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Application of nanofiltration in the process of the separation of model fermentation broths components

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The effectiveness of nanofiltration with the use of ceramic membranes in the process of concentration and separation of fumaric acid or succinic acid from glycerol and citric acid from erythritol was evaluated. It was found that the retention of sodium salts of the acids investigated increased strongly with increasing the pH of the feed solution (depending on the degree of dissociation), while the retention degrees of di- and tricarboxylic acids, erythritol or glycerol were lower than 2%, irrespective of the initial concentration of the solution to be filtered. The results obtained showed that nanofiltration can be considered as one of the purification steps in the process of recovery of salts of organic acids from fermentation broth.

Keywords: nanofiltration, ceramic membrane, fumaric acid, succinic acid, erythritol, glycerol.

INTRODUCTION

Development and improvement of biotechnological processes and preference for low-waste technologies have stimulated much interest in the fermentation processes. In many cases bioconversion is a safer and more cost-effective alternative to the production of chemicals. Moreover, a biosynthetic process usually takes place in milder conditions than traditional chemical synthesis and also requires less energy. Biocatalysts and reactants of biosynthesis (substrates, intermediates and final products) are biodegradable. In most biotechnological processes water is used as the solvent, which makes a great difference between bioconversion and chemical conversion in which biodegradable toxic chemicals and catalysts are generally used. Although biotechnological processes have many advantages, they are not free from drawbacks, of which the most important is the problem of purity of the final product. However, this problem can be successfully solved by suitable purification techniques of the reactive mixture. There are many methods proposed for the separation and concentration of low molecular weight organic compounds from fermentation broths, they include crystallization, precipitation, distillation, liquid-liquid extraction, ion exchange[1–3]. Alternative to the traditional methods of purification are membrane techniques, for example nanofiltration, which is the subject of the study presented.

It is expected that the significance of biodiesel and thus its use will soon increase not only in Poland but globally. The raw materials for its production are primarily vegetable oils, but also the fraction of fatty waste, waste fats and substances containing triglycerides. Currently, the most important raw materials for the production of biofuels are rapeseed oil and soybean oil[4]. In the biodiesel production process, significant amounts of by-products are generated, including (in the most quantity) glycerol phase in amounts not less than 12% relative to the esters obtained. This phase contains mainly glycerol (propane-1,2,3-triol) (50–60%), methanol, mono-, diacylglycerols, free fatty acids and soaps. Management of this waste could contribute to a significant reduction of fuel prices. Utilization of waste glycerol by microbial bioconversion seems to be most favorable both from the economic and environmental points of view. By using appropriate microbial fermentation of glycerol it is possible to obtain commercially useful metabolites, such as carboxylic acids (e.g., fumaric acid – (*E*)-butenedioic acid, succinic acid – butanedioic acid), polyols (e.g. propane-1,3-diol) or sugar alcohols (e.g. erythritol – (2*R*,3*R*)-butane-1,2,3,4-tetraol). Bioconversion of glycerol is also used for the production of microbial fat, biosurfactants, amino acids: L-lysine and L-glutamine, pigments such as astaxanthin or prodigiosin and polyhydroxyalkanoates (PHA) – exemplary of biodegradable polymer[5, 6].

The paper reports on the possibility of separation of selected components of the model fermentation broths after conversion of glycerol in the process of nanofiltration with the use of ceramic membranes. The effect of concentration and pH is taken into account.

EXPERIMENTAL PART

Materials

The model solutions of the individual components of the fermentation broth (fumaric acid, succinic acid, citric acid, erythritol, glycerol, Sigma-Aldrich) and solutions containing a mixture of dicarboxylic acids with glycerol, and tricarboxylic acid with erythritol were studied. Nanofiltration was carried out without adjusting the pH of the solutions, and for the solutions with a pH corrected by addition of sodium hydroxide (POCH SA).

Experiment and operating conditions

Two nanofiltration modules were used in this study: laboratory scale: Spirlab cross-flow module (Tami Industries) and pilot scale (Intermasz). The parameters of these modules and membranes, given by the producers, are presented in Table 1. The experiments were performed with recycling of the retentate into the feed vessel and collecting the permeate solution into a permeate test-tube.

