HYDROLYSIS PROCESS OF SORGHUM AND MISCANTHUS BIOMASS USING CELLULOLYTIC ENZYMES FOR ETHANOL PRODUCTION

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The process of producing bioethanol from lignocellulosic materials can be divided into a few stages: pretreatment, enzymatic hydrolysis and ethanol fermentation. The synergistic action of enzymes in the hydrolysis process involves their attack on cellulose by attaching to cellulose fibres on amorphous sites, the cleavage of cellulose chains, cutting off large chain fragments, followed by their decomposition until glucose is obtained. The aim of the study was to compare the action of enzymatic preparations on lignocellulosic raw materials, i.e. sorghum and miscanthus. The plant material was disintegrated and exposed to alkaline treatment. The study included assessing the effect of selected enzymatic mixtures on the content of reducing sugars, according to Miller's method, which enabled to determine their usefulness in enzymatic hydrolysis. Then, the parameters of the hydrolysis process were determined by Response Surface Methodology for a specific enzymatic preparation – the enzyme dose, pH, temperature and hydrolysis time.

Keywords: lignocellulosic biomass, sorghum, miscanthus, enzymatic hydrolysis, ethanol production

INTRODUCTION

Renewable sources of energy and innovative technologies determine the economic development to a continuously increasing degree. Currently, the basic alternative source of energy is bioethanol produced from vegetable biomass. The process of bioethanol production from raw lignocellulosic materials is divided into several stages. Pretreatment of the raw material in order to prepare it for enzymatic hydrolysis and ethanol fermentation determine the final result of the whole process. The process of pretreatment of the plant biomass consists of fragmentation of the material and its chemical treatment.

A technological bottleneck involves the complexity of the chemical structure of lignocellulosic materials. A lignocellulosic complex includes cellulose (a glucose homopolymer) – a fraction of the biomass that is most useful for bioethanol production, hemicelluloses (a copolymer of monosaccharides) – an only partially useful fraction, and also lignin (a polymer built from phenyl propane derivatives) – a fraction hindering the processing of the biomass and completely useless in the bioethanol production. The most commonly applied chemical treatment of the biomass is the alkaline processing, which allows the removal of the lignin and the partial degradation of hemicelluloses, enabling the preparation of the cellulosic fraction for enzymatic digestion.[1,2,3,4] Then, it is crucial to make simple sugars, obtained in enzymatic hydrolysis, accessible to yeasts in the fermentation process. The synergistic action of enzymes in this process involves the attack on the cellulose by binding with cellulose fibres (microfibrils) in amorphous places, the cleavage of cellulosic chains, cutting off considerable fragments, and then degrading them until the glucose monomer is obtained.

The study aimed at comparing the effect of combinations of commercially available preparations, i.e.:

- Flashzyme Plus 200 – a multi-enzyme complex with endo-glucanase, cellobiohydrolase, cellobiase, xylanase, mannanase (AB Enzymes),
- Celluclast 1.5L – cellulase from *Trichoderma reesei* with endo-1,4-β-D-glucanase activity (Novozymes),
- ACx3000L – a complex of cellulase/xylanase with both endo- and exo-cellulase activity, i.e.: β-glucosidase, β-(1,4 and 1,6) glucanase,

and also of cellobiohydrolase and xylanase activity (Enzyme Supplies),
- Novozyme 188 – cellobiase from *Aspergillus Niger* with β-glucosidase, xylanase, ß-xylosidase activity (Novozymes),
- xylanase from *Thermomyces lanuginosus*, endo-1,4-β-xylanase,

on the lignocellulosic material, which in our case was sorghum and miscanthus biomass.

The plant material was subjected to fragmentation and alkaline chemical treatment at elevated temperature. Using Miller's method, the study examined the influence of selected enzymatic mixtures on the content of released reduced sugars, which allowed determining their usefulness in the process of the enzymatic hydrolysis during bioethanol production. The following parameters were determined for the hydrolysis: time, temperature, pH and the dose of the main enzyme, according to the Response Surface Methodology (RMS).

