ADVANCES & PROSPECTS IN THE FIELD OF WASTE MANAGEMENT

Effect of alkaline pretreatments on the enzymatic hydrolysis of wheat straw

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Abstract

Lignocellulosic materials are mainly consisted of lignin, cellulose, and hemicellulose. Lignin is recognized as the main obstacle for the enzymatic saccharification of cellulose towards the fermentable sugars' production. Hence, the removal of lignin from the lignocellulosic feedstock is beneficial for reducing the recalcitrance of lignocellulose for enzymatic attack. For this purpose, various different alkaline pretreatments were examined in order to study their effect on the enzymatic saccharification of wheat straw, as a typical lignocellulosic material. Results revealed that the alkaline pretreatments promoted delignification reactions. Regarding the removal of lignin, the most efficient pretreatments were alkaline treatment with hydrogen peroxide 10% and NaOH 2% autoclave with delignification efficiencies of 89.60% and 84.86% respectively. X-ray diffraction analysis was performed to enlighten the structural changes of raw and pretreated materials. The higher the delignification of the raw material, the higher the conversion of cellulose during enzymatic saccharification. In all cases after enzymatic saccharification, the cellulosic conversion was much higher (32–77%) than the untreated wheat straw (8.6%). After undergoing alkaline peroxide 10% pretreatment and cellulase treatment, 99% of the initial raw straw was eventually solubilized. Thus, wheat straw could be considered as an ideal material for the production of glucose with proper pretreatments and effective enzymatic hydrolysis.

Keywords Agro-residues · Alkaline peroxide · Cellulases · Crystallinity · Delignification efficiency · Lignin

Introduction

The quest for environmental and sustainable energy sources has grown significantly during the last 20 years paving the way for biofuels' production originating from renewable resources. These resources are usually classified in following groups: residues from industrial, domestic, or agricultural origin, energy crops from dedicated farming, and wood. The utilization of these renewable feedstocks for the production of biofuels has formed two distinct generations in the field of biofuels. The 1st biofuel generation originates from food and/or grain resources. However, the competition between food and energy crops has brought about a food-over-fuel conflict worsened by the increased food prices (Sanchez et al. 2011).

The 2nd generation of biofuels originates from non-food and non-grain resources such as lignocellulosic biomass. Currently, the latter is either burnt towards the production of electricity and heat, or it is utilized for the production of liquid biofuels. However, by-products and wastes from industrial and/or agricultural processes such as rice husks, coconut shells, corn stover, sugarcane bagasse, and corncob are produced every day all over the world and are scarcely utilized. These by-products and wastes contain abundant amounts of hemicellulose and cellulose that are considered suitable raw materials for the fermentation process towards the production of biofuels. On the other hand, feedstocks such as maize starch or sugarcane are not so abundant. Cellulose and hemicellulose are well inside the plant's cell wall and are encased by lignin (Solarte-Toro et al. 2018). The latter's aim is to protect the plant and thus acts as an impenetrable obstacle for the penetration process. Consequently, an efficient pathway to overcome this barrier and to boost the plant's digestibility is to either degrade or separate lignin by a process called delignification. This process may be performed by chemical means such as solvents, alkalis,

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or acids promoting by this way the selective solubilization of lignin or carbohydrates. When the means are alkaline, lignin is the target component that solubilizes. On the contrary, when the means are acidic, then the structural carbohydrates solubilize. The latter are also solubilized even when solvents are used; that is organosolv pretreatment (Sanchez et al. 2011). Even though the use of acids is quite efficient in degrading lignocellulose, several toxic by-products are produced during degradation, mainly when high pressures and temperatures and low pH are applied. Apart from formic acid, acetic acid, levulinic acid and other free aliphatic acids and/or vanillin, 4-hydroxybenzoic acid, or other phenolic derivatives originating from hemicellulose and lignin degradation, hydroxymethylfurfural (HMF) and furfural (FF) may be also produced causing severe inhibition to enzymatic saccharification and fermentation. HMF and FF are the products of dehydration reactions of C-6 sugars (mainly glucose, galactose, and mannose) and C-5 sugars (such as arabinose and xylose) respectively. The inhibition caused by HMF and FF on the yeast growth as well as the way that these compounds reduce ethanol productivity and yield has been reported extensively in literature. Besides these, other weaknesses of this process are the need for neutralization of the acidic conditions before any downstream processes along with the difficulties in the market exploitation of the condensed lignin (Jaisamut et al. 2016).

As far as organosolv pretreatment is concerned, there are some inherent drawbacks. The pretreated solid phase should always be washed so as to prevent the precipitation of lignin that has already been solubilized. This makes necessary tedious and time-consuming cleaning and washing procedures. The high cost of organic solvents impels energy consuming recovery processes (Zhao et al. 2009). In addition, solvents inhibit the process of enzymatic saccharification thus removing them is crucial for hydrolyzing biomass efficiently. Besides, extra costs are created by the organic solvents' removal (Chaturvedi and Verma 2013). Moreover, the organosolv process should be fully and efficiently controlled since the organic solvents used are highly volatile. Given the inherent explosion and fire hazards, leakages from the digesters cannot be afforded. Thus, for the time being, organosolv processes are quite exorbitant to be applied for the pretreatment of biomass (Zhao et al. 2009).

