PhD thesis

POSSIBILITY OF THE UTILIZATION OF AGRICULTURAL WASTES AND RECOVERY OF THE APPLYED ENZYME

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1. BACKGROUND OF RESEARCH

Second-generation bioethanol has the potential to be the major source of renewable energy in the world. Displacement of gasoline with cellulosic ethanol can reduce Green House Gases emission substantially and mitigate climate change significantly. Besides, there is a vast source of biomass feedstock for this environment-friendly biofuel throughout the world. Hence, second-generation bioethanol is regarded as the best solution for security in the future. Sugarcane-based ethanol is dominating the market and will remain for quite some time in the future.

2. AIMS OF RESEARCH

The main aim of my thesis was the enzymatic saccharification of cellulose and hemicellulose wastes and conversion to ethanol, as well as recycling of the applied enzymes in the process.
I examined various pretreatment processes (grinding, steam-explosion, acid-alkali treatment, microwave energy input, thermal treatment) and different parameters of the treatment conditions (treatment time, temperature, enzyme/substrate ratio, grinding degree) and the way of the conversion process (simultaneous saccharification and fermentation, separate saccharification and fermentation), in order to increase the efficiency of the enzymatic degradation process and to determine the impact on the degradation process. To increase the economic efficiency my goal was a chemical-free, environmentally friendly and cost-effective bioethanol production process where the applied enzymes with a minimal loss become recyclable in the process.

3. APPLIED METHODES

The experiments were performed in the University of Szeged, Department of Process Engineering, Faculty of Engineering, and in the Norwegian University of Life Sciences (UMB, Ås, Norway). For feedstock in the food industry and in the agriculture resulting cellulosic wastes were study: sugar beet pulp, sugar beet pellet, biomass tobacco, industrial tobacco waste, birch chips. The mechanical pre-treatment, grinding of the raw materials was done with laboratory cutter, and the thermal pre-treatment of the fermentation broth was performed with using heated laboratory mixer (ARE Heat Magnetic Stirrer).

The microwave pre-treatments were study with a laboratory (Labotron 500, Bucher-Guyer AG) microwave equipment in addition at 250, 500, 800 W, and in different treatment period. The pre-treatment of the birch chips was made with a steam-explosion unit (Cambi AS, Asker, Norway).

In my study in each case the first determination was the reducing sugar content of the enzymatic exploration samples by spectrophotometry (NANOCOLOR® UV / VIS, Macherey-Nagel) method (by 3, 5 dinitro-salicylic acid color reaction). In the case of birch chips in addition to the spectrophotometric measurement, RI Detector Dionex Ultimate 3000 HPLC-method was used as well. The separate hydrolysis and fermentation or simultaneous saccharification and fermentation experiments were carried out in laboratory fermentation unit (Labfors Minifors, Belgium), and with shake flask method (Tecator 1024). The alcohol content of the fermentation broth which obtained from the hydrolysis was determined with two methods. One of the methods was a classical laboratory distillation unit and the alcohol content was measured with a refractometer type of Refracto 30GS, contained internal ethyl-alcohol calibration (Mettler Toledo, Switzerland). The other method was carried out a gas chromatography with Stabilwax column (GC), Daniel Master. For the purpose of enzyme recovery and recycling was used a stirred batch laboratory ultrafiltration unit (MEUF - Micellar Enhanced Ultrafiltration) (Millipore, USA, 2002). The efficiency of the filtration was examined with using ultrasound field which was related with the membrane device, in 60% amplitude and 3.5 bar trans-membrane pressure, and 350 RPM. The membrane separation was carried out with a 4 kDa cut off value thin film (TF) and with a 5 kDa, 7kDa, 50 kDa polyethersulfone (PES) ultrafiltration membranes. Classical filter paper test was used for the control of activity of the separate enzymes.

4. NOVEL SCIENTIFIC RESULTS

1. In the case of sugar beet pellets the flux values were higher with using ultrasound field in membrane separation, due to the significant reduction of the fouling resistance.

Marietta Ábel, Gábor Szabó, Oriane Poser, Zsuzsanna László, Cecilia Hodúr. ***Enzyme recovery and fouling mitigation by ultrasound-enhanced ultrafiltration,*** *Desalination and Water Treatment, 51 (25-27) (2013), pp. 4921-4926. IF: 1,18*

