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Membrane separation and sonication in bio-ethanol production

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ABSTRACT

The importance of biofuels is growing for both environmental and economic point of view. The usage of wastes in bio-ethanol production is investigated in our paper. Cellulose contained wastes i.e. byproduct of tobacco is used for experiments. The paper is focused mainly on the investigation of the enzyme recovery that followed the saccharification process. The membrane filtration combined with sonication was used for enzyme recovery. Our results show that recovered enzyme did not lose its activity. The patterns were originated from fermented solutions made from tobacco byproducts. Based on our results, the ultrasound accompanied by a membrane separation would be a possible and effective operation of bio-ethanol production technology.

Keywords: Tobacco; Enzymatic hydrolysis; Ultrafiltration; Ultrasound

1. Introduction

The fuel ethanol can be obtained from lignocelluloses that contain biomass, but that production method is very complex and a little bit expensive. Nowadays, great efforts are being made to diminish the production costs of lignocelluloses, ethanol, and other bio-fuels [1,2]. Bio-ethanol can be produced from raw materials containing fermentable sugars, especially sucrose-containing feed stocks such as sugarcane, sugar beet, or tobacco. Tobacco plants *(Nicotiana rustica, Nicotiana tabacum)* produce abundant biomass in more than 100 countries could be used to produce abundant biofuels [3]. Tobacco is an ideal crop for bio-based products; it is a perennial herbaceous plant. It is found only in cultivation where it is the most commonly grown of all plants in the *Nicotiana* genus and its leaves are commercially grown in many countries to be processed into tobacco.

Tobacco grows to heights between 1 and 2 m [4] 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 80–85% and soil without a high level of nitrogen are also optimal [4]. Tobacco has potent oil biosynthesis machinery, which produces up to 40% oil per seed dry weight [5]. Recently, tobacco seed oil has been successfully tested for its potential as a fuel for diesel engines [6]. Tobacco leaves contain 1.7–4% oil per dry weight [7], which is extractable as fatty acid esters, the major component of biofuel oil [8].

Tobacco produces both high-value products and an enormous amount of biomass which can be

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converted into food products or industrial raw materials. Tobacco produces more biomass than virtually any other agricultural crop. Tobacco naturally produces large volumes of starches and sugars [9].

These starches and sugars are valuable both in the food ingredients industry and as industrial raw materials which can replace petroleum products. Tobacco produces many complex chemicals with potential high-value niche uses, for example, nicotine is used as a natural insecticide. About one quarter of the tobacco plant is cellulosic material [10]. This biomass appears attractive for conversion to ethanol because it contains very low amounts of the hard-to-convert woody material lignin. Cultivation of tobacco for biomass is very different from conventional tobacco. While conventional tobacco production is labor-intensive, biomass tobacco is largely mechanized. Thus, production costs are substantially reduced. The plants are grown much more closely together and for biomass tobacco it is possible to obtain multiple harvests in a single season from a single crop of tobacco, where the plants will re-grow following a harvest [10].

Lignocelluloses biomass is made up of very complex biopolymers. The main components are cellulose, hemicelluloses and lignin in addition to a small amount of extractives, acids and minerals. For its conversion into ethanol a complex process of pretreatment and hydrolysis is done in order to transform the carbohydrate polymers (cellulose, hemicelluloses) into fermentable sugars [11]. Tobacco represents an attractive and promising energy plant platform, and could also serve as a model for the utilization of other high-biomass plants for biofuel production [12].

Enzyme recovery and recycling is one of the most important and effective ways of increasing the efficiency of the enzymatic hydrolysis process by lowering the enzyme costs [13]. The possible separation processes are the membrane filtration processes.

In this case, the best way is ultrafiltration (UF) because the membranes pass through the sugar components and the protein fractions were retained. Among the specific membrane processes of value for biorefining, ultrafiltration appears to be particularly suitable for enzyme separation by virtue of its molecular weight cut-off (MWCO) value.

In the biological industries, fouling results in a significant decline of the permeate flux in case of UF. Many techniques are applied to overcome fouling, such as vibration [14], gas sparging [15], back-flushing [16], and pulsatile flow [17], but the knowledge available on membrane cleaning still seems insufficient for practical membrane filtration systems [18].

Ultrasound (US) has been widely used as a method of cleaning materials because of the cavitation phenomenon [19]. The US applied increases the flux by breaking the concentration polarization and cake layer at the membrane surface. The liquid jet serves as the basis for cleaning, but there are also other cavitational mechanisms which lead to particle release from the fouled membrane. The effectiveness of US treatment is influenced by various parameters. Damage due to US irradiation on the membrane surface has been discovered in some researches, whereas even the frequent use of US in other studies did not affect the membranes. US-enhanced UF filtration has not yet been widely commercialized.

The main reasons for the delay in the breakthrough are the stagnation in the development of transducer technology for membrane filtration and the control of membrane erosion [20].

2. Material and methods

2.1. Raw material

"Experimental" and "By-products" tobacco samples were got 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 kept in deep frozen until hydrolysis. One part of the sample was cut by cutter to reduce the size of particles before hydrolysis.