To the contrary, dilute alkali processes enhance the formation of pores in lignocellulosic material since the ester bonds between molecules are saponified, the crosslinks among lignin, xylan, and other hemicellulosic compounds are removed, acetyl groups are also removed, and just a few inhibitory compounds are produced. Low capital costs are required since alkaline pretreatments are usually carried out at lower temperatures and with less specialist equipment than acidic alternatives. A high pH environment also helps in maintaining sterile conditions during pretreatment, avoiding the consumption of carbohydrates (Vasmara et al. 2017).

About 750×10^6 tonnes of wheat were produced globally in 2016. Among cereals, wheat is ranking third in production universally. Thus, it could be considered as an abundant renewable feedstock. The lignin levels in wheat are lower than the respective of corn stover which is the residue from the production of corn. On the contrary, the concentrations of cellulose and hemicellulose are much higher (Jaisamut et al. 2016). Since it is a type of grass, the structure of wheat straw is quite looser than that of wood, exhibiting huge potential for the delignification processes. Extensive research studies have focused on developing technological schemes that utilize the sugar content of wheat straw towards the production of biofuels (Chaturvedi and Verma 2013; Jaisamut et al. 2016; Vasmara et al. 2017; Zhao et al. 2009).

To this end, even though wheat straw has been recognized as a representative and promising lignocellulosic feedstock for biofuels production and the need for its pretreatment is undisputable, a proper efficient pretreatment delignification technique has not yet been established. In this context, the primary objective of this paper is to study ten different alkaline pretreatment methods so as to render the enzymatic saccharification of wheat straw more efficient.

Materials and methods

Biomass

Wheat straw (WS), as a representative lignocellulosic agricultural residue, was harvested in the vicinity of Aspropyrgos province, Greece. The raw material was milled by use of a knife mill FRITSCH Cutting mill (Pulverisette 15) and then it was sieved in order to attain particles that were smaller than 1 mm. The milled straw mainly composed in dry basis of 33.8% cellulose, 45.1% hemicellulose, 16.4% lignin (15.4% Klason lignin (acid-insoluble lignin AIL) and 1.0% acid-soluble lignin (ASL)), and 4.7% ash. The ground samples were kept in airtight bags at ambient temperature.

Wheat straw chemical composition

Moisture, extractives, ash, cellulose, hemicellulose, and lignin in untreated and pretreated materials were analyzed according to the experimental protocols of NREL (National Renewable Energy Laboratory) (Sluiter et al. 2008). All chemicals used were obtained from Sigma-Aldrich, were of analytical grade, and were utilized as received without any further purification. All solutions were prepared with deionized water of resistivity not less than 18.5 MΩ/cm. The commercial enzymatic formulation CelliCTec2 was kindly donated by Novozymes (Denmark) and was used without any further purification. Total cellulase activity (FPU) was measured according to the standard methods and was estimated equal to 223 FPU/mL. In

the liquid phase, volatile fatty acids (VFA), phenolic compounds, and total organic carbon (TOC) were also measured based on the standard methods while the glucose concentration was estimated by use of a marketable kit (Biosis S.A., Athens, Greece) that is based on the Glucose Oxidase–Peroxidase (GOX–PER) method. All analyses were performed in triplicate.

The crystallinity structure of the raw and pretreated WS was characterized by X-ray diffractometry. A Bruker D8-Advance X-ray diffractometer (Bruker-AXS, Karlsruhe, Germany) was employed where radiation CuKα (λ = 1.5418 Å) was the X-ray source employed, with 10 mÅ and 20 kV applied, measured at angle 2θ, ranging between 5° and 45° with a scanning rate of 0.1° s⁻¹.

Pretreatment of wheat straw

Ten different alkaline pretreatments were applied in this study (Table 1). The operational conditions for each pretreatment were selected based on the optimum experimental results reported in other literature studies (Bolado-Rodríguez et al. 2016; Feng et al. 2014). In autoclave pretreatments, WS was slurried for 5 min with alkaline solution in autoclavable bottles with a solid to liquid ratio of 1 to 10 w/w, and afterwards, the samples were autoclaved at 121 °C for 1 h. The same experiments were conducted under milder thermal conditions (50 °C, 96 h). In alkaline peroxide pretreatment, WS was mixed for 5 min with 5% or 10% w/w H_2O_2, in a solid to liquid ratio of 1 to 20 w/w, the pH was corrected to 11.5 with 8% NaOH, and the whole sample was kept in a rotary shaker for 1 h at 120 rpm and 50 °C.

When the samples were cooled down to ambient temperature, a slurry was the outcome of every pretreatment. Filtration was applied in order to separate the residual solids which were then oven dried for 48 h at 45 °C. A composition analysis of solid and liquid samples was performed. Then, the solid samples were set as the feedstock for the subsequent enzymatic saccharification step. All experimental runs were performed thrice and the average values are presented.

