K- 115691

final report

Environmental protection and sustainability have a high demand for new technologies that allows for the most efficient use of available resources. Potential resources include organic waste and by-products accumulated in agricultural economy and food industry. Therefore, the main goal of our research is to develop, model and optimize the recovery/reuse processes of these materials, as well as efficiency-enhancing pre-treatment operations/processes as microwave energy transfer, application of ultrasound, pH change, membrane separation).

In the course of our research, we mapped a wide range of wastes containing organic matter in terms of usability. Several cellulose / lignocellulose-containing wastes were examined, e.g. wastes containing corn cob, wood chips, tobacco biomass, but also other potentially recoverable organic matter produced in significant quantities, such as e.g. sewage sludge from the meat industry or sewage sludge from the dairy industry. Biogas and bioethanol production were considered to be the possible areas of their utilization. Efficiency of our treatments was followed by determination of the change of biodegradability and solubility and, of course, the amount of biogas produced , the reducing sugar content or the amount of ethanol formed.

The goal of microwave pre-treatment research is to determine, model, and optimize raw material-specific operating parameters in batch and continuous laboratory and semi-industrial scale processes. The most significant task was to combine the determining parameters, i.e., specific microwave power intensity, treatment time, and mass flow, and microwave treatment with chemical methods and to examine their interactions. The aim of the related membrane separation research was to develop an enzyme recovery process based on ultrafiltration during the enzymatic hydrolysis of cellulose fractions pre-treated with microwave energy transfer, which significantly influences the economics of the technology; modelling the deceleration of permeate flux decreases, considering the specifics of the raw material. To increase the bioethanol yield during fermentation, different types of reducing sugars were fermented with different types of fungi.

The results are presented on the basis of the "expected results" in the application.

- Main determinative process parameters and their interaction for cellulose degradation during microwave pre-treatment. Feed dependent optimum ranges of process parameters
- Mathematical model to describe the microwave pre-treatment with combination of chemical process and to estimate the efficiency of pre-treatments
- Model to describe and optimize the process parameters of microwave combined chemical pre-treatment methods

The effect of NaOH dosing and the reported specific amount of microwave energy was investigated by the combined, continuous microwave and alkaline treatment of meat industry sewage sludge by the changes of the following parameters:

- degree of disintegration (DF) was calculated on the basis of changes in chemical oxygen demand

$$DF = \frac{SCOD_1 - SCOD_0}{TCOD - SCOD_0} 100\ [\%] \qquad (1)$$

where SCOD – soluble chemical oxygen demand, TCOD- total chemical oxygen demand

- Biodegradation index BDI) calculation based on the determination of the biochemical oxygen demand (BOD) characteristic of aerobic degradability

$$BDI = \frac{\left(BOD_5 SCOD^{-1}\right)_1 - \left(BOD_5 SCOD^{-1}\right)_0}{SCOD_{max}^{-1}} \qquad (2)$$

- dielectric constant (ε').

Combined alkaline-microwave treatment is suitable for increasing both DF and BDI. In the applied treatment range (between 0.15 - 0.6 $g/g_{dry\ matter}$ NaOH addition and 2221 - 9661 Jg^{-1} specific microwave energy level), the degree of disintegration increased with increasing amount of NaOH at low irradiation energy level. The highest disintegration (DF) value (45%) can be achieved with high specific microwave specific energy and medium and high amount of added NaOH.

Based on the analysis, Equation 3 is the mathematical model created, where Y_1 denotes the degree of disintegration, x_1: NaOH addition, x_2: specific microwave energy.

$$Y_1 = -10{,}2 + 135{,}8x_1 - 15{,}77_1^2 - 0{,}05x_2 \qquad (3)$$

Range of interpretation of the equation: NaOH addition: 0.15-0.6 g/g $_{dry\ matter}$, specific microwave energy level: 2221-9661 Jg^{-1}.

