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Effect of pretreatment on enzymatic hydrolysis process

BUZAŁA KAMILA*, PRZYBYSZ PIOTR*, KALINOWSKA HALINA**, KUCNER MARTA*, DUBOWIK MARCIN*
*Institute of Papermaking and Printing, Technical University of Lodz, Poland
** Institute of Technical Biochemistry, Technical University of Lodz, Poland

Abstract: Pretreatment is an essential step in the biotechnology conversion of lignocellulosic materials, which are substrate to the production the second generation of biofuels and other bioproducts. The aim of the pretreatment is to facilitate enzymatic hydrolysis of polysaccharides contained in lignocellulosic materials. This work shows the influence of the species of wood material on efficiency of hydrolysis process. Obtained results are compared with the ones for wood, previously chemically treated. Sulphate method is chosen as pretreatment. This method in the papermaking industry is known for over 150 years.

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

INTRODUCTION

The main factor determining the suitability of lignocellulosic materials for processing in biorefineries is their chemical composition. The lignocellulosic biomass is characterized by a high content of cellulose and hemicelluloses. These components are the source of monosaccharides, which are used to produce biofuels and other products.

Among the many lignocellulosic materials, the potential economic importance has such materials as straw, wood, technological waste of paper production, wastepaper and plantations of energy plants [1].

The conversion of lignocellulosic materials needs some stages. The first step is pretreatment, the second one – enzymatic hydrolysis, next ones monosaccharides separation, fermentation and products separation. In this way we can get different products, especially bioethanol. Nowadays, a major obstacle in the use of lignocellulosic materials by biotechnological methods is the lack of adequately effective methods of pretreatment process [2]. The aim of the pretreatment is to facilitate the enzymatic hydrolysis of polysaccharides contained in lignocellulosic materials. This process usually involves grinding the raw material, removing the lignin and cellulose crystallinity decreased [3]. Pretreatment process should not cause the loss of sugars or produce products that are inhibitors of enzymatic hydrolysis and fermentation. Pretreatment is the most important step in the biotechnology conversion of lignocellulosic materials. The industrial success, both technologically and economically, depends on effective pretreatment process [4].

THE GOAL OF THE RESEARCH

The aim of the study was to determine influence of degree fragmented different kind of wood and pulps on efficiency of hydrolysis process.

EXPERIMENTAL

Chips of birch, lime, oak, beech, pine and spruce woods were prepared. The size of chips was in the range 0.43÷0.8 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 6.0 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, 48h and 72h. For comparison, there were prepared pulps of poplar, beech, birch and pine by the sulphate method. The number of kappa equal respectively 24.2, 25.8, 28.3, 31.4. Pulps were hydrolyzed for 48h. All samples of enzymatic hydrolysates were filtered through a medium-fast filter paper and the filtrates were subjected to analyses. In each test the concentration of glucose and reducing sugars was measured.

METHODS

Wood pulps

Cellulosic pulps of various Kappa numbers were prepared by the sulfate method as described in Modrzejewski et al. [5] from woodchips (20 mm x 15 mm x 8 mm), containing 7-8% humidity. The chips were obtained from wood after mechanical removal of bark and knots. The disintegrated materials were kept in hermetically closed vials to avoid any changes in the humidity before the treatment with NaOH and Na2S solutions, which were prepared freshly before the usage. Delignification processes were conducted in 2.5 L stainless steel reactors with regulation of temperature. Suspensions of woodchips were heated for 120 min to achieve the temperature of 160^0C and incubated at this temperature for the next 120 min. Then the temperature was decreased to the ambient one using cold tap water and the insoluble residue was separated by filtration on Buchner funnel, washed several times with demineralized water and incubated overnight in demineralized water to remove residues of the alkali-soluble fractions. The solids were disintegrated for 3 min in a laboratory propeller pulp disintegrator and the fibers were collected by centrifuging and weighed. Triplicate samples of these fibers were analyzed for the humidity and residual lignin (Kappa number) contents.

Determination of glucose was tested using GOD-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

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 chips and pulps were hydrolyzed. However, both the dynamics of these processes and the performance differ significantly. Among wood chips the best performance was obtained for the reaction of beech (29.59%). In turn, reducing sugars yields for oak chips was 6.23%. In pulps hydrolysate the yields of glucose and reducing sugars reached approximately 80 and 110%. An apparently higher weight of reaction products compared to the dry weight of pulp samples results from incorporation of water into reaction products (water is the second substrate in hydrolysis reactions).