Enzymatic hydrolysis

Enzymatic saccharification of untreated and pretreated solid samples was executed in 100 mL Erlenmeyer flasks at 50 °C (orbital shaker, frequency 135 rpm) that contained 10% w/w dry solids and the cellulolytic formulation, Cellic CTec2 (Novozymes, Denmark), at a loading of 15 μL g⁻¹ dry solid for 96 h. A buffer solution of sodium citrate 0.05 M and phosphoric acid 0.1 M was employed in order to adjust the pH to 5.0. The enzymatic saccharification took place in a rotary shaker incubator at 300 rpm and 50 °C for 96 h. After the enzymatic saccharification, the treated liquid samples were analyzed regarding their glucose concentration. All experimental trials were repeated three times and the mean values are presented.

Results and discussion

Effect of pretreatment schemes on wheat straw composition

As far as the composition of the solid fraction is concerned, in respect to the different pretreatment techniques, the lignin, hemicellulose, and cellulose content varied in comparison with the raw material. The degradation efficiencies of total solids and structural components of wheat straw were estimated using the equation presented below and presented in Table 2.

%degradation efficiency of X component

$$= \frac{X_o - X_f}{X_o} \times 100\%$$

where X_o is the initial mass of X component and X_f is the final mass of X component after pretreatment.

For all the pretreatment schemes, cellulose and hemicellulose solubilization and decomposition were significantly lower than the respective for lignin, leading to a corresponding rise in cellulose content in the solids in comparison with the raw material. The higher the solubilization and decrease of lignin, the higher the increase in the cellulose content of the pretreated solids. The higher cellulose content and lower lignin content of the solid fraction after delignification will probably induce an improvement of the enzymatic accessibility of wheat straw in comparison with the non-delignified straw. The consequences of lignin degradation were apparent in the chemical composition of the pretreated agricultural residues.

Table 1 Alkaline pretreatments applied on wheat straw

A/A	Reagent	Concentration (% w/w)	T (°C)	Autoclave	Solid:liquid (w/w)	Time (h)
i	H_2O_2	5	50		1:20	1
ii	H_2O_2	10	50		1:20	1
iii	NaOH	2	50		1:10	96
iv	NaOH	2	121	✓	1:10	1
v	CH_5N	25	50		1:10	96
vi	CH_5N	25	121	✓	1:10	1
vii	Na_2CO_3	5.3	50		1:10	96
viii	Na_2CO_3	5.3	121	✓	1:10	1
ix	NH_3	25	50		1:10	96
x	NH_3	25	121	✓	1:10	1

Table 2 Solid fractions' composition after the different pretreatments of wheat straw: (i) alkaline peroxide 5%, (ii) alkaline peroxide 10%, (iii) dilute NaOH 2%, (iv) dilute NaOH 2% autoclaving, (v) methylamine 25%, (vi) methylamine 25% autoclaving, (vii) Na_2CO_3 5.3%, (viii) Na_2CO_3 5.3% autoclaving, (ix) ammonia 25%, (x) ammonia 25% autoclaving

	% total solids (TS) hydrolysis	% cellulose degradation	% AIL degradation	% ASL degradation	% hemicellulose degradation
i	11.68 ± 0.02	11.06 ± 0.96	31.63 ± 0.06	0.76 ± 2.87	5.22 ± 0.49
ii	28.05 ± 0.20	1.88 ± 1.37	89.60 ± 0.68	43.99 ± 0.9	17.69 ± 2.21
iii	30.07 ± 0.36	4.45 ± 1.11	75.06 ± 5.03	36.48 ± 4.66	30.66 ± 8.26
iv	36.47 ± 2.78	33.52 ± 3.44	84.86 ± 0.45	49.86 ± 5.72	9.29 ± 7.89
v	29.84 ± 0.03	22.3 ± 5.63	76.38 ± 1.06	99.22 ± 0.09	8.66 ± 9.46
vi	26.09 ± 4.73	24.85 ± 2.33	70.78 ± 4.23	99.03 ± 0.19	3.84 ± 2.61
vii	11.59 ± 0.37	4.87 ± 1.76	38.51 ± 6.92	16.99 ± 2.16	1.31 ± 1.72
viii	11.05 ± 8.65	3.71 ± 1.10	59.81 ± 4.15	38.93 ± 6.63	0.50 ± 3.75
ix	26.75 ± 1.49	44.41 ± 7.35	57.31 ± 2.53	99.21 ± 0.01	0.90 ± 1.10
x	25.00 ± 2.16	17.11 ± 1.17	44.83 ± 7.55	99.24 ± 0.04	24.41 ± 2.71

Acid-soluble lignin degradation ranged from 0.76 to 99% whereas acid-insoluble lignin from 31.63 to 89.60%.