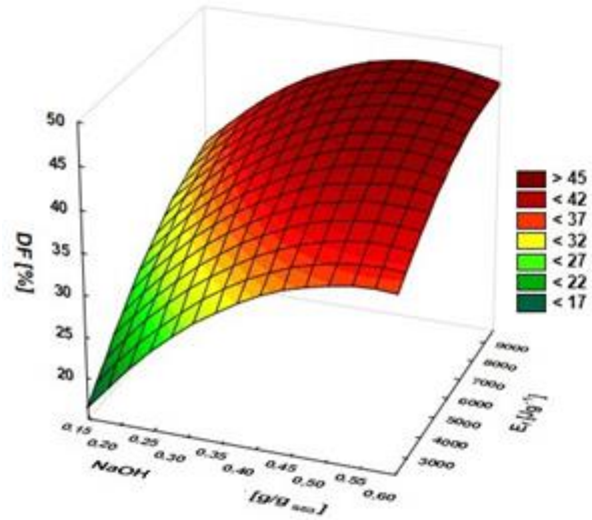

Figure 1: Degree of disintegration (DF) as a function of the relationship between NaOH addition and specific microwave energy (Ef)

For the biodegradation index (BDI), similar trends can be observed for low and medium (2221 - 9661 Jg^{-1}) specific microwave energy input as for the degree of disintegration. However, the increase measured at high energy levels was observed only with low NaOH (0.15 g / g $_{dry\ matter}$) dosing, while a decrease was observed with medium and high NaOH (0.6 g / g $_{dry\ matter}$) dosing. The highest BDI value (27.4%) is achieved with a medium specific microwave energy level and NaOH addition.

Equation 4 was obtained as a result of the modelling describes the results of the biodegradation index and the combined treatments, where Y_2 denotes the biodegradation index, x_1: NaOH addition, x_2: specific microwave energy:

$$Y_2 = -5{,}6 + 55{,}9x_1 - 0{,}005x_2 - 0{,}00011x_2^2 \qquad (4)$$

Range of interpretation of the equation: NaOH addition: 0.15-0.6 g/g $_{\text{dry matter}}$, specific microwave energy level: 2221-9661 Jg^{-1}.

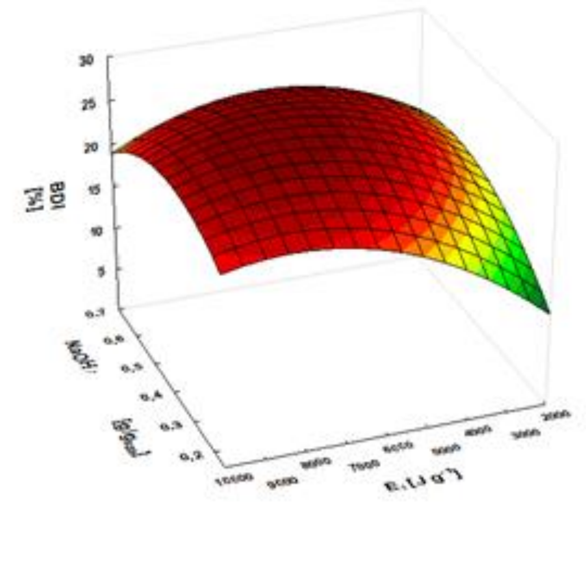

Figure 2: Biodegradation index (BDI) as a function of NaOH addition and specific microwave energy content (Ef)

Dielectric constant measurement provides a fast, real-time monitoring option, which makes it possible to estimate the efficiency of the treatment process, as well as the possibility of immediate intervention. Therefore, we investigated the correlation between the change of the dielectric constant and the degree of disintegration as well as the biodegradation index (Fig. 3). The paralel trends can be observed for the change of the dielectric constant and the degree of disintegration. Based on the high value of determination coefficient of the function obtained from the analysis of the relationship (R2 = 0.9517), it can be stated that in the case of the studied meat sewage sludge, the NaOH addition and the change of specific microwave energy and dielectric constant show a strong correlation. Based on these, it can be stated that by measuring the dielectric constant, we can get a good estimate of the degree of disintegration, which is achievable with sludge treatments (Fig.4), thus this is clearly an important parameter for feasibility.

