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OZONE PRETREATMENT OF WHEAT STRAW AND ITS EFFECT ON REDUCING SUGARS IN HYDROLYZATE

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Abstract

The aim of this contribution is to measure the effect of the pretreatment of lignocellulosic phytomass utilization for bioethanol production. The first step of bioethanol production from lignocellulosic phytomass is pretreatment of raw material. The next step is hydrolysis, and then the fermentation of sugars follows. The physical (grinding, breaking) and chemical (ozonization) processes were used as pretreatment. Ozone was applied to the aqueous suspension of lignocellulosic phytomass before and during the hydrolysis. Ozone pretreatment did not perform as effectively as expected. The results of study, which are focused on evaluation of reducing sugars are included in this contribution.

Key words

lignocellulosic phytomass, pretreatment, particle size, ozone, reducing sugars

Introduction

The increased concern for the security of the oil supply and the negative impact of fossil fuels on the environment, particularly greenhouse gas emissions, has put pressure on society to find renewable fuel alternatives. The most common renewable fuel today is ethanol produced from sugar or grain (starch); however, this raw material base will not be sufficient. Consequently, future large-scale use of ethanol will most certainly have to be based on production from lignocellulosic materials (1).

Bioethanol production

Bioethanol can be produced by fermenting sugary crops (sugar cane, sugar beet), starch-containing materials (corn, potatoes, wheat) (2, 3) and lignocellulosic biomass (wood, agricultural and forest residues – corn stover, cereal straws, bagasse) (4, 5). Bioethanol

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production depends on the used entry materials, because conversions of sugar, starch and lignocelluloses to ethanol are different.

Lignocellulosic biomass, including forestry residue, agricultural residue, yard waste, wood products, animal and human wastes, etc., is a renewable resource that stores energy from sunlight in its chemical bonds. It has great potential for the production of affordable fuel bioethanol, since it is less expensive than starch (e.g. corn) and sucrose (e.g. sugarcane) producing crops and available in large quantities (6). Lignocellulose composed of cellulose (40 – 50 %), hemicelluloses (25 – 35 %) and lignin (15 – 20 %) is extremely resistant to enzymatic digestion (7).

Production of bioethanol from lignocellulosic biomass contains three major processes, including pretreatment, hydrolysis and fermentation (6, 8).

The prerequisite in the utilization of lignocellulose for bioethanol production is to efficiently yield a fermentable hydrolyzate rich in glucose from the cellulose content present in the feedstock. The purpose of pretreatment is to break down the shield formed by lignin and hemicellulose, disrupt the crystalline structure and reduce the degree of polymerization of cellulose. Pretreatment has been viewed as one of the most expensive processing steps within the conversion of biomass to fermentable sugar. With the advancement of pretreatment technologies, the pretreatment is also believed to have great potential for the improvement of efficiency and reduction of cost (6). Pretreatment is required to alter the biomass macroscopic and microscopic size and structure as well as its submicroscopic structural and chemical composition to facilitate rapid and efficient hydrolysis of carbohydrates to fermentable sugars.

Hydrolysis refers to the processes that convert the polysaccharides into monomeric sugars (6).

The hydrolysis of lignocellulose is limited by several factors. Several researchers conclude that crystallinity of celulose is just one of the factors. Other factors are degree of polymerization, moisture content, available surface area and lignin content (9). The fermentable sugars obtained from hydrolysis process could be fermented into ethanol by ethanol producing microorganisms, which can be either naturally occurred or genetically modified (6).

Pretreatment processes

Physical (e.g. mechanical comminution, hydrothermal pretretment), physico-chemical (e.g. steam or CO_2 explosion, ammonia fiber explosion), chemical (e.g. ozonolysis, acid or alkaline hydrolysis) and biological processes (with microorganisms such as fungi) can be used for pretreatment of lignocellulosic materials. Each pretreatment has its own effect(s) on the cellulose, hemicellulose and lignin; the three main components of lignocellulosic biomass (9). The goal of pretreatment is to remove lignin and hemicellulose, reduce cellulose crystallinity, and increase the porosity of the material. Successful pretreatment can significantly increase the quantity of reducing sugars and improve the hydrolysis (10).

Lignocellulosic phytomass can be comminuted by a combination of chipping, grinding and milling to reduce cellulose crystallinity. This reduction facilitates the access of cellulases to the biomass surface increasing the cellulose conversion. The energy requirements of mechanical comminution of lignocellulosic materials depend on the final particle size and biomass characteristics. Although mechanical pretreatment methods increase celulose reactivity towards enzymatic hydrolysis, they are unattractive due to their high energy and capital costs (11).