As it can be seen from Table 2, NaOH pretreatment (iv) resulted in slight degradation of hemicellulose (9.29%), while the lignin contents after the alkaline pretreatments were quite low, demonstrating that lignin was successfully removed (84.86%) by alkaline solutions by rupturing ester bonds. In so doing, the biomass porosity is increased. Nevertheless, a part of cellulose of wheat straw (33.52%) was also degraded during pretreatment, since alkaline pretreatment is a non-selective chemical pretreatment. These outcomes are in accordance with Zheng et al. (2018a), who reported that after 2% NaOH pretreatment at 121 °C for 1 h, hemicellulose and lignin degraded by 31.4% and 84% respectively. Furthermore, cellulose was also slightly degraded (19.5%).

Xin et al. (2019) also used NaOH for the delignification of rice straw but obtained poorer results (21.28–40.03%) despite the higher concentrations of NaOH used (from 3 to 9%) and the longer experimentation period (7 days). This could possibly be attributed to the lower temperature applied (28 °C).

Similar results were also reported by Feng et al. (2014), who studied the effect of five alkaline media (calcium hydroxide, sodium carbonate, sodium hydroxide, methylamine, and ammonia) on the pretreatment of wheat straw at 140–200 °C. They found that the loss of cellulose varied from 4 to 13% and hemicellulose removal varied from 15 to 25% at 140 °C. For the pretreatments with methylamine, ammonia, and sodium hydroxide, just 50% of the acid-insoluble lignin (AIL) was recovered. Sodium carbonate induced even lower AIL removal than all other alkaline media. This was also the case for our study despite the lower temperatures applied (121 °C instead of 140 °C).

The glucose, VFA, and phenolic compounds release during the ten pretreatment schemes as indicative end-products of degradation reactions of the structural constituents of wheat straw are presented in Table 3. For most cases, almost all cellulose was retained in the solid phase, and from 0 to 2.1 mg glucose/g straw were released into the pretreated liquid phase. These results are lower than what is reported in some studies in literature; 2.6% glucose (w/w of raw wheat straw) was released when dilute acid pretreatment was applied and 2.26% and 0.96% glucose (w/w of wheat straw) was released in similar alkaline peroxide and dilute alkaline autoclave pretreatment schemes, respectively (Toquero and Bolado 2014). Even in the cases where a fair cellulose degradation was observed (pretreatments iv and ix), the respective glucose production was very low implying that the depolymerization of cellulose did not end up to its monomeric form; glucose.

Lignin is composed of cross-linked polymers of phenolic compounds, and thus, it constitutes a large, with a high degree of complexity, molecular structure. It is therefore evident that the decomposition of lignocellulosic material will lead to the

Table 3 Liquid fractions' composition after different pretreatment schemes of wheat straw. (i) alkaline peroxide 5%, (ii) alkaline peroxide 10%, (iii) dilute NaOH 2%, (iv) dilute NaOH 2% autoclaving, (v) methylamine 25%, (vi) methylamine 25% autoclaving, (vii) Na_2CO_3 5.3%, (viii) Na_2CO_3 5.3% autoclaving, (ix) ammonia 25%, (x) ammonia 25% autoclaving

	Glucose (mg/g straw)	VFA (mg/g straw)	TPC (mg/g straw)
i	0.88 ± 0.04	18.10 ± 0.28	0.27 ± 0.01
ii	0.15 ± 0.03	34.50 ± 0.57	0.68 ± 0.02
iii	0.02 ± 0.03	98.30 ± 14.99	2.13 ± 0.04
iv	0.62 ± 0.06	33.55 ± 1.63	5.60 ± 0.14
v	1.50 ± 0.14	64.10 ± 39.46	5.56 ± 0.37
vi	2.10 ± 1.27	28.10 ± 4.95	4.60 ± 0.76
vii	n.d. ± 0.00	28.20 ± 3.39	1.07 ± 0.07
viii	0.70 ± 0.94	80.85 ± 4.45	2.91 ± 0.16
ix	0.90 ± 0.14	38.30 ± 9.76	3.63 ± 0.44
x	1.20 ± 0.00	35.50 ± 1.27	3.61 ± 0.38

release of phenolic compounds (TPC) in the liquid phase. Indeed, phenols release in the liquid fraction ranged from 0.27 to 5.60 mg phenol/g straw. Apart from the alkaline peroxide pretreatments, for the rest of the cases, the higher the achieved delignification, the higher the phenolics release. For the alkaline peroxide pretreatments, the concentration of phenolics in the liquid was low even for high delignification efficiency, probably due to subsequent oxidation reactions of phenolic compounds by the hydroxyl radicals formed during the hydrolysis of hydrogen peroxide (Ho et al. 2019).

Furthermore, it is expected that volatile fatty acids will also appear in the supernatant, as a result of hemicellulose degradation. From the experimental results (Table 3), it was verified that the highest hemicellulose degradation (30.66%) resulted in the highest VFA concentration (98.30 mg VFA/g straw) for pretreatment (iii).

As far as lignin degradation is concerned, the most efficient pretreatments were (ii) and (iv) with delignification efficiencies of 89.60% and 84.86% respectively. From the viewpoint of glucose recovery in the solid phase, these schemes would appear preferable as pretreatment schemes prior to enzymatic saccharification of the solid phase, as glucose release in the liquid phase is almost negligible. In addition, for pretreatment method (ii), the hemicellulose and cellulose contents of the solid fraction were very high.

