Corp., Milford, MA) according to NREL/TP-510-42618 [21]. Carbohydrate standards were prepared at ~1 mg/ml each and evaluated under the same conditions. Integrated peak areas for each of the monosaccharides were used to evaluate the data and quantify the sugar content [21]. Three replicate measurements were conducted for each sample.

2.6 Statistical analysis

In this study, response surface methodology (RSM) was used to design experiments, build models, and evaluate the effect of factors. User-Defined Design (UDD) was used to study the effect of independent variables (microwave heating time or soaking time and alkali concentration) on the response and factor interactions with different combinations of variables (cellulose, hemicellulose and lignin) [19]. The analysis of variance (ANOVA) or quadratic model was applied to the polynomial ($p < 0.01$ and $p < 0.05$) to evaluate the effect of each independent variable against the responses.

2.7 SEM analysis of biomass sample

Analysis of the ground and pretreated canola straw and oat hull structure was carried out using a JEOL-6010LVscanning electron microscope (JEOL USA Inc., Peabody, MA) at 5 kV. All samples were sputter-coated with a thin layer of gold before imaging at 250 and 500x magnification.

3. Results and discussions

3.1 Chemical composition of biomass samples

Lignocellulosic biomass is composed essentially of cellulose and lignin matrix-bound by hemicellulose. The pretreatment process helps to disintegrate the cell wall structure, allowing enzymes to access the carbohydrate polymers for microbial utilization [7, 28]. The chemical compositions of MW-alkali pretreated and alkali-treated canola straw and oat hull are presented in **Table 1**. MW heating and alkali treatment enhanced the breakdown of the lignin and hemicellulose in alkali solutions [29, 30]. The proportional content of cellulose increased with increasing alkali concentration and microwave pretreatment time, while the lignin content was inversely related to microwave pretreatment time and alkali concentration. Alkali treatment caused an apparent increase in the proportional cellulose content with

Sample	Treatment method	Alkali concentration (%)	Treatment time (min)	Composition[a] (%)		
				Cellulose	Hemicellulose	Lignin
Canola straw 1.6 mm	A	0.75% NaOH	6	79.9 (1.9)	8.5 (7.5)	7.9 (1.4)
	A	0.75% NaOH	18	69.7 (5.1)	6.0 (5.2)	6.6 (4.1)
	A	1.5% KOH	12	69.2 (1.0)	9.8 (8.6)	8.8 (1.2)
	A	1.5% KOH	18	61.3 (16.7)	8.2 (9.0)	8.3 (1.4)
	MWA	1.5% NaOH	18	59.1 (0.5)	9.4 (8.3)	4.3 (1.2)
	MWA	1.5% NaOH	6	37.8 (3.1)	7.2 (6.5)	4.7 (0.6)
	MWA	0.75% KOH	12	53.6 (9.2)	10.6 (9.2)	5.8 (0.3)
	MWA	1.5% KOH	6	56.9 (17.0)	7.7 (9.0)	4.6 (0.5)
Oat hull 1.6 mm	A	0.75% NaOH	18	67.9 (23.1)	14.4 (14.6)	11.5 (0.8)
	A	1.5% NaOH	12	62.4 (0.1)	21.4 (23.6)	11.2 (2.7)
	A	0.75% KOH	6	64.7 (1.4)	12.0 (11.0)	10.9 (2.1)
	A	0.75% KOH	12	37.0 (18.8)	10.3 (10.6)	13.4 (1.8)
	MWA	0.75% NaOH	18	42.8 (11.3)	15.6 (13.8)	6.3 (1.0)
	MWA	1.5% NaOH	18	37.1 (8.5)	14.3 (12.6)	4.2 (1.2)
	MWA	1.5% KOH	18	56.4 (17.9)	16.0 (13.8)	4.8 (0.9)
	MWA	1.5% KOH	6	41.8 (14.0)	12.9 (11.5)	5.7 (1.6)
Canola straw 3.2 mm	A	1.5% NaOH	6	54.1 (6.7)	10.4 (11.2)	9.2 (1.6)
	A	1.5% NaOH	18	82.2 (3.9)	7.3 (7.0)	6.9 (3.0)
	A	0.75% KOH	6	68.1 (8.4)	9.2 (9.1)	9.1 (0.9)
	A	1.5% KOH	6	46.6 (1.5)	9.7 (8.4)	8.2 (1.2)
	MWA	0.75% NaOH	12	54.2 (2.3)	6.7 (5.8)	5.1 (0.6)
	MWA	0.75% NaOH	6	38.2 (2.7)	8.7 (7.5)	5.3 (0.3)
	MWA	0.75% KOH	12	30.8 (2.9)	13.8 (13.0)	5.0 (1.6)
	MWA	1.5% KOH	6	63.4 (35.0)	10.3 (9.2)	4.4 (0.5)
Oat hull 3.2 mm	A	0	6	41.5 (2.1)	9.5 (8.4)	11.7 (1.3)
	A	1.5% NaOH	6	66.9 (8.3)	20.1 (17.4)	9.8 (3.4)
	A	1.5% NaOH	18	57.0 (3.5)	14.1 (13.2)	11.8 (0.6)
	A	0.75% KOH	12	57.2 (17.2)	24.8 (23.7)	13.7 (2.3)
	MWA	0.75% NaOH	6	22.7 (11.0)	12.9 (14.4)	6.8 (2.2)
	MWA	1.5% NaOH	18	48.7 (8.3)	14.4 (13.3)	5.1 (0.8)
	MWA	0.75% KOH	12	47.9 (18.2)	16.0 (16.0)	5.4 (0.6)
	MWA	1.5% KOH	18	62.6 (2.0)	10.4 (18.0)	6.4 (1.3)