In the equation obtained as a result of the modeling (5), Y_3 denotes the dielectric constant (f = 2450 MHz), x_1: NaOH addition, x_2: specific microwave energy:

$$Y_3 = 62{,}84 + 63{,}35x_1 + 0{,}0033x_2 - 44{,}17x_2^2 \qquad (5)$$

Range of interpretation of the equation: NaOH addition: 0.15-0.6 g/g $_{\text{dry matter}}$, specific microwave energy level: 2221-9661 Jg^{-1}.

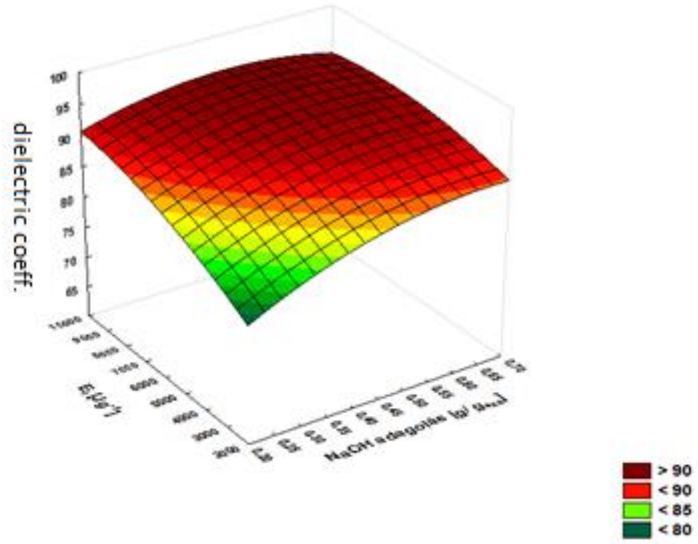

Figure 3: DA dielectric constant (f = 2450 MHz) as a function of specific microwave energy (Ef) and NaOH addition

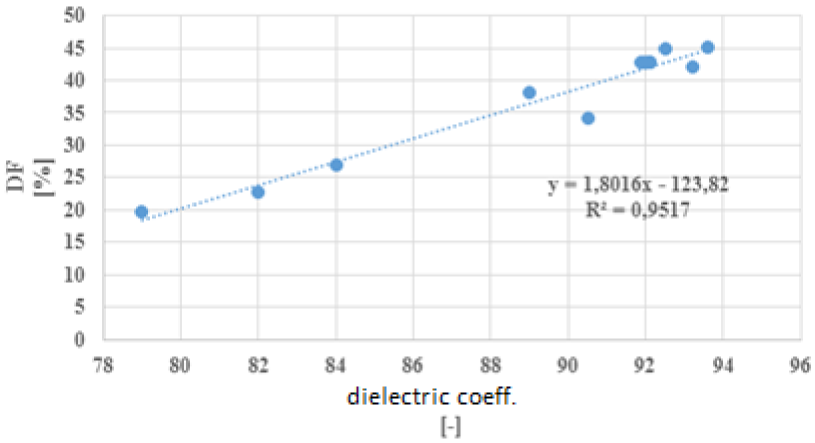

Figure 4: Relationship between dielectric constant and degradation index

Low-level (<10 kJg^{-1}) specific microwave energy intake alone, but in an alkaline medium, increases the solubility of organic molecules even more, and thus their biodegradability. These effects clearly lead to an increase in biogas / bioethanol yields. By measuring the values of the dielectric constant and analyzing the correlation, it can be proved that the result of a fast and non-destructive method which does not require a chemical reagent during the determination, shows a clear correlation with the progress of decomposition (disintegration)., It is a significant advantage in industrial implementation.