Yet, when utilizing methylamine as alkaline medium (pretreatments (v) and (vi)), in the pretreatment liquid phase, part of the initial sugars was released and a substantial percentage of cellulose was degraded. Thus, the solid/liquid separation of the pretreated slurry could be considered in some cases of enzymatic hydrolysis unnecessary as long as no inhibitory compounds are produced or that their influence is minimal.

The crystallinity measurement may offer a qualitative estimation of the crystalline and amorphous regions/compounds of biomass. X-ray diffraction was applied as a means to evaluate qualitatively the impact of the pretreatment schemes. Because of the structure of the lignocellulosic materials, any pretreatment could possibly improve the cellulase's diffusion and influence considerably the accessibility of the enzymatic formulations on lignocellulosic biomass. The crystallinity index (CrI) is strongly affected by the structural complexity of biomass, where lignin and hemicellulose stand as the amorphous components, while cellulose as the crystalline one (Haque et al. 2012). The CrI is a crucial parameter that is strongly correlated with the enzymatic digestibility since it may illustrate vividly the crystallinity of the entire biomass complex. Thus, it may reveal, in an indirect way, the amorphous phase signal of the wheat straw such as cellulose, hemicellulose, and lignin regions (Zheng et al. 2018b).

The CrI was calculated using a method introduced by Segal et al. (1959), acknowledged as peak height or Segal method. In this method, CrI is calculated by dividing the maximum interference, I_{200} (that is the height of (200) peak) and the minimum height between the (110) peak and (200) peak (I_{am} is the intensity at $2\theta = 18°$) (Karimi and Taherzadeh 2016; Segal et al. 1959; Zheng et al. 2018b):

$$CrI\ (\%) = (I_{200} - I_{am})/I_{200} \times 100\%$$

At the present time, the peak height method is the most commonly applied method for the evaluation of CrI in lignocellulosic materials, either untreated or pretreated, destined for biofuels' production (Karimi and Taherzadeh 2016).

The XRD patterns along with CrIs of raw and pretreated WS are shown in Fig. 1. The main impact of pretreatment schemes was the removal of lignin and hemicellulose, which are amorphous in structure, as was also presented in Table 2. The CrI of all samples were calculated revealing the relative quantity of crystalline cellulosic material in the entire complex biomass of wheat straw. Among the three major constituents, the amorphous regions include lignin and hemicellulose apart from amorphous cellulose. Thus, the XRD spectra reveal the crystallinity of the entire complex biomass. The crystallinities of wheat straw after different pretreatments were in all cases greater than that of raw wheat straw, since the amorphous components (such as xylan and lignin) were removed (Hou et al. 2017; Xu et al. 2016; Yu et al. 2014; Zhao et al. 2018). The more the lignin removal, the greater the CrI values. The pretreatments presenting the highest CrI were (ii) and (iii) with delignification efficiencies of 89.12% and 78.61% respectively. After the pretreatment schemes, the crystallinity of the lignocellulosic material was increased, and hence, this could lead to an elevated cellulose conversion rate due to the fact that lignin and hemicellulose were partly degraded during the pretreatments. Similarly, Tsegaye et al. (2018) reported the increase of CrI from 37.2 to 48.5% after the partial removal of lignin through biotreatment by the *Ochrobactrum oryzae* BMP03 bacteria strain.

Notwithstanding, the outcomes from enzymatic saccharification should also be studied so as to further enlighten the way the different pretreatment schemes affect the glucose recovery of wheat straw.

Effect of pretreatments on saccharification

Table 4 presents the response of the different pretreatment schemes on the degradation efficiencies of the structural polysaccharides after 4 days of enzymatic saccharification.