A: Alkali treatment; MWA: Microwave-assisted alkali pretreatment.
[a]Mean ± standard deviation of three replicates.

Table 1.
Chemical composition (% dry basis) of alkali treatment and microwave-assisted alkali pretreatment of canola straw and oat hull 1.6 mm screen size.

decreasing alkali concentration and lower soaking times, while the hemicellulose and lignin contents decreased with longer soaking times and decreasing alkali concentrations. The lignocellulosic changes suggest that there is a breakdown in the lignin structure, which enhances surface area accessibility for enzyme utilization

[19, 28–31]. The solubilization of lignin in microwave alkali pretreated canola straw and oat hull samples were lower than the corresponding alkali-treated biomass. The decrease in lignin content suggests that the aqueous alkaline solution solubilized the lignin. The apparent increase in cellulose content is likely explained by the solubilization of other cell wall components in the alkali solution. The microwave alkali pretreatment was more effective in solubilizing hemicellulose and lignin in canola straw compared to oat hull samples. Hence, similar results were observed with alkali treatment. MW-assisted alkali pretreatment of canola straw and oat hull resulted in higher solubilization of cellulose, along with a decrease in lignin and hemicellulose. The MW heating pretreatment results support structure breaking reactions [19]. Singh and Bishnoi [7] observed a similar effect when optimizing MW alkali pretreated wheat straw for ethanol production. Canola straw samples showed higher solubilization with the alkali solutions compared to oat hull in MW-assisted alkali pretreatment and alkali treatment processes. Furthermore, the NaOH solution was more effective in MW-assisted alkali pretreatment and alkali treatment of canola straw and oat hull.

3.2 Estimation of the chemical composition of biomass

Results obtained showed the highest lignocellulosic solubilization from canola straw samples under both treatment conditions. The response variable obtained was expressed as a function of independent variables reported by Agu et al. [19]. The ANOVA p-value and R^2 results of the quadratic models suggested that the models were significant in both samples (**Tables 2** and **3**). The independent variables had a significant influence ($p < 0.05$) on the solubilization of cellulose, hemicellulose and lignin in both samples. The highest R^2 values 0.899 and 0.868 for cellulose, 0.883 and 0.882 for hemicellulose, and 0.876 and 0.817 for lignin demonstrate the accuracy of the model. The high R^2 values suggested better precision and reliability of the experimental values obtained [32, 33]. The interaction effect of variables on cellulose, hemicellulose, and lignin were studied by plotting three-dimensional surface curves to depict levels of interaction of each variable for maximum response. Representative surface plots showing the interaction of a pair of factors on the chemical composition of MW-assisted alkali pretreated and alkali treated canola straw and oat hull are given in **Figures 1** and **2a, b**.

Sample/screen size (mm)	MW-alkali pretreatment	Parameters	C	H	L
Canola straw 1.6 mm	NaOH	Model	0.030	0.033	0.024
		Alkali conc.	0.052	0.024	0.035
		MW time	0.021	0.039	0.041
		R-Square	0.868	0.852	0.805
Oat hull 3.2 mm	NaOH	Model	0.049	0.026	0.045
		Alkali conc.	0.011	0.049	0.040
		MW time	0.033	0.048	0.042
		R-Square	0.838	0.882	0.817

C: Cellulose; H: Hemicellulose; L: Lignin; Alkali conc.: Alkali concentration.

