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Effect of microwave pretreatment on lignocellulosic degradation of corn cob

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Abstract

Lignocellulosic biomass is a widespread and renewable alternative to fossil resources from which valuable products such as biofuels and chemicals can be obtained. The usual biotechnological way of lignocellulose processing involves pre-treatment of biomass and enzymatic hydrolysis of complex carbohydrates (cellulose and hemicellulose) to simple sugars. The aim of this study was to examine the effect of microwave pretreatment on lignocellulosic degradation of corn cob. Biomass will be treated in a microwave oven (Bosch Serie 2, Germany) using hydrogen peroxide, under different conditions (pH and treatment time). After treatment, the solid fraction was subjected to enzymatic hydrolysis using the commercial enzyme CellicCTec2 (Novozymes, Denmark). The effect of the treatment was determined by determining the surface area of cellulose and lignin (based on the equation of Langmuir adsorption isotherm for adsorption of Congo red and Azure B, respectively), and the crystallinity index (iodine adsorption method). Enzymatic hydrolysis of biomass was monitored using spectrophotometric methods to determine the concentration of reducing sugars (DNS method), total hexose sugars (Antron method) and total pentose sugars (Orcinol method). The results of the treated samples were compared with the untreated ones in order to determine the most efficient treatment conditions for the degradation of lignocellulosic biomass.

Keywords: pretreatment; hydrolysis; lignocellulose, reducing sugars; treatment condition

1. Introduction

Plant derived material or biomass is the most abundant and renewable material on the earth used as a source of fuel for numerous reasons. When biomass is converted to renewable fuels, it is in effect of carbon neutralization because utilization of the carbon dioxide produced during such fuel production and consumption is neutralized by the subsequent utilization of the same quantity of carbon dioxide via the photosynthetic generation of plant material during growth cycles [1].

Geographically there are many types of plant materials that can be managed wisely and used as a hydrocarbon fuels source, eradicating the necessity for long distance fuel transport. Most of these types of biomass are naturally heterogeneous, specifically lignocellulosic biomass [2]. Lignocellulose biomass feedstocks for energy production can be categorized into four groups: hard wood, softwood, agricultural wastes and grasses.

These categories of lignocellulose are supplied by agricultural, industrial and forestry sectors. Globally agricultural sectors produce large amounts of residues in billions of tons each year. However, the majority of these residues are discarded in landfills or burned which results in no contribution to any economic benefit to the industry and not completely utilizing the biomass in spite of its abundance in mass. Having said that, lignocellulosic wastes produced from agriculture and food processing used as feedstock are promising as second generation of sustainable biorefineries. Biomass feedstocks from agriculture waste and forest residues are comparatively of lower cost than industrial waste [3, 4].

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Corn cob is one of the sources of plant biomass produced from agricultural waste generated from maize and remains part of the ear on which the kernel grows. Corn cob is known to be a potential energy resource that can be used in gasification systems for energy production, and its advantages outweigh other biomass feedstocks, not excluding its dense and even nature as well as its high energy content, low sulfur and nitrogen concentration [5,6]. Corn cob is mainly composed of cellulose, hemicellulose and lignin [7]. These polysaccharides are polymers of sugar, which are converted into fermentable sugars, making it easier for enzymatic hydrolysis to separate the lignin and hemicellulose structures to free the concealed cellulose [8, 9].

Pretreatment steps are the most critical part of biomass utilization prior to conversion due to the intricate structure and recalcitrant nature of the lignocellulose biomass. For a biomass resource to be considered 'efficient', the pretreatments should not be complicated but rather should be easy, cost effective, and economically feasible. Indeed, the process should not generate inhibitory compounds or loss in the portion of interest [10].

Currently research on pretreatment is focused on identifying, assessing, developing, and demonstrating promising methods that mainly support the subsequent enzymatic hydrolysis of treated biomass with lower dosages of enzyme and shorter bioconversion times. In the last few decades, a vast number of pretreatment approaches have been investigated on an extensive variety of feedstocks types. This has generated a wealth of new publications that offer a general impression of the field [11, 12, 13, 14].

Pretreatment methods must be carefully selected to suit the type of lignocellulosic biomass because the physicochemical properties of the lignocellulose material differ. The choice of pretreatment will have a huge impact on subsequent phases in the conversion process in terms of cellulose digestibility, production of toxic compounds, potentially inhibitory for yeast, stirring, power requirement, energy demands in the downstream process and waste-water demands [15].

Consequently, the aim of this research work was to investigate the effect of microwave pretreatment on lignocellulosic degradation on corn cob at different process conditions, followed by enzymatic hydrolysis of cellulosic material from biomass using CellicCTec2 commercial enzyme which was monitored by spectrophotometric methods to determine the concentration of reducing sugars, total hexose sugars and total pentose sugars. Also, the effect of the treatment was evaluated by determining the surface area of cellulose, lignin and crystallinity index. The results of the treated samples were compared with the untreated samples to determine the most effective treatment conditions for degradation of the lignocellulose biomass.

2. Materials and Methods

2.1. Sample collection and preparation

Corn cob was obtained from a local farm in Vojvodina province, Serbia. It was grounded and sieved and a fraction with particle size of 400 μm-1 mm was used in experiments. Composition, % of dry matter: cellulose 43.74 %, hemicellulose 43.29 %, lignin (as acid detergent lignin) 5.83 %.

2.2. Methods

2.2.1. Pretreatments of biomass (corn cob)

The biomass was pretreated to break down the lignin structure and disrupt the crystalline structure of cellulose.

2.2.2. Microwave pretreatment of biomass with hydrogen peroxide (H_2O_2)

3 grams of the biomass was weighed in three (3) separate bottles and 120ml of 3% hydrogen peroxide (H_2O_2) solution was added. The mixtures were treated in the microwave oven at different treatment time intervals (30 s, 60 s and 120 s) with power of 360 W. The solutions of each sample were filtered to separate the solid (residues) fraction from the liquid fraction. The residue of each sample was put into a Petri dish and dry at a temperature of 90 ℃ for 12 hours. The moisture content of the dry samples was determined with a moisture analyzer.

2.2.3. Microwave pretreatment of biomass with alkaline H_2O_2

3 grams of the biomass was weighed in two (2) separate bottles and 120 ml of 3 % alkaline hydrogen peroxide (H_2O_2) solution which pH was adjusted to 11.5 was added. The mixtures were treated in a microwave oven for 30 s and 60 s, longer treatment time (for 120 s) was not performed because of sample boiling and difficulties to control the

temperature inside the oven. The solutions of each sample were filtered to separate the solid (residues) fraction from the liquid fraction. The residue of each sample was put into a Petri dish and dry at a temperature of 90 ℃ for 12 hours. The moisture content of the dry samples was determined with a moisture analyzer.

