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Chemical Oxidation with Ozone as Pre-treatment of Lignocellulosic Materials for Bioethanol Production

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The main results derived from the ozone pre-treatment of grain straw are shown. The use of ozone reduced acid insoluble lignin content in grain straw, whereas acid soluble lignin increased. This fact resulted in a more accessible structure to enzymes leading to higher enzymatic hydrolysis yield. Ozone consumption after 2.5 h reaction time was about 0.10-0.12 g/g DS, independently of the type of cereal straw. Wheat and rye straw showed the highest sugar release after enzymatic hydrolysis: about 2-2.5 g of glucose was released per gram of ozone consumed and 1.1-1.4 g of xylose/g of ozone consumed in the ozonation stage. Moreover, about 1.5 g of glucose/g of ozone consumed and 1.2 g of xylose/g of ozone consumed were released to the hydrolysis medium when barley and oat straw were pretreated with ozone.

1. Introduction

Lignocellulosic materials, especially agricultural and forestry residues, offer a great potential as cheap and abundant feedstock for biofuels production. Enzymatic hydrolysis of the cellulose fraction yields glucose that is fermented to obtain fuel-grade ethanol. The main disadvantage to produce economically ethanol from lignocellulosic materials is its low digestibility due to the complex chemical structure. Lignocellulosic materials are formed by cellulose, hemicellulose and lignin, closely linked. Lignin significantly impacts enzymatic hydrolysis of lignocellulosic biomass. Therefore, a pre-treatment step has to be considered because of the high crystallinity of the cellulose and the presence of lignin. The pre-treatment step should improve the accessibility of hydrolytic enzymes to cellulose. Moreover, the pretreatment should avoid degradation of hemicellulose and cellulose because sugar degradation not only decreases ethanol yield but also produces degradation products that are inhibitory to the yeast used in the subsequent fermentation.

Different alternatives have been used as pre-treatment of lignocellulosic materials, involving the use of physical, chemical, physicochemical and/or biological methods, e.g. steam explosion, hot water extraction (Rosgaard et al., 2007), sulfuric acid, sodium hydroxide, hydrogen peroxide, peracetic acid, ozonolysis (Silverstein et al., 2007; Zhao et al., 2008; García-Cubero et al, 2009) ammonia fiber explosion, AFEX (Sendich et al.,

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2008; Teymori et al., 2005) and wet oxidation (Hendriks and Zeeman, 2009; Sun and Cheng, 2002).

Among them, chemical oxidation with ozone offers numerous advantages. Ozone may degrade lignin producing soluble compounds of much lower molecular weight. Moreover, ozonation reduces the formation of degradation products that might interfere with subsequent hydrolysis or fermentation stages.

The aim of this work was to investigate the influence of the chemical pre-treatment with ozone on the production of fermentable sugars in the hydrolysis stage. Straws from different crops such as rye, wheat, oat and barley were used as raw material.

2. Materials and Methods

2.1 Raw material

Straws were kindly donated by the Institute of Technological Agriculture of Castilla y León. Straws were ground in a blender, sieved to obtain particle sizes about 3-5 mm and kept in an oven at 45 °C. Before ozonation, water content was set to 40 % w/w, according to Garcia-Cubero et al. (2009). The characteristics of raw materials are summarized in Table 1.

Table 1: Characterization of raw material (in % w/w)

	Wheat straw	Rye straw	Oats straw	Barley straw
Cellulose (as glucose, %)	32.4	25.9	32.9	39.2
Hemicellulose (as xylose, %)	19.1	21.5	21.7	21.5
Acid Lignin (%)	21.3	27.1	18.1	22.9
Ash (%)	6.4	3.1	3.9	4.4
Moisture (%)	6.9	5.9	7.1	6.2