Table 2.
ANOVA P-value and R^2 for the response surface methodology quadratic model for the chemical composition of MW-assisted alkali pretreated canola straw and oat hull.

Sample/screen size (mm)	MW-alkali pretreatment	Parameters	C	H	L
Canola straw 1.6 mm	KOH	Model	0.016	0.018	0.014
		Alkali conc.	0.025	0.028	0.023
		Soaking time	0.059	0.051	0.053
		R-Square	0.883	0.853	0.865
Canola straw 3.2 mm	KOH	Model	0.025	0.012	0.016
		Alkali conc.	0.016	0.045	0.038
		Soaking time	0.038	0.018	0.025
		R-Square	0.899	0.883	0.856
Oat hull 3.2 mm	NaOH	Model	0.039	0.038	0.039
		Alkali conc.	0.047	0.043	0.046
		Soaking time	0.012	0.013	0.013
		R-Square	0.875	0.878	0.876

C: Cellulose; H: Hemicellulose; L: Lignin; Alkali conc.: Alkali concentration.

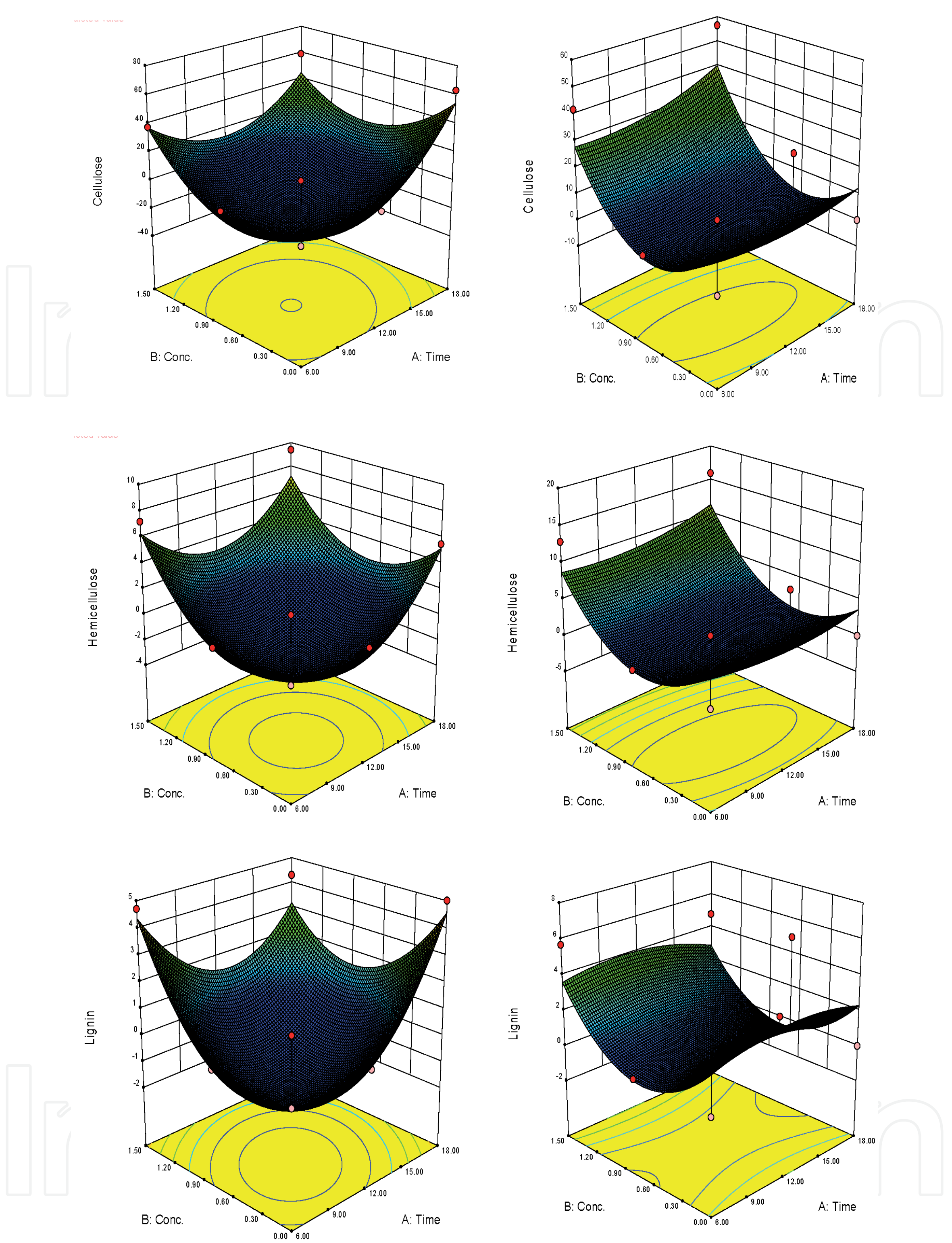
Table 3.
ANOVA P-value and R^2 for the response surface methodology quadratic model for the chemical composition of alkali-treated canola straw and oat hull.