- Suitable indicators to energetic analysis and optimization both of cellulose hydrolysis and ethanol fermentation

In addition to microwave energy transfer, we also examined the pretreatment effect of ultrasound-mediated energy in addition to the tasks undertaken. The degraded substrate was maize cob grist (Cobex). The biological effect of ultrasound is already well known, especially in living organisms, but in our case its effect on the rate of hydrolysis was investigated through enzyme kinetic characteristics. Enzyme kinetic parameters are shown in Table 1 for different ultrasonic amplitudes using the Lineweaver-Burk method

Table 1. v_{max} and Michaelis-Menten constant of samples treated at different amplitudes

	60%	80%	100%	Control
v_{max}	77,519	37,594	36,630	26,316
K_M	36	16,654	15,817	7,266

The maximum velocity values (v_{max}) are the smallest for the control samples. During sonication at 80-100% amplitude, i.e. with significant energy input, the maximum rate of velocity is slightly higher than that of the control, but at 60% amplitude, its value increases significantly, more than double the values of the control samples. The value of the Michaelis-Menten constant (K_M) is a characteristic value of the enzyme reaction. In the control samples, the K_M value shows the characteristic value of the native enzyme without sonication. It changed as a result of sonication, almost equally at 80% and 100% applied ultrasonic amplitude, doubling the Michaelis-Menten constant, but at 60% amplitude there was a further significant increase, similar to the maximum velocity value. The rate of increase is five times that of the control.

The results thus show that ultrasound influences both the substrate and the enzyme as well, but this effect is not linear; it shows a maximum at the energy input indicated by 60% amplitude at a treatment time of 30 minutes. The accuracy of the measurement is confirmed by the fact that a direct proportionality can be observed between these two enzyme kinetic parameters - K_M and v_{max} – according the classical Michaelis-Menten enzyme kinetic reactions.

- Ultrafiltration method to recycle the enzymes to hydrolysis step of the process
- Mathematical model to describe the flux behavior and fouling dynamic of ultrafiltration and nanofiltration applied in cellulose hydrolysis and fermentation stages of bioethanol production technology
- Technology for full scale energetic utilization of cellulose contented wastes applying microwave pre-treatment and two step membrane separation process

The importance of recovery of enzymes used in the hydrolysis of lignocellulose-based biomass is justified by the cost of the enzymes. This represents a significant proportion of the total cost, energy and chemicals are required during their production, and their release into the environment may be concerned. These damaging effects on the environment, as well as the cost of the hydrolysis process, can be significantly reduced by using a method that allows the reuse of enzymes, namely ultrafiltration.

Due to the experimental ultrafiltration equipment conversion, an ultrasound generator can be connected to the filter cell, thus making it possible to combine ultrafiltration and ultrasound processes and reduce the decrease in permeate flux, which is a significant obstacle in the case of ultrafiltration of proteins. In order to delay and reduce the formation of blockages, to restructure the blockage mechanism, on the one hand, increased intensity of the cross-flow (Reynold's number) and on the other hand, sonication was used. The effect of increasing membrane-sonotrode distance and Reynold's number on the recovery of both xylanase and cellulase-cellobiase enzymes was examined.

It was found that sonication increased the permeate flux in all cases, but there was no significant difference in case of different sonotrode distances (2, 3, and 4 cm) from the membrane. Increasing the Reynold's number (max. Re = 6000) also had a positive effect on the permeate flux, and this effect proved to be greater than the value achieved with sonication. However, the ratio of reversible and irreversible resistances is more favourable using sonication and mixing together at 60 % intensities than applying them separately.

Stirring is preferred for flux values, but ultrasound is preferred for retention (based on Kjeldahl protein retention values).

