ENZIME RECOVERY BY ULTRAFILTRATION FROM BROTH

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ABSTRACT

Recycling waste products of food industry is important: one side because of the environmental aspects and the other side because of the economic reasons. The one of the most preferred basic material for second generation bio-fuels might be the tobacco (Ábel et al. 2011). The cost of the process depends on the cost of the hydrolysis of cellulose/lignocelluloses i.e. and the cost of the enzymes. These enzymes are very expensive and that is why it is so important to find a good enzyme recovery method.
In my research the membrane separation was used for enzyme recovery. Different polyether-sulphone membranes with cut-off value of 7 kDa (PES7) and 10 kDa (PES10) were used for separation the hydrolyzate.

Keywords: recycling, enzymes, membrane

1. INTRODUCTION

Tobacco plants *(Nicotiana tabacum)* produce abundant biomass and could be used to produce abundant biofuels. Tobacco grows to heights between 1 to 2 meters and it is sensitive to temperature, air, ground humidity and type of land. Temperatures of 20-30 °C are best for adequate growth, an atmospheric humidity of 80 to 85%. Tobacco produces high-value products and an enormous amount of biomass, which can be converted into food products or industrial raw materials and naturally produces large volumes of starches and sugars. Tobacco represents an attractive and promising energy plant platform and could serve as a model for the utilization of other high-biomass plants for biofuel production.

Enzyme recovery and recycling is one of the most important and effective way of increasing the efficiency of the enzymatic hydrolysis process by lowering the enzyme costs.

A considerable number of enzyme recovery/separation methods are known, but the low energy consumption, good separation efficiency and high quality of the final product are the main attractions of membrane separation processes in bio-refining and bioenergy production [de Morais et al. 2009; Szélpál et al., 2013]. Among the specific membrane processes for biorefining ultrafiltration (UF) appears to be particularly suitable for enzyme separation. Its molecular weight cut-off (MWCO) value might be the same as the applied enzyme –complex average molar weight. In the biological industries, fouling results a significant decline of the permeate flux in course of UF. Many techniques are applied to overcome fouling, such as vibration [Hodúr et al., 2009;], gas sparging [Cui et al., 2003], back-flushing [Srijaroonrat et al., 1999] and pulsatile flow [Finngan et al., 1989] but the knowledge available on membrane cleaning still seems insufficient for practical membrane filtration systems [Hilal et al., 2005].

2. MATERIAL AND METHODS

Raw material: “Experimental” and “By-products” tobacco samples were get from a Hungarian tobacco plant cultivation. The “experimental” (EX) samples were the whole plant, the stem and leaves at all. Meanwhile the “by-product” (BY) consisted mainly on the stem, the part of plant after tobacco-processing. The samples were cut and frozen after harvesting immediately and were keeping in deep frozen until hydrolysis. One part of the samples was cut by cutter to reduce the size of particles before hydrolysis.

Enzymatic saccharification: The hydrolyzate was made from experimental (EX) and by-product (BY) tobacco samples. It was prepared in a 2L fermentation unit (Labfors Minifors, Belgium) at 30°C±0.2 and pH 4.5±0.1. The enzyme was endo-1,4-β xylanase (Sigma Aldrich) from *Trichoderma longibrachiatum* and the dose was 2000 mg/L

Modell solution: 200g of sugar, 1L of water and 4g enzyme endo-1,4-β xylanase (Sigma Aldrich) from *Trichoderma longibrachiatum*

Ultrafiltration: Separation was carried out stirred cell devices with capacity of 400 cm^3 or 100 cm^3, equipped with a 0.004534 m^2 or a 0.001734 m^2 polyether-sulphone (PES) membrane with an MWCO of 7 and 10 kDa. The sample was mixed continuously with a magnetic stirrer during separation.

The selectivity of a membrane for a given solute and the efficiency of the process were expressed by the retention (R):

$$R = \left(1 - \frac{c}{c_0}\right) \cdot 100 \quad (\%) \tag{1}$$

Where c is the concentration of the permeate phase (% or mg dm^{-3}), and the c_0 is the concentration of the feed (% or mg dm^{-3}).
The permeate flux (J) can be described as a function of time:

$$J = J_0 t^{-K} \quad (Lm^{-2}h^{-1}) \tag{2}$$

J_0 is the initial permeate flux (L m^{-2} h^{-1}), t is the filtration time [h], and K is the fouling index.
The membrane resistance (R_M) was calculated as:

$$R_M = \frac{\Delta p}{J_w \cdot \eta} \quad (m^{-1}) \tag{3}$$

Where J_W is the flux of water (m^3 m^{-2} h^{-1}) through the clean membrane, and η is the water viscosity (Pas). The fouling resistance (R_f) of the membrane can be measured by washing the gel layer from the membrane. R_f and the resistance of the gel layer (R_g) can be calculated as:

$$R_f = \frac{\Delta p}{J_{WW} \cdot \eta} - R_M \quad (m^{-1}) \tag{4}$$

$$R_g = R_T - (R_M + R_f) \quad (m^{-1}) \tag{5}$$

Where J_{WW} is the flux of water (m^3 m^{-2} h^{-1}) through the fouled/washed membrane.
Reynolds' number in the case of mixing can be calculated via the equation (6):

$$Re_{mix} = \frac{d^2 n \rho}{\eta} \quad (-) \tag{6}$$

Where ρ is the retentate density (kg m^{-3}), n is the rotation rate of the stirrer (s^{-1}), η is the viscosity of the retentate (Pas), and d is the diameter of the stirrer (m).

Protein content: The protein quantity was determined by the Kjeldhal method (KJELTEC 2300 FOSS, Based on Tecator Technology). The method is applicable to the determination of nitrogen occurring in the trinegative state in food and raw materials.

