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ENZYME RECOVERY BY MEMBRANE SEPARATION METHOD FROM WASTE PRODUCTS OF THE FOOD INDUSTRY

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ABSTRACT: Recycling waste products of food industry become more and more important: one side because of the environmental matter the other side because of the economic reasons. The most preferred basic material for second generation bio-fuels is the waste products, food industrial waste products such as sugar chip, straw or bagasse. The cost of the process depends on the cost of the hydrolysis of cellulose/lignocelluloses i.e. the cost of the enzymes. These enzymes are very expensive that's why it's so important to find a good enzyme recovery method. In our research programme the membrane separation was used for enzyme recovery. Different ultra-filtration membranes such as a polyether-sulfone membrane with a cut-off value of 5 kDa, (PES5) and thin-film membrane with a cut-off value of 4 kDa (TF4) was used for separation the hydrolyzatethe aim of our work was to determine the optimal conditions for the enzymes separation, the value of the fluxes and the resistances values and the investigate the effect of the ultrasound on the membrane separation. We found that the fluxes are enhanced and the fouling resistance is decreased due to the ultrasound application and we also found that the gel layer resistance is increased during the processing.
KEYWORDS: bioethanol, ultrafiltration, enzyme recovery

INTRODUCTION

In the food industry and in the agribusiness, the waste management is becomes more and more important. These sectors produce a lot of biologically infectious or contaminated waste products (Mold, insects etc.) but these wastes contain lot of useful organic components such as cellulose, sugar, etc which could be useful for bio-fuel production. The amount of waste become reducable on this way and it's also possible to replace one part of the fossil fuels (Mabee et al. 2011).

Cellulose-containing waste is very common for example in sugar industry: molasses (sugar-beet pulp without sugar) or bagasse (sugar cane without sugar). The main problem with the cellulose is the necessity of hydrolysis before fermentation since it cannot be transformed directly into bioethanol; first, it should be transformed (with hydrolysis) into glucose and after the glucose should be fermented into ethanol (Buaban et al, 2010).

It's also possible to do these two processes in the same time: it's called Simultaneous Saccharification and Fermentation (SSF) (Morales-Rodriguez et al, 2010). Different ways had been tested to make the cellulose more accessible for fermentation. One method is the heating of the cellulose with steam or another common method is the acidic hydrolysis with sulfuric acid (H_2SO_4).

These two methods cannot be applied in practice because the elevated temperature improved the enzymatic digestion but unfortunately, this high temperature leading to a significant reduction in total sugar recovery (Kahar et al, 2010). Kahar proposed another method: using enzymes (like cellulase and ß-glycosidase and a few other fermentation enzymes too, like cell-wall degrading enzymes including xylanase, pectase, ligninase as suggest Shunichi et al 2008 and Mübeccel et al, 2000.) in addition of light chemical treatment 0,1% of H_2SO_4 and physical treatment: milling and heating at 120°C for 20 min in an autoclave.

The enzymes cannot be immobilized for a maximal efficiency but have to be free in the solution (Ruchi et al, 2011). The advantages of the enzymes were experienced after the use but the costs of the enzymes are high for using in this bioethanol production method. So it is very important to find a way to recover and recycle those enzymes after use. During the research programme it was found that the best recovering procedure is the membrane separation. (Lipnizki, 2010).

There are different kinds of membrane separation. The membrane process is based on the membrane itself, which is a perm-selective barrier between two phases.

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These membranes are passed certain components through and held back some other components or molecules. The membranes can be categorized by their thickness, construction, charge or according to their origin. The membrane science knows four different membrane separation processes: micro-filtration, ultra-filtration, nano-filtration and reverse osmosis. The main difference is the pore size of the membranes and the applied differential pressure. Micro-filtration has pore size between 0.1 and 10μm and the used pressure is 0.2-0.6 MPa, in ultra-filtration the pore size is between $10^{-3}$and 0.1μm and the used pressure is 0.2-1 MPa; the nano-filtration has pore size between 1 and 10 nm and has applied pressure between 1-4 MPa. In the reverse osmosis process, it's only the water which can pass through the membrane. The reverse osmosis membrane separation process used 0.1-1 nm pore size membranes with 3-10 MPa pressure.