3.3 Effect of interaction on response variables

The response surface plots in **Figure 1a** and **b** show the effect of the interactions between MW heating time and alkali concentration on cellulose, hemicellulose, and lignin of canola straw and oat hull pretreated in NaOH and KOH solutions. The plots show that there were notable interactions among the variables in both samples. Increasing the alkali concentration and MW heating time showed higher solubilization of cellulose, hemicellulose, and lignin contents in canola straw and oat hull. The response surface plots in **Figure 2a** and **b** show the effect of the interactions between soaking time and alkali concentration on chemical cellulose, hemicellulose, and lignin of canola straw and oat hull soaked in NaOH and KOH solutions. The 3D response surface plots of the samples show different shapes of interactions when compared with MW pretreated samples. The interaction effect between soaking time and alkali concentration on both samples significantly influenced the response variables. Decreasing the alkali concentration and soaking time increased the proportional content of cellulose. The hemicellulose and lignin contents plots resulted in longer soaking times and decreased alkali concentrations. Generally, the interaction plot curves of canola straw and oat hull samples significantly influenced the hemicellulose and lignin contents regardless of the alkaline concentration, MW heating or soaking time.

3.4 Glucose yield

Microwave-assisted pretreatment was investigated due to its rapid heating efficiency in disintegrating the ultrastructure of cellulose. Several studies have combined the technique with alkali pretreatment. The results showed an accelerated chemical reaction rate in lignin removal and partial degradation of hemicellulose depending on the type of feedstock used [34]. NaOH and KOH at various concentrations with MW heating of canola straw and oat hull at different heating and soaking times were investigated. The samples pretreated with MW-assisted alkali and alkali



a.   b.

Figure 1.
Surface plot of the effects of alkali concentration and MW heating time on chemical composition. (a) canola straw (NaOH) and (b) oat hull (KOH).

were subjected to cellulase hydrolysis to convert cellulose to glucose. The data shown in **Figures 3** and **4** indicate that the microwave-assisted alkali pretreated samples yielded a higher level of reducing sugars compared to alkali-treated samples. **Figure 3** shows the highest glucose yield (110.05 mg/g for one-gram canola straw) obtained after pretreatment with 1.5% NaOH concentration for 18 min. The glucose yield (96.77 and 110.05 mg/g for one-gram canola straw) increased after 6 and 12 min of pretreatment, respectively. In alkali treatment, the glucose yields recorded

