

Effect of wood grinding on enzymatic hydrolysis process

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Abstract: *Effect of wood grinding on enzymatic hydrolysis process.* Wood is an object of interest of researchers as a possible raw material for production of second generation biofuels. One of the basic operations that is necessary in biofuel production is to divide polysaccharides into simple sugars. In the scientific literature there are many methods presented how to perform this hydrolysis. Currently, the most interesting for scientists and technologists is enzymatic hydrolysis. The biggest problem in this process is to enable access of enzymes to wood so as to reduce protective effect of lignin. This work presents the influence of fineness of wood material on the kinetics and efficiency of the enzymatic degradation and compared it with the preliminary results obtained for previously chemically treated wood. Preliminary results suggest that, irrespective of the degree of fragmentation, chemical treatment of wood is a necessary step for this process to be efficient.

Keywords: wood, enzymatic hydrolysis, polysaccharides, simple sugars,

INTRODUCTION

Pulp and paper industry is a modern and ecological sector of the economy annually processing large amounts of lignocellulosic biomass. Practical implementation of new technologies increases the profitability of production and ensure its adaptation to the ever-increasing environmental requirements. The main directions of development of technologies for the use of lignocellulosic biomass come down to biorefinery processes, as a result of which you new products like biofuels, bio-materials and chemicals can be obtained. [1,2,3]

Lignocellulosic wastes contain polymers of sugars and five- or sixcarbons, characterized by a high resistance to physical, chemical and biological factors. This makes fermentation process to be preceded by pre-treatment and hydrolysis.[6]

Cellulose is the most widespread completely biodegradable polymer in nature. The main role in the bioconversion play microorganisms, which produce cellulases, enzymes responsible for degradation of cellulose into simple sugars.[5]

Over the years, for the enzymatic treatment, physical methods, physico-chemical and chemical processes were used.

Physical pretreatments include methods of machining (grinding material) and thermal decomposition. Physico-chemical pretreatments include: steam explosion, ammonia fiber explosion, catalyzed CO₂ steam explosion. Chemical pretreatments include: ozonolysis, acid treatment, alkali treatment and wet oxidation.[2,3]

After the pretreatment of lignin-cellulose materials can be proceed to the next stage – enzymatic hydrolysis. Enzymatic hydrolysis is carried out using enzymes capable of degrading cellulose and hemicelluloses.[7]

The classical scheme for cellulose degradation involves the synergistic action of three classes of enzymes:

- Endoglucanases - which cleave at a randomly point bonding β -1,4-glycosidic bonds in the cellulose chain
- Cellobiohydrolases – exo-1,4- β -glucanases attack the reducing or nonreducing end of cellulose polymer
- B-glucosidases convert cellobiose to glucose. [4]

Hydrolysis of cellulose can be divided into three steps. In the first stage there is adsorption of cellulase to cellulose surfaces, in the second – degradation of cellulose to simple sugars, the third - cellulase desorption from the surface of the material to be hydrolyzed. [7] The choice of the process variables depends on the type of enzyme used, but enzymatic hydrolysis is usually carried out at 50°C and pH = 5.

THE GOAL OF THE RESEARCH

The aim of the study was to determine influence of degree of fragmentation of wood on efficiency of hydrolysis process.

EXPERIMENTAL

5 samples of poplar chips with different degrees of fineness were prepared. The largest chips have size of 1.6÷2.0 mm, while the smallest ones have size below 0.43 mm. Weighed on an analytical balance, weight of the corresponding sample was about 0.3 g. Samples were smeared with sodium acetate buffer in an amount of 20 ml. Then, for each of the samples 1 ml of the enzyme from Novozymes, which was previously diluted 4.5 times was added. Samples were inserted into a water bath at temperature equal to 50°C. The first test samples were collected immediately after the addition of enzyme. The next ones, respectively after 1h, 3h, 6h, 24h and 48h. Maximum duration of the process was 48h. That time was chosen as the maximum time which can be applied in industrial scale. In each test the concentration of glucose and reducing sugars was measured. Sample, which was additionally hydrolyzed was prepared from poplar cellulose pulp and contained 2.3%, determined by the number of kappa equal to 15.4.

METHODS

Determination of glucose was tested using GOD-POD

Glucose test used for this determination is based on two enzymatic reactions catalyzed by glucose oxidase (GOD) and peroxidase (POD).

Samples prepared by mixing 0.1 ml of the appropriate dilution of the liquid after hydrolysis, and 2 ml of working reagent. The reference test was prepared which contained 0.1 ml of distilled water instead of the liquid after hydrolysis. Thus prepared sample was thoroughly mixed and incubated at room temperature for 15 minutes. Then the absorbance of the sample was measured on a spectrophotometer at a wavelength of 500 nm.