The method consists of three steps: 1) Digestion of the sample in sulphuric acid with a catalyst. The nitrogen contained in the sample is converted to ammonia; ammonium sulphate being formed. 2) Distillation of ammonia released from ammonium sulphate by addition of an excess of sodium hydroxide; ammonia being trapped in a trapping solution (sulphuric acid). 3) Back titration of the excess of the trapping solution. The percentage of nitrogen found in the original sample can now be calculated by:

$$CP = \%N * 6.25 \tag{7}$$

It is also possible to calculate the amount of crude protein in the sample. Although there are differences between different samples, the amount of crude protein (CP) can be found by multiplying the percent Nitrogen by a factor (usually 6.25).

3. RESULTS AND DISCUSSION

The flux vs. time data are shown in Fig. 1. There is a big difference between the separations with 7 kDa and 10 kDa membranes due to their cut-off values.

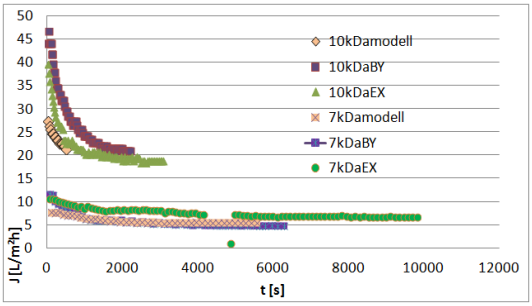

Figure 1. Flux values of the model, EX and BY tobacco samples with 7 kDa and 10kDa PES membrane

The differences between the samples are not so sophisticated, even between the model solution and the samples also. It is show that the component of the model solution was selected in a right way, the most important components are added to the model solution.

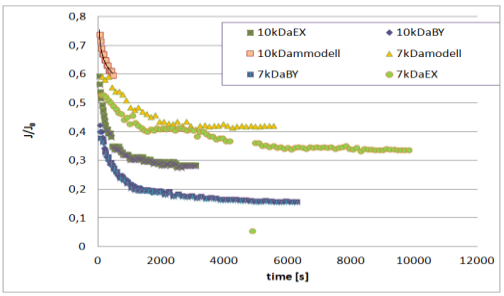

Figure 2. Relative flux values of the model, EX and BY tobacco samples with 7 kDa and 10kDa PES membrane

The relative flux values (Fig. 2.) shows better view of the separation. The relative flux values give us an information about the decreasing tendency/velocity if the flux value. These data shows the steepest decline is at the BY samples, the lowest rate of decline at the models. What means that the BY samples consist of the most smallest components which can foul the pores inside of the membrane or can make less porous structure to the gel layer.

These phenomenon is confirmed by the fouling indexes as well (Fig. 3). the fouling indexes are calculated by (2).

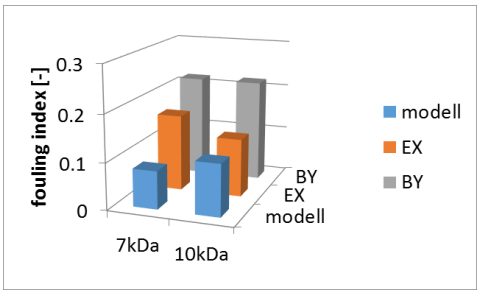

Figure 3. Fouling index values of the model, EX and BY tobacco samples with 7 kDa and 10kDa PES membrane

Three different membrane resistance values were measured during the experiments, first the membrane resistance (Rm), second the fouling resistance (Rf) and finally the resistance of the gel layer on the surface of the membrane (Rg) (Fig. 4).

In this measurements the 7 kDa cut-off value membrane has a higher resistance values and the BY samples give the highest among them.

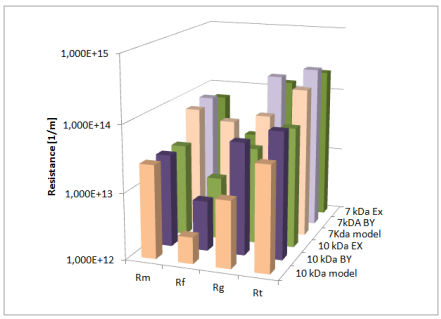

Figure 4. Resistance values of the model, EX and BY tobacco samples with 7 kDa and 10 kDa PES membrane

The protein retention was measured as an indicator of enzyme recovery. The retention values show (Fig. 5) that the enzymes could be separated into the concentrate phase. The best separation is shown by the model solution, since it has only the enzyme as and N-content component, i.e. the permeate has hardly any protein content. Meanwhile the BY and EX samples has other protein and protein-type components, so the ratio between the protein content (N – content) of the permeate and the feed originated not only from the enzymes. The retention is better at the smallest cut-off values, and better of the BY samples at any case.

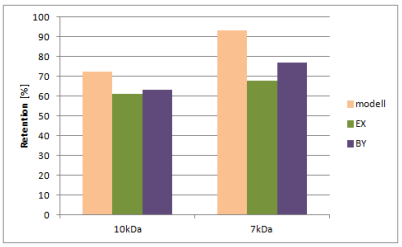

Figure 5. Retention values of the EX and BY tobacco samples with 7 kDa and 10 kDa PES membrane

4. SUMMARY

The cellulose content waste recycling for bioethanol production might be successful even economically as well if the key elements of the technology are well designed. One of the key elements is the enzymatic saccharification. When the enzyme would be reusable, the price of the unit operation is less.

Two different by-product samples and model solutions were used as examples. The samples came from the tobacco industry – BY samples, and from the tobacco cultivation – EX samples. The fermented samples were separated by 7 kDa and 10 kDa membranes for recovering the enzymes. Our data show the ultrafiltration itself is a possible method for enzyme retention, but the cut-off values might be too big for achieving a real cost related enzyme retention.

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