The lignin, hemicellulose, and cellulose contents of the pretreated samples varied nonconformably depending on the pretreatment schemes. The cellulose and hemicellulose degradation ranged from 13.29 to 100% and 37.29 to 100% respectively. Cellulose and hemicellulose degradations were in most cases higher than those of the control experiment, except for the pretreatment with ammonia. Acid-insoluble lignin
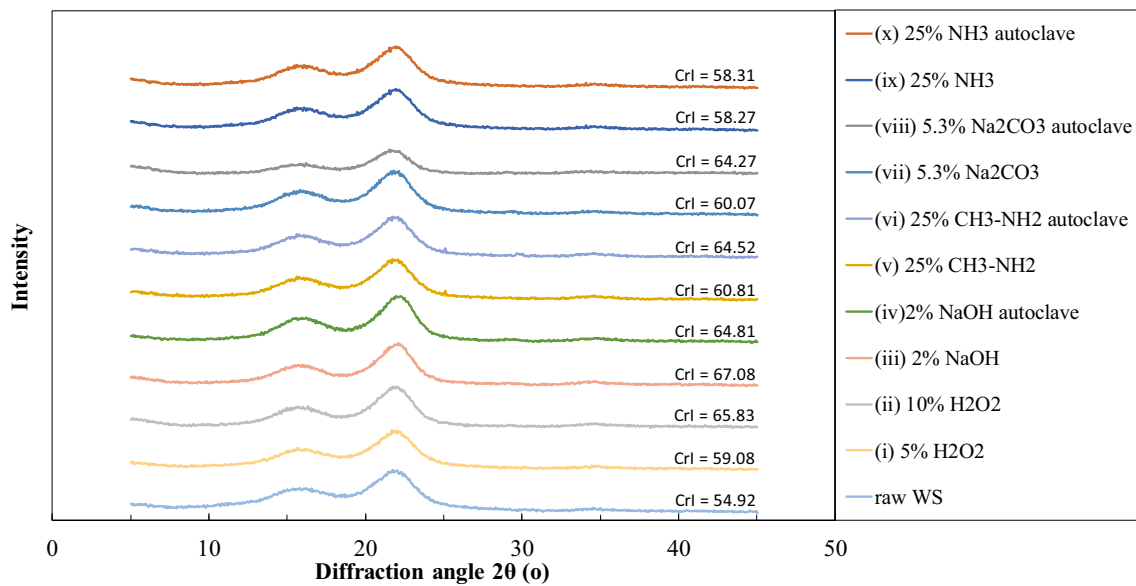
Fig. 1 XRD patterns for all wheat straw samples after different pretreatments (i) alkaline peroxide 5%, (ii) alkaline peroxide 10%, (iii) dilute NaOH 2%, (iv) dilute NaOH 2% autoclaving, (v) methylamine 25%, (vi) methylamine 25% autoclaving, (vii) Na$_2$CO$_3$ 5.3%, (viii) Na$_2$CO$_3$ 5.3% autoclaving, (ix) ammonia 25%, (x) ammonia 25% autoclaving

degradation ranged from 1.15 to 100% whereas acid-soluble lignin from 33.61 to 100%.

After alkaline peroxide 10% pretreatment, treatment of the slurry with CelliCTec2 cellulolytic formulation bioconverted almost all the cellulosic content of the sample to simple monomeric. The insoluble part of the sample was very slight (Table 4). In other words, 99% of the raw wheat straw sample was eventually solubilized by the sequence of alkaline peroxide (10%) addition and cellulolytic enzymatic treatment. The complete solubilization of agro-residues was also reflected in the degradation efficiencies (100%) of the structural components of wheat straw (cellulose, hemicellulose, and lignin)

implying the total elimination of polymeric forms. This is in accordance with Gould (1984) who reported that the degradation of lignin of agro-residues by use of alkaline peroxide presents higher efficiencies in the cases where the ratio of alkaline peroxide solution to lignocellulose is more than 0.25 g H$_2$O$_2$/g substrate.

In the experimental runs using 15 μL g^{-1} (CellicCTec2) of dry solid, the sugar content in the non-delignified sample was just 30 mg/g straw, while it was over 110 mg/g straw in the delignified material in all cases (Fig. 2). It is obvious that lignin had an inhibitory effect on the enzymatic saccharification of the non-delignified samples, and this was possibly

Table 4 Degradation of the total solids and structural polysaccharides of the solid fractions of raw and pretreated wheat straw after 4 days of enzymatic saccharification with 15 μL g^{-1} dry solid Cellic CTec2: (i) alkaline peroxide 5%, (ii) alkaline peroxide 10%, (iii) dilute NaOH 2%, (iv) dilute NaOH 2% autoclaving, (v) methylamine 25%, (vi) methylamine 25% autoclaving, (vii) Na$_2$CO$_3$ 5.3% autoclaving, (ix) ammonia 25%, (x) ammonia 25% autoclaving

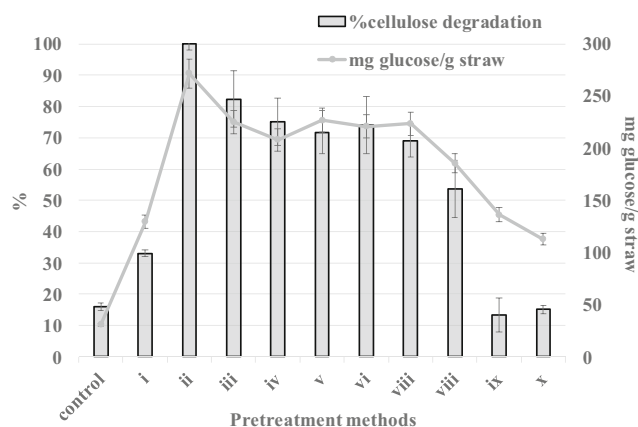|	% TS hydrolysis	% cellulose degradation	% AIL degradation	% ASL degradation	% hemicellulose degradation
Control	23.61 ± 0.62	15.89 ± 1.21	13.42 ± 0.15	33.61 ± 2.16	37.43 ± 8.12
i	31.18 ± 0.12	33.03 ± 1.08	20.18 ± 0.08	53.74 ± 1.87	46.56 ± 0.99
ii	95.82 ± 0.25	100.00 ± 2.02	100.00 ± 0.78	100.00 ± 0.87	100.00 ± 3.11
iii	77.39 ± 0.39	82.40 ± 8.92	18.40 ± 4.13	78.72 ± 5.68	82.43 ± 6.15
iv	80.72 ± 3.18	75.20 ± 7.58	9.94 ± 0.63	80.67 ± 8.65	91.81 ± 10.12
v	74.08 ± 1.13	71.79 ± 6.98	3.12 ± 0.95	74.08 ± 2.17	85.68 ± 9.26
vi	69.58 ± 2.53	74.03 ± 9.21	13.93 ± 1.11	65.38 ± 3.84	77.59 ± 7.68
vii	10.36 ± 2.42	53.73 ± 5.36	10.25 ± 1.18	44.49 ± 2.02	37.29 ± 7.98
viii	65.61 ± 6.85	69.13 ± 9.13	1.15 ± 2.21	62.62 ± 6.21	63.88 ± 5.68
ix	40.50 ± 1.87	13.29 ± 5.45	2.16 ± 1.23	45.47 ± 1.65	66.23 ± 6.21
x	36.17 ± 1.68	15.12 ± 1.27	5.73 ± 1.35	46.17 ± 1.74	61.16 ± 2.68