Dry matter (DM) was determined by drying the samples overnight at 105°C.

2.2. Enzymatic saccharification

Saccharification of the samples was carried out in duplicate using 50 cm^3 reaction volumes in 150 cm^3 Erlenmeyer glass flask with shaking water bath (1024 TECATOR) at 3 rpm and at 50°C. The pH in the reactions was adjusted by 0.1 M citrate buffer, pH 5.

The substrate concentration in the glass flask was 80 g_{DM}/L. The enzyme dose was determined by experimental design and was applied dosed in a different concentrations of cellulose (0.466 and 0.372 cm^3) (Cellulast 1.5 L, Novozymes A/S, Denmark; 700 U/g) from *Trichoderma reesei* (Sigma Aldrich) and cellobiase (0.386 and 0.458 cm^3) (Novozym 188, Novozymes A/S, Denmark; 250 U/g) from *Aspergillus niger* (Sigma Aldrich), at pH 5 and 50°C and glucose release was monitored after 4, 24, 48, and 72 h.

2.3. Sugar content

The sugar content was determined spectrophotometrically using 3, 5-dinitrosalicylic acid (DNS) method, after calibration. This method tests for the presence of free carbonyl group (C=O), the so-called reducing sugars.

This involves the oxidation of the aldehyde functional group present in, for example, glucose and the ketone functional group in fructose. Simultaneously, 3, 5-dinitrosalicylic acid (DNS) is reduced to 3-amino, 5-nitrosalicylic acid under alkaline conditions because dissolved oxygen can interfere with glucose oxidation, sulfite, which itself is not necessary for the color reaction, and is added in the reagent to absorb the dissolved oxygen [21].

All samples were diluted 10 times and subsequently 300 μL of DNS was added to 300 μL of samples. The mixtures were heated at 90°C for 10 min to develop the red-brown color. After the heating, 100 μL potassium sodium tartrate (Rochelle salt) was added in all samples and thereafter the samples were put in a cold water bath and the absorbance was recorded with a spectrophotometer (Nanocolor UV/Vis, Macherey-Nagel) at 540 nm [21].

The sugar content was measured at the received ferment juice and it was given per unit dry material weight basis.

2.4. Protein content

The protein content was determined spectrophotometrically. The absorbance of samples was measured at 280 nm. The average molar extinction coefficient of proteins is 1.6×10^5 mol/cm. Distilled water was reset and using the Lambert–Beer law to determine the concentration using Eq. (1).

$$A = \varepsilon \times c \times L \tag{1}$$

where A is the measured absorbance at 280 nm, ε is the molar extinction coefficient, c is the concentration, and L is the path length of the light in cm (in this case the thickness of the cuvette). Molar extinction coefficient *(ε)* is a measurement of how strongly a chemical specie absorbs light at a given wavelength.

2.5. Cellulose filter paper test

The enzyme activity was measured in each case and the concentrate was added 45 cm^3 to 0.5 g cutted cellulose filter paper. The digestion was set to 50°C and stirring under hourly sampling.

2.6. Ultrafiltration

Separation was carried out by stirred cell devices with capacity of 400 or 100 cm^3, equipped with a 0.004534 or a 0.001734 m^2 polyether-sulfone (PES) membrane with an MWCO of 5 kDa. The sample was mixed continuously with a magnetic stirrer during separation. The relevant data on the membranes are presented in Table 1.

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

$$R = \left(1 - \frac{c}{c_0}\right) \times 100\,(\%) \tag{2}$$

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:

$$J = J_0 t^{-K}\,(\mathrm{L m^{-2} h^{-1}}) \tag{3}$$

where 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:

$$R_M = \frac{\Delta p}{J_w \cdot \eta}\,(\mathrm{m^{-1}}) \tag{4}$$

where J_W is the flux of water (m^3 m^{-2} h^{-1}) and η is the water viscosity (Pas) at 25°C. 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:

$$R_f = \frac{\Delta p}{J_W \cdot \eta} - R_M\,(\mathrm{m^{-1}}) \tag{5}$$

$$R_g = \frac{\Delta p}{J_W \cdot \eta} - R_M - R_f\,(\mathrm{m^{-1}}) \tag{6}$$

where η (Pas) is the viscosity of the filtered solution.

Reynolds' number in the case of mixing can be calculated via the Eq. (7).

$$\mathrm{Re_{mix}} = \frac{d^2 n \rho}{\eta}\,(-) \tag{7}$$

Table 1
Characteristics of membranes used

Membrane	Maximum pressure (bar)	MWCO (g mol^{-1})	Maximum temperature (°C)	Recommended pH range
PES5	7–17	5,000 Da	90	2–12

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).

2.7. Sonication

A pin US transducer (UP 100H Ultrasonic Processor-Hielscher, US Technology) with 60% amplitude, 3.5 bar pressure, and 350 RPM was submerged on the feed side.

3. Results and discussion

Fig. 1 shows that the initial values of the filtration were the same, but the values of the filtration from the byproduct and experimental tobacco samples are well separated from each other.