2.2 Ozonation pre-treatment

The ozonolysis pretreatment of wheat and rye straw was performed in a fixed bed reactor (glass column 50 cm in height and 2.7 cm in diameter) under room conditions. Reactor was filled with ground material (50-55 g) after setting the humidity to 40%. Ozone production (ozone generator model Sander 301) was controlled by varying either the air flow rate or the electrical power supply. The inlet gas flow rate was 60 L/h. Ozone concentration in the gas phase was 2.7% w/w. Ozone was supplied at the bottom of the fixed bed through a gas diffuser. Ozone concentration in the gas phase was determined according to the iodometric method (Standard Methods for the Examination of Water and Wastewater, 1995). Two ozone traps containing 2 % potassium iodine solutions were connected in series with the reactor to determine the ozone concentration in the outlet gas stream. The ozonated straw was dried in an oven at 45 °C, stored in a freezer and used for the subsequent enzymatic hydrolysis experiments and/or for composition analysis. Ozonation experiments were conducted in duplicate. The mean value and standard deviation were calculated.

2.3 Enzymatic hydrolysis

Enzymatic hydrolysis experiments were performed using a mixture of cellulase complex (NS50013) and β-glucosidase (NS50010), enzymes kindly donated by Novozymes (Denmark). The hydrolysis step was performed at 50 °C during 96 h. Test flasks were shaken in a rotary incubator at 300 rpm. After hydrolysis, 600 μL samples were withdrawn, filtered with 0.22 μm filter and stored for sugar analysis. Tests were conducted in triplicate. The mean value and standard deviation were calculated.

2.4 Analytical methods

Acid insoluble lignin (AIL), acid soluble lignin (ASL), cellulose and hemicellulose in the raw materials were estimated following NREL laboratory analytical procedures Lap 003, 004 and 002 respectively. A Bio-Rad HPX-87C ion-exclusion column was used to determine sugar concentrations. Water was used as mobile phase at a flow rate of 0.6 mL min-1 and 60 °C. The detector was a refractive index. Sugars from enzymatic hydrolysis were also analyzed by HPLC using the Aminex HPX-87C column (Bio-Rad, Hercules, CA) under the operating conditions previously indicated.

3. Results and Discussion

3.1 Determination of reaction time

A set of experimental runs using wheat straw as raw material were firstly performed to select the reaction time (Figure 1). From the experimental results it was observed that chemical oxidation with ozone exerted a great impact on both soluble and insoluble lignin content. Ozonation pretreatment seems to solubilize lignin more than degrade it. An ozonation time of 2.5 h reduced acid insoluble lignin content from 22.1 % (w/w) to 12.3% whereas acid soluble lignin content increased from 3.2 % to 8.1 %. Longer reaction times did not further reduce insoluble lignin content. Therefore, from these results a reaction time of 2.5 h was selected for subsequent trials.

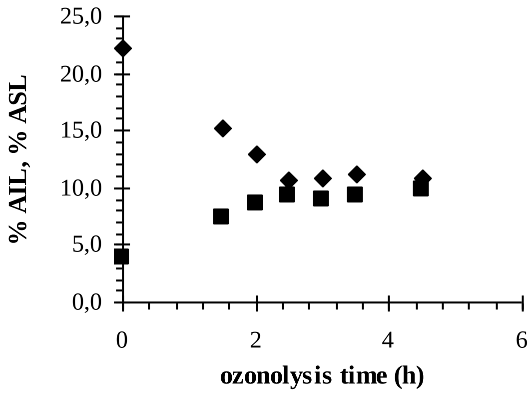

Figure 1: Effect of reaction time on lignin content. ♦ Acid Insoluble Lignin (AIL); ■ Acid Soluble Lignin (ASL).

3.2 Effect of ozone on enzymatic hydrolysis yield

After selecting the reaction time, a set of experimental runs were performed to analyze the influence of ozone pretreatment on hydrolysis efficiencies. The contents on acid insoluble lignin and acid soluble lignin before and after the ozonation stage were compared. Glucose and xylose concentrations were also compared to analyze the influence of ozonation pretreatment on hydrolysis. Results are summarized in Table 2.