The effect of ozone pretreatment has been found to be essentially limited to lignin degradation. Hemicellulose is slightly attacked, while cellulose is hardly affected. Ozonation has been widely used to reduce the lignin content of both agricultural and forestry wastes (12).

Experiment

Pretreatment is the first and the most important step in the experiment. Hydrolysis is the next step. The physical (grinding, breaking) and chemical (ozonization) processes were used as pretreatment. Impact of pretreatment on sugars (defined as reducing sugars) occurring in the hydrolyzate was studied. Hydrolyzate was accrued after hydrolysis from raw materials as the main product for bioethanol production. Scheme of the experiment is shown in Figure 1. For the comparative study of different pretreatment methods, the best pretreatment conditions of each method was first selected on the basis of quantity of total reducing sugars of the pretreated sample.

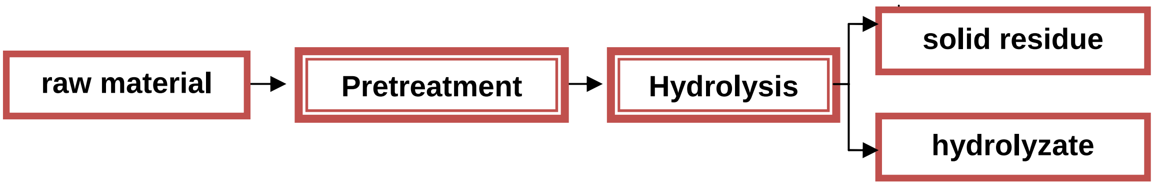


Fig. 1 *Scheme of experiment*

Raw material

Lignocellulosic phytomass – wheat straw, variety Bardotka, was used as a raw material. The straw was grown, harvested and stored in hay after a drought period in Slovakia during the summer 2010. The straw was cut into 40–100 mm pieces and stored in plastic bags at room temperature until pretreatment. The chemical composition of the untreated wheat straw can be seen in Table 1.

CHEMICAL COMPOSITION OF UNTREATED
WHEAT STRAW USED IN THE EXPERIMENT

Table 1

Total solids		96.16 %
Cellulose		44.38 %
Lignin	**insoluble (Klason)**	22.07 %
	soluble	2.56 %
Ash		5.1 %

Pretreatment

The first type of the straw pretreatment was breaking and grinding. The straw was pretreatment into 1–30 mm pieces. Pretreated straw was divided according to the sieve analysis (size: ≤ 1 mm, 1–12 mm, 15–30 mm). After pretreatment, the straw was hydrolyzed with acid.

The second type of straw pretreatment was pretreatment by ozone. Ozonizator DEZOSTER, machine of company HIVUS, Žilina was used for pretreatment. Ozone was produced from air, which was sucks into equipment by pneumatically by means of pump. Ozone was applied to the aqueous suspension of lignocellulosic phytomass before and during the hydrolysis.

Ozonization before hydrolysis

The straw with particle size ≤ 1 mm, 1–12 mm, 15–30 mm was used for experiment. Suspension was prepared in the ratio of 1 part straw and 8 parts distilled water in a boiling flask. This suspension was pretreated with ozone for 5, 10, 15, 30, 45, 60 minutes at a room temperature and ozone flow rate 3.61 mg minutes^{-1}. After pretreatment, the suspension was hydrolyzed with acid.

Ozonization during hydrolysis

The straw with particle size ≤ 1 mm, 1–12 mm, 15–30 mm was used for experiment. Suspension was prepared in a boiling flask under the conditions of acid hydrolysis. This suspension was pretreated with ozone for 5, 10, 15, 30, 45, 60 minutes with the ozone flow rate of 3.61 mg minutes^{-1}. At the same time, this suspension was hydrolyzed with acid.

Hydrolysis

The hydrolysis was carried out with sulphuric acid. Acid hydrolysis was conducted under the following conditions: hydrolysis duration - 60 minutes, 10 % (w/w) straw, sulfuric acid 20 % v/v aqueous solution in an amount of 30 % for landfills phytomass.