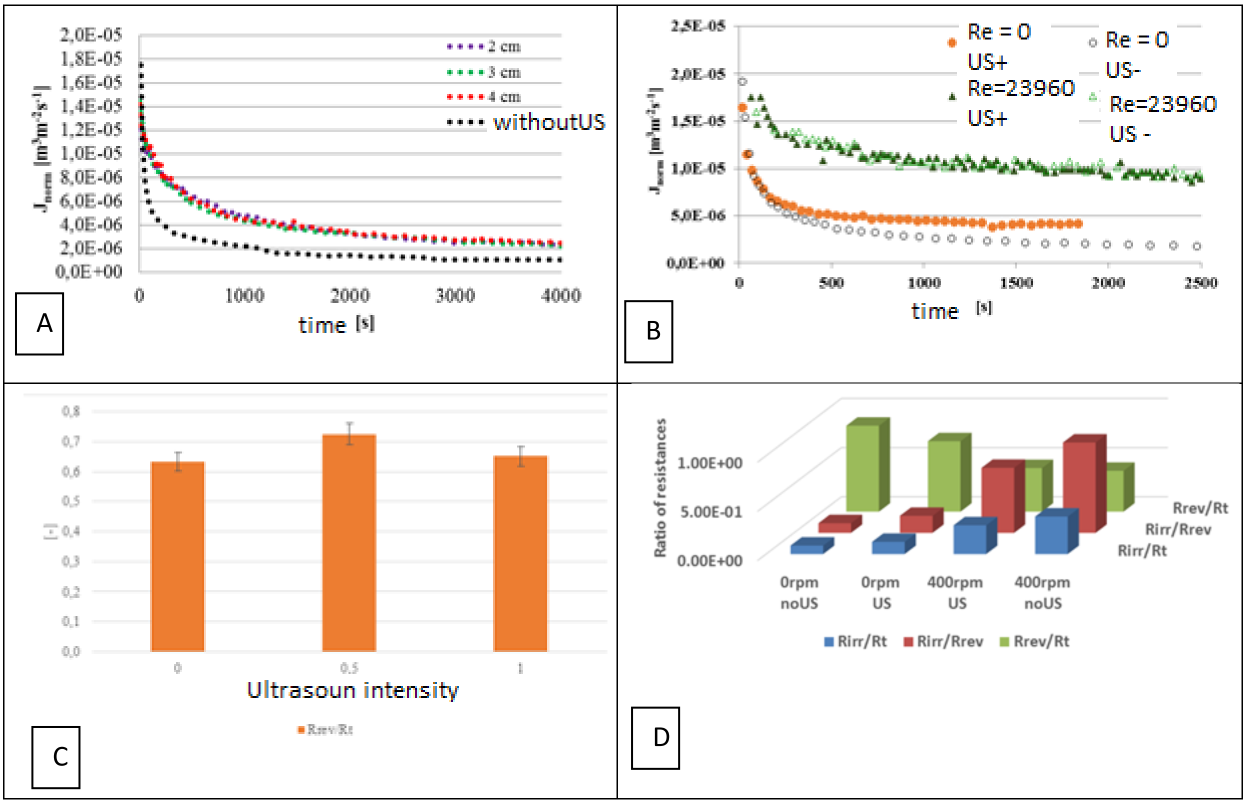

Figure 6: A - Effect of sonication and treatment head distance on permeate flux, B - combined effect of agitation and sonication on flux, C - Reversible resistance ratio to total resistance using agitation and different ultrasonic intensities, D - Resistance ratios for different procedures

Monitoring the activity of the enzymes used in the process and then recovered by ultrafiltration (either in mixed or ultrasound-coupled process) is essential for their subsequent use. Our measurements clearly demonstrated that the permeates did not show enzyme activity, i.e., all enzymes were separated and that the enzymes that went through the separation process retained their activity regardless of agitation and ultrasonic settings. Thus, neither the mechanical effect of the agitation nor the specific ultrasonic power of $4.08 \cdot 10^{-5}$ W / cm^3 reduced the activity of the enzymes.