Regarding the ozonation stage, ozonation efficiencies is defined as the percentage of ozone fed into the reactor that is consumed after a given time. In all the experimental runs, ozonation efficiencies after 2.5 h reaction time reached values about 85-95 %. Ozone consumption was about 0.10-0.12 g O_3/g of dry matter, independently of the type of cereal straw. As consequence of the ozonation pretreatment, it was observed that the acid insoluble lignin content was considerably reduced from about 20-24 % (w/w) to 11-13 % whereas acid soluble lignin content increased from about 3-4 % to 7-8 % after 2.5 h ozonation time.

Regarding the hydrolysis stage, the pretreatment with ozone increased considerably the concentration of fermentable sugars in hydrolysis stage in comparison with the non-pretreated raw materials. Higher yields were obtained for ozonated rye and barley straw, the raw materials with the higher lignin content, whereas the straw with the lower lignin content (oat straw) led to the lower yield for hydrolysis, in accordance with previous experimental results (Garcia-Cubero et al., 2009). In all cases, the pretreatment with ozone of raw materials enhanced the yield of hydrolysis. From the different types of straws studied, rye and barley were easier to hydrolyze than wheat, even without ozone pretreatment. Enzymatic hydrolysis yield increased from 13.9% corresponding to untreated raw material to 40.1 % for pretreated wheat straw. Acid insoluble lignin content was reduced from 21.3 % to 13.2 %. For rye straw, the enzymatic hydrolysis yield improved from 15.4 % (27.1 % AIL) corresponding to untreated material to 53.7 % (13.5 % AIL) for pretreated samples whereas for barley straw the enzymatic hydrolysis yield improved from 19.6 % (22.9 % AIL) corresponding to untreated material to 59.4 % (13.5% AIL) for pretreated straw. Oat straw led to the lowest saccharification yield, improving from 6.2 % (18.1 % AIL) for untreated material to 18.8 % (10.8 % AIL) for the pretreated straw.

Similar results were found for hemicellulose hydrolysis. Although enzymes mixture contains cellulose and β-glucosidase, a partial hydrolysis of hemicellulose was obtained. Higher xylose yields were obtained for rye, barley and wheat straws whereas oat straw led to lower xylose yield.

These results can be explained because ozonation pretreatment removes a higher percentage of acid insoluble lignin in rye and barley than in wheat straw, although the remaining lignin content in the pretreated samples was similar for all raw materials (around 13–13.5 %). A fraction of acid insoluble lignin is probably solubilized or degraded. Therefore cellulose is more accessible to enzymes. However, there may be other fraction of lignin refractory to ozonation pretreatment.

With regard to the amount of sugar released as a function of ozone consumed, about 2-2.5 g of glucose was released per gram of ozone consumed and 1.1-1.4 g of xylose/g of ozone consumed in the ozonation stage. The ozonation pretreatment of barley straw and oat straw was also able to enhance fermentable sugar concentrations. About 1.5 g of glucose/g of ozone consumed and 1.2 g of xylose/g of ozone consumed was released to the hydrolysis medium.

Table 2: Lignin content (%) of ozone pretreated material and yield of hydrolysis

	AIL (%)	ASL(%)	Enzymatic hydrolysis	
			glucose yield (%)	xylose yield (%)
Wheat straw	13.2	7.0	40.1	33.9
Rye straw	13.5	7.6	53.7	31.3
Oat straw	10.8	7.7	18.8	15.2
Barley straw	13.5	7.4	59.4	34.7

4. Conclusions

From the results, it was shown than the chemical pretreatment with ozone enhanced the production of fermentable sugars in the subsequent hydrolysis stage. It can be concluded that ozonolysis is an efficient pretreatment for cereal straw. Ozone degrades and/or solubilizes lignin and slightly solubilizes the hemicellulose fraction, improving the subsequent enzymatic hydrolysis. Negligible losses of cellulose were detected. Although rye and barley straws yielded higher efficiencies, about 53-57 % of glucose and 31-35 % of xylose, ozone pretreatment was also able to increase the fermentable sugar concentrations released by wheat straw (about 40 % glucose and 34 % of xylose). Oat straw was the raw material that led to the lowest hydrolysis yields (about 19 % glucose and 15 % of xylose).

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