The most frequently used methods are nano-filtration and ultra-filtration. Ultra-filtration is used to clarify fruit juice or to filter protein and retain casein in the diary industry. Nano-filtration is mostly used in the water treatment industry: to filter antibiotics or pesticides, softening and reducing the salt content in water. (Daufin 1998)

There are some advantages the work can be affected at ambient temperature, no chemical has to be added and the process can be continued. But there are also drawbacks: risks of fouling, limited selectivity and lifetime. For continuous processes the pumps can be very expensive. (Bimbenet 2001)

In our work, our first purpose was to find the best parameters to recover enzymes with membrane ultra-filtration, and our second objective was to investigate the effect of ultrasound of filter parameters.

MATERIAL AND METHODS

All experience was carried out under optimal conditions for the enzymes. This reported 26°C ± 0.2, and they were repeated twice.

The model solution was prepared from 5% glucose and from 2% of cellulase of Trichoderma reesei (Cellulast 1.5L, Novozymes A/S, Denmark; 700 U/g) and from cellobiase of Aspergillus niger (Novozym 188, Novozymes A/S, Denmark; 250 U/g).

The hydrolyzate was made from sugar-beet pulp. It was prepared in a 2L fermentation unit (Labfors Minifors, Belgium) at 26°C±0.2 and pH 4±0.1. Enzymes used are the same than above at concentration of 200, 400 and 600 μL $g_{\ of\ solution}^{-1}$. Polyether sulfone (PES) membranes with a cut-off value at 5kDa and thin-film membranes with a cut-off value at 4kDa were used in a micellar enhanced ultra filtration (MEUF) device.

During the measurements, 3.5 bar pressure was applied and the feed solution was stirred with a magnetic stirrer at 350 rpm to prevent fouling of the membranes and to facilitate the formation of micelles. The permeates and the concentrates were analyzed after the measurements and the sugar and protein content were measured too.

It had been calculated the different components of total membrane resistance.

The retention (R) of the model and the hydrolyzates were calculated by the following formula [1]:

$$R = \left(1 - \frac{c}{c_0}\right) \cdot 100 \qquad [1]$$

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 value of the fouling coefficients was determined from the analysis of the flux-time functions [2]:

$$J = J_0 t^{-K} \qquad [2]$$

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 from the following correlation [3]:

$$R_M = \frac{\Delta p}{J_w \eta} \qquad [3]$$

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 was determined by washing the gel layer from the membrane. The fouling resistance [4] and the resistance of the polarization layer (R_g) were calculated as [5]:

$$R_f = \frac{\Delta p}{J_W \eta} - R_M \qquad [4]$$

$$R_g = \frac{\Delta p}{J_W \eta} - R_M - R_f \qquad [5]$$

where Δp is the pressure difference between the two sides of the water (Pas), η [Pas] is the viscosity of the filtered solution.

The Reynolds' number was calculated as [6]:

$$Re_{mix} = \frac{d^2 n \rho}{\eta} \qquad [6]$$

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].

The protein quantity was determined by the Kjeldhal method, and the glucose content was calculated by colorimetric method with a spectrophotometer. The ultrasonic treatment was used at the same time of the membrane separation.

RESULTS AND DISCUSSION

The measured fluxes were showed almost the same values on smaller pressure values. These fluxes were showed exponential raise after the 2.5 bar pressure value on higher pressure values, but this raise was slowed after the 3.5 bar value. The 3.5 bar pressure was chosen to our experiences, because an outlier data of the flux value was measured in this pressure value (Table 1).

