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Enzyme recovery and fouling mitigation by ultrasound-enhanced ultrafiltration

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ABSTRACT

The development of second-generation biofuels from cellulosic/lignocellulosic biomass has advantages from energy and environmental aspects, but the overall cost of the process is mainly dependent on the cost of the enzymes. Enzyme recovery and recycling is one of the most important and effective means of increasing the efficiency of enzymatic hydrolysis processes by lowering the enzyme costs. The primary objective of this study was to investigate the possibilities of enzyme recovery by membrane separation. Ultrafiltration (UF) membranes with various cut-off values and materials were used to recycle cellulase and cellobiase in model solutions and cellulosic hydrolysates. The membrane separation process was followed by determination of the flux, and its efficiency of it was measured via sugar and protein retention, and the resistances were also calculated. A polyether–sulfone membrane with a cut-off value of 5 kDa, (PES5) operated at 26.8 $L\,m^{-2}\,h^{-1}$ with 87.3% protein rejection while a thin-film membrane with a cut-off value of 4 kDa (TF4) operated at 26.3 $L\,m^{-2}\,h^{-1}$ with 92.4% of protein rejection, allowing the free transmission of glucose. Large differences were measured between the distrubutions of various kinds of resistances for the PES5 and TF4 membranes; 65% of the total resistance was due to the fouling mechanism in the case of the PES5 membrane, whereas the fouling resistance amounted to only 41% for TF4 membrane. Ultrasound (US) treatment during the UF of a hydrolysate increased the flux and changed the proportions of fouling resistance and the gel resistance.

Keywords: Enzyme recovery; Biofuels; Cellulosic biomass; Ultrasound; Membrane separation

1. Introduction

From the aspect of the production of ethanol as an alternative fuel, cellulosic biomass is of great potential as an abundant renewable energy source [1]. Cellulosic material is converted to ethanol in a two-step process: the hydrolysis of cellulose to fermentable reducing sugar, and the fermentation of the reducing sugar to alcohol. The hydrolysis step usually involves enzy-

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matic saccharification, while the fermentation is generally carried out by the action of yeasts or bacteria [2].

Hydrolysis by the cellulase system primarily depends on three enzymes: beta-1,4-endoglucanase, beta-1,4-exoglucanase and cellobiase [3]. Cellulose is broken down beta-1,4-endoglucanase and beta-1,4-exoglucanase to cellobiose units, which are hydrolysed by cellobiase to produce glucose. However, the enzyme costs are among the major obstacles to the large-scale commercialization of the enzymatic hydrolysis of cellulose [4], accounting for as much as 60% of the overall process costs [1] or by [5] approximately 50% of the costs of the hydrolysis process and 20% of the overall costs of ethanol production [5].

Two main strategies may be applied to reduce the enzyme costs: (1) enhancement of the cellulase specific activity through molecular manipulation, and via the productivity and yields of various cellulolytic fungi and bacteria, and (2) recovery and recycling of the cellulases after hydrolysis [6]. 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.

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 bio-energy production [7]. Among the specific membrane processes of value for bio-refining ultrafiltration (UF) appears to be particularly suitable for enzyme separation by virtual of its molecular weight cut-off (MWCO) value. In the biological industries, fouling results in a significant decline of the permeate flux in course of UF. Many techniques are applied to overcome fouling, such as vibration [8], gas sparging [9], back-flushing [10], and pulsatile flow [11], but the knowledge available on membrane cleaning still seems insufficient for practical membrane filtration systems [12].

Ultrasound (US) has been widely used as a method of cleaning materials because of the cavitation phenomenon [13]. An US-generating transducer produces US waves in a fluid through changes in concert with an electrical signal oscillating at US frequency. This creates compression waves that "tear" the fluid apart, leaving behind many millions of microscopic "voids" or "partial vacuum bubbles" (cavitation). These bubbles collapse with enormous energy; temperatures and pressures of the order of 5,500°C and 50 MPa are achieved [14]. The bubbles are so small that they need more than clean and remove surface contamination, that is, they can remove fouling particles from the surface or from the inside of a membrane.

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 that 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 [14].