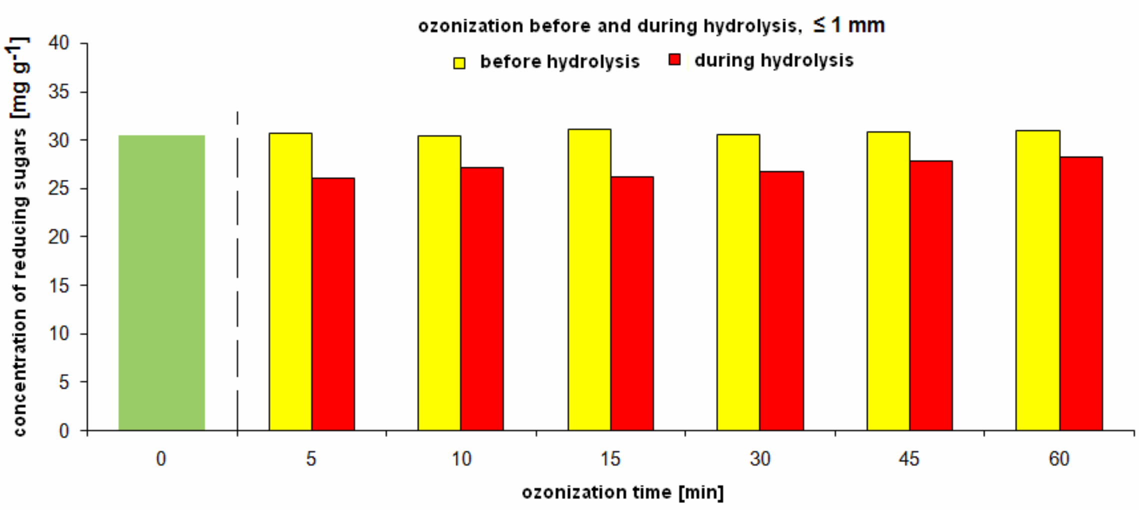
Analytical methods

The total solids content in the solid fraction was determined by drying app. 1 g of the sample overnight at 105 °C. The ash content of the solid fraction was determined by incineration of app. 1 g of dried sample at 575 °C for 7 hour. The cellulose content of the solid fraction was determined by oxidative hydrolysis. Acid insoluble lignin and acid soluble lignin in the raw material were estimated following NREL laboratory analytical procedures. The reducing sugars in the hydrolyzate was determined by Miller (1959) with 3.5-Dinitrosalicylic acid.

Results and discussion

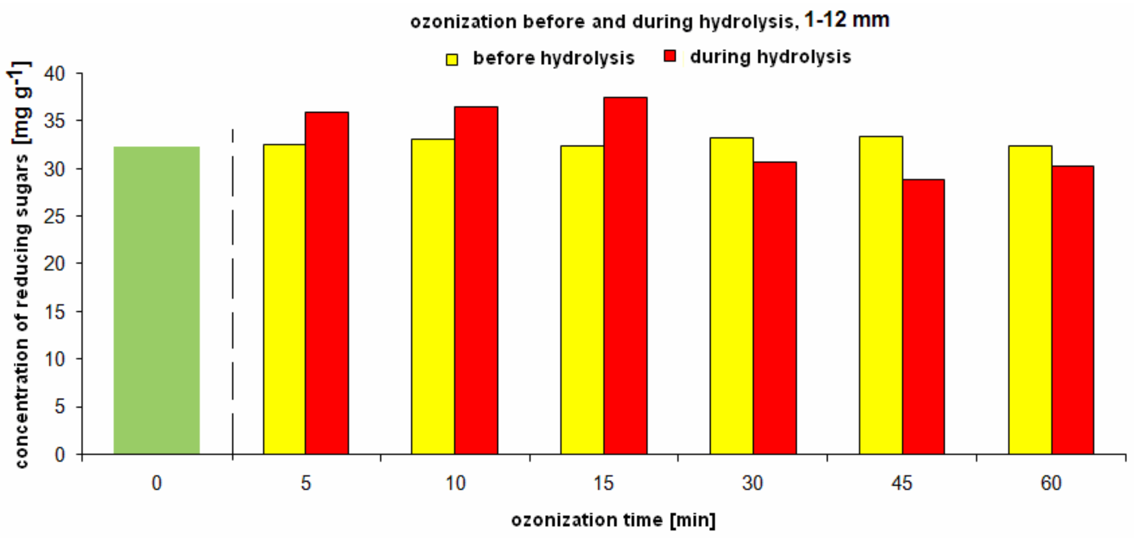
The effect of pretreatment on sugars (defined as reducing sugars) occurring in the hydrolyzate was analyzed. In the next graphs, the concentration of reducing sugars in hydrolyzate can be seen. The concentration of reducing sugars evidently depends on the conditions of pretreatment (ozonization before or during hydrolysis).

As seen in Graph 1, ozonization of straw (particle size ≤ 1 mm) during hydrolysis is a preferable method of pretreatment compared with ozonization before hydrolysis. Both kinds of pretreatment, ozonization before and during hydrolysis, are not appropriate for pretreatment, as the concentration of reducing sugars in pretreated straw are similar or lower than the concentration of reducing sugars in the straw which was not pretreated.