Determination of reducing sugars by Miller

Miller's method allows to determine all reducing sugars, contained in the solution under examination.

For measurement of reducing sugar 0.5 ml of the dinitrosalicylic acid (DNS) reagent was added to 0.5 ml of an appropriately diluted sample, and the mixture was placed in boiling water for 5 min. The reference test was prepared which contained 0.1 ml of distilled water instead of the liquid after hydrolysis. After cooling and adding 4 ml of distilled water, the absorbance was measured at 540 nm.

RESULTS

All the tested samples of poplar chips were hydrolyzed. However, both the kinetics of these processes and the performance remained at a low level. The best performance was

obtained for the reaction of poplar dust. Its yield was up to 11%. In turn, yield for chips at size of 1.2-1.6 mm and 1.6-2.0 mm was 9%. For chips at size range of 0,43÷0,8 mm yield was 10%. Poplar pulp containing 2,3% lignin, showed much higher performance compared to other tests. For this sample obtained yield was almost 98%.

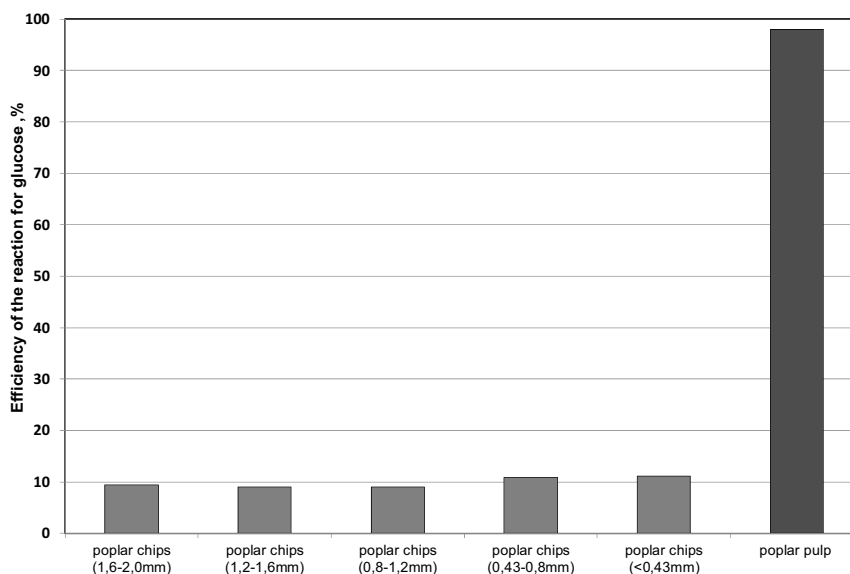


Fig.1. Performance tests of enzymatic hydrolysis of poplar chips and poplar pulp

CONCLUSION

The above-described studies show that the degree of fragmentation of the timber has practically no effect on the kinetics and efficiency of the enzymatic hydrolysis process. Reducing the particle size increases the surface area of contact of the enzyme with the raw material. However, the efficiency of this process is not satisfactory. In addition, in order to properly crush wood material, large amount of energy is needed and the right equipment. A very good way to increase the efficiency of hydrolysis is to use cellulose pulps instead of wood. Cellulose pulps can be obtained by sulphate method – well tested, and efficient in industrial scale technology. This pretreatment has many advantages. The biggest of which is full recovery of chemicals, and thus minimal impact on the environment.

Comparison of the kinetics and efficiency of hydrolysis of pulps and wood chips and an introduction to future research. Looking at the first results of this process for pulps appears to be very promising.

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Streszczenie: *Wpływ stopnia rozdrobnienia drewna na przebieg procesu hydrolizy enzymatycznej.* Drewno stanowi obiekt zainteresowania badaczy jako jeden z możliwych surowców do produkcji biopaliw II generacji. Jedną z podstawowych operacji umożliwiających otrzymanie biopaliw jest rozkład polisacharydów zawartych w drewnie do cukrów prostych. W literaturze naukowej przedstawiono wiele sposobów dokonania tej hydrolizy polisacharydów i obecnie największym zainteresowaniem cieszy się hydroliza oparta na działaniu enzymów. Największym problemem w tym procesie jest utrudniony dostęp enzymów do surowca drzewnego i ochronny wpływ ligniny. W pracy tej przedstawiono wpływ stopnia rozdrobnienia materiału drzewnego na kinetykę i wydajność procesu enzymatycznego rozkładu oraz porównano to z wstępnymi wynikami otrzymanymi dla drewna, uprzednio poddanego obróbce chemicznej. Wstępne wyniki sugerują, że niezależnie od stopnia rozdrobnienia drewna obróbka chemiczna jest etapem koniecznym by proces ten przebiegał wydajnie.

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