After each experiment, the membrane was washed with a view to recovering its initial permeability with the following procedure: i) washing with water (3–5 minutes at 30–50°C), ii) alkaline bath – about 2% strength sodium hydroxide (15–45 minutes at 70–80°C), iii) washing with

Table 1. The parameters of modules and membranes

	Laboratory scale module	Pilot scale module
Kind of membrane	Flat, Tami Industries	tubular monochannel, Inopor
Cut off, Da	1000	450
Area, m^2	0.0064	0.0125
Dimensions of membrane	diameter 90 mm	internal diameter 7 mm, external diameter 10 mm, length 600 mm
Lp, m/h · MPa	0.144	0.333
Circulation flow, l/h	25	360
Transmembrane pressure, MPa	0.4	0.8

water (3–5 minutes at 40–50°C), iv) washing with water (3–5 minutes at 25–50°C) until the pH neutralization, v) acid bath – about 1% aqueous phosphoric acid (5–10 minutes at 50°C), vi) washing with water (3–5 minutes at 20–50°C), vii) washing with water (3–5 minutes at 20–50°C) until the pH neutralization. Moreover, prior to each filtration process the membrane was re-filtered with deionized water over 30 minutes and the permeate flux was then checked and compared with the initial one. Usually, there were no apparent differences between these fluxes.

Analysis

The concentration of fumaric acid and its sodium salt was determined using polarograph 797 VA Computrace (Metrohm)[7]. Other components were determined using the reversed-phase high performance liquid chromatography (HPLC). The HPLC system consisted of an LKB BROMMA 2150 solvent pump with a SSI LO-Pulse Damper, a Waters 517 Plus autosampler and a RIDK-102 refractometric detector. A Chromeleon (Gynkotek) integration system was used for data acquisition. The column used was a Rezex ROA – Organic Acid H+ (Phenomenex), 150 mm x 7.8 mm stainless steel analytical column with 8 μm particle size. The mobile phase was water containing 5 mM of sulfuric acid. The separation was carried out by isocratic elution with a flow rate of 0.5 ml · min^{-1}. All samples were acidified using the five times diluted sulfuric acid (200 μl per 2 ml sample).

RESULTS AND DISCUSSION

In the first stage of this study, the retention of individual components in the process of nanofiltration with ceramic membrane module was examined. The results showed that glycerol, fumaric acid, succinic acid, citric acid and erythritol were not retained in the nanofiltration process of aqueous solutions of these compounds, irrespective of the initial concentration used in the feed solution. No pH adjustment was made, so it was about 3 for the solutions of carboxylic acids and about 7 for the aqueous solutions of glycerol or erythritol. The retention of individual components did not exceed 2%. As follows from a comparison of the size of the molecule studied and the membrane cut off, these compounds must have had poor retention by the membranes investigated.

Because of the negative results of separation another approach was taken. Organic acids are in general weak and undergo dissociation at high pH. As the retention of weak acids and bases is highly dependent on pH, their retention in the NF process should be greater in the ionised form. It was found that the degree of retention of acids investigated significantly increased with increasing the pH of the solution. The effect observed can probably be explained by the increase in electrostatic interaction (repulsion) between the ionized form of acids and the surface charge of membrane's material. Van der Bruggen et al.[8] reported that only the rejection of negatively charged organic solutes with a molecular size close to the pore size of a nanofiltration membrane is driven more by sieving than electrostatic repulsion. The results of nanofiltration process of aqueous solutions of fumaric acid are shown in Fig. 1. According to them the rejection of fumaric acid changed from 1.5 to 98% over pH variation in the range 3–11, implying that the separation mechanism of fumaric acid by the ceramic membrane may involve only electrostatic repulsion because of the molecular size much smaller than the cut off of the membrane used. A similar relationship was observed by Choi et al.[9] during the separation of formic acid in the nanofiltration by polyamide membrane.