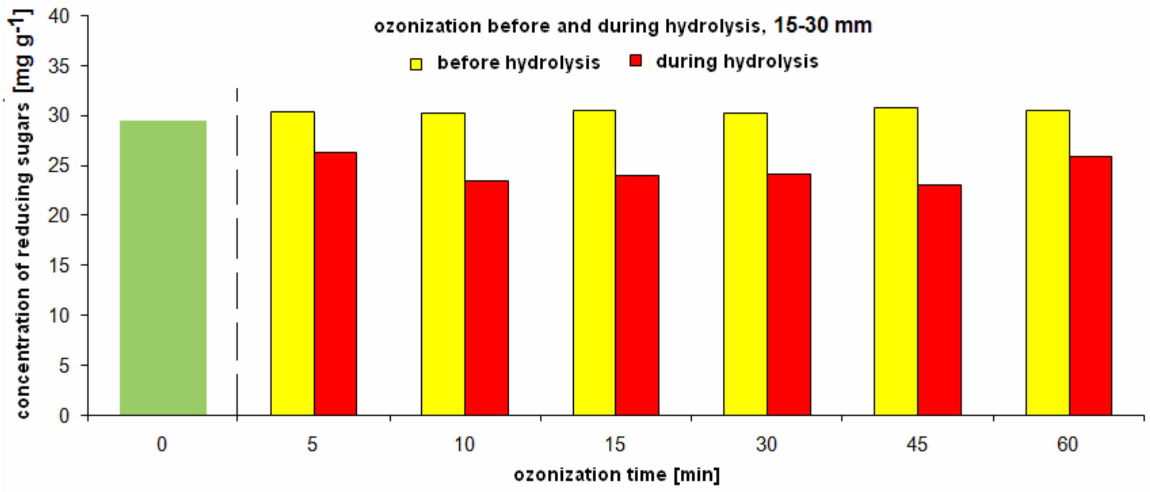
Graph 1 *Concentration of reducing sugars – ozonization before and during hydrolysis, particle size ≤ 1 mm*

In the next graph, the concentration of reducing sugars in hydrolyzates which was obtained from pretreated straw with size 1 – 12 mm can be seen. The most appropriate method of pretreatment is ozonization during hydrolysis for 5, 10, 15 minutes. Here, we obtained the highest concentration of reducing sugars and this type pretreatment was used for pretreatment. The concentration of reducing sugars is lower for ozone pretreatment during hydrolysis for 30, 45 a 60 minutes. The concentration of reducing sugars is similar for ozone pretreatment before hydrolysis for all periods. Therefore, this method of pretreatment cannot be considered appropriate.



Graph 2 *Concentration of reducing sugars – ozonization before and during hydrolysis, particle size 1 – 12 mm*

In the next graph, the concentration of reducing sugars in hydrolyzates which was obtained from pretreated straw with size 15 – 30 mm can be seen. The concentration of reducing sugars as for ozone pretreatment before hydrolysis for all treatment time, as well as that without ozone pretreatment is similar. The concentration of reducing sugars is lower for ozone pretreatment during hydrolysis than that of reducing sugars without ozone pretreatment. The concentration of reducing sugars after pretreatment of straw is similar to or lower than the concentration of reducing sugars in hydrolyzate for not pretreated straw. Therefore, this method of pretreatment with this straw size is not appropriate.



Graph 3 *Concentration of reducing sugars – ozonization before and during hydrolysis, particle size 15 – 30 mm*

A cotton stalks were pretreatment by ozone for 30, 60, 90 minutes. Ozone did not cause any significant changes in lignin, xylan, or glucan contents over time by Silverstein (12).

Conclusion

The aim of ozonization pretreatment was to break the complex structure of lignocellulosic materials. We can state that this method of pretreatment is appropriate only for specific conditions. In certain circumstances, the best method of pretreatment is ozonization during acid hydrolysis for 5, 10 a 15 minutes for particle size of straw 1 – 12 mm. The concentration of reducing sugars was increased by 3,59 – 5,22 mg g^{-1} dry compared with that of reducing sugars in not pretreated straw. Ozone pretreatment for other treatment time is not appropriate. Ozone pretreatment for particle size of straw $\leq$ 1 mm and 15 – 30 mm is not appropriate, as the concentration of reducing sugars is lower than or similar to the concentration obtained from not pretreated straw. When assessing the effectiveness of pretreatment methods, we should considered, whether minimum increase the concentration of reducing sugars is favorable due to the energy consumption.

Ozone pretreatment did not perform as effectively as expected. Possible explanations include unsuitable pretreatment conditions (low ozone concentration, uneven distribution of ozone throughout the sample) and hydrolysis conditions.

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