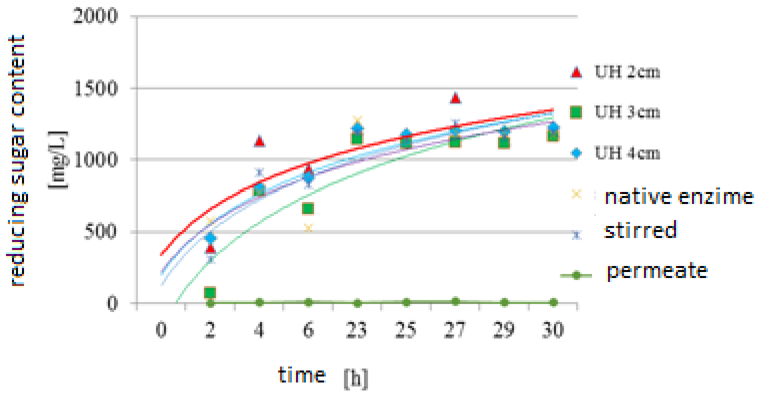

Figure 7: Reducing sugar contents as a function of time measured during enzyme activity tests

Table 2: Specific energy requirements and flux increase index (E)

$Re_{stir.}$ [-]	ultrasound [+/-]	P_{spec} [W/cm^3]	E [-]
2995	-	$1{,}1\cdot10^{-5}$	3,6±0,28
5990	-	$8{,}56\cdot10^{-5}$	4,9±0,43
11980	-	$6{,}43\cdot10^{-4}$	4,39±0,39
17970	-	$2{,}08\cdot10^{-3}$	4,3±0,37
23960	-	$4{,}78\cdot10^{-3}$	4,94±0,41
29950	-	$8{,}61\cdot10^{-3}$	5,12±0,42
0	+	$4{,}08\cdot10^{-5}$	2,17±0,29
23960	+	$4{,}78\cdot10^{-3}+4{,}08\cdot10^{-5}$	4,43±0,35

Examining the effect of agitation and sonication separately, it was found that in terms of flux increase both methods have a positive effect on permeate flux, but the effect of agitation is bigger, overriding the effect of ultrasound when used simultaneously. In the case of ultrasonic treatment, on the other hand, the ratio of irreversible / reversible resistance is lower, which is an important information in terms of the lifetime and cleaning of the membrane; it is essential for the success of further industrial applicability and scaling processes. The flux-increasing effect of sonication has been proven, but based on its extent it can be stated that it does not represent an advantage over mixing even energetically, so it is suggestible to use only in cases where mixing cannot be established, e.g. spiral-wound modules.

In the modelling of membrane filtration processes, especially ultra- and nano-filtration processes, several liquids with significant organic matter content (dairy wastewater, vegetable milk substitutes, hydrolysates and fermentation juices) were examined for more accurate modelling.

The characteristic of each filtration arrangement was determined by mathematical models describing the three types of membrane blockages: the resistance in series model, the Hermia blockage model, and the Makardij model. Comparing the results of the models to describe the clogging mechanism, it was found that in the case of ultrafiltration and the related cross-flow or sonication, the cake filtering (cake) model is the most satisfactory way to properly describe the processes. When the two filtration-intensifying methods are applied simultaneously, the permeate flux varies according to the dynamics described by the intermediate blocking model.

This statement was supported by the results of resistance-in-series model and Hermia model as well as the relatively rarely used Makardij model. In this case, it was proved that the semi-empirical coefficients of the model, i.e. k_1 is a permeation cross-flow depending coefficient, k_2 the coefficient of permeation inhibitory effects, well define the role of two mechanisms influencing flux, but the independence of the coefficients from flow rate is not always true. In our measurements, the dependence on the cross-flow rate can be clearly demonstrated in the case of fermentation broths containing dissolved components, which can be classified in a wide range of sizes. This indicates that the model provides a reliable value only under narrower application boundary conditions, moreover, the mechanism of gel layer formation may consist of several sub-mechanisms with different influencing roles on the flux value.