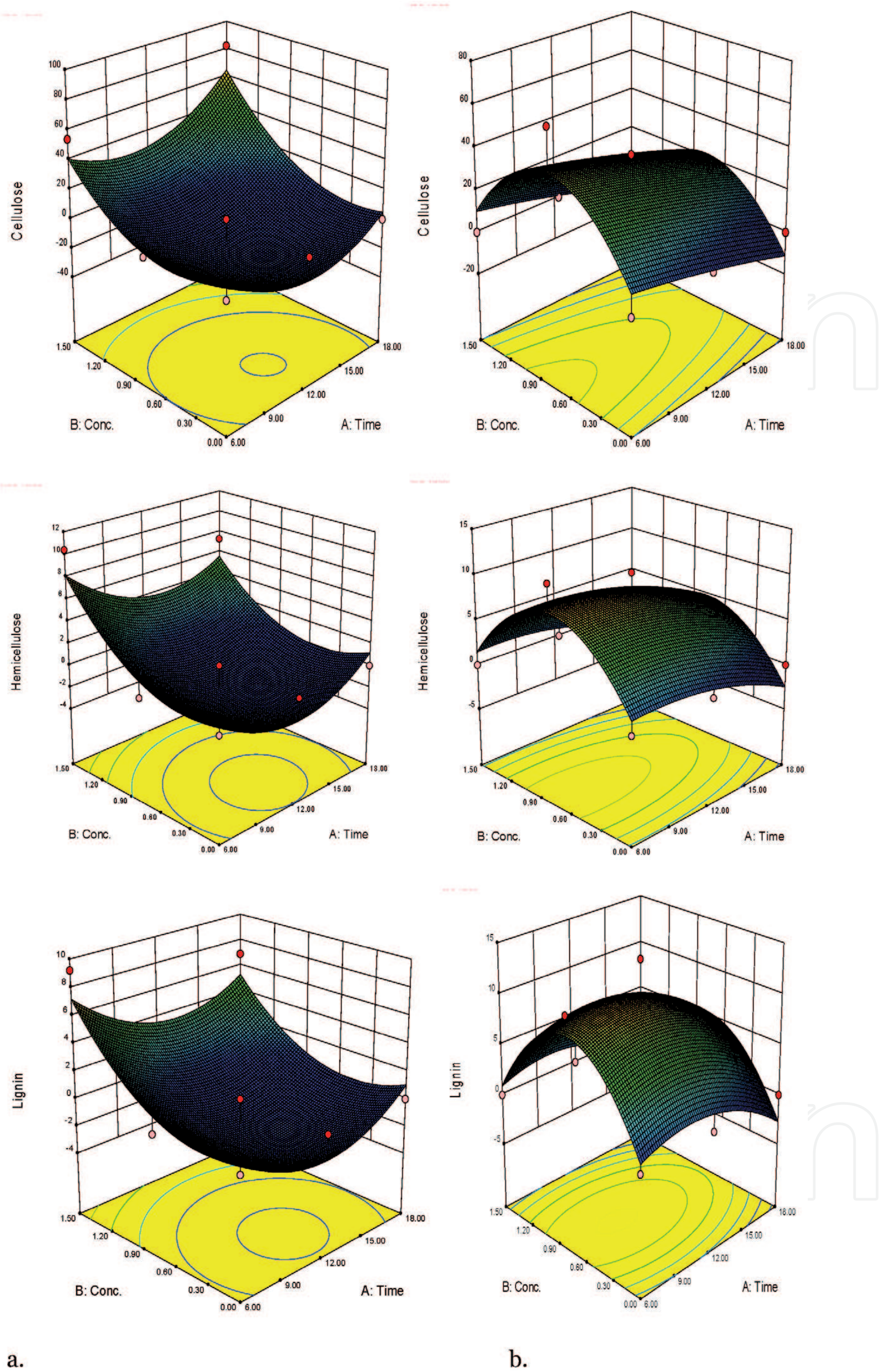


Figure 2.
Surface plot of the effects of alkali concentration and soaking time on chemical composition. (a) canola straw (NaOH) and (b) oat hull (KOH).

similar results after treatments with 0.75% and 1.5% NaOH and KOH concentrations with longer soaking time for canola straw ground using 1.6 mm screen size.

Figure 4 shows the highest glucose yield (99.10 mg/g for one-gram oat hull sample) obtained after pretreatment with 0.75% NaOH concentration for 18 min. Extending the residence time from 6 to 18 min increased the sugar yields in

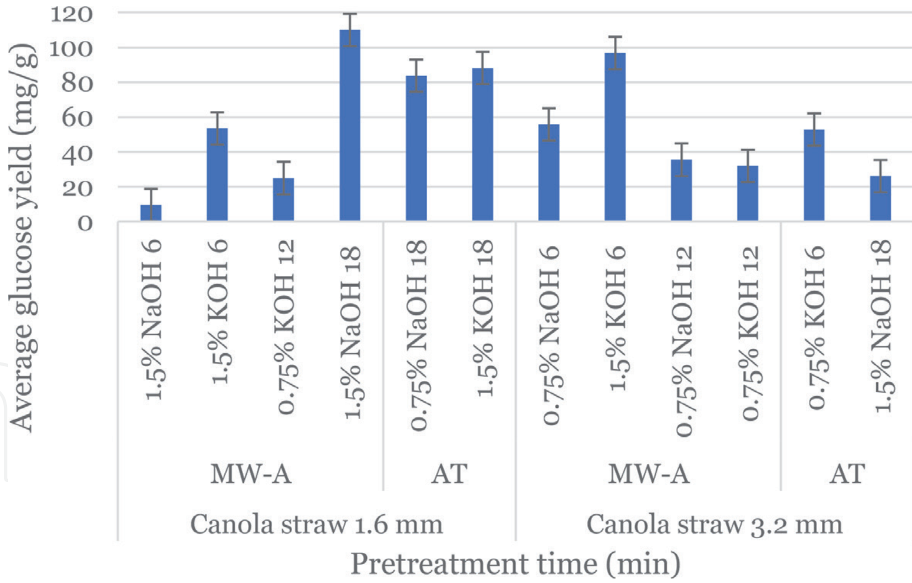


Figure 3.
Glucose production from enzymatic saccharification of microwave-assisted alkali pretreated and alkali treated ground canola straw. MW-A: Microwave-assisted alkali pretreated; AT: Alkali treated.