2. Materials and methods

The fermented liquid originated from the enzymatic fermentation of sugar beet pulp. For enzymatic hydrolysis, cellulase (Cellulast 1.5L, Novozymes A/S, Denmark; 700 U/g) from *Trichoderma reesei* (Sigma) and cellobiase (Novozym 188, Novozymes A/S, Denmark; 250 U/g) from *Aspergillus niger* (Sigma) were applied, in concentrations of 200, 400, and 600 μL g_{TS}^{-1}. The conditions of enzymatic hydrolysis were controlled at 26 ± 0.2°C and pH 4.0 ± 0.1. Pellets saccharified were in a 2 L laboratory fermentation unit (Labfors Minifors, Belgium).

The model solution used contained 5% sugar, together with 2 mg cellolase and 2 mg cellobiase per 100 cm^3.

Separation was carried out micellar enhanced ultrafiltration (MEUF) devices with capacity of 400 or 100 cm^3, equipped with a 0.004534 or a 0.001734 m^2 polyether–sulfone membrane (PES) with an MWCO of 5 kDa or a thin-film (TF) membrane with a MWCO of 4 kDa. The sample was mixed continuously with a magnetic stirrer during UF. 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):

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

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 can be described as a function of time:

$$J = J_0 t^{-K} \quad (2)$$

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
TF4	3–4	4.000 Da	70	2–11

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:

$$R_M = \frac{\Delta p}{J_w \times \eta} \tag{3}$$

where J_W is the flux of water [$m^3\,m^{-2}\,h^{-1}$], and η is the water viscosity 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:

$$R_f = \frac{\Delta p}{J_W \times \eta} - R_M \tag{4}$$

$$R_g = \frac{\Delta p}{J_W \times \eta} - R_M - R_f \tag{5}$$

where η [Pas] is the viscosity of the filtered solution.

Reynolds' number in the case of mixing can be calculated via the Eq. (6).

$$\mathrm{Re}_{\mathrm{mix}} = \frac{d^2 n \rho}{\eta} \tag{6}$$

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].

A pin US transducer (UP 100H Ultrasonic Processor-Hielscher, Ultrasound Technology) with varying power level in the interval 30–90 W, and frequency of 30 kHz was submerged on the feed side. All experiments were performed in duplicate and the average values were reported.

3. Results

3.1. Fluxes

The flux values of the PES5 and TF4 membranes differed somewhat in the separation of the model solution, but not in the separation of hydrolysate. This is not surprising, in view of the very small, (only 1 kDa) differences in between the MWCO values of the membranes, and while the composition of the hydrolysate covered a very wide range of molecules, the model solution consists only of disaccharides and enzymes.

Fig. 1 reveals that the average flux for the PES5 membrane was 26.8 $L\,m^{-2}\,h^{-1}$, while that for theTF4 membrane was 26.3 $L\,m^{-2}\,h^{-1}$. The protein retention on the PES5 membrane was 87.3%, and that on the TF4 membrane was 92.4%, the free transmission of glucose being allows in both cases. (The retention was calculated via Eq. (1), and the protein concentration was measured by the Kjehldahl method.).

3.2. Resistances

The membrane resistance (R_m), the gel resistance (R_g), the fouling resistance (R_f) were calculated by means of Eqs. (3)–(5). The results are shown in Fig. 2.

Appreciable differences were measured between the proportions of the various resistances with the PES5 and TF4 membranes, mainly in the case of the hydrolysate (RTF4, RPES5). Sixty five percentage of the total resistance was due to the fouling mechanism in the case of the PES5 membrane, whereas the fouling resistance accounted for 41% of the total with the TF4 membrane.

As a similar tendency was demonstrated by the model solution, but the fouling resistance amounted to only 42 and 33% with the PES5 and TF4 membranes, respectively. The membrane resistances appear

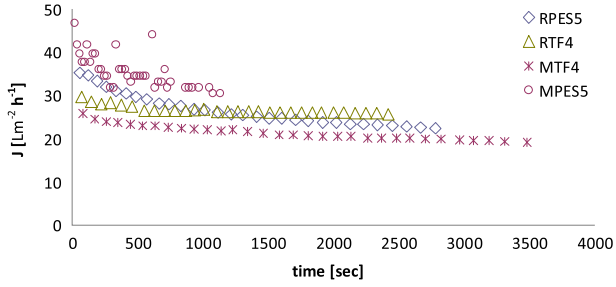

Fig. 1. Flux values of the hydrolysate and the model solution on different membranes. (Pressure: 0.35 MPa, temperature: 26 ± 0.5 °C, 350 rpm, RPES5 – hydrolysate on PES5 membrane, RTF4 – hydrolysate on TF4 membrane, MTF4 – model solution on TF4 membrane, MPES5 – model solution on PES5 membrane).

to be greater for the model solution, but this is relative since the total resistance of the model solution is lower as it contains fewer components than the hydrolysate.