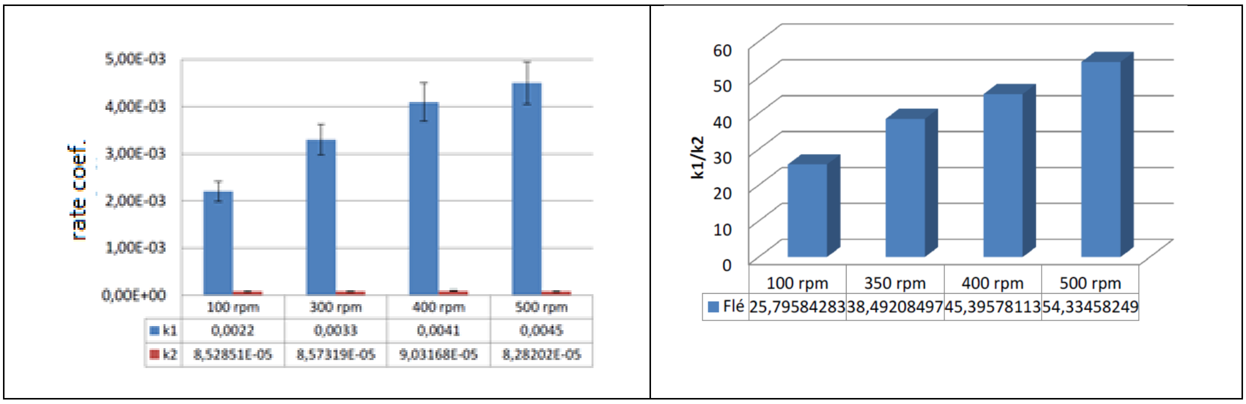

Figure 8: Coefficients of the Makardij model in the study of fermentation broth, resp. k1 / k2 values at different speeds

In another series of experiments the applicability of vibrational membrane separation was investigated. Our aim was to examine the effect of applied module vibration and transmembrane pressure as operating parameters to increase the filtration efficiency achieved by reducing membrane clogging, such as higher permeate fluxes and lower specific energy values, while not significantly changing the retention values.

The value of the specific energy consumption, i.e. the energy required to extract one cubic meter of permeate, without vibration is 0.70 $kWhm^{-3}$, with intermediate vibration (module vibration amplitude 1.27 cm) 0.43 $kWhm^{-3}$, and with maximum vibration (vibration amplitude 2.54 cm) was 0.38 $kWhm^{-3}$. The retention of the membrane in terms of organic matter content is as follows: without vibration 70.8%, with intermediate vibration 73.1%, with maximum vibration 71.3%.

Based on these data, it can be clearly concluded that the use of vibration has a significant advantage in reducing resistance values, i.e. an increase in permeate flux, while there is no significant change in retention values, which at the industrial level may mean increasing fluxes and reducing energy requirements. it is worth applying the module vibration.

- Nanofiltration process for concentration of sugar content in hydrolysis stage for increasing the efficiency of subsequent ethanol fermentation

The lignocellulose-containing biomass from the large quantities of agricultural waste available to us produces fermentable sugars after enzymatic hydrolysis, which can be converted to ethanol by the *Saccharomyces cerevisiae* used in our study. The formed alcohol can then be recovered from the medium by distillation.
The tobacco-based aqueous suspension hydrolyzed with cellulase and cellobiase gave results close to the theoretical ethanol concentration values, with a conversion sugar efficiency of 0.97 at the highest sugar concentration of 9.54%.