2.3. Determination of enzymatic hydrolysis of treated and untreated biomass with CellicCTec2

0.6 grams of samples (Untreated, Alkaline H_2O_2 30 s, Alkaline H_2O_2 60 s, H_2O_2 30 s, H_2O_2 60 s, H_2O_2 120 s) were measured into a conical flask and 9 ml of buffer (citrate) was added. After that, 30 µl of CellicCTec2 was added to the solution and incubated for 2 days at 50 ℃. The samples were taken from the incubator and centrifuge in an Eppendorf tube and the supernatants were collected to determine sugar concentrations (reducing sugars, hexoses and pentoses).

2.3.1. Determination of reducing sugar concentration in obtained hydrolysates by dinitro salicylic acid (DNS) method

Reducing sugars have the property to reduce many of the reagents. One such reagent is 3,5-dinitrosalicylic acid (DNS). 3,5-DNS in alkaline solution is reduced to 3-amino-5-nitro salicylic acid. The sample is diluted with distilled water depending on the expected sugar concentration, then 1.0 ml of the sample is added to 1.0 ml of DNS reagent. A blank is prepared the same way using distilled water instead of a sample. The reaction is conducted with heating at 90 °C in a water bath for 5-15 min. Afterward, 333 µl of sodium potassium tartrate is added to the hot solution and mixed well. The solution was cooled to room temperature before its absorbance was measured at a wavelength of 505 nm because absorbance is sensitive to temperature. The concentration of reducing sugar expressed as glucose is calculated using the equation of calibration curve. The calibration curve is constructed by measuring the absorbances of standard glucose solutions (0.05-0.4 g/l) at 505 nm [15]. Figure 1 presents the calibration curve for estimation of reducing sugars with DNS at 505 nm.

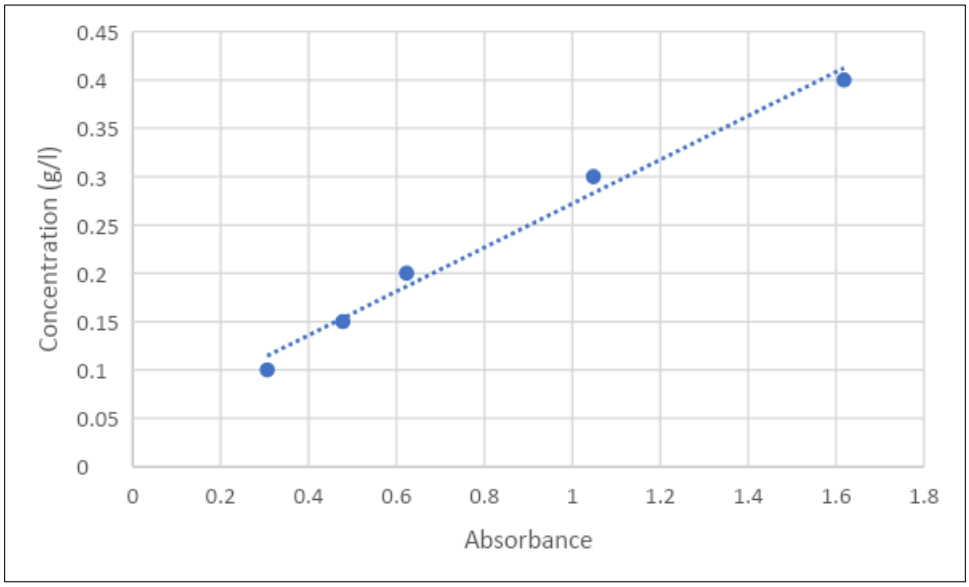

Figure 1 Calibration curve for estimation of reducing sugars with DNS at 505 nm

2.3.2. Determination of total hexoses concentrations by anthrone assay

200 µl of the sugar solution of the samples were diluted with water in a test-tube depending on the expected sugar concentrations and 0.2 % of anthrone (2.0 ml) in conc. H_2SO_4 was added, mixed well and heated in a boiling water bath for 5 mins, allowed to cool, vortex again and its absorbance were measured at a wavelength of 620 nm and 660 nm. The standard curves for hexoses were plotted using the absorbances of standard glucose solution [16]. The standard curve is presented in Figure 2.

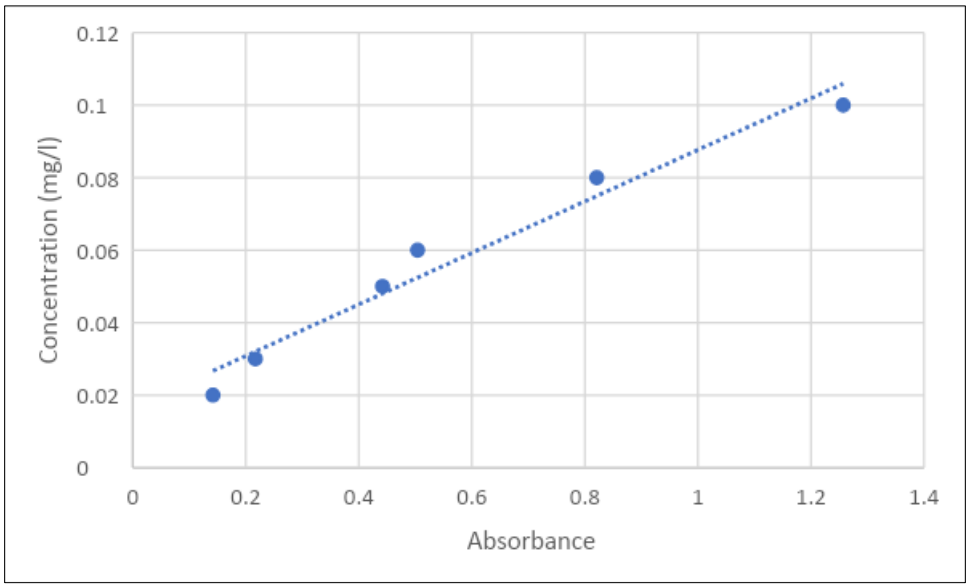

Figure 2 Calibration curve for estimation of total hexoses at 620 nm

2.3.3. Determination of total pentoses concentrations by orcinol assay

200 µl of the sugar solution of the samples were diluted with water in a test-tube depending on the expected sugar concentrations and 6 % of orcinol (134 µl) in 95 % ethanol was added, followed by 2 ml or 200 µl of $FeCl_3$ in concentrated HCl, mixed well and heated in a boiling water bath for 20 mins, allowed to cool, vortex again and its absorbances were measured at a wavelength of 665 nm. The standard curves for pentoses were plotted using the absorbances of standard xylose solutions [16], and presented in Figure 3.

