Effect of alkaline pretreatments on the enzymatic hydrolysis of wheat straw

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Introduction

Lignocellulosic materials are mainly composed of cellulose, hemicellulose and lignin. Lignin is considered to be the major barrier to the enzymatic hydrolysis of cellulose towards the production of fermentable sugars. Therefore, removing lignin from the lignocellulosic raw materials is favorable to reducing the recalcitrance of lignocellulose for enzymatic attack. For this purpose, various pretreatment technologies have been developed to improve the removal of lignin from the compact structure of plant cell walls and enhance the digestibility of cellulose by enzymes. Wheat straw is an abundant agriculture residue worldwide. In this work, wheat straw was firstly alkaline pretreated under selected chemicals and then subsequently it was enzymatically hydrolysed.

Materials and Methods

Wheat straw (WS) was obtained from Aspropyrgos province, Greece. The wheat straw was milled (FRITSCH Cutting mill Pulverisette 15) to 1 mm. The milled straw mainly composed of 33.8% cellulose, 45.1% hemicellulose, 16.4% lignin (15.4% Klason lignin and 1.0% acid-soluble lignin), and 4.7% ash. All chemical reagents were of analytical grade and used without further purification. The commercial enzymatic formulation CelliCTec2 was kindly donated by Novozymes (Denmark). Total cellulase activity (FPU) was measured against filter paper by the standard IUPAC method and found to be 223 FPU/mL. Moisture, extractives, ash, cellulose, hemicellulose and lignin in raw and pretreated materials were analysed following National Renewable Energy Laboratory's (NREL) standard analytical procedure. In the liquid phase, TOC, VFA and phenolic compounds were also measured according to standard methods while glucose concentration was determined using a commercially available kit (Biosis S.A., Athens, Greece) that employed the Glucose Oxidase–Peroxidase (GOX–PER) method. All analyses were performed in duplicate.

Pretreatment of wheat straw: Ten different alkaline pretreatments were applied in this study: (i) alkaline peroxide 5%, (ii) alkaline peroxide 10%, (iii) dilute NaOH 0.5M, (iv) dilute NaOH 0.5M autoclaving, (v) methylamine 25 %w/w, (vi) methylamine 25 %w/w autoclaving, (vii) Na_2CO_3 0.5M, (viii) Na_2CO_3 0.5M autoclaving, (ix) ammonia 25 %w/w, (x) ammonia 25 %w/w autoclaving. The operational conditions were selected according to the optimal experimental settings previously reported for each pretreatment (Bolado-Rodríguez et al., 2016; Feng et al., 2012). In autoclave pretreatments, WS was slurried for 5 min with alkaline solution in autoclavable bottles with a solid:liquid ratio of 1:10 w/w, and then autoclaved at 121°C for 60 min. The same experiments were conducted under milder thermal conditions (50°C, 96 h). In an alkaline-peroxide pretreatment, WS was mixed for 5 min with 5% or 10% w/w H_2O_2, in a solid:liquid ratio of 1:20 w/w, the pH was then be adjusted to 11.5 with 2 M NaOH and the mixture was placed in a rotatory shaker at 50°C and 120 rpm for 60 min.

Enzymatic hydrolysis: Enzymatic hydrolysis of untreated raw material and pretreated solids was performed in 100 mL Erlenmeyer flasks containing 10% w/w dry solid and 15μL g^{-1} (CellicCTec2) of dry solid. A buffer solution of sodium citrate 0.05M and phosphoric acid 0.1M was used for pH adjustment (5.0). Hydrolysis was performed in a rotatory shaker at 50°C and 300 rpm for 96 h. After hydrolysis, samples were collected and stored for analysis of sugars. All experiments were carried out in triplicate and average data are shown.

Results and discussion

Effect of pretreatments on solid composition

Table 1. Degradation of main components of solid fractions after different pretreatments of wheat straw. (i) alkaline peroxide 5%, (ii) alkaline peroxide 10%, (iii) dilute NaOH 0.5M, (iv) dilute NaOH 0.5M autoclaving, (v) methylamine 25 %w/w, (vi) methylamine 25 %w/w autoclaving, (vii) Na_2CO_3 0.5M, (viii) Na_2CO_3 0.5M autoclaving, (ix) ammonia 25 %w/w, (x) ammonia 25 %w/w autoclaving.

