SONICATED MEMBRANE SEPARATION

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Abstract

Membrane separation process is currently a proven technology in many areas. The main limitation of this process is the accumulation of matter at the membrane surface which leads to two phenomena: concentration polarization and membrane fouling. Numerous authors published that permeate flux is increased by sonication. Our work focuses on separation of real broth by sonicated ultrafiltration. The broth was originated from hydrolysis of grounded corn-cob by xylanase enzyme. The filtration was carried out in a laboratory batch stirred cell with sonicator. In our work the effect of the stirring, the intensity of sonication and the membrane-transducer distance was studied on the efficiency of the ultrafiltration and on the quality of separated enzymes. Results reveal that xylanase enzyme can be effectively separated from real fermentation broth by ultrafiltration and enzyme keeps its activity after processes. Enzyme activity tests show that low energy sonication is not harmful to the enzyme.

Keywords: enzyme, ultrafiltration, ultrasound, hydrolysis, xylanase

Introduction

Biomass energy has become a very crucial subject on maintaining the sustainable energy without causing environmental pollution and reducing the effects of fossil fuels on the natural environment and human health (Kaplan, Aydin and Fidan, 2009). Biomass is a renewable energy resource with high potential that is derived from current or recently living organisms and is mostly produced plant-based. One of the most important implementations of biomass energy systems is the conversion of lignocellulosic biomass to bioethanol. Bioethanol can be produced from numerous sources such as grain, molasses, sugar beets, sugarcane extract, corn and cellulose processed with biological sugar fermentation by microorganisms and continuously obtained by distillation (Hossain et al., 2008; Mills and Ecklund, 1987). Bioethanol has favourable combustion of characteristics of alcohols, namely clean burning with high octane performance, which makes it an excellent melding component. With these characteristics of bioethanol, providing less combustion duration, it is considered to be a

relatively good alternative to gasoline with high performance and efficiency in properly designed automotive systems (Kumar, Singh and Prasad, 2010; Berg, 2004).

Theory

Lignocellulose is a planta complex composed of cellulose microfibrils, hemicellulose and pectin in plant cell walls. Lignocellulosic biomass is the most abundant raw material in the world. Conversion of lignocellulose to bioethanol helps to increase the energy availability, decrease air pollution and diminish the atmospheric CO_2 accumulation in a profitable and eco-friendly manner. Therefore in the last two decades, extensive research has been carried out on this conversion which consists of two processes: (i) hydrolysis of cellulose in the lignocellulosic materials to fermentable reducing sugars and (ii) fermentation of sugars to ethanol (Balat, 2007). The high-cost of enzymes makes the process more uneconomical. For this reason, in our study, we used the membrane separation which is considered to be the best enzyme recovering procedure (Prasad et al., 2007).

Being highly selective, energy-saving and low-cost, membrane separation takes an important place in bioethanol industry not only for starch-based but also for second generation technology of bioethanol. The main reason that leads to use the membrane separation systems is that it works with no chemicals added (Szélpál, Poser and Ábel, 2013). However, it should be remembered that membrane separation, despite being a very successful application in production of bioethanol, still has to face with some challenges. The most important among these is the fouling phenomena. This fouling can restrict the permeate rate and can essentially make the situation unsuitable for the application (Kang et al., 2014).

Nowadays, there are many different procedures applied in membrane separation field. For instance, ultrasound-assisted membrane filtration which has recently been started to use in membrane separation processes for the reason that the application of ultrasound in membrane systems the flux enhancement and prevents fouling on a large scale. The main experimental parameters that alter the ultrasonic filtration can be counted as external pressure, power density, cross-flow velocity, frequency and temperature (Muthukumaran et al., 2006). As Csoka et al. concluded the closer the horn to the bottom of the solid surface, the higher the efficiency in the horn type ultrasonic system (Csoka, Katekhaye and Gogate, 2011).

The aim of our work was to separate the enzymes from fermentation liquid produced from “Cobex” (corn cob grist) using the stirred batch filter and ultrasound-assisted membrane separation methods and later analyse the enzyme activity under optimal temperature, pressure and pH conditions in order to make the bioethanol production process economically appropriate.

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Materials and Methods

Membrane filtration Separation was carried out with a stirred ultrafiltration batch device with a capacity of 400 cm^3, equipped with a 40 cm^2 polyethersulphone (PES) membrane with a cut-off value of 10 kDa. During filtration, the sample was mixed continuously with a magnetic stirrer, or an ultrasound processor (UP100H) was applied.