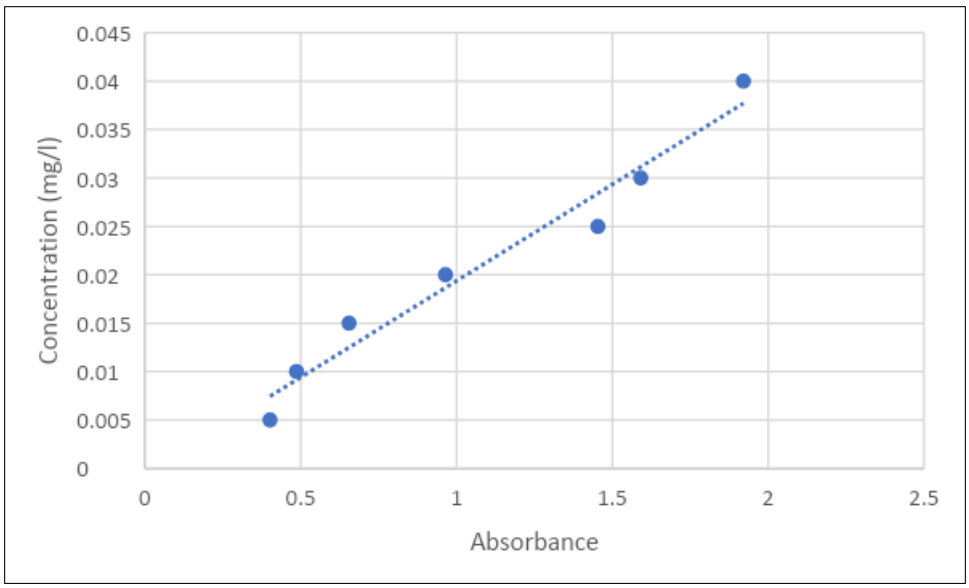

Figure 3 Calibration curve for estimation of pentoses at 665 nm

2.3.4. Sugar yield percentage (%)

After the determination of reducing sugars, total hexoses and pentoses concentrations, the corresponding sugar yields were calculated as follows:

$$\text{Reducing sugar yield (\%)} = \frac{amount\ of\ reducing\ sugar\ produced\ after\ enzymatic\ hydrolysis\ (\frac{g}{l})\ x\ 0.9\ x\ 100}{amount\ of\ hemicellulose\ (g) + amount\ of\ cellulose\ (g)\ in\ sample}$$

$$\text{Hexose yield (\%)} = \frac{amount\ of\ total\ hexoses\ produced\ after\ enzymatic\ hydrolysis\ (\frac{g}{l})\ x\ 0.9\ x100}{amount\ of\ cellulose\ (g)+amount\ hemicellulose\ (g)\ in\ sample}$$

$$\text{Pentose yield (\%)} = \frac{amount\ of\ total\ pentoses\ produced\ after\ enzymatic\ hydrolysis\ (\frac{g}{l})\ x\ 0.88\ x100}{amount\ of\ hemicellulose(g)\ in\ sample}$$

2.4. Determination of physical characteristics of untreated and treated samples

In this part of the experiment the physical characteristics such as surface area and crystallinity are determined.

For determination of crystallinity, 0.3 g of the treated and untreated samples without moisture was weighed into a conical flask and 2 ml of KI_3 solution was added and left for 3 mins, after which 100 ml saturated sodium sulfate was added and stirred. The solution was incubated in the darkness for an hour and the solid was separated from the liquid. 50 ml of the liquid (solution) was measured into another conical flask and diluted with 50 ml distilled water and used for titration. The iodine concentration of the samples and blank was determined by titration with 0.2 sodium thiosulphate until a yellow color was obtained and 1% of starch solution (indicator) was added and titration continued till the endpoint was obtained. The iodine sorption values (ISV) in mg I_2 per g of the sample was calculated as follows:

$$\text{ISV (mg/g)} = \frac{(V_b - V_s)\times 2{,}04\times 2{,}54}{m\times(1-\frac{w}{100})}$$

V_b = volume (ml) of $Na_2S_2O_3$ solution for blank titration

V_s = volume (ml) of $Na_2S_2O_3$ solution for the titration of sample solution

m = weight of the sample (g)

w = moisture content (%) of the sample

According to Schwertassek, absorption takes place in the amorphous phase. A ratio of ISV per g cellulose to 412 (mg iodine absorbed per 1 g of methylcellulose) determines the amorphous fraction. The crystallinity index (CrI) was calculated using the following equation:

$$\text{CrI (\%)} = 100 - (\frac{ISV}{412} \times 100)$$ [17].

2.4.1. Determination of surface area of cellulose and lignin by dye adsorption

Langmuir adsorption isotherm was used to determine the amount of adsorbed dye molecules on the surface of the adsorbent (biomass) at a given time. Initially, the isotherm proposed by Langmuir in 1918 was generally appropriate for describing the chemisorption process when ionic or covalent chemical bonds are formed between the adsorbent and the adsorbate, the equation is obeyed in many systems with moderately low coverage and can be easily extended to describe the behavior of the binary adsorption system. Also, the Langmuir was based on the assumption that:

- The adsorption consists entirely of a monolayer at the surface.
- There is no interaction between molecules on different sites and each site can hold only one adsorbed molecule.
- The heat of adsorption does not depend on the number of sites and is equal for all sites.

The Langmuir adsorption isotherm is of limited application since for real surfaces the energy is not the same for all sites and interactions between adsorbed molecules cannot be ignored. The surface areas of cellulose and lignin in treated and non-treated biomass were determined by dye adsorption methods, using Congo Red and Azure B which are specific for dyeing cellulose and lignin, respectively. The solid loading in dye adsorption experiments for cellulose was 0.7% (w/v) and for lignin 0.2% (w/v).

2.4.2. Congo Red adsorption for determination of cellulose surface area

35 mg of untreated and treated biomass were measured into conical flask and dissolved in 5ml solution of the Congo Red dye in 0.03 M phosphate buffer (pH = 6) with 1.4 Mm NaCl and with the following dye concentration of 2, 1.5, 1, 0.75, 0.5, 0.25, and 0.1 mg/ml. All the samples were incubated in a shaking incubator at 60 °C and 200 rpm for 24 hrs to reach the adsorption equilibrium. After the incubation, the supernatants were collected by centrifugation (8000 ×g for 5 min) and the concentration of the unabsorbed Congo Red was measured at a wavelength of 498 nm. The initial stock

solution of Congo Red was prepared and diluted to the following concentrations (10, 20, 30, 40, 70, and 90 mg/l) to get a standard equation for the calibration curve as presented in Figure 4. [18].