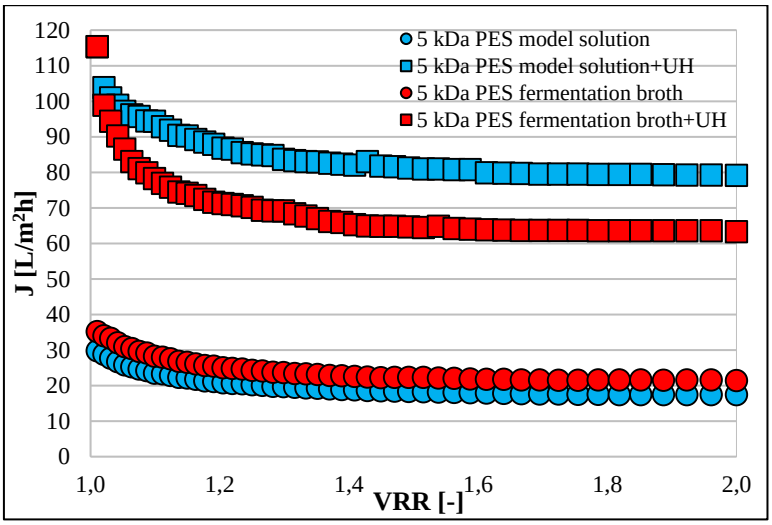

Figure 1.: Flux values of the model solution and fermentation broth with using 5 kDa PES (polyethersulphone) membrane, in nornal and ultrasound field

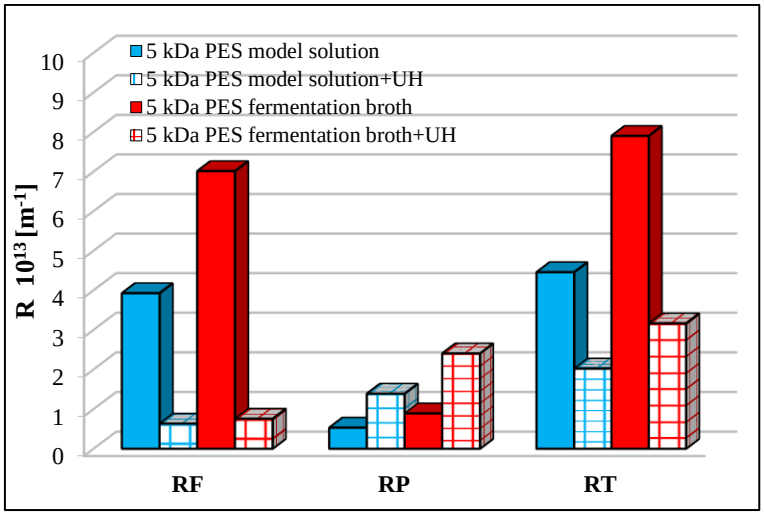

Figure 2.: Resistance values of the model solution and fermentation broth with using 5 kDa PES (polyethersulphone) membrane, in nornal and ultrasound field

2. **The enzyme activity made by classical filter paper test demonstrated that the application of ultrasound has not affect to the recycling of the enzymes.**

Marietta Ábel, Gábor Keszthelyi-Szabó, Dóra Vitay, Cecilia Hodúr. ***Membrane separation and sonication in bio-ethanol production,*** *Desalination and Water Treatment (2015), pp. 1-6. IF: 0,93*

In case of reuse of the ultrasound field exposed enzymes the activity was not decreased, moreover sometimes was little higher the degradation of cellulose, than in case of without using of ultrasounded enzyme.

3. **It has been demonstrated, in case of sugar beet pellets, that the particle size and the rate of enzyme has a combined effect on the sugar yield production.**

There was higher sugar yield when the particle size was increased (max. 0.63 mm) in a given enzyme ratio. Presumably, since the smaller particle size there is a long-term grinding process which would make a significant physical and chemical changes caused differences in suspended ability, aldose-ketose rate, etc.

Table 1.: The maximum sugar yield values of sugar beet pellets in given enzyme rate and particle size

CLA/CLB	***Particle size of sugar beet pellets [mm]***	***Maximal sugar yiled values [mg_{sugar}/g_{DM}]***
1.4	0.63	23.788
1.4	0.40	14.550
1.0	0.315	8.036
0.8	0.50	7.370
0.7	0.80	20.421
0.7	0.25	15.019
0.7	0.20	8.316
0.4	1.00	11.804

4. **It was proved that the microwave pretreatment increases the enzymatic degradation of the tobacco plant samples, and it was proved also that the applied microwave power level is the most determinant parameter opposite the microwave energy and its efficiency depends on the composition of the samples.**

Marietta Ábel, Kinga Drenda, Balázs Lemmer, Sándor Beszédes, Gábor Keszthelyi-Szabó, Cecilia Hodúr. ***Combined pre-treatment for saccharification,*** *Acta Technica Corviniensis – Bulletin of Engineering, 8 (4) (2015), pp. 111-114.*

The microwave energy loosens the structures of the cellulose/lignocellulose/lignin molecules thus the hydrolysis will be more efficient and the cellulose molecules become more accessible to the enzymes. The maximum sugar yield at biomass tobacco samples was given at 250 W and 5 minutes, while 500 W and 10 minutes at the by-product tobacco samples

This difference could be explained that the biomass tobacco samples contain the complete tobacco crop, and the by-product tobacco samples contain less of the finer structure leaves and it contains more thick cellulose bundles of the leaf, stem and veins, thus the samples has different lignin-cellulose ratio. The breakdown of lignocellulose patterns of the by-product tobacco samples is required a higher microwave powe level.