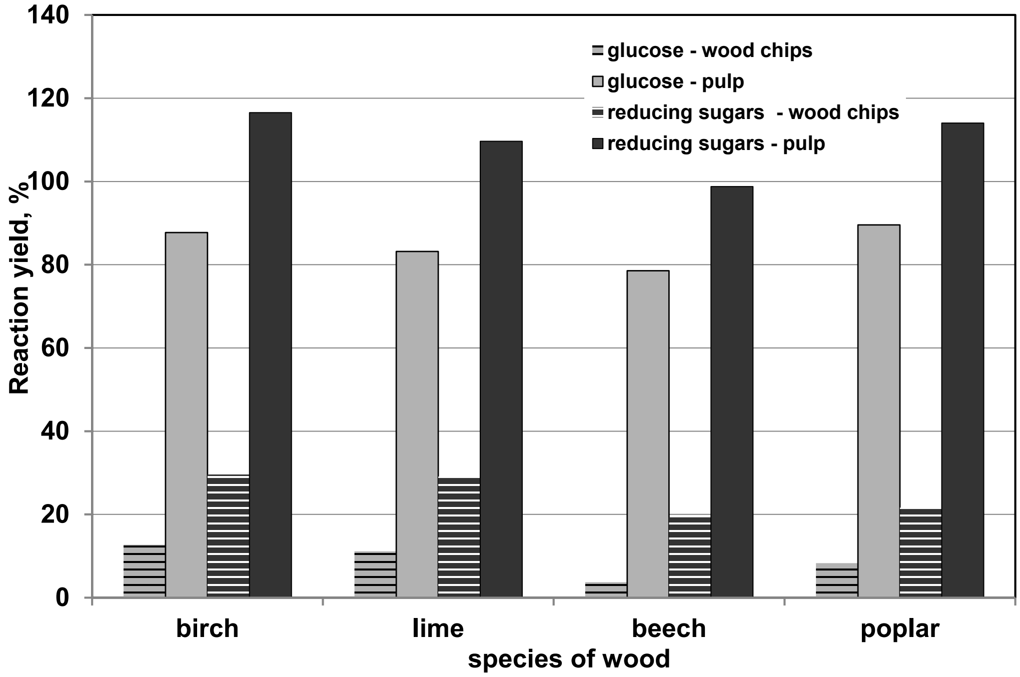

Figure 1. Performance tests of enzymatic hydrolysis of wood chips.

CONCLUSION

The above-described studies show that the choice of pretreatment process is very important. Fragmentation of different kind of wood has practically very small effect on the dynamics and efficiency of the enzymatic hydrolysis process. The efficiency of this process is not satisfactory. A very good step to increase the efficiency of hydrolysis is to use cellulose pulps. Cellulose pulps can be obtained by sulphate method. This method is very well technologically elaborated. Moreover it is selective, inexpensive and this way we can convert a wide range of plant materials. The use of cellulosic pulps is a new and easy source to produce of biofuels.

REFERENCES:.

1. Bansal M., KariMi I. A., Srinivasan R.: 2007. *Optimal contract selection for the global supply and distribution of raw materials.* Industrial and Engineering Chemistry Research, 46/20, 6522-6539
2. Chandel A. K., Es C., Rudravaram R., Narasu M. L., Rao L. V., Ravindra P.: 2007. *Economics and environmental impact of bioethanol production technologies: An appraisal.* Biotechnol. Molec. Biol. Rev., 2, 14-32
3. Kobayashi K., Kimura S., Kim UJ., Tokuyasu K., Wada M.: 2012. *Enzymatic hydrolysis of cellulose hydrates*. Cellulose 19, 967-974
4. Mosier N., Wyman C., Dale B., Elander R., Lee YY., Holtzapple M., Laddisch M.: 2005. *Features of promising technologies for pre-treatment of lignocellulosic biomass.* Bioresource Technology, 96, 673-686
5. Modrzejewski K, Olszewski J, Rutkowski J (1969) In: *Analyses in papermaking industry*. Editorial Office of the Lodz University of Technology, Lodz, pp. 60-89, 206-250

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Streszczenie: *Wpływ obróbki wstępnej na przebieg procesu hydrolizy enzymatycznej.* Obróbka wstępna jest kluczowym etapem biotechnologicznego przerobu surowców ligninocelulozowych, na których oparta jest produkcja biopaliw II – generacji oraz innych bioproduktów. Celem obróbki wstępnej jest ułatwienie hydrolizy enzymatycznej zawartych w surowcach ligninocelulozowych polisacharydów. W pracy tej przedstawiono wpływ rodzaju materiału drzewnego na wydajność procesu enzymatycznego rozkładu oraz porównano to z wynikami otrzymanymi dla drewna, uprzednio poddanego obróbce chemicznej. Jako metodę obróbki wstępnej wybrano metodę siarczanową, która w Papiernictwie jest znana od ponad 150 lat.

Corresponding author:

Buzała Kamila
Wolczanska 223
90-924 Lodz, Poland
e-mail: kamila.buzala@wp.pl