3.3. Sonication

Numerous publications have reported data on the effects of the US on membrane separation, and Kyllönen et al. [13] emphasized the elimination of fouling by US treatment. We prepare to learn how US acts when applied simultaneously with membrane separation, not separately as a cleaning method.

A magnetic mixer provided the cross flow during the separation with the membrane filter equipment used. Will the mechanism of action of the sonication known, we measured the combined effect of mixing and sonication at different power levels. The data were illustrated in Fig. 3.

The plotted data reveal a significant difference between the flux only when stirring was applied without sonication. When US treatment was applied, there was no difference between the stirred and unstirred samples.

3.4. Relative fluxes

We observed a flux-enhancing effect of US during UF. The flux of the hydrolysate was elevated due to the cavitation caused by US, and the flux decline was much lower, (i.e. the flux ratios (J/J_0) were much more larger, as shown in Figs. 4 and 5. This effect was not seem when the feed was the model solution, that is, when the feed solution contained only sugar and enzymes. In this case, there is no difference between

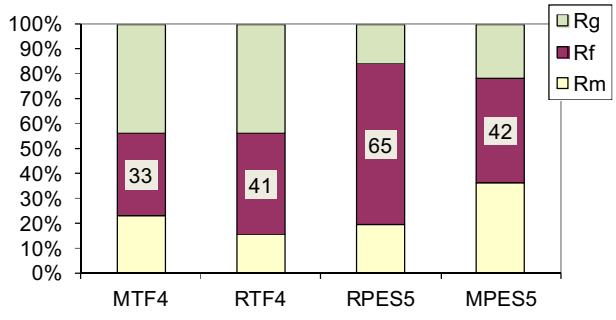

Fig. 2. Resistances of separation of model solution and hydrolysate with PES5 and TF4 membranes. (RTF4 – hydrolysate on TF4 membrane, MTF4 – model solution on TF4 membrane, RPES5 – hydrolysate on PES5 membrane, MPES5 – model solution on TF4 membrane, R_g – gel resistance, R_f – fouling resistance, R_m – membrane resistance).

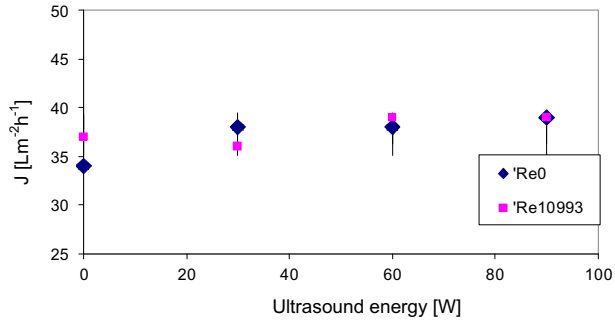

Fig. 3. The effects of the US energy applied energy and the Reynolds number on the flux.

the flux values of the samples treated with or and without US.

3.5. Changes in resistance

The effects of US on membrane-, gel- and fouling resistances are plotted in Figs. 6 and 7 for the model solution and the hydrolsyate.

The fouling resistance predominated in the processes when the separation was made in the classical mode, without sonication (RWoUSPES5). This effect was mainly developed at PES5 membranes with MWCO 5 kDa, than in the case of the TF4 membrane (4 kDa). The difference indicated that the hydrolysate is rich in components and fragments in the range 4–5 kDa. The fouling resistance decreased from 65 to 27% due to the cavitation effect of US. This technique is very applicable when a material with a tendency to clog must be separated.

The tendency observed in the effects of sonication was the same when TF4 membranes were used as a separation medium (see Fig. 7).