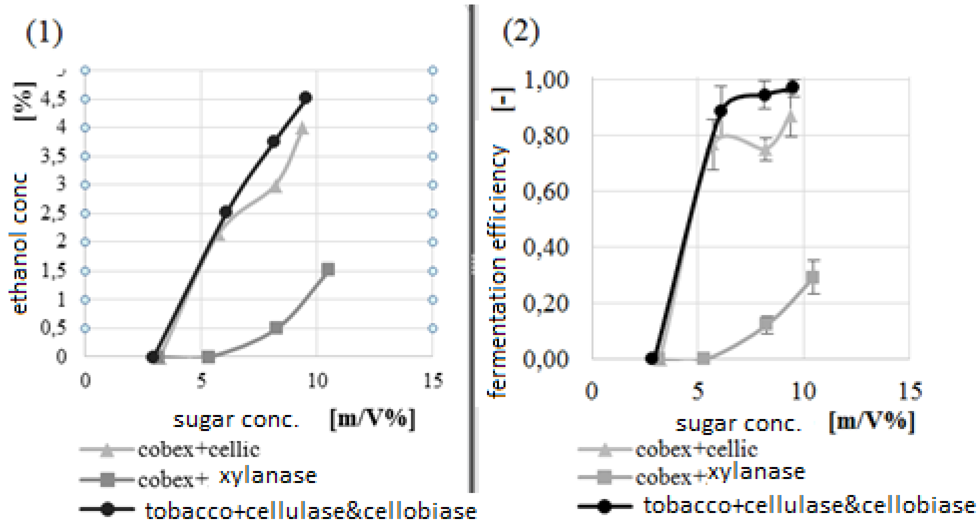

Figure 9: ethanol concentration and (2) fermentation efficiency as a function of sugar concentration

In the case of fermentation broth hydrolyzed with Cellic enzyme mixture containing Cobex Feeds, the yeast had a lower efficiency because Cellic enzyme mixture also contains hemicellulase, which, when digested hemicellulose, also removes pentoses which unusable for yeast. Therefore, other microorganisms that also ferment pentoses, *Kluyrveromyces marxianus* and *Pichia stipitis* species were also examined in our ethanol fermentation measurements.
In the study of *Kluyrveromyces marxianus* species, the cobex substrate hydrolyzate degraded with xylanase alone showed lower fermentation efficiency than the mixed lignocellulose substrate hydrolyzed with both cellulase, cellobiase and xylanase enzymes. This is because, although *Kluyveromyces marxianus* is able to use pentoses, it prefers glucose and therefore has a higher rate of ethanol production; more ethanol was formed in the solutions containing mixed reducing sugars.
In the case of *Pichia stipitis*, the trend was completely the same as in case of *Kluyveromyces marxianus*, but the fermentation efficiency was lower.

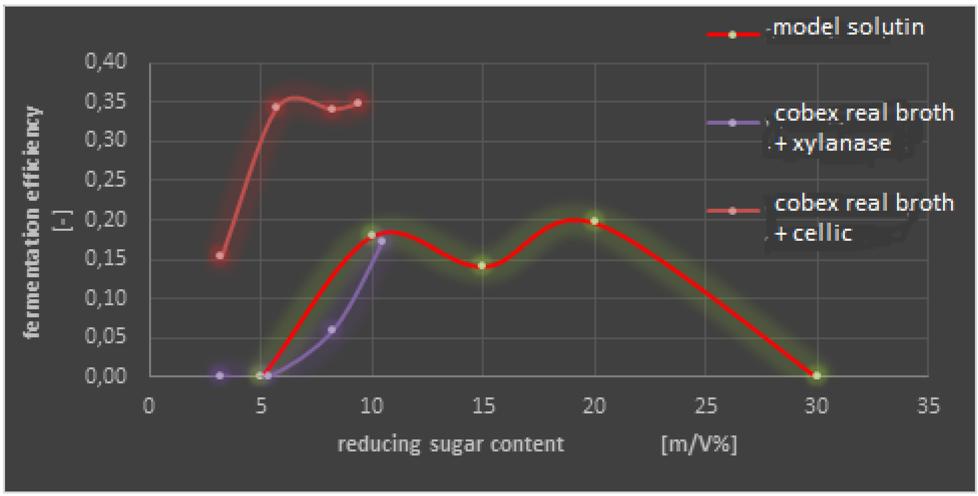

Figure 9: Fermentation efficiency as a function of reducing sugar conten.

- Technology for full scale energetic utilization of cellulose contented wastes applying two step membrane separation process.

In the case of both the thickening of fermentation broths or even other liquids with a high organic content, it is advantageous from the point of view of treatment efficiency and specific input if a smaller volume must be agitated / treated / cooled / fermented / distilled. Therefore, the study of membrane separation as a gentle concentration-increasing / volume-decreasing process, including vibration-operated modules, is organically related to the project topic.