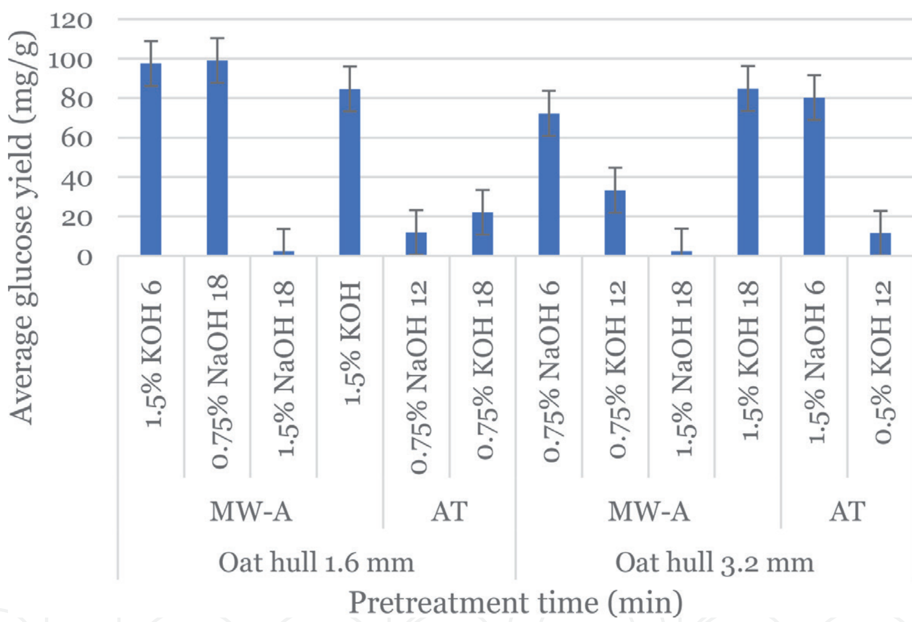


Figure 4.
Glucose production from enzymatic saccharification of microwave-assisted alkali pretreated and alkali treated ground oat hull. MW-A: Microwave-assisted alkali pretreated; AT: Alkali treated.

treatments using 0.75% NaOH and in both ground samples. Similar results were reported in Agu et al. [19] using MW-assisted alkali and distilled water pretreated canola straw and oat hull biomass. For alkali-treated samples, a shorter soaking time with 1.5% NaOH concentration resulted in high glucose yield in samples ground with a 3.2 mm screen size. Aguilar-Reynosa et al. [15] studied MW-assisted pretreatment of corn stover and reported the glucose yields achieved at reduced residence time (10–50 min). On the other hand, Rodrigues et al. [34] studied cashew apple bagasse MW-assisted pretreatment, and Hu and We [35] reported on switchgrass pointing out that glucose yield in both studies increased with increasing alkali concentration and with pretreatment time. To further evaluate the pretreatment alkali reagents, total glucose yields and glucose digestion from the enzymatic saccharification of pretreated biomass substrates compared with alkali-treated biomass substrates are given in **Figures 5** and **6**. Overall glucose yields of microwave

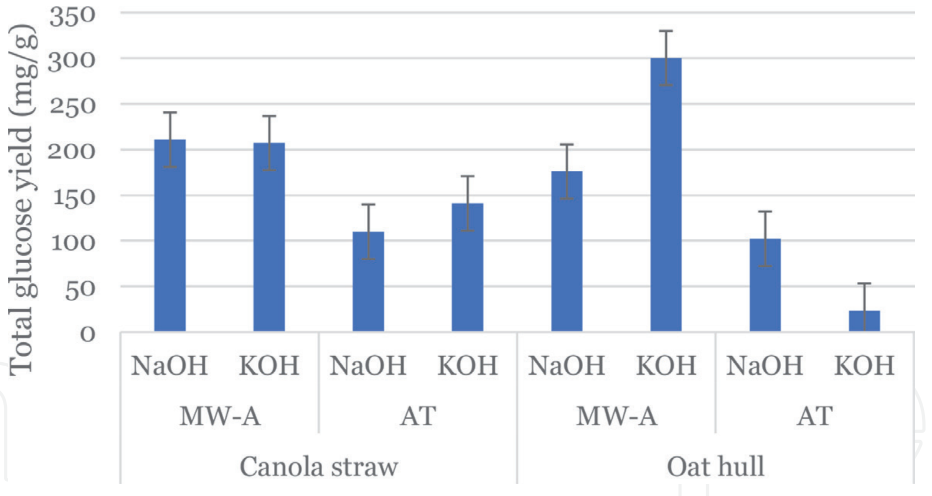


Figure 5.
Glucose production from enzymatic saccharification of microwave-assisted alkali pretreated and alkali treated canola straw and oat hull. MW-A: Microwave-assisted alkali pretreated; AT: Alkali treated.