	%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 ± 0.2	1.88 ± 1.37	89.60 ± 0.68	43.99 ± 0.9	17.69 ± 2.21
iii	30.07 ± 0.36	4.45 ± 10.11	75.06 ± 5.03	36.48 ± 4.66	30.66 ± 10.26
iv	36.47 ± 2.78	33.52 ± 15.44	84.86 ± 0.45	49.86 ± 10.72	9.29 ± 10.89
v	29.84 ± 0.03	22.3 ± 10.63	76.38 ± 1.06	99.22 ± 0.09	8.66 ± 10.46
vi	26.09 ± 4.73	24.85 ± 32.33	70.78 ± 14.23	99.03 ± 0.19	3.84 ± 31.61
viii	11.05 ± 8.65	3.71 ± 13.1	59.81 ± 4.15	38.93 ± 6.63	0.50 ± 3.75
viii	11.59 ± 0.37	4.87 ± 6.76	38.51 ± 6.92	16.99 ± 2.16	1.31 ± 12.72
ix	26.75 ± 1.49	44.41 ± 7.35	57.31 ± 2.53	99.21 ± 0.01	0.90 ± 12.10
x	25.00 ± 2.16	17.11 ± 21.17	44.83 ± 7.55	99.24 ± 0.04	24.41 ± 22.71

In terms of solid fraction composition, different variations were observed in cellulose, hemicellulose and lignin compared to the raw material depending on the pretreatment. These results are shown in Table 1. For all the pretreatments tested, cellulose and hemicellulose solubilisation and degradation proved much lower than was experienced by lignin, resulting in a relative increase in cellulose percentage in the solid compared to raw material. The higher the solubilisation and reduction of lignin, the greater the cellulose composition increase of pretreated solid. The increase in cellulose content and decrease in lignin content achieved after delignification are expected to lead to an enhancement in the enzymatic convertibility of the wheat straw compared to non-delignified straw. The effect of the delignification was evident in the chemical composition of the pretreated wheat straw. Acid soluble lignin content decreased from 0.76 to 99% whereas acid insoluble lignin from 31.63 to 89.60%.

In the experiments using 15μL g^{-1} (CellicCTec2) of dry solid, glucose concentration in the non-delignified material was only 0.5 mg/g straw, whereas it was more than 110 mg/g straw in the delignified material. It is apparent that lignin inhibited the enzymatic hydrolysis of the non-delignified material, and this was probably due to adsorption of cellulases on lignin. Lignin appears to reduce cellulose hydrolysis by non-productively binding cellulolytic enzymes.

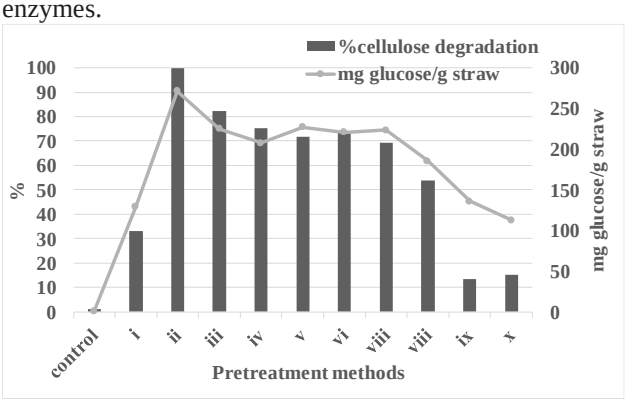

Figure 1. Cellulose degradation and glucose recovery after enzymatic hydrolysis at 10% w/w dry solid using 15μL g^{-1} (CellicCTec2) at 50°C for 96 h of pretreated and non-pretreated solids. (i) alkaline peroxide 5%, (ii) alkaline peroxide 10%, (iii) dilute NaOH 0.5M, (iv) dilute NaOH 0.5M autoclaving, (v) methylamine 25 %w/w, (vi) methylamine 25 %w/w autoclaving, (vii) Na_2CO_3 0.5M, (viii) Na_2CO_3 0.5M autoclaving, (ix) ammonia 25 %w/w, (x) ammonia 25 %w/w autoclaving.

After alkaline peroxide 10% pretreatment, treatment of the residue with CelliCTec2 cellulase converted essentially all of the cellulose to glucose, leaving a small amount of insoluble material (Figure 1**).** In other words, 99% of the original straw sample was ultimately solubilized by the combination of alkaline peroxide 10% and cellulase treatments. This is in accordance with Gould (1983) who reported that delignification of agricultural residues by alkaline peroxide proceeds most efficiently when the ratio of peroxide to lignocellulosic substrate is at least 0.25 g H_2O_2/g substrate.

Conclusions

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% w/w (ii) and NaOH 0.5M (iii) yield glucose with 73% and 60% efficiency respectively, based upon the cellulose content of the raw material. These data indicate these pretreatments are efficient for enhancing the enzymatic digestibility of lignocellulosic crop residues to levels approaching the theoretical maximum.

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