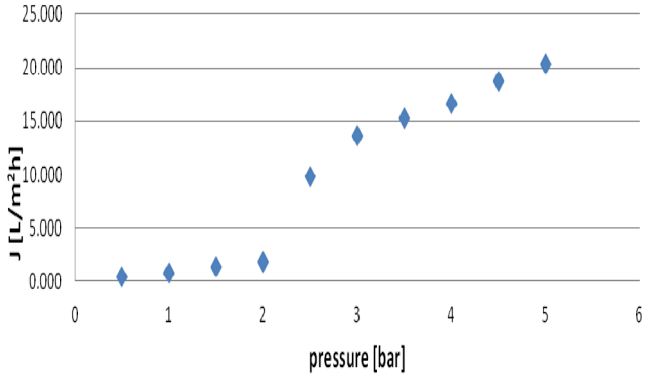

Table 1: the flux changes as a function of the pressure changes on TF4

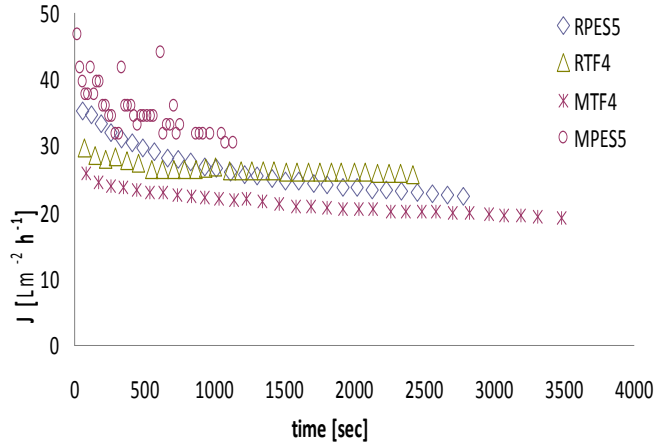

Table 2: Fluxes values for PES-5 and TF-4 with model and hydrolyzate. (RPES5 - Hydrolyzate on PES5 RTF4 - Hydrolyzate on TF4 , MTF4 - Model solution on TF4, MPES5 - Model solution on PES5)

On the TF-4 membrane almost the same flux values had been received when the hydrolyzate and the model solution were filtered. These two solutions were produced very different flux values on the PES-5 membranes. The hydrolyzate did not give us adequate informations as the model solution. High-dispersion data had been received (Table 2).

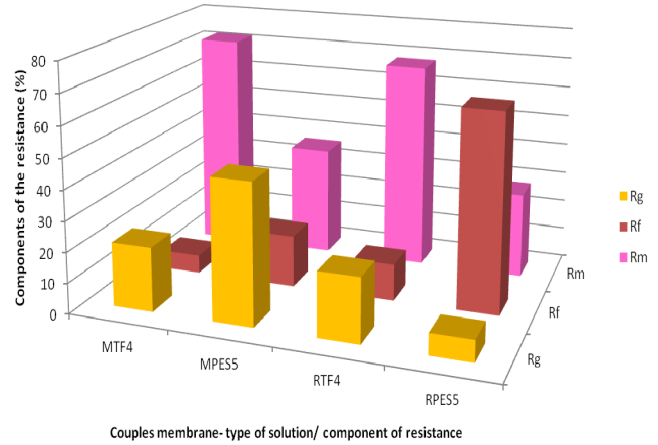

Table 3: Resistance composition depending on the solutions and membranes. (RPES5 - Hydrolyzate on PES5 RTF4 - Hydrolyzate on TF4 , MTF4 - Model solution on TF4, MPES5 - Model solution on PES5)

Tree different membrane resistance values were measured during the experiments, first the membrane resistance (Rm), second the resistance of the gel layer on the surface of the membrane (Rg), and finally the fouling resistance (Rf).
The Rm resistance showed higher values on the TF-4 membranes against the PES-5 membranes. This difference came from the different pore sizes of the membranes. The 4 kDa cut-off value size membrane can hold back more components of the solution as the 5 kDa cut-off value size PES-5 membrane (Table 3).
The model solution Rm resistance values (72% & 68%) were higher than the hydrolyzate values (36% & 28%). The lot of small components (molecules & amino acids) were fouled the pores of the membrane in the model solution. The hydrolyzate's big components, like the proteins were occluded fast the pores of the membrane.
In the preliminary measurements we didn't find significant difference between the resistance values of the membranes, but the 5kDa cut-off value membrane had a higher flux value that's why we continued our measurements with these membranes. The total resistance values were showed the same; the 5 kDa membrane had a little bit higher total resistance value as the 4 kDa membrane. This difference was observed between the two different solutions too (Table 4).

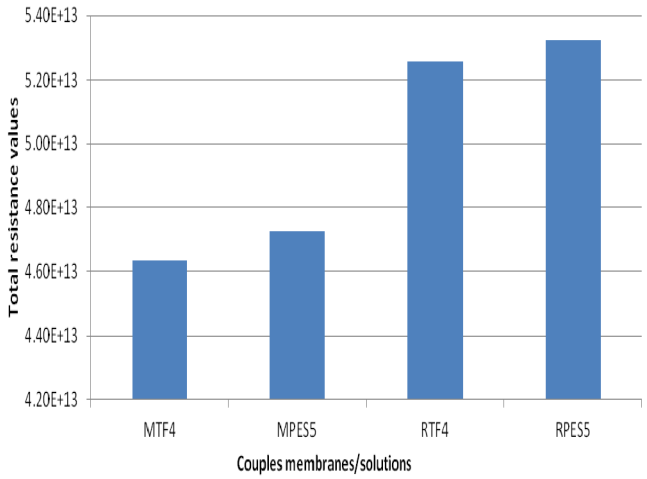

Table 4: Total resistance values depending of the couples solutions/ types of membranes