Fig. 2 Cellulose degradation and glucose recovery after enzymatic hydrolysis at 10% w/w dry solid using 15 μL g^{-1} (CellicCTec2) at 50 °C for 96 h of pretreated and non-pretreated solids: (i) alkaline peroxide 5%, (ii) alkaline peroxide 10%, (iii) dilute NaOH 2%, (iv) dilute NaOH 2% autoclaving, (v) methylamine 25%, (vi) methylamine 25% autoclaving, (vii) Na$_2$CO$_3$ 5.3%, (viii) Na$_2$CO$_3$ 5.3% autoclaving, (ix) ammonia 25%, (x) ammonia 25% autoclaving

because cellulases are adsorbed on lignin. Lignin appears to negatively affect the hydrolysis of cellulose since cellulolytic enzymes bind un-productively to lignin.

The latter is also confirmed by Fig. 3 where the delignification efficiency expressed as acid-insoluble lignin degradation from the pretreatment step is plotted against the cellulose conversion achieved at the saccharification step. Cellulose conversion was calculated by:

$$Y_{glu/cel} = \frac{C_{glu}}{C_{cel} \times f} \times 100(\%)$$

where $Y_{glu/cel}$ is the conversion of cellulose to glucose, C_{glu} is the glucose concentration after 4 days of enzymatic saccharification in grams per liter, C_{cel} is the initial cellulose concentration used in grams per liter, and f is a factor used to correct the water addition to 1 unit of hexose during enzymatic
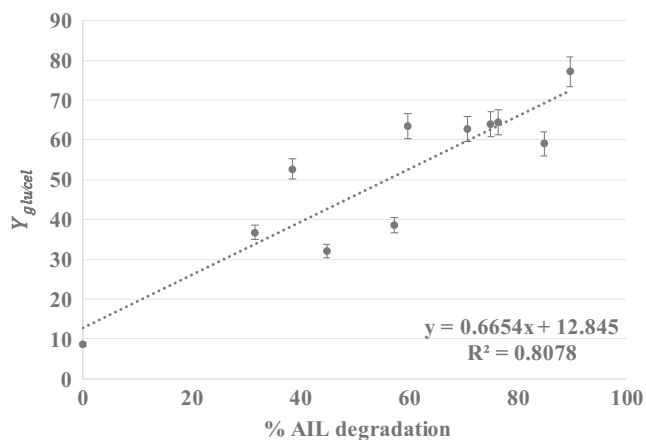

Fig. 3 The effect of acid-insoluble lignin (AIL) degradation efficiency from the pretreatment schemes on the cellulose degradation of enzymatic saccharification

saccharification, that is $f = \frac{180}{162} = 1.11$) (Jaisamut et al. 2016).

An almost linear correlation of these two parameters is evident, proving that the higher the delignification of the raw material, the higher the cellulose conversion. During the enzymatic saccharification, the cellulolytic enzymes have the tendency to attach to surfaces that are rich in lignin. This fact has adverse effects on the cellulose hydrolysis process and also negatively affects the possibility of recycling cellulolytic enzymes (Rahikainen et al. 2013). The proposed inhibitory effects of lignin include physical barrier, nonproductive adsorption, and possible deactivation of enzymes (Yang and Pan 2016). Lignin and hemicellulose adversely influence enzymatic saccharification by attaching to cellulose, and thus hindering its access to the enzyme. The decomposition of lignin and hemicellulose could cause an increase in the size of the pores and to the accessible surface of the wheat straw that in turn improves the accessibility to cellulose, and therefore, the cellulose conversion rate is increased (Zheng et al. 2018a).