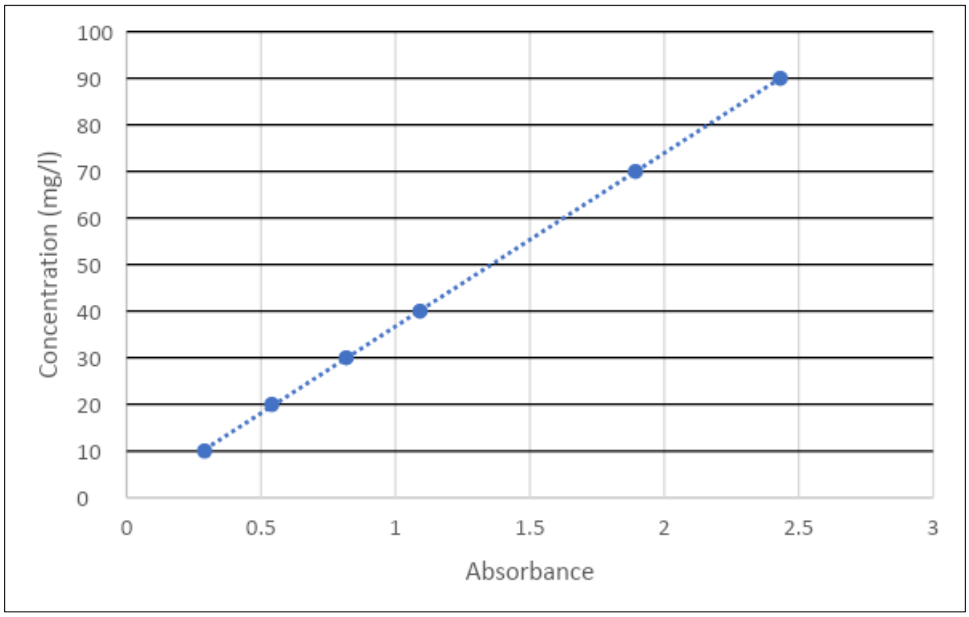

Figure 4 Calibration curve for estimation of Congo Red concentration at 498 nm

Equilibrium adsorption capacity (mg/g) was calculated from the equation:

$$q_e = \frac{V_0(C_0 - C_e)}{m_{adsorbent}}$$

Where V_0(l) = volume of Congo Red solution, C_0 and C_e are the respective concentrations (mg/l) of Congo Red in the solution without and with biomass, a_{dsorbent} (g) is the dry weight of the adsorbent (biomass).

The respective maximum adsorption capacity of Congo Red was determined by fitting data to Langmuir adsorption isotherm:

$$q_e = \frac{q_m K_L C_e}{1 + K_L C_e}$$

q_e(mg/g) = equilibrium adsorption capacity (the amount of adsorbed dye)

q_m (mg/g) = maximum adsorption capacity (the amount of dye adsorbed when saturation is attained)

K_L (ml/mg) = Langmuir equilibrium constant

C_e (mg/l) = equilibrium concentration (the amount of non-adsorbed dye).

The *linear equation* for *Langmuir isotherm* is expressed as follows:

$$\frac{C_e}{q_e} = \frac{1}{q_m} \cdot C_e + \frac{1}{K_L \cdot q_m}$$

Using the Langmuir equation described above, the amount of adsorbed dye was calculated from a plot of C_e/q_e versus C_e [18].

2.4.3. Azure B adsorption for determination of lignin surface area

30 mg of untreated and treated samples were measured into conical flasks and dissolved in 15ml of Azure B dye with the following concentrations (0.01, 0.05, 0.1, 0.2, 0.4, 0.6, 0.8 mg/ml). All samples were incubated in a shaking incubator

at 150 rpm for 24 h to reach the adsorption equilibrium. After incubation, the supernatants were collected by centrifugation and the solutions with unabsorbed Azure B dye concentrations were diluted to reduce the level of the concentration and its absorbances were determined at a wavelength of 647 nm. The initial stock solution of Azure B was prepared and diluted to the following concentrations (2, 4, 6, 8, 10, 12, and 14 mg/ml). Absorbances of these solutions were measured at 647 nm and the calibration curve was constructed (Fig.5) to get a standard equation for calculating dye concentration [19].

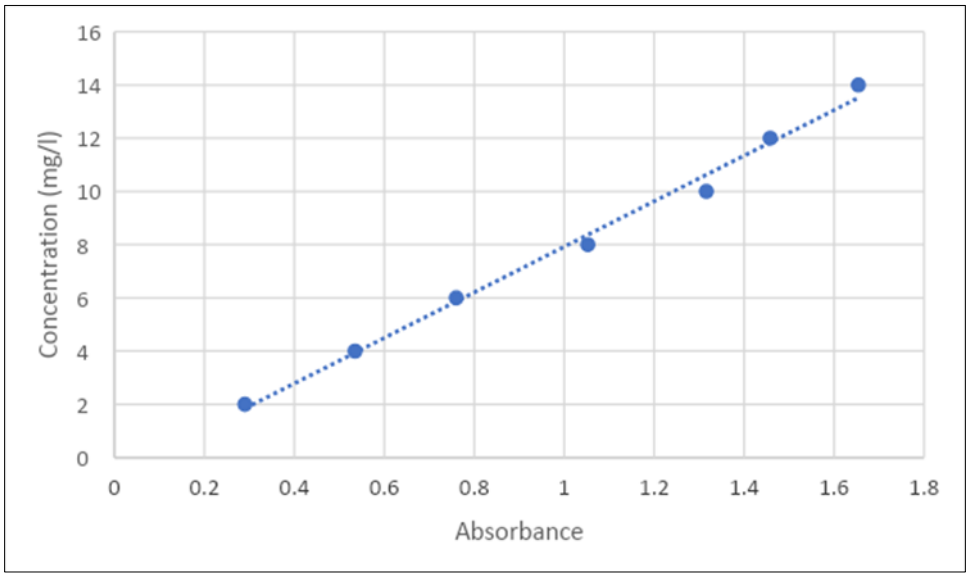

Figure 5 Calibration curve for estimation of Azure B concentration at 647 nm

The respective maximum adsorption capacity of Azure B to lignin was determined by fitting data to Langmuir adsorption isotherm as previously described for Congo Red. The surface area of cellulose was calculated with the assumption that 1 g of the adsorbed dye represents 1055 m^2 of cellulose area, and lignin surface area was obtained from the maximum Azure B adsorption capacity with the mass unit (per gram) of Azure B representing 1297 m^2 of lignin surface area.

3. Results and Discussions

3.1. Microwave pretreatment of samples with hydrogen peroxide (H_2O_2) and alkaline hydrogen peroxide (H_2O_2)

Figure 6 shows the appearance of pretreated biomass at different process conditions such as pH and treatment time.

The lighter color of the samples treated with alkaline H_2O_2 for 30s and 60s is a result of efficient removal of lignin from the biomass. Many reports have confirmed that alkaline pretreatment removes acetyl and uronic acid groups present on hemicellulose and thus enhances the accessibility of the enzyme that degrades hemicellulose [20]. Also, ester linkages that join xylan and hemicellulose residues are also hydrolyzed in alkaline pretreatment [21].