The protein and the sugar were showed lower values in the permeate of the 5kDa membrane against the 4 kDa membrane. This means that the membrane and the gel on the surface of the membrane could hold back the proteins and glucose fragments and the enzyme molecules too.
The protein retention values (what we measured) were showed that the enzymes or proteins could be separated in the concentrate.
The next table (Table 5) shows that the two different membrane how to retain the proteins or enzymes. In here we could see that the PES-5 membrane knew to retain the protein that's why the value of the proteins in the permeate is very small value.

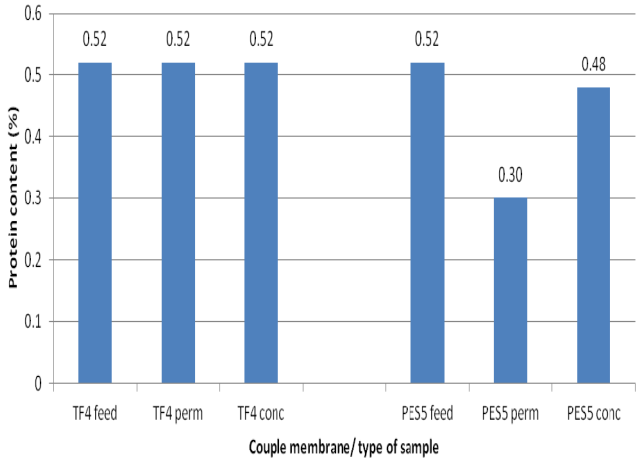

Table 5: The protein retention contents measured in the different solutions (TF-4 permeate, TF-4 concentrate; TF-4 and PES-5 feed: PES-5 permeate, PES-5 concentrate)

The enzymes and proteins were being able to hold back the gel on the surface of the 5 kDa membrane and the pore size of the membrane. The 4 kDa membrane was showed the same values of the proteins in the permeate solution and in the concentrate as in the feed. Because of this dates the 5 kDa membrane was chosen to use to our work and continued our measurements with it. In the next step the enzyme was separated and recovered them with using the 5 kDa membrane for the separation process. The Ultrasound was used in this process to.

We tried to measure that the ultrasound can be help to recover the enzymes or increase the retention values of the membrane. Higher flux values were produced the use of ultrasound than the membrane separation without ultrasound (Table 6).

The protein molecules were fractured the ultrasound and raised the amount of these fragments in the feed of the model solution because the ultrasound had an anti-fouling and an anti-gel layer effect. This means that the ultrasound could disrupt the structure of the gel on the surface of the membrane and prevent the formation of this gel.

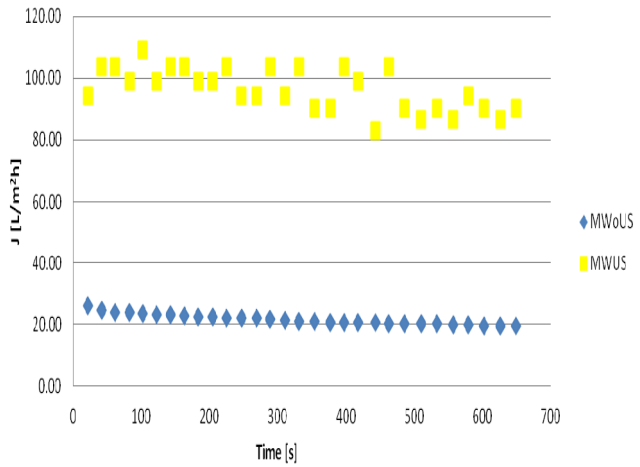

Table 6: The model solution flux values are graphed as a function of time with or without US on PES-5 membrane

It can be seen on the table 7 that the flux values are lower with using ultrasound than the ultrafiltration of the hydrolyzate without using ultrasound.

This gel was disrupted the ultrasound on the surface of the membrane, but also the molecules of the enzymes and the proteins were disrupted the ultrasound too, that's why the pores of the membrane were obturated these fragments and lower fluxes were measured with ultrasound. Lot of small, disrupted items and molecules were generated the treatment which the pores of the membrane were obturated these items and the concentration of the feed was increased too.