The filtration time and volume was measured and the membrane permeability is expressed as the permeate flux through the membrane (J) was calculated as:

$$J = \frac{dV}{d\tau}\frac{1}{A} = K_M\left(\Delta p - \Delta\pi\right) = \frac{\Delta p}{\eta \cdot R} \qquad (1)$$

where J the flux [m^3/m^2s], A surface of the filter [m^2], V volume of the filtration [m^3], τ the time [s], K_M permeability coefficient [m^3/m^2sPa], Δp the pressure difference between the two side of the membrane [Pa], Δπ the osmotic pressure [Pa], R resistance of the process [1/m].

The total resistance consists of three parts: the resistance of the membrane (R_m), the resistance of the irreversible flux decreasing mechanisms (R_{irr}), the resistance of reversible flux decreasing mechanisms (R_{rev}):

$$R_t = R_m + R_{irr} + R_{rev} \qquad (2)$$

The R_m was calculated from the flux of clean water through native membrane, the R_{irr} was calculated from the flux of clean water through rinsed membrane followed the separation.

Samples for our work, fermentation liquid prepared from the hydrolysis of corn cob grist with the Xylanase enzyme was used as feed for membrane separation to analyse the enzyme recovery and followed enzyme activity.

Different separation methods were applied and the obtained permeates and concentrates were analysed after filtering. The different separation methods are shown in the Table 1.

Table 1: Sonication and stirring parameters of samples

samples		horn distance	sonication intensity	stirring rpm
		[cm]	[-]	[min^{-1}]
A1	nostr 0.5US2cm	2	0.5	no
A2	nostr 0.5US3cm	3	0.5	no
A3	nostr 0.5US4cm	4	0.5	no
B1	str noUS	no	no	350
B2	str 0.5US3cm	3	0.5	350
B3	str 1US3cm	3	1.0	350

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Fouling ratio (FRR) were calculated with below formulas:

$$FRR\% = (1 - \frac{J_{w1}}{J_{w2}})100 \qquad (3)$$

where J [m^3/m^2s], is the equilibrium flux at normal filtration, J_{US} [m^3/m^2s], is the equilibrium flux at sonicated filtration, J_{w1} [m^3/m^2s], is the water flux followed the separation, J_{w2} [m^3/m^2s], is the initial water flux.

Enzyme activity test was carried out in thermostatic laboratory stirred fermenters with capacity of 100 cm^3. Permeate and concentrate samples from filtering experiments were used after diluting them to similar protein content, pH was set to 5.4. The substrate for the enzyme was 5g Cobex corn-cob grist. The total volume of the samples was 100cm^3. Fermentation was kept for 2 days in 40°C. Samples were taken daily from the liquids and placed in freezer until measuring reducing sugar content. Sugar yield was proportional to enzyme activity.

Reducing sugar content was determined spectrophotometrically with using of the 3,5-dinitrosalicylic acid (DNSA) method. DNSA 3,5-(dinitrosalicylic acid) and 50 µl of the sample were mixed and heated at 90º C for 15 minutes. After cooling in water bath the absorbance was measured at 575 nm. This method test for the presence of free carbonyl group(C=O), the so-called reducing sugars.

Results

The effect of the transducer distance from the membrane surface on the flux is presented in the Figure 1. To summarize briefly it can be said that the flux values under different transducer distances are quite similar but the rate of decreasing is different a little bit. This means that the components which cannot pass through the membrane and thus remain on the membrane surface cause a fall in flux values in all.

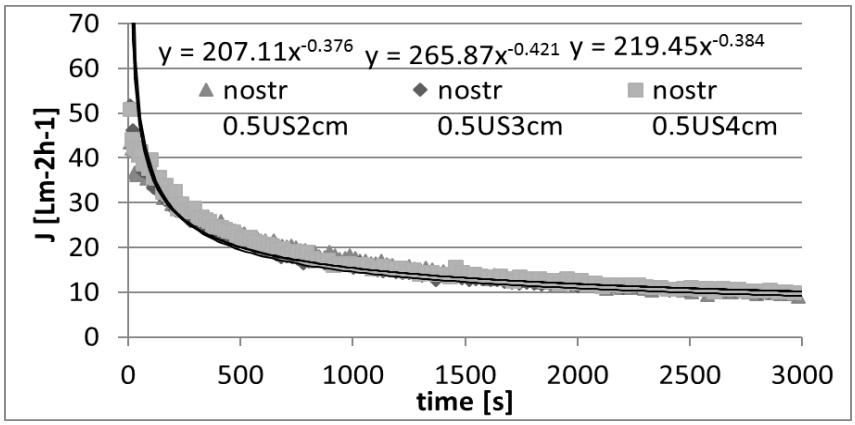

Fig 1: *Permeation flux as a function of time without stirring*

There is no very noticeable difference between the samples in Figure 1 but after the analysation the measuring data and calculation of the resistances (Eq.1), (Eq2), and followed analysation of FRR (Eq.3) valuable differences emerged (Figure 2).

