NaOH alkaline pretreatment for Improvement of Enzymatic Digestibility of wheat straw as Alternative Sugar Source

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Keywords: alkaline pretreatment; delignification; enzyme loading; scale-up; wheat straw
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Introduction

Lignocellulosic materials, such as straw, 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. Alkaline pretreatments have been proved to promote delignification reactions It has been reported that hydrolysis of the insoluble fraction of straw with cellulases after alkaline treatment with NaOH yields high cellulose degradation efficiencies accompanied by high saccharification yields. Nevertheless, in order to turn the whole process line viable, process optimisation is more than necessary. To this end, this paper aims to optimise the NaOH pretreatment process along with the enzymatic hydrolysis in order to obtain more fermentable sugars and glucose recovery.

Materials and Methods

Wheat straw (WS) was obtained from Aspropyrgos province, Greece. For the comminution of straw, a FRITSCH Cutting mill Pulverisette 15 was used in order to achieve homogeneous and easy handlable raw material. By using proper sieves, the desired particle sizes (1mm, 1-2cm) were obtained. For coarse particle size of 3-5cm, a grinder was used. 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.

For the chemical pretreatment, wheat straw was slurried for 5 min with NaOH 0.5M solution in autoclavable bottles with a solid to liquid ratio of 1 to 10 w/w. NaOH pretreatment was tested with two different operational set ups; autoclaved at 121°C for 1 hour and under milder thermal conditions (50°C, 96 h).

The enzymatic hydrolysis of the pretreated solid samples was performed in 100 mL Erlenmeyer flasks at 50 ºC after pH adjustment to 5 with H_2SO_4 (0,1M). The reaction mixtures that contained 10% w/w dry pretreated solids and the cellulolytic formulation, Cellic CTec2 (Novozymes, Denmark) were incubated at 50ºC and 300 rpm for 96 h in a rotary shaker incubator. Enzyme loadings of 5, 10, 15 and 20 μL/g pretreated straw were used.

Moisture, extractives, ash, cellulose, hemicellulose and lignin in raw and pretreated materials were analysed following National Renewable Energy Laboratory's (NREL) standard analytical procedure. All chemical reagents were of analytical grade and used without further purification. 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.

Results and discussion

In order to maximise glucose release from straw, the following process parameters were studied: straw particle size, enzyme loading, NaOH pretreatment kinetics, recycling of alkaline solution and scale-up factor.

Particle size

By using proper sieves, the desired particle sizes (1-3mm and 1-2cm) were obtained and tested. From the experimental data it was obvious that the particle size in the range of 1 to 20mm didn't affect significantly the performance of the pretreatment scheme, since both chemical pretreatment and enzymatic hydrolysis presented similar efficiencies within the statistical error of the experimental trials. Thus, all the experimental trials beyond this point were conducted using coarse straw particles (10-20mm).

Enzyme Loading

In order to examine the effect of enzyme loading of the NaOH autoclaved straw on the solubilization efficiency, different enzyme loadings of CellicCTec2 were tested. Enzyme loadings of 5, 10, 15 and 20 μL/g pretreated straw were used. It was concluded that for enzyme loadings up to 15 μL/g pretreated straw, there is an almost linear correlation between enzyme loadings and performance indicators of straw hydrolysis such as cellulose degradation, soluble organic carbon and glucose released in the liquid phase. On the other hand, further increase of enzyme loading resulted in similar cellulose degradation efficiency. Nevertheless, a noticeable decrease in concentration of soluble compounds was also observed. Thus, 15 μL/g pretreated straw was selected as the optimum cellulase dosage.

Pretreatment Kinetics

NaOH pretreatment was studied with two different operational set ups; autoclaved at 121°C for 1 hour and under milder thermal conditions (50°C, 96 h). Hydrolysis of the insoluble fraction with CellicCtec2 cellulase after alkaline treatment with NaOH 0.5M (50°C, 96 h) and NaOH 0.5M autoclaving yielded similar cellulose degradation efficiencies, 82.4% and 75.2% respectively. These elevated cellulose degradation efficiencies were accompanied by high saccharification yield ranging from 207 to 225 mg glucose/g straw. Since both performances were similar, but the cost infrastructure and energy needs of the two pretreatment approaches differ in magnitude, the kinetics of NaOH pretreatment of straw under milder thermal conditions was studied. From this experimental set it was proved that after 6h of pretreatment (up to 96h), the delignification efficiency remained nearly constant.

Recycling

The possibility of alkaline solution recycling was also investigated. It was proven (Table 1) that the solution could be recirculated at least 4 times before being "saturated" although after each experiment it needed to be supplemented with some fresh solution.

Table 1. Delignification efficiencies after recycling of alkaline solution.

Cycle No.	1	2	3	4	5
% delignification	86.4	79.5	72.2	76.5	66.0

Scale-up

Up-scaling of the whole treatment scheme from 5g straw to a capacity of 0.5kg straw was studied. Both delignification and saccharification efficiencies remained in the same range, implying that scale-up factor of 100 could be applied in the process successfully. Further up-scaling to a capacity of 3kg straw was also successful.

Conclusions

Conclusively the optimal parameters for saccharification of wheat straw are: particle size up to 2cm, alkaline pretreatment with NaOH 0.5M at 50ºC for 6h followed by enzymatic hydrolysis at enzyme loading of 15 μL CellicCTec2 s/g pretreated straw. The alkaline solution could be recycled up to 4 times without compromising the delignification efficiency. Up-scaling process capacity from 5g to 3 kg of straw presented similar yields, implying that the whole process could be incorporated in full scale systems.

Acknowledgements

The authors acknowledge funding through European Horizon 2020 NoAW (No Agro Waste, Grant no. 688338) project for supporting this work.

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