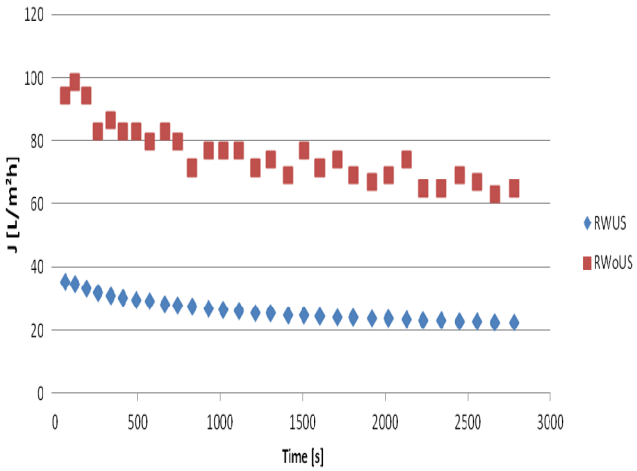

Table 7: The hydrolyzate flux values are graphed as a function of time with or without US on PES-5 membrane

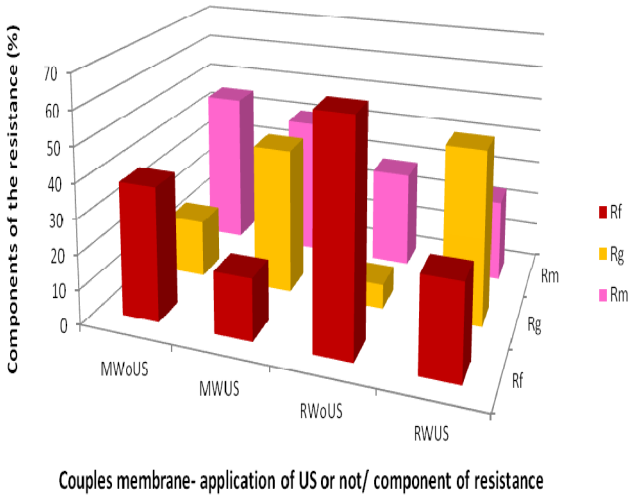

Table 8: The total resistance values as a function of depending of the application or not of US and of the solution

When the ultrasound was used for the filtration the total resistance values were become higher in the fouling resistance and in the membrane resistance. When the ultrasound wasn't used in the process the model solutions flux values were become smaller. The fragments of the proteins and the enzymes were made a gel on the surface of the membrane because of the effect of concentration polarization, and the concentration of the feed was increased the thickness of this gel. Since the concentration at the membrane

surface is larger than the feed side of the main mass, and therefore a movement in the opposite direction in order is generated that reduces the flux. That's why the total resistance shown lower values with using ultrasound in the fouling resistance and in the membrane resistance too.

The gel resistance showed a difference because the values are much bigger when we used ultrasound than when we not used it. These values mean that the ultrasound obturated the protein and enzyme fragments.

As we can see on the above diagram, when increased the number of small, obturated fragments due to this the gel resistance increased too. We could see than the different solutions showed the same effects.

CONCLUSIONS

In our research project we tried to find the best way of enzyme recovery by membrane separation. We thought that the membrane filtration could help recovering the enzymes which we used in the fermentation of sugar-beet to creating bioethanol. Model solution and hydrolyzate's were used for the measurements which were contained 2% cellulose and cellobiase enzymes.

Our first step was to find the best conditions for the separation process. We found that the temperature between 24-28 degree, 3.5 pressure and 4-4.2 pH were the best conditions for the enzymes to work and for the separation process to recovery the most of the used enzymes.

Our second step was to find the appropriate membrane. Tests were made with two different pore size membranes, the TF-4 and the PES-5. We found that the PES-5 membrane is better to recovery most of the used enzymes and better not to obturating fast the pores of the membrane.

A deposit of gel was made the concentration polarization effect on the surface of the membrane that's why a stirrer was used during the separation process. The stirrer could help for us to slow down the gel formation.

The same flux and resistance intervals were showed the model solution and the hydrolyzate during the tests. After this we wanted to see if we using ultrasound in the separation process how it is going to change the different values.

A lot of small fragments from the proteins and enzymes were made the ultrasound and the membrane was obturated these small fragments and the speed of the gel formation on the surface of the membrane was increased it too. The flux values were augmented the gel because the concentration of the feed became lower.

In the end of our experiences we can say that the membrane separation is a good process to separate the enzymes in the feed and recover them.

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