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The rate of decreasing is expressed by the index of the fitted power equation and the fouling ratio in Figure 2. The index expresses the flux rate reduction caused by total resistances, whereas FFR refers only decreasing irreversible resistance caused. Strong correlation is found between FRR% and index of fitted power equations in Figure 2; meaning both of the total resistance and the irreversible resistance is the biggest at distance 3 cm comparing the other investigated distances.

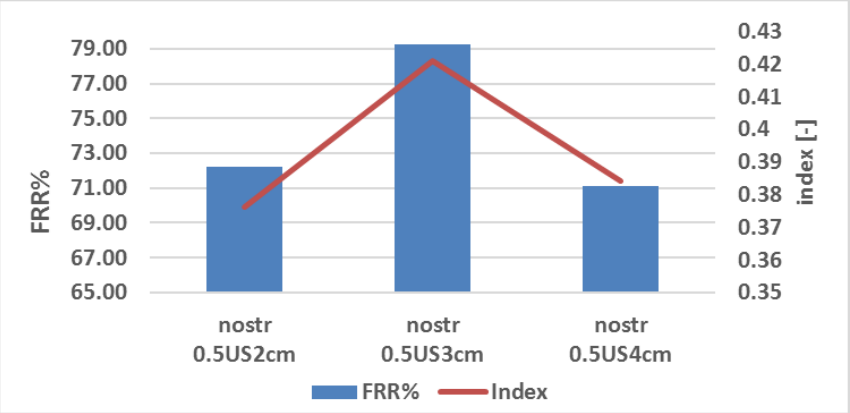

Fig 2: *Fouling ratio (FRR%) and index of fitted power equations*

When we investigate the FRR calculated on Eq.4. we can realise the importance of the distance between transducer and the membrane surface when there is no stirring effect.

This positive effect is caused by the ultrasound generated periodic mechanical motion. This periodic motion transfers energy into the solution and causes alterations in pressure leading to the creation of small rapidly growing bubbles. These bubbles are enlarged during the negative pressure cycles, finally collapse violently which generates high pressures, temperatures and shear forces. This effect can case the low FRR %, since the medium size molecules are pushed through the pores by them. The investigation of the resistances enhanced the previous explanation (Figure 3).

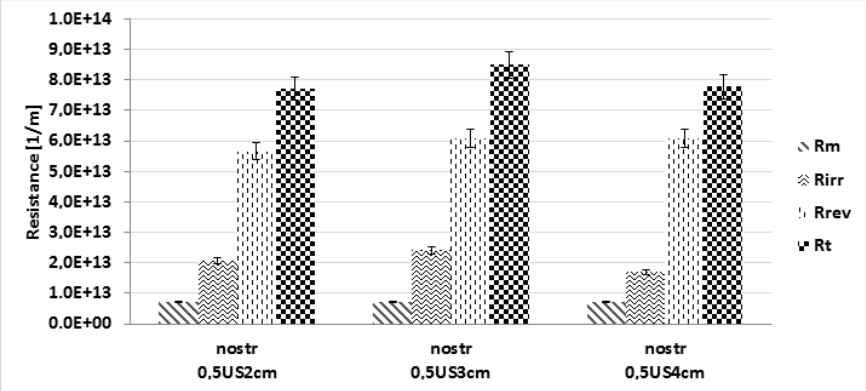

Fig 3: *Resistance values obtained without stirring*

The biggest total resistance is detected at 3 cm distance but there are no significant differences between the samples. Meanwhile regarding the irreversible resistance the biggest, the most

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unfavourable is at “nostr 0.5US3cm” samples; the reason of it is the previous mentioned ultrasound generated high pressure and share force, since these are able to push the medium size molecules via pores, but some of these molecules are trapped by the tortuous path.