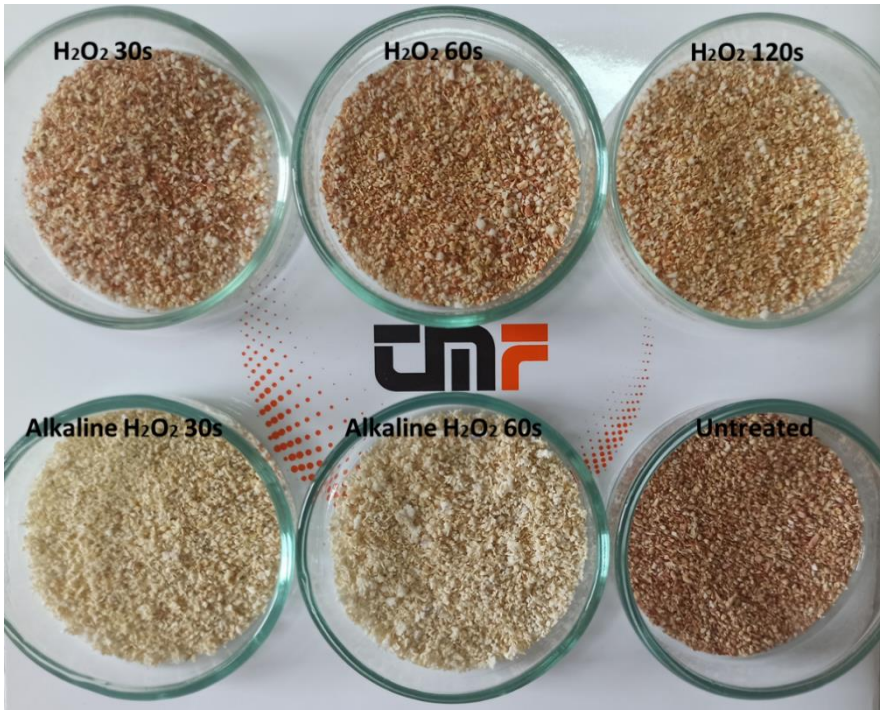

Figure 6 Appearance of biomass after pretreatment (H_2O_2 30s: biomass obtained from microwave assisted pretreatment with hydrogen peroxide for 30 seconds, H_2O_2 60s: biomass obtained from microwave assisted pretreatment with hydrogen peroxide for 60 seconds, H_2O_2 120s: Biomass obtained from microwave assisted pretreatment with hydrogen peroxide for 120 seconds, Untreated: biomass obtained without treatment, Alkaline H_2O_2 30s: biomass obtained from microwave assisted pretreatment with alkaline hydrogen peroxide for 30 seconds, Alkaline H_2O_2 60s: biomass obtained from microwave assisted pretreatment with alkaline hydrogen peroxide for 60 seconds)

3.2. Effect of degree of crystallinity on enzymatic hydrolysis

The crystallinity index of treated and untreated samples was determined after obtaining the iodine sorption values, according to the description in section 2.4. The obtained results are shown in Figure 7.

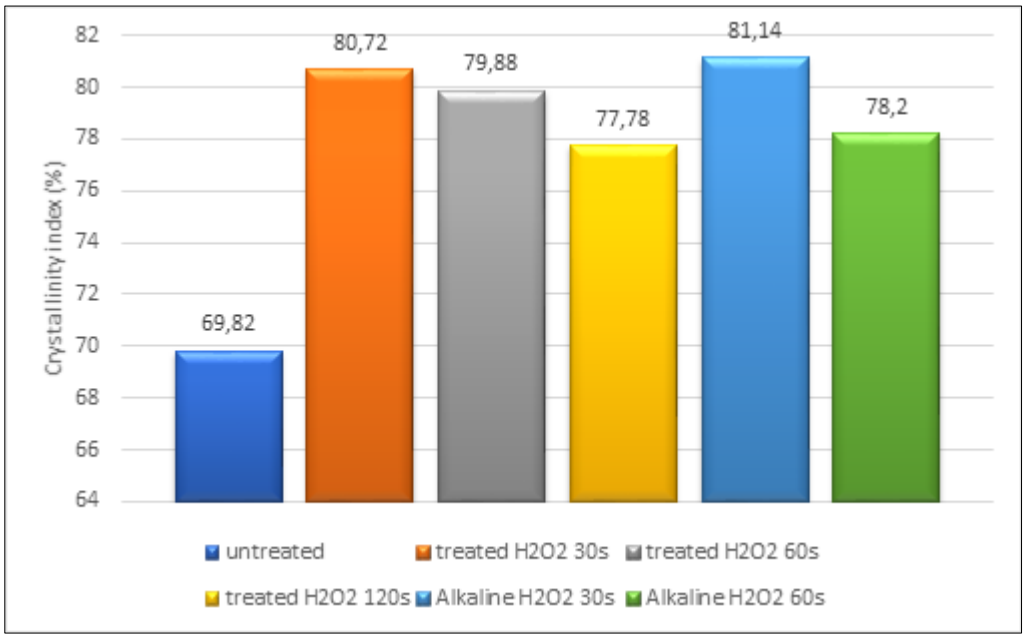

Figure 7 Degree of crystallinity of treated and untreated samples

The crystallinity index of untreated, treated H_2O_2 120s, alkaline H_2O_2 60s, treated 60s, treated H_2O_2 30s and alkaline H_2O_2 30s corn cob was 76.1, 77.78, 78.2, 79.88, 80.72 and 81.14 % respectively. This increasing trend of crystallinity index of cellulose confirmed that the corn cob biomass was altered during the pretreatment process. Therefore, the increased crystallinity index after pretreatments might be due to hydrolysis of glycosidic linkages in the cellulose accessible regions. The percentage of crystallinity of treated corn cob was higher due to the removal of hemicellulose and amorphous parts of cellulose in the hydrolysate and due to the hydrolysis of glycosidic linkages in the cellulose accessible regions.

Also, pretreatments affect the crystallinity of cellulose in different ways, being decreased by the disruption of crystal hydrogen bonding, and increased by degradation of amorphous cellulose. The degree of crystallinity has been more regularly defined as the first crystalline index of the total material obtained by X-ray diffraction [22]. Thus, the crystalline index has usually not been related to the actual cellulose content [23, 24, 25], which has led to some ambiguity in the perception of the effect of pretreatment of cellulose crystallinity [26].

Pang et al. [27] and Pang et al. [28] both reported increases in crystallinity index following microwave assisted steam explosion of corn stover. Increased crystallinity was more attributed to pronounced effect of microwave irradiation on the amorphous area than crystalline area of cellulose while reduced lignin and hemicellulose content was also cited as one of the reasons for increased crystallinity [27, 28, 29, 30].

3.3. The amount of adsorbed dye molecules on the surface of the adsorbent (biomass) at a given time.

The surface areas of cellulose and lignin in treated and non-treated biomass were obtained by using Langmuir adsorption isotherm to determined amount of dye adsorbed on the surface of a biomass according to the descriptions in section 2.4.1, the obtained results of Langmuir adsorption isotherms for the adsorption of Congo Red are shown in Figure 8 and that of Azure B in Figure 9.

The surface area of cellulose and lignin, expressed as 1 gram of absorbed dye, represents 1055 m^2 of cellulose area and 1297 m^2 of lignin area. These values were obtained using the linear equation for the Langmuir isotherm, as described in section 2.4.2. The results are presented in Table 1.