Asghar et al. (2015) reported that pretreatment with 2.5% NaOH autoclave at 121 °C for 90 min had a pronounced effect on wheat straw, resulting in a hemicellulose content of 10.5%, a cellulose content of 83%, and a decomposition of lignin of 81%. The saccharification yield that was achieved after 8 h of incubation at 50 °C was 52.93%. Similarly, Akhtar et al. (2001) reported that after 20 h of enzymatic hydrolysis of wheat straw pretreated with 1% NaOH for 4 h at 121 °C, the saccharification yield was almost 30%. On the other hand, after 96 h of enzymatic saccharification of wheat straw that had been pretreated with 2% NaOH at 121 °C for 1 h the saccharification yield reached up to 60%. This is in accordance with Zheng et al. (2018a) who studied the effect of pretreatment with NaOH for concentrations from 0.5 to 4% and reported the increase in the conversion yield from 38.1 to 65.8%.

According to Feng et al. (2014), for pretreatments at 140 °C of wheat straw with sodium carbonate, ammonia (15% w/w), and methylamine, the glucose yields after 168 h of enzymatic hydrolysis that were reported were 47, 80, and 84% respectively. These yields are higher than those achieved in our study and this could be attributed to the increase of the conversion rate of cellulose due to higher temperatures.

With regard to CrI, the higher the CrI, the higher the cellulose conversion during enzymatic hydrolysis (Fig. 4). In comparison with the CrI of raw wheat straw (54.92%), the CrI of NaOH-pretreated and 10%H$_2$O$_2$-pretreated WS was significantly greater (67.08% for NaOH-pretreated WS and 65.83% for H$_2$O$_2$-pretreated WS, respectively). The cellulose conversion rates of these two pretreatments were very elevated (82.4% and 100.0%, respectively, calculated on the remaining cellulose after the delignification pretreatment). This fact
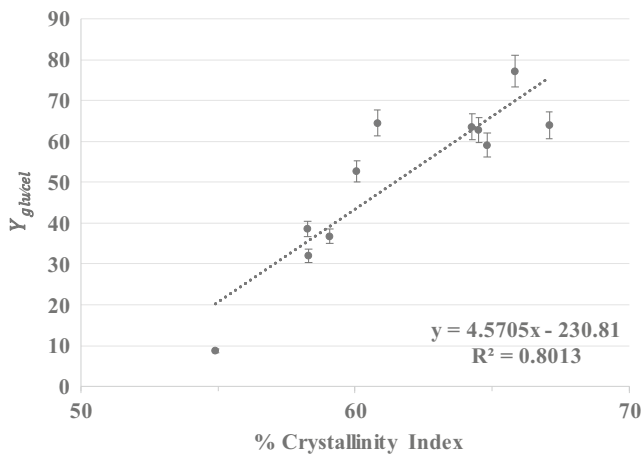
Fig. 4 Influence of crystallinity index (CrI) from various pretreatment methods on the cellulose conversion of enzymatic hydrolysis

may be is ascribed to the partial degradation of lignin and hemicellulose after the pretreatments (Table 2). Similar results were also reported by other studies. Zheng et al. (2018b) reported that the crystallinity index of raw wheat straw (48.27%) was significantly lower than the respective value of alkaline-pretreated (61.84%) and acid-pretreated (61.51%) wheat straw, even though the cellulose conversion rates of these two pretreatments were considerably elevated (87.2% and 86.6%). In addition, Haque et al. (2012) investigated the pretreatment of WS with NaOH (0.5%, 1.0%, 1.5%, and 2.0%) at 105 °C and concluded that the pretreatment with NaOH induced a pronounced effect on the crystallinity index of samples resulting in a considerable increase (52.3%, 57.9%, 65.3%, and 71.5% respectively) in comparison with raw wheat straw (45.0%). The respective reducing sugar yields also improved from 45 to 78% as the NaOH concentration and CrI increased.

Conclusions

An efficient biorefinery must be capable of using all the components in lignocellulosic residues resourcefully for the production of fuels in addition to other chemicals and products. Although there is a wide range of chemical means, alkaline pretreatment in sequence with by enzymatic saccharification of wheat straw may induce the removal of lignin. The latter acts as a protective media for the polysaccharides inhibiting microbes and/or enzymes accessibility. Hence, this procedure could lead to an increment of sugars monomers and consequently to elevated biofuels' yield. In this paper, the effect of different alkaline pretreatments on cellulose digestibility of WS was investigated. Alkaline pretreatments tested proved to promote delignification reactions. The higher the delignification efficiency at the pretreatment, the higher the glucose recovery at the enzymatic hydrolysis. Hydrolysis of

the insoluble fraction with CellicCtec2 cellulase after alkaline treatment with hydrogen peroxide 10% (ii) and NaOH 2% (iii) yielded glucose with 73% and 60% efficiency, respectively, based on the content of cellulose of the raw wheat straw. The CrIs of biomass increased after pretreatments, particularly that using NaOH 2% and H_2O_2 10%, which resulted in an enhancement of enzymatic hydrolysis given that cellulose degradation rates of these two pretreatments were high (100.0% and 82.4% respectively). Furthermore, there exists a definite correlation between polysaccharide conversion and crystallinity index for all pretreatments due to the fact that the higher the delignification efficiency, the higher the cellulose conversion. These data indicate that these pretreatments are efficient for improving the enzymatic digestibility of lignocellulosic materials to levels approaching the theoretical maximum.

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