5. **Degradation of the biomass and by-product tobacco samples was proven efficient when xylanase enzyme was used instead of cellulase/cellobiase enzymes.**

The xylanase enzyme, as it was expected, had a greater effect to the by-product tobacco samples, and this difference was more pronounced in the simultaneous saccharification and fermentation (SSF) technology. The xylanase enzyme yields significantly more sugar in all case. The amount of dry matter per unit of ethanol in addition to 20-30 times higher at xylanase application comparing the same settings of optimum CLA/CLB enzymes values.

6. **The steam explosion pre-treatment for the birch chips was effective treatment, and the dosage of the reducing agent does not increase the sugar production.**

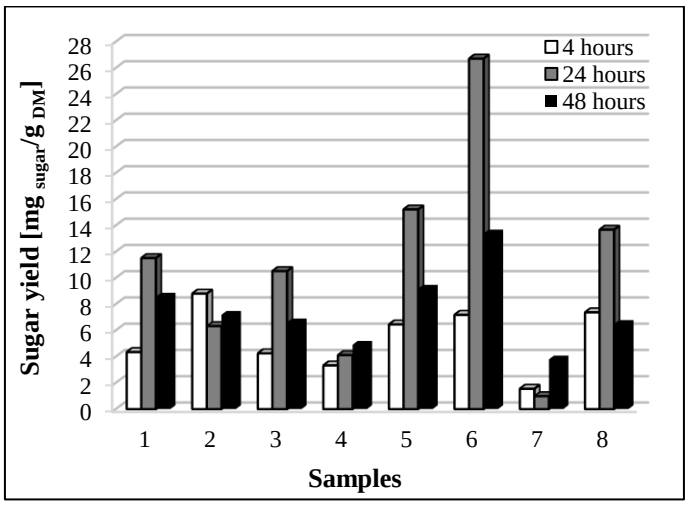

Figure 3.: Sugar yield of the pre-treatment and without pre-treatment samples (pH 5; T=50 °C)

The steam explosion is a suitable physical pre-treatment because redound the hydrolysis of the hemicellulose and enhance the enzymatic degradation of cellulose in the biomass.

Table 2.: Parameters of the hydrolysis of birch samples

Samples	*500 mM EDTA [cm³]*	*500 mM glutathione [cm³]*	*500 mM Cu [cm³]*	*enzyme(cellic CTec2) [cm³]*
1	0	0	0	0,3
2	0	0	0,01315	0,3
3	0	0,0526	0	0,3
4	0	0,0526	0,01315	0,3
5	0,1315	0	0	0,3
6	0,1315	0	0,01315	0,3
7	0,1315	0,0526	0	0,3
8	0,1315	0,0526	0,01315	0,3

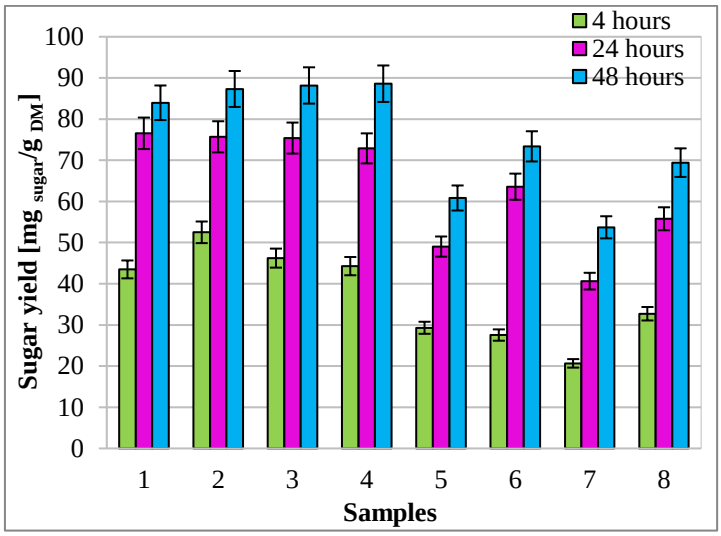

Figure 4.: Sugar yiled of the steam explosion pretreated birch (pH 5; T=50 °C)

There is no significant different between the sample that contain just the enzyme (1.) and the samples which contain additives (2.-4.). Therefore taking account into the economic considerations, there is not appropriate to enhanced the hydrolysis with reducing agents.

5. SCIENTIFIC PUBLICATIONS RELATED TO THE THESIS

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Független idéző: 3, Függő idéző: 3, Összesen: 6

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Sándor Beszédes, Marietta Ábel, Zsuzsanna László, Gábor Szabó, Cecilia Hodúr
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Independent citations: 4, Dependent citations: 1, Összesen: 5