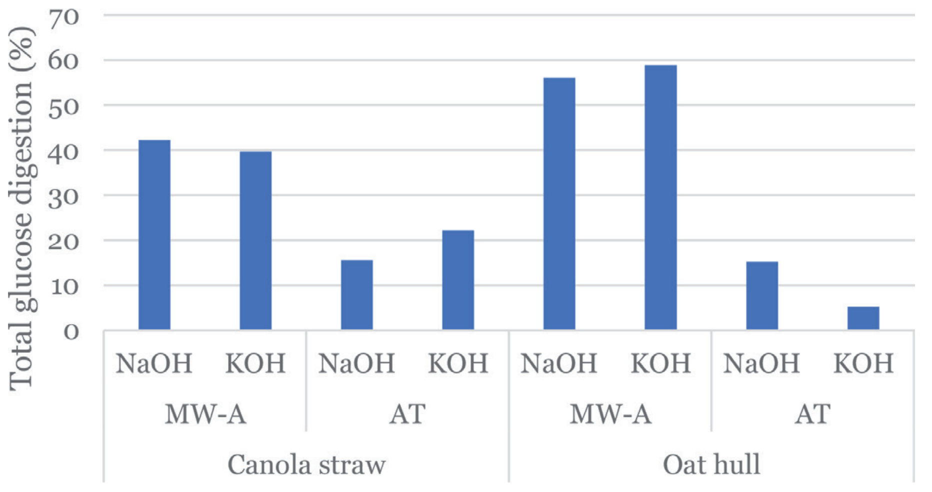


Figure 6.
Glucose digestion from enzymatic saccharification of microwave-assisted alkali pretreated and alkali treated canola straw and oat hull. MW-A: Microwave-assisted alkali pretreated; AT: Alkali treated.

pretreated canola straw with NaOH and KOH pretreatments were 210.75 and 207.07 mg/g, and oat hull total glucose yields with NaOH and KOH pretreatments were 175.96 and 300.30 mg/g. These yields are substantially higher than the yield from alkali-treated canola straw and oat hull with NaOH and KOH pretreatments (109.88 and 140.91, and 102.28 and 23.47 mg/g). For both feedstocks, total glucose yield and digestion overall were higher with KOH pretreatments over pretreatment with NaOH. A similar result was obtained with alkali treatments.

3.5 Scanning electron microscopy analysis

Figures 7 and **8(a-c)** show the SEM images for the cross-sectional area of untreated ground, alkali-treated, and MW-assisted alkali pretreated canola straw and oat hull biomass at 250x and 500x magnifications, respectively. It was observed that both untreated ground canola straw and oat hull samples showed undamaged surfaces, smooth and no cracks (**Figures 7a** and **8a**). Alkali-treated samples in **Figures 7(b)** and **8(b)** show many induced physical changes on the surfaces of the biomass. Soaking of canola straw and oat hull in alkali solution caused breakage and cracks of the cell walls, and slight erosion of micro-fibrils, especially on oat hull samples. MW-assisted alkali pretreated samples in **Figure 7(c)** shows detached fibres with an altered fibrillar structure of the