The outcomes regarding cellulose and lignin surface area after various treatment conditions indicate that alkaline hydrogen peroxide had a more significant impact on the biomass surface compared to an acidic peroxide solution. The most substantial increase in cellulose surface area was observed in the sample treated with alkaline H_2O_2 for 60s, reaching 119.89 m^2/g. On the other hand, different treatment durations with the acidic hydrogen peroxide solution had varied effects, resulting in unchanged surface area (30s treatment), slightly higher surface area (120s treatment), or lower surface area (60s treatment) of cellulose compared to untreated biomass.

Table 1. The surface area of cellulose and lignin obtained by maximum adsorption capacity of Congo Red and Azure B dye.

Biomass	**Cellulose surface area (m^2/g)**	**Lignin surface area (m^2/g)**
Untreated	60.98	60.33
Treated alkaline H_2O_2 30s	109.90	216.17
Treated alkaline H_2O_2 60s	119.89	270.21
Treated 30s	60.29	80.56
Treated 60s	49.53	79.57
Treated 120s	71.77	67.20

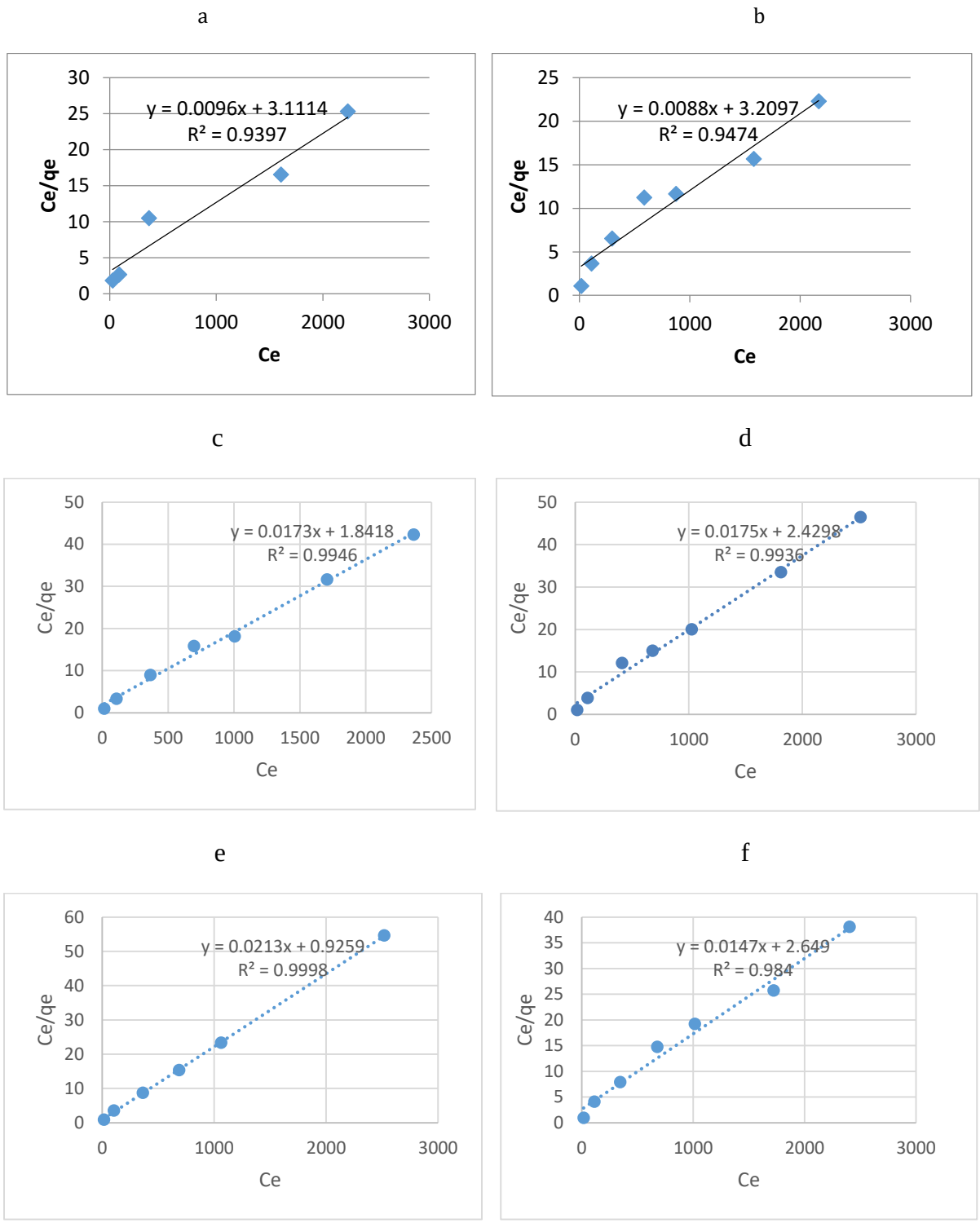

Figure 8 Langmuir adsorption isotherms for the adsorption of Congo Red on a) alkaline H2O2 30s, b) alkaline H2O2 60s, c) untreated sample, d) treated 30s, e) treated 60s, f) treated 12