EXPERIMENTAL

Material and method

The material examined was sorghum and miscanthus biomass subjected to pretreatment according to the following parameters: the desiccation of initially crushed biomass at a temperature of 50 °C and humidity below 15%. Then, the material was subjected to further fragmentation in a knife mill with a sieve mesh size of 4 mm. After the mechanical pretreatment, the raw material was exposed to the action of 1.5% NaOH at a temperature of 90 °C for 5 hours.

The sorghum and miscanthus biomass, pretreated as described above, was subjected to a high-temperature enzymatic (SHF) hydrolysis with the use of commercial enzymatic preparations: Flashzyme Plus 200, Celluclast 1.5L, ACx3000L, Novozyme 188, and pure xylanase. The hydrolysis was carried out at a temperature of 55 °C for 24 hours in a citrate buffer of pH 4.8 and with the enzyme dose of 10 FPU/g.

The tests were carried out for several combinations of commercial enzymes:
- Combination 1 – 10 FPU/g cellulolytic enzyme,
- Combination 2 – 10 FPU/g cellulolytic enzyme + 20 CBU/g ß-glucosidase,
- Combination 3 – 10 FPU/g cellulolytic enzyme + 500 XU/g xylanase,
- Combination 4 – 10 FPU/g cellulolytic enzyme + 20 CBU/g ß-glucosidase + 500 XU/g xylanase.

Analytical method

The parameters of the hydrolysis were determined experimentally using High Performance Liquid Chromatography equipment, Agilent Technologies 1200 series, equipped with automatic sample feeder (G1329B), double pump (G1312B), RID detector (G1362A) and Rezex ROA-Organic Acid H + column (300 x 7.8 mm). A flow of 0.6 mL/min, the isocratic mode, and injection of 10 µL at 40 °C were used. The obtained results were then analysed according to the Response Surface Methodology (RMS).

The efficiency of the conducted enzymatic hydrolysis was examined by determining the content of reducing sugars in the hydrolysate by Miller's method with 3,5 dinitrosalicylic acid (DNS).

RESULTS AND DISCUSSION

The tests on the commercial enzymatic preparations were preceded by process optimization of the enzymatic hydrolysis according to the Response Surface Methodology (RMS). The objective included maximum shortening of the hydrolysis, maximum reduction of the dose of the enzymatic preparation and the use of low temperature. The obtained results allowed concluding that the best efficiency was observed for the process carried out at a temperature of 55 °C, at pH 4.8, for 24 hours with an enzyme dose of 10 FPU for both raw materials (Figure 1 and Figure 2).

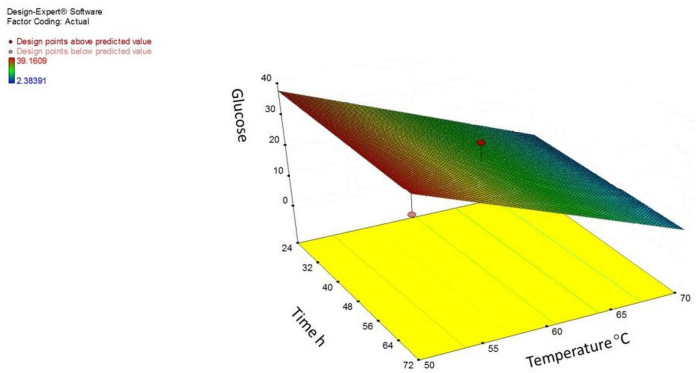

Figure 1: Parameters of enzymatic hydrolysis for the sorghum biomass according to RMS