In order to increase the relatively low flux levels —particularly in order to reduce the resistance of the gel layer—US was created in the input side of the filtration, but unfortunately the results did not reflect the expectations. The values of the membrane separation with US did not show higher values than without US.

The membrane resistance (R_m), the gel resistance (R_g), and the fouling resistance (R_f) were calculated by means of Eqs. (4)–(6). The results are shown in Fig. 2. The resistance values were bigger with US than without US. Probably the US-induced cavitations chopped the cellulose fibers and the resulting fragments significantly increased both the gel layer and the fouling resistance as well. The membrane resistance appears to be bigger for the BY with US and without US. It can be concluded that the enzymatic hydrolyzate of the cellulose content of EX contains several smaller fragments than the BY samples. These small particles can penetrate in the membrane pores and increase the fouling resistance. The EX samples contain the whole plant and not only the stem and petiole, so the EX samples have specifically less thick cellulose fibers.

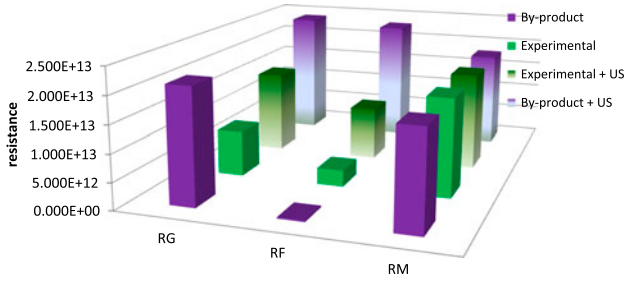

Fig. 2. Resistance values of the different tobacco samples with US and without US.

Fig. 3 shows the protein retention values during with and without US membrane separation. The protein retention was significantly lower with US than without US treatment; therefore, more enzyme passed through the permeate.

Filter-paper test was used to exam the enzyme activity following the separation. The Fig. 4 shows that the sugar content of samples are increasing vs. time, so the enzymes keep its activity during the membrane separation even by sonication. The function

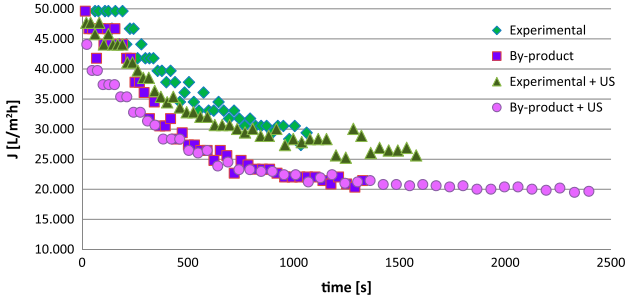

Fig. 1. Flux values of the hydrolyzate solution with the use of US and without US.

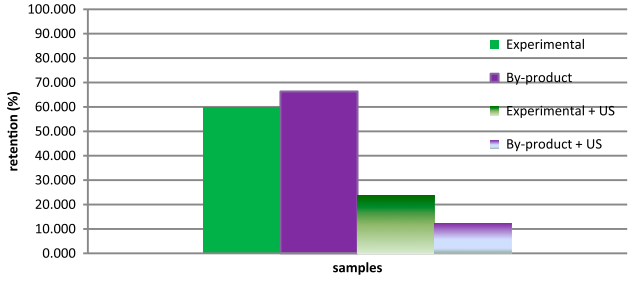

Fig. 3. Protein retention values with and without US membrane separation at tobacco samples.

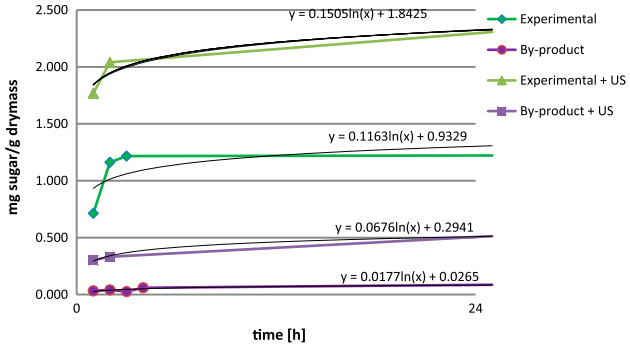

Fig. 4. The results of the enzymatic hydrolysis of cellulose with recovered enzymes.

coefficients show that in the enzyme activity, the rate of the sugar production is mainly independent on the conditions of the membrane separation, where a quite high sugar production rate was measured at the US-separated samples as well. The sugar production rate difference was bigger between the EX and BY samples.

4. Conclusion

The main goals of our experiments were to investigate the enzyme recovery from the enzymatic hydrolyzate of EX and BY tobacco samples. This study shows that the membrane separation is a possible method to recover the enzymes. The patterns were originated fermented solution and it was made from byproduct and experimental tobacco. The enzyme separation was not enhanced by the sonication, the flux and the retention values were even smaller by sonicated membrane filtration.

The enzymes kept their activity, even followed the membrane separation by US treatment as well, but sonicated samples have smaller enzyme activity.

Based on our results, the US accompanied by a membrane separation would be a possible and effective operation of bio-ethanol production technology in the future.

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