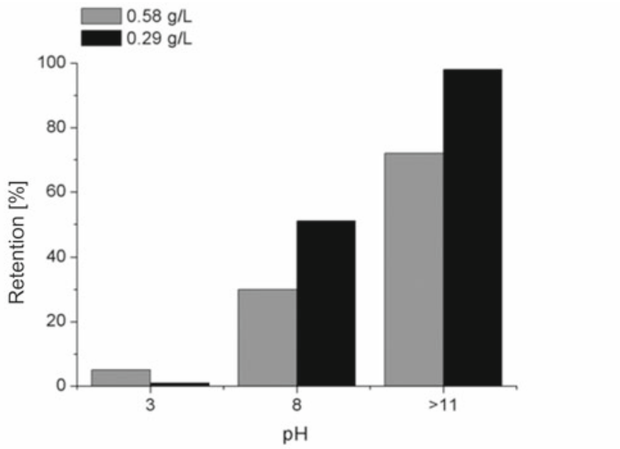

Figure 1. Retention of fumaric acid or sodium fumarate solution in different concentration and various pH (laboratory scale module)

The retention of sodium fumarate also depends on the initial concentration. Higher values of retention were observed at lower concentration. A similar relationship was observed for the other sodium salts of acids investigated. It is particularly evident in Table 2 which shows the results of filtration of aqueous solutions of succinic acid sodium salt, at pH 11 and for concentrations varied from 0.5 to 30 g/L. With increasing the initial concentrations of sodium succinate, the degree of retention decreases from 40% to about 2.5%. The lower retention at higher concentration of sodium salts of carboxylic acids could be explained by the transfer of charged solute which depends on a combination of steric hindrance effects and electrostatic interactions (interaction, attraction or repulsion forces, between the charged solute and fixed charge on the mem-

Table 2. Retention of sodium salts of succinic acid, pH = 11

Concentration [g/L]	R [%]
0.5	40
5	14
10	5
30	2.5

brane surface)[10]. At low concentrations, the electrostatic repulsions are dominant. At higher salt concentrations, the electrostatic repulsions become weaker and the salt retention decreases. It is called "the screening effect". Moreover, at high concentrations the effect of concentration polarization is important.

There are no significant differences in the retention for other components of model fermentation broth - glycerol and erythritol – with increasing the pH of the aqueous solution. For example, the average retention of erythritol at pH 11 does not exceed 2% (Table 3). Generally, the retention of uncharged solute, as glycerol or erythritol, only results from the steric-hindrance effect, which is determined by the hydrodynamic radius of the solute (Stokes radius) and the mean pore size of the membrane[11].

Table 3. Retention of erythritol, pH = 11

Concentration [g/L]	R [%]
25	2
50	1.8

In the next step of the study, a possibility of the separation of low molecular weight compounds from binary model aqueous solutions mixture was studied. Three types of mixtures were considered: i. succinic acid and glycerol, ii. fumaric acid and glycerol, iii. erythritol and citric acid. It was found that the addition of glycerol to the aqueous solution of dicarboxylic acids or their salts causes a slight decrease in the retention of acids or their salts in the case of the laboratory scale module. The exemplary results for a mixture of fumaric acid and glycerol are shown in Figure 2. A similar relationship was observed for a mixture of glycerol and succinic acid. However, as the pH was higher, almost the whole amount of glycerol permeated through the membrane and the two dicarboxylic acids in the form of their salts were retained in the nanofiltration process. As follows, the separation of glycerol from sodium fumarate or succinate is expected. It is not possible to separate directly dicarboxylic acids from glycerol. Citric acid as well as both dicarboxylic acids tested, could be retained in the nanofiltration at high pH, thus in the ionised form. Moreover as mentioned above, the retention of erythritol does not exceed 2%, irrespective of pH. Thus, nanofiltration process could be used to separate erythritol from sodium citrate.