The sonication intensity refers to the ratio of time when the US processor is working on. The distance between the horn and the membrane surface was fixed as high as 3 cm, the stirrer was used 350 rpm and the intensity of US was changed between 0.5 and 1.0, i.e US energy was emitted only half time (0.5), or full time (1.0) during irradiation.

The data show (Figure 4) there is no significant difference between the samples regarding the flux as a function of the time; there is need further data analyses.

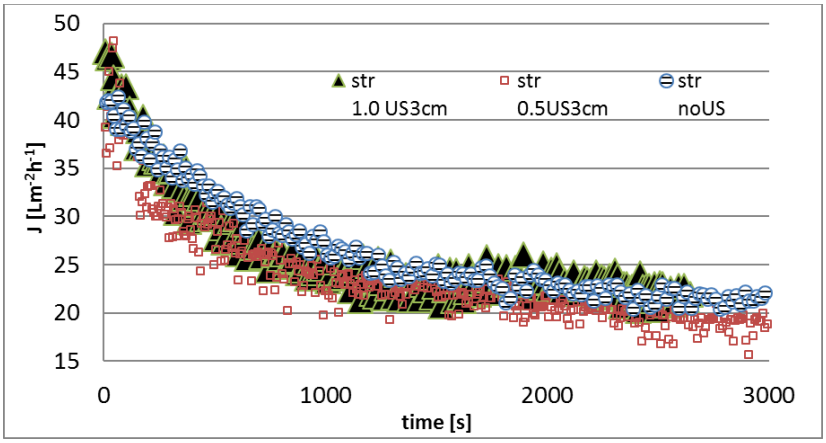

Fig 4: *Permeation flux as a function of time with stirring*

There is seem to be a contradictory relationship in the Figure 5, since examining of stirred samples irradiated with half intensity of US, the index is the biggest the FRR is the smallest one, meanwhile at no stirring samples these parameters have had positive correlation.

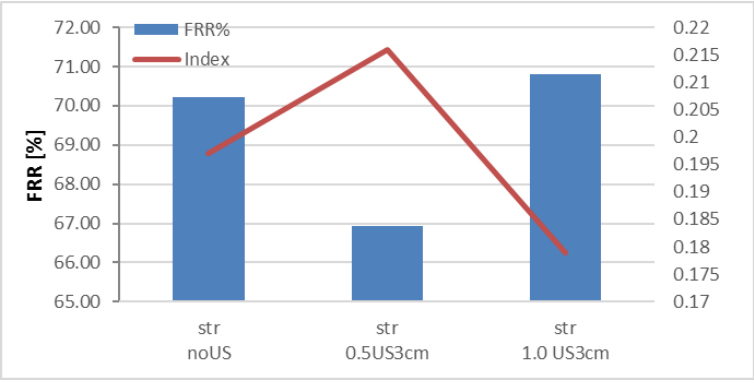

Fig 5: *Fouling ration at different stirring samples*

But when the resistance values are analysed detailed we can realize that there are differences between the irreversible and reversible ratios (Rirr/Rrev) (Figure 6).

While the Rirr/Rrev ratio is the highest at “no stirring0.5US3cm” samples, i.e. the irreversible resistance is the determinant, in the case of stirring samples the Rirr/Rrev ratio is the smallest at the “str0.5US3cm” sample. This ratio difference is the reason of the apparent contradiction.

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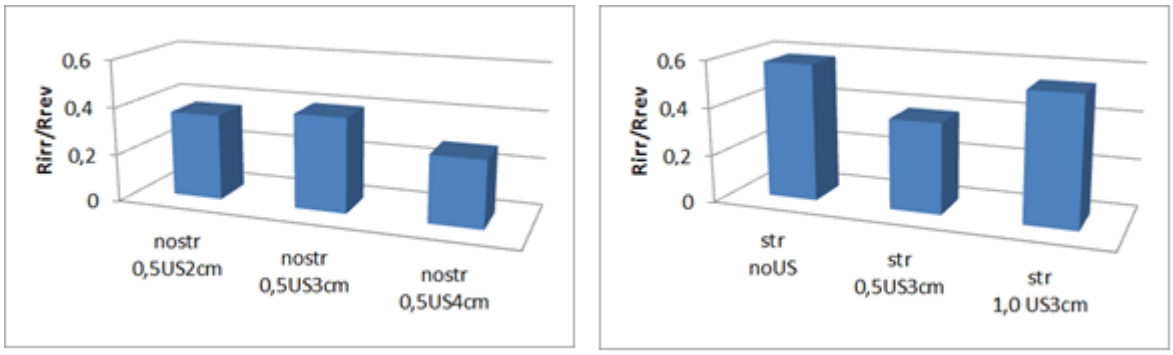

a) b)

Fig 6: *Ratios of the irreversible and reversible resistances a) without stirring, b) with stirring*

Figure 7 illustrates different resistance values depending on different filtration applications. Overall, it can be clearly seen that the membrane resistance is stable in all methods as expected while irreversible and reversible resistance show a change in different applications of stirring apparatus and ultrasound with different intensity.