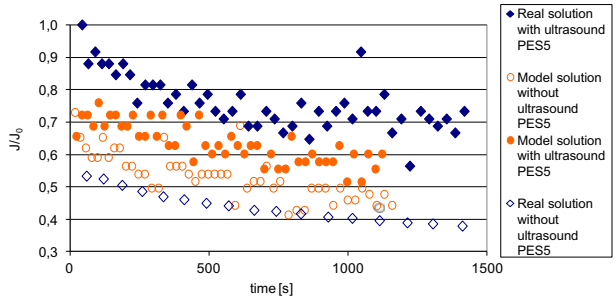

Fig. 4. Relative flux values of samples separated on the PES5 membrane.

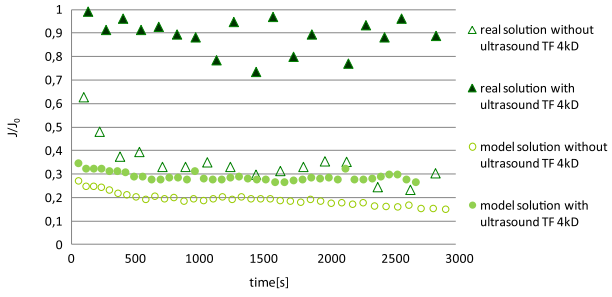

Fig. 5. Relative flux values of samples separated on the TF4 membrane.

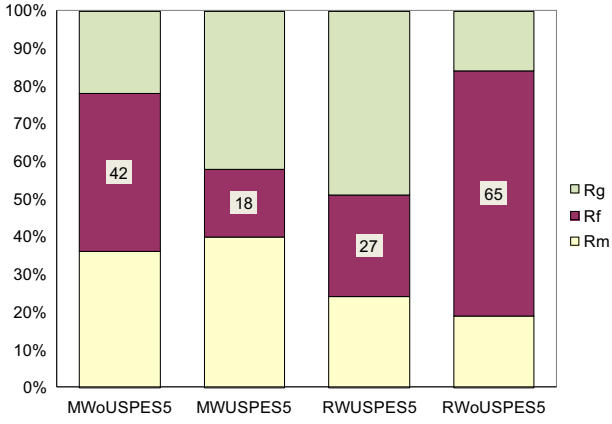

Fig. 6. Percentages of resistances during the separation of the model solution and the hydrolysate with the PES5 membrane. (MWoUSTF – model solution without sonication, MWUSTF – model solution with sonication, RWUSTF – hydrolysate with US treatment, RWoUST – hydrolysate without US treatment, R_g – gel resistance, R_f – fouling resistance, R_m – membrane resistance).

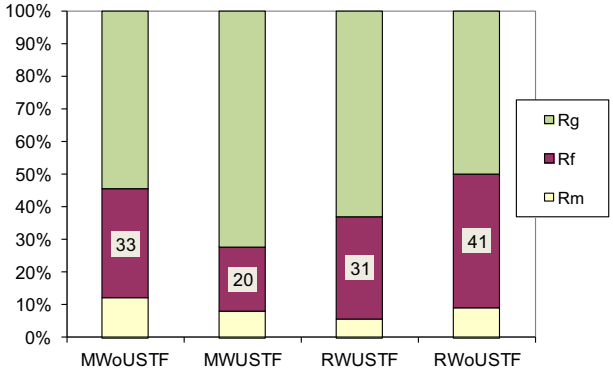

Fig. 7. Percentages of resistances during the separation of the model solution and the hydrolysate with the TF4 membrane. (MWoUSTF – model solution without sonication, MWUSTF – model solution with sonication, RWUSTF – hydrolysate with US treatment, RWoUSTF – hydrolysate without US treatment, R_g – gel resistance, R_f – fouling resistance, R_m –membrane resistance).

4. Conclusion

This study has highlighted the effects of US during the UF of model and fermented solutions. Although the role of US was earlier investigated, mainly for the cleaning period in the present study we evaluated the effect of sonication on the separation itself.

With a pin transducer submerged into the upper side (i.e. the feed side) of the separation space during sonication experiments, US treatment during UF increased the flux of a hydrolysate and changed the proportion of fouling resistance and gel resistance.

US exhibited a flux-enhancing effect when fouling resistance was the major factor determining the overall mechanism. The relative flux (J/J_0) values vs. time functions decreased much less when US treatment was applied during the separation, and also when the hydrolysate was used as a sample. These data are very important from the aspect of the planning of a long-term process.

Stirring, with increase in the Reynolds number had no effect on the flux during sonicated membrane separation, confirming the finding of Kyllönen et al. [13].

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