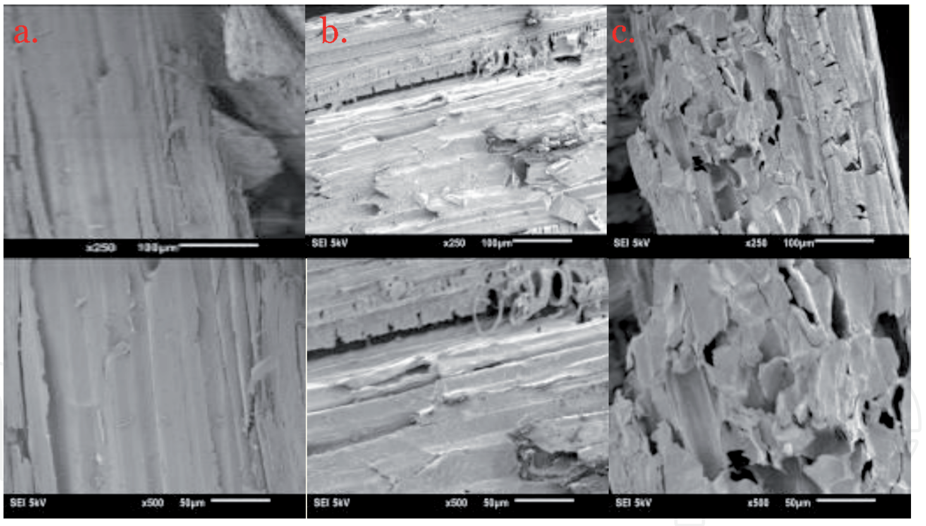


Figure 7.
SEM images of canola straw at magnifications 250 and 500x. (a) untreated sample; (b) alkali pretreated; (c) microwave-assisted alkali pretreated.

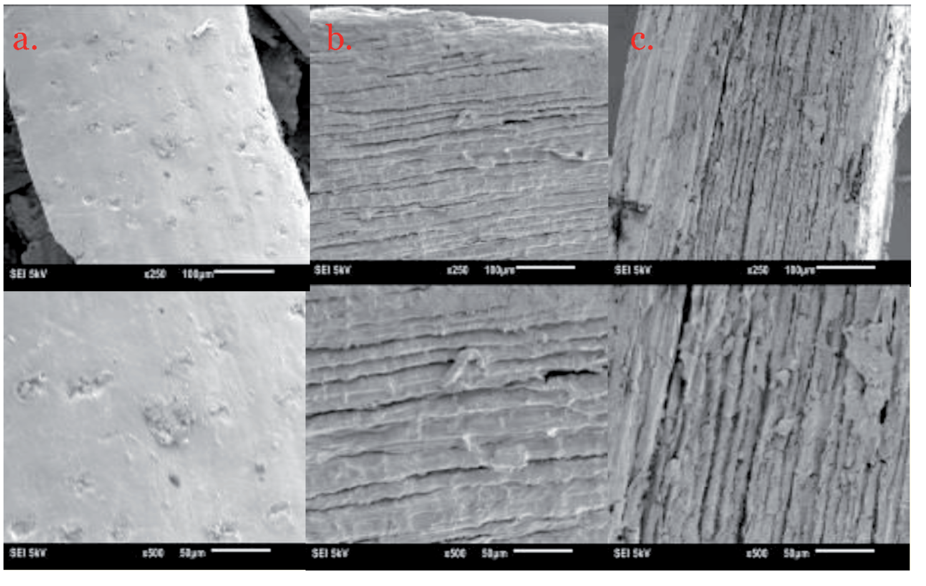


Figure 8.
SEM images of oat hull at magnifications 250 and 500x. (a) untreated sample; (b) alkali pretreated; (c) microwave-assisted alkali pretreated.

distorted cell walls for canola straw samples, and **Figure 8(c)** for the oat hull sample shows porous development on surfaces. Hence, the SEM images revealed the disruptive effects of the MW-assisted alkali pretreatment and alkali treatment (no heating) on the surface of the samples, which subsequently changed the canola straw and oat hull compositions. These observations were concordant with the positive effects of microwave-assisted alkali pretreatment on the improved enzymatic digestibility of canola straw and oat hull [19, 30, 31]. Furthermore, the chemical treatment using alkali solutions (NaOH/KOH) developed deep cracks on the biomass, increasing the surface area to facilitate lignocellulose disruption, a prerequisite to improving enzymatic reactions. Similar observations have been reported in various studies [36, 37].

4. Conclusions

This study showed that microwave-assisted alkali pretreatment of canola straw and oat hull enhanced the enzymatic digestibility of these substrates compared to alkali pretreatment alone. MW-assisted alkali pretreatment and alkali treatment methods were effective in disrupting the lignocellulose structure of the biomass by inducing changes in their chemical compositions. The MW-assisted alkali pretreatment of biomass increased the glucose yields upon enzymatic saccharification. Total glucose yield overall was higher with KOH pre-treatment compared to pre-treatment with NaOH in both feedstocks. Therefore, based on the results presented, the MW-assisted alkali pretreatment was an efficient pretreatment method of canola straw or oat hull substrate for bioethanol production. Subsequently, treatment variables of MW-assisted alkali pretreatment will be optimized to improve glucose digestibility in the future.

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Conflict of interest

The authors declare no conflict of interest.

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