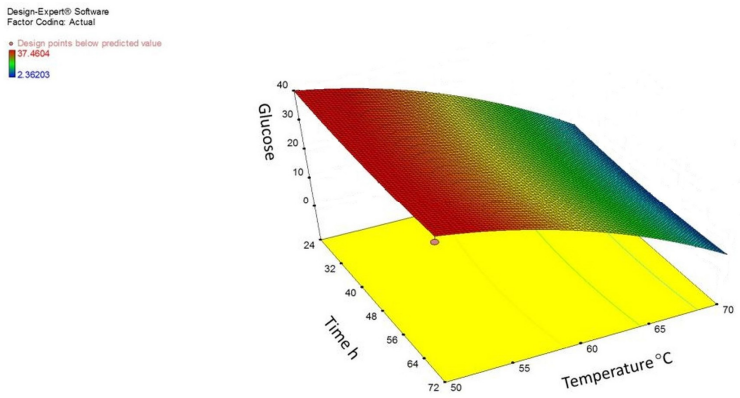

Figure 2: Parameters of enzymatic hydrolysis for the miscanthus biomass according to RMS

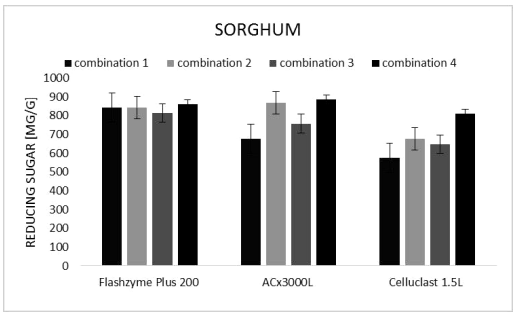

Figure 3: Content of reducing sugar in sorghum hydrolysates after enzymatic treatment

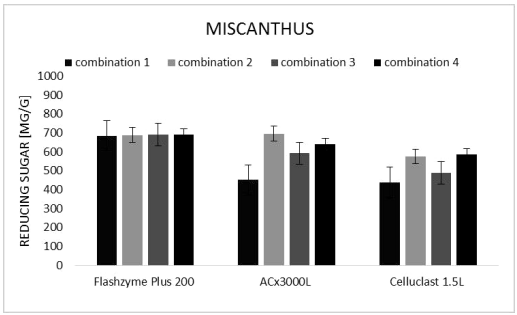

Figure 4: Content of reducing sugar in miscanthus hydrolysates after enzymatic treatment

The best results of the enzymatic hydrolysis were observed in the case of sorghum, for combination 4 with ACx3000L composed of: FPU/g ACx3000L + 20 CBU/g Novozyme 188 + 500 XU/g xylanase; and for miscanthus, combination 2 also including ACx3000L composed of 10 FPU/g ACx3000L + 20 CBU/g Novozyme 188. When comparing all examined combinations (1-4), the best results for both types of the biomass were observed with the use of Flashzyme Plus 200, whereas the least effective turned out to be the enzymatic cocktails with Celluclast 1.5L. Flashzyme Plus 200 is the enzymatic preparation with the most complex composition containing endo-glucanase, celobiohydrolase, cellulase, xylanase and mannase. This permitted the use of the preparation Flashzyme Plus 200 alone without additives with the same effect as the best combinations achieved for remaining enzymatic preparations (the difference below 5%). The preparation ACx3000L was also characterized by a rich composition, yet the results fluctuated and differed depending on the raw material. The least efficient preparation turned out to be the preparation with the shortest list of components, but in the case of sorghum, the addition of ß-glucosidase and xylanase gave similar results as other examined preparations (Figure 3 and Figure 4).

CONCLUSION

1. The synergism between enzymes is a commonly observed phenomenon in biomass hydrolysis. Studies indicate the highest levels of saccharification are achieved for the most complex enzymatic preparations.
2. In the case of sorghum and miscanthus biomass, the best results were obtained when using ACx3000L enzyme at specific combinations, i.e.:
   - sorghum biomass – the addition of ß-glucosidase and xylanase;
   - miscanthus biomass – the addition of ß-glucosidase.
3. In this study, Flashzyme Plus 200 without additions was found as the optimum enzymatic

preparation for the hydrolysis of sorghum and miscanthus.

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