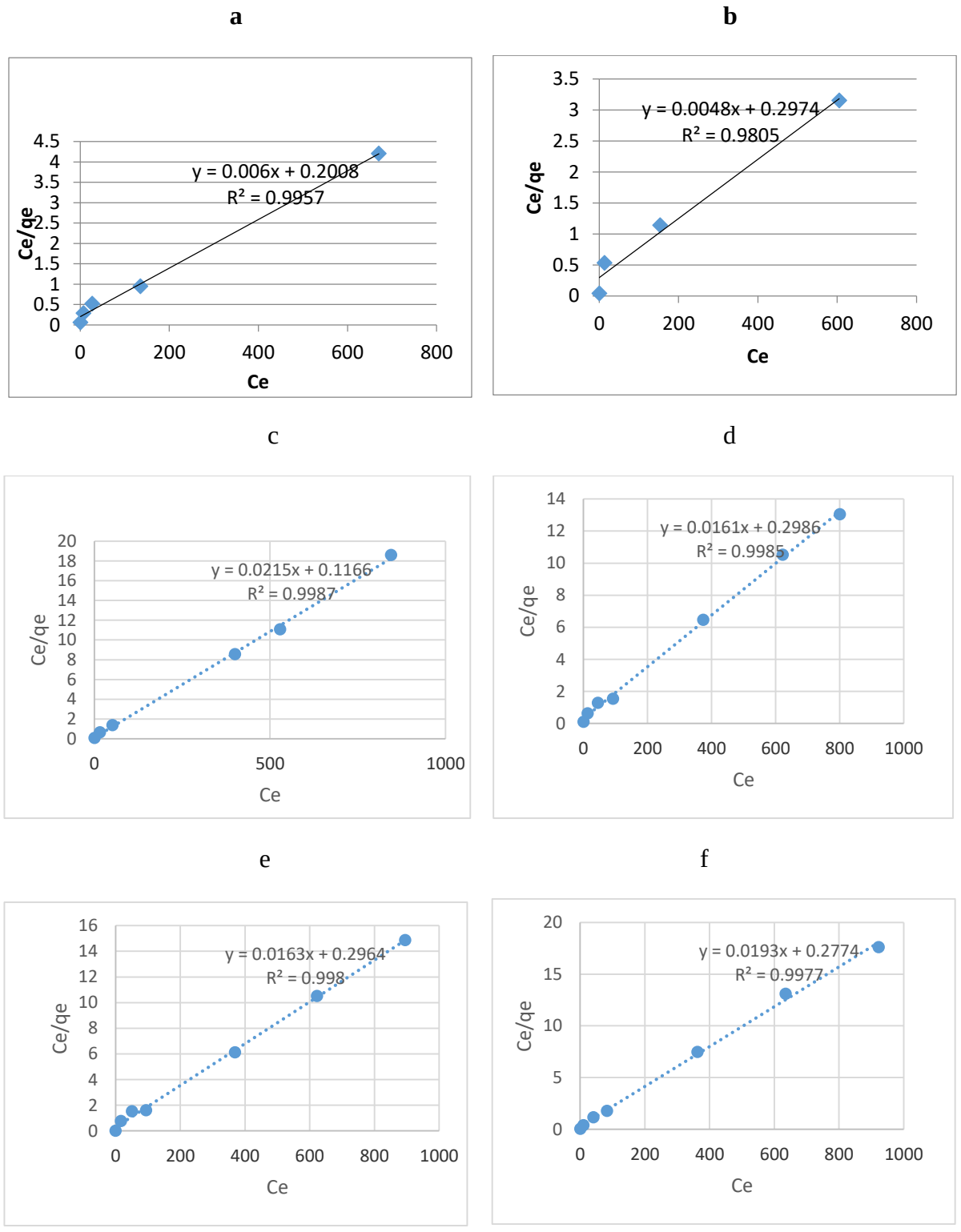

Figure 9 Langmuir adsorption isotherms for the adsorption of Azure B on a) alkaline H_2O_2 30s, b) alkaline H_2O_2 60s, c) untreated sample, d) treated 30s, e) treated 60s, f) treated 120s

While certain treated samples exhibited an increase in cellulose surface area, the results for lignin surface area suggest that the pretreatment processes were insufficient to remove abundant lignin. For a more comprehensive understanding of the treatment effects on biomass constituents (cellulose, hemicellulose, and lignin), additional chemical and or physical analyses are necessary. Previous studies have also indicated that oxidative treatments could introduce numerous anionic groups on biomass surfaces, which serve as new adsorption sites for Azure B [31]. This suggests that the increase in lignin surface area observed in this study could potentially lead to misinterpretations. Azure B dye binds to the phenolic hydroxyl groups of lignin, thereby more accurately representing the surface of accessible phenolic hydroxyls than the physical area. Furthermore, the rise in lignin surface area may signify changes in the chemical surface properties of the remaining lignin, resulting in increased lignin hydrophilicity [31, 32]. Azure B acts as a base that deprotonates acidic hydroxyl groups, such as phenolic OH groups, leading to an ionic bond between protonated Azure B and the phenoxide anion [22].

The increase in lignin surface area is a consequence of the corn cob pretreatment process, and this augmentation in cellulose surface area influences the efficiency of enzymatic hydrolysis of the substrate [33]. Other studies have reported that lignin and hemicellulose cover the cellulose surface within the cell wall of lignocellulosic materials, impacting the adsorption of cellulase on cellulose [34]. However, the greater removal of these components during pretreatments increases the surface area of cellulose, thereby enhancing its accessibility to cellulase systems [35].

3.4. Effect of enzymatic hydrolysis on sugar concentrations and sugar yield

After the pretreatments, the concentrations of reducing sugar, pentose sugar and hexose sugar of the obtained hydrolysates were determined by the procedure described in 2.3, the sugar concentrations of each sample were estimated. The obtained results are shown in Figure 10.

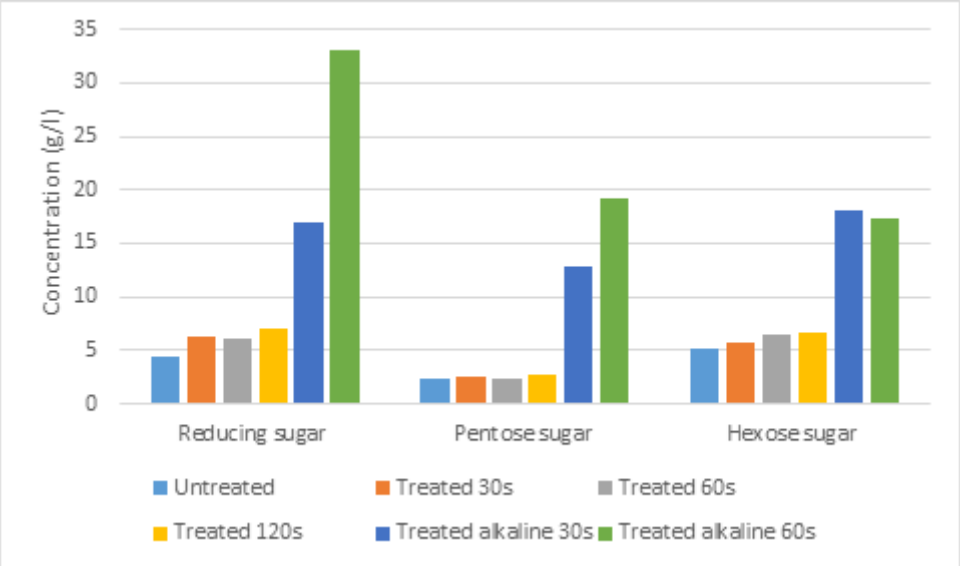

Figure 10 Concentrations of reducing sugar, pentose sugar and hexose sugar obtained in enzymatic hydrolysis of treated and untreated samples

After enzymatic hydrolysis, the concentrations of reducing sugar, pentose sugar, and hexose sugar were measured. The concentrations of these sugars were higher in samples treated with alkaline solution for 60s and 30s compared to untreated biomass and samples treated with unadjusted H_2O_2. Pentose sugar concentration remained low in untreated biomass and samples treated with H_2O_2, indicating rapid sugar release in alkaline H_2O_2 treated samples and slower sugar release in untreated biomass and H_2O_2 treated samples. This observation aligns with findings from other studies, which have shown that using H_2O_2 without pH correction results in less efficient sugar release during enzymatic hydrolysis. Mishima et al. [36] conducted a comparative study on the chemical pretreatment of aquatic plants used in water purification, and their results demonstrated that H_2O_2 pretreatment alone was ineffective in improving enzymatic hydrolysis (10% w/v total solids; 1% v/v H_2O_2; room temperature for 2 hours). However, combining H_2O_2 with NaOH solution yielded the best enzymatic hydrolysis results (1% w/v NaOH, room temperature for 12 hours, followed by the addition of 1% w/v H_2O_2 at room temperature for 12 hours).