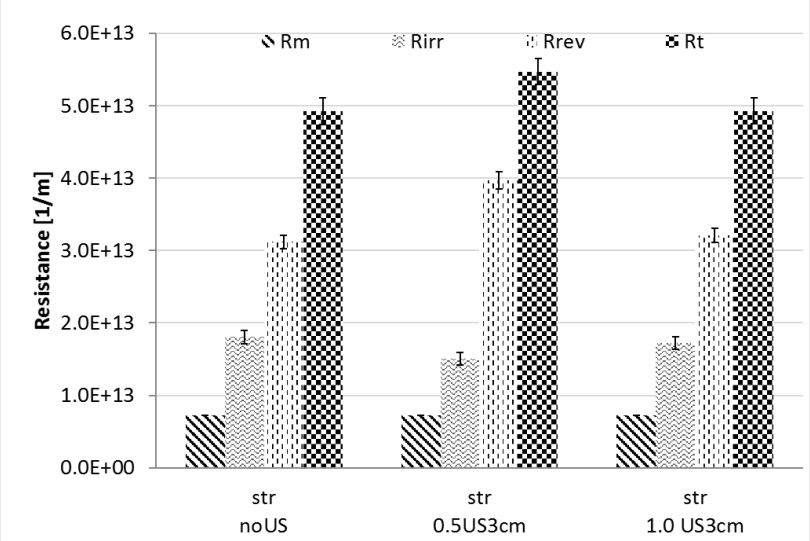

Fig 7: *Resistance values obtained without stirring*

Effect of the processing on the enzyme activity is seen in the Figure 8, there is no growth in reducing sugar content when permeate is used for hydrolysis. The measured sugar content is a residual amount in the permeate phase from the previous fermentation. Significant sugar yield was detected, when the concentrate is used. It means our theory is proved; neither the ultrafiltration, nor the ultrasound energy has any effect on the enzyme activity.

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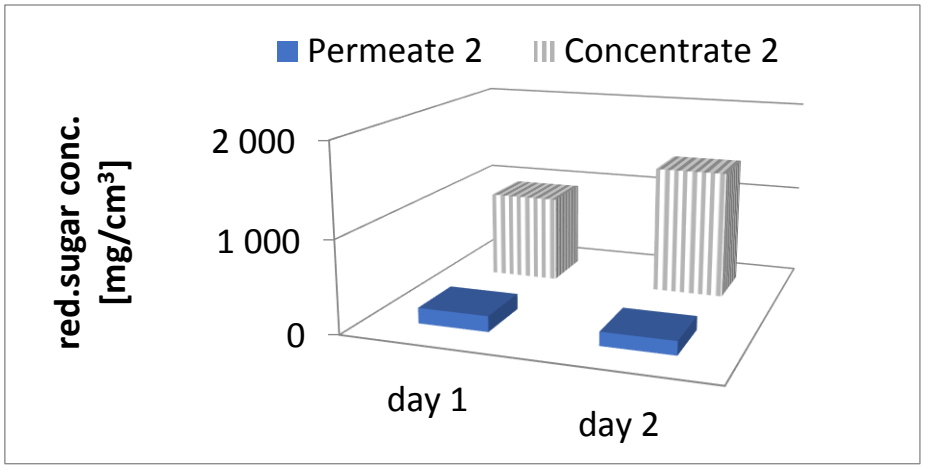

Fig 8: *Results of enzyme activity test*

Conclusion

The aim of our work was to separate xylanase enzyme from real fermentation broth by ultrafiltration and study the effect on filtering behaviour of sonication and stirring. Results have revealed that xylanase enzyme can be effectively separated from real broth and low energy ultrasound is not injurious for the enzyme. Full intensity sonication can increase permeate flux when stirring is used but membrane fouling is more intensive. When stirring is not used and sonication intensity is 0.5, membrane-transducer distance has no significant effect neither on permeation flux nor resistances.

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