Alkaline hydrogen peroxide has proven efficient in pretreating various lignocellulosic biomass, exhibiting high efficiency rates for enzymatic hydrolysis when used alone or in combination with other pretreatment methods. Additionally, high solubilization values of lignin and hemicellulose in the liquid fraction support the viability of this pretreatment method in biorefinery processes. The efficacy of alkaline H_2O_2 pretreatment depends on pH conditions, as it facilitates hemicellulose removal and peroxide oxidation breaks down lignin bonds [37]. Kim et al. [38] reported that treating empty palm fruit bunches with acid/alkaline pretreatment followed by enzymatic saccharification increased fermentable sugar production.

Alkaline peroxide treatment yields highly digestible cellulose and generates low concentrations of inhibitors during pretreatment. Studies by Duan [39] indicate that the effect of a specific amount of biomass after acid pretreatment increases hydrolysis time, leading to enhanced sugar yield. Consequently, untreated biomass demonstrated limited sugar yield during enzymatic hydrolysis. This result suggests that pretreatment improves enzyme access to cellulose within the biomass, facilitating rapid release of reducing sugars from the pretreated biomass. Moreover, the duration of pretreatment also impacts sugar yield, with longer treatment times leading to increased cellulose availability and higher yields.

Results in Figure 11 shows the yield of reducing sugar, pentose and hexose sugars expressed in percentage by each sample were obtained by the calculations described in section 2.3.4.

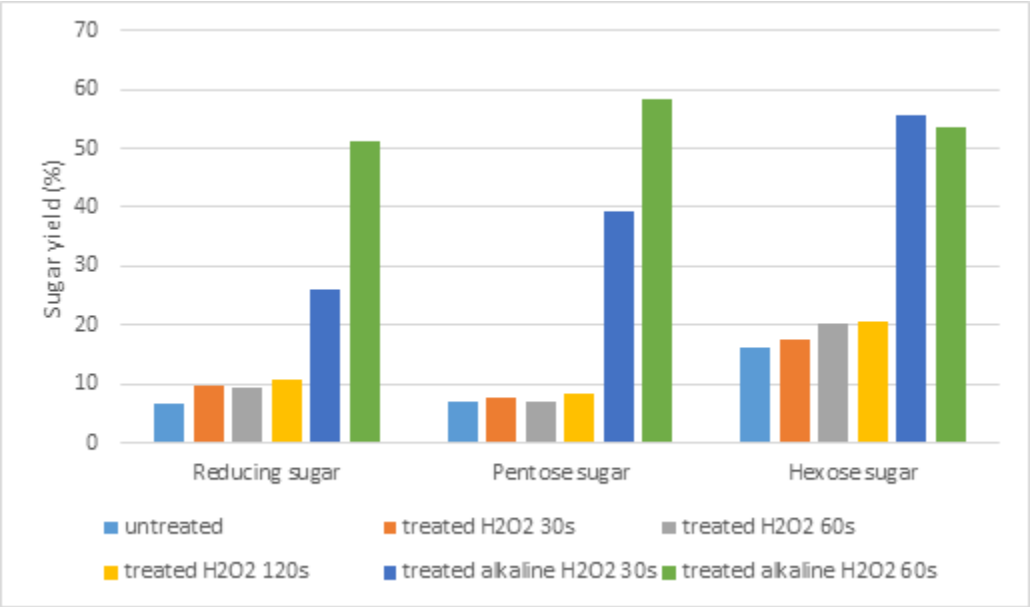

Figure 11 Effect of the pretreatment methods on reducing sugar, pentose and hexose sugar yield

The sugar yields (%) for reducing sugar, pentose, and hexose sugar were higher in biomass samples treated with alkaline H_2O_2 for 60s and 30s, and lower in untreated biomass and samples treated with H_2O_2 without pH correction. Hexose sugar yield (%) was higher in untreated biomass and samples treated with H_2O_2 than the pentose sugar yield (%) in untreated biomass and samples treated with H_2O_2. These results indicate a rapid release of sugars in samples treated with alkaline H_2O_2 and a slower release of sugars in untreated biomass and H_2O_2 treated samples. Additionally, these findings suggest that alkaline pretreatment is efficient because more cellulose was converted to glucose during enzymatic hydrolysis, leading to a significant increase in sugar yields (reducing sugar, hexose, and pentose sugar). The alkaline H_2O_2 treatment's pH adjustment enhances the efficiency of sugar release during pretreatment. Gould [40] demonstrated that the optimal pH value is 11.5.

In a study by Li et al. [41], alkaline H_2O_2 was employed for the pretreatment of seaweed biomass with varying pH levels (4, 6, 8, and 10). This resulted in higher yields of reducing sugar and glucose in the pretreated sample groups compared to the control groups. The optimal pH was found to be 4.0, which exhibited the highest rate of reducing sugar production, while the lowest glucose yield was observed at pH 6.0. However, smaller increases were observed at pH 8.0 and 10.0. The authors suggested that, when compared to other lignocellulosic biomass, the optimal condition is pH 11.5. This preference could be attributed to the lower lignin content, which makes the yield of sugar reduction more responsive to pretreatment pH.

4. Conclusion

The current study explored the impact of microwave pretreatment on lignocellulosic corn cob degradation using acidic H_2O_2 and alkaline H_2O_2 treatments. Alkaline H_2O_2 pretreatment demonstrated higher efficacy in corn cob degradation, exhibiting elevated efficiency rates for enzymatic hydrolysis and resulting in a correspondingly higher sugar yield (%), thus confirming increased cellulose availability. Conversely, acidic H_2O_2 pretreatment led to a reduced sugar yield (%) following enzymatic hydrolysis when compared to the alkaline treatment.

Furthermore, the sugar yield from the enzymatic hydrolysis of untreated biomass closely resembled that of biomass treated with acidic hydrogen peroxide, indicating that the acidic solution did not induce significant alterations in biomass composition or structure. The sugars analyzed included reducing sugar, pentose, and hexose sugar, all of which yielded significantly higher amounts in biomass treated with alkaline H_2O_2, thereby presenting a promising source for effective biofuel production.

Further characterization of untreated biomass, samples treated with alkaline H_2O_2, and samples treated with H_2O_2 revealed distinct influences on the crystallinity index due to the different conditions applied during corn cob treatment.

Additionally, the calculation of cellulose and lignin surface areas through the maximal adsorption capacity of Congo Red and Azure B dye exhibited substantial alterations in biomass surface due to the treatments. Nonetheless, comprehensive data and analyses are imperative for establishing precise and reliable conclusions.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest statement to be disclosed.

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