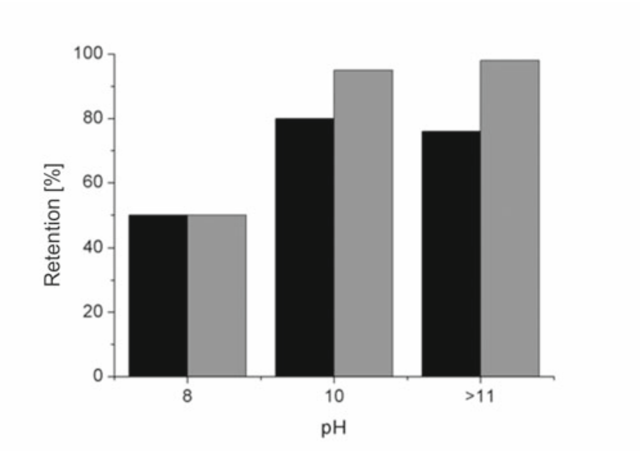

Figure 2. Comparison of the degree of retention of sodium fumarate: grey bar – aqueous solution of sodium fumarate (c = 0.0025 g/L), black bar – binary mixture of sodium fumarate (c = 0.0025 g/L) and glycerol (c = 2.5 g/L), depending on the pH (laboratory scale module)

According to the results, there is a slight reduction in the degree of retention of fumaric acid in the presence of glycerol in the mixture filtrated, at the next stage of the study the impact of addition of other substances (inorganic salt, other carboxylic acids) on the NF membrane separation capacity was examined. Figure 3 shows the results of retention of fumaric acid in the multicomponent solution. The composition of the solutions studied is shown in Table 4. Based on the results obtained, it was found that the retention of sodium fumarate by the ceramic membrane was lower for the ternary model solution containing acetic acid (mixture 4) than the degree of the retention of sodium fumarate in the case of NF separation of mixture with glycerol and citric acid (mixture 5) at the same concentration of fumaric acid and pH. This effect can be explained by a different structure of these acids and their molecular size, i.e. citric acid has three carboxylic groups, while acetic acid only one. As shown, fumaric acid can be effectively concentrated in the nanofiltration process on the module with a ceramic membrane.

CONCLUSIONS

The retention of a neutral solute such as glycerol, erythritol or carboxylic acids is mainly due to the steric

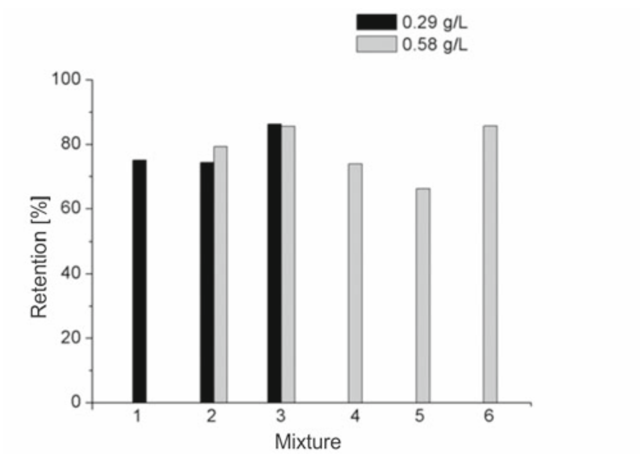

Figure 3. Retention of fumaric acid in its sodium salt form in the model fermentation broths (pilot scale module)

Table 4. Composition of the solutions tested (pH = 11)

	Concentration [g/L]					
	1	2	3	4	5	6
Fumaric acid	0.29	0.29 0.58	0.29 0.58	0.58	0.58	0.58
NaCl	–	5.8	5.8	5.8	5.8	5.8
Glycerol	–	–	2.5	–	–	2.5
Acetic acid	–	–	–	0.03	–	0.03
Succinic acid	–	–	–	–	–	0.06
Citric acid	–	–	–	–	0.1	0.1

effect which follows from the ratio of the solute to the pore radii. The transfer of a charged solute (sodium salts of acids investigated) results from a combination of the steric effect and electrostatic interactions.

The results obtained indicate that the nanofiltration process with ceramic membranes can be applied to concentrate fumaric acid, succinic or citric acid from aqueous solutions under the following conditions - low initial concentrations of these acids and the pH above the acid dissociation constants.

Because glycerol is not retained in the nanofiltration process, it can be separated from the fermentation broth, e.g. from fumaric acid or succinic acid in the form of their salts. In the studies of separation from a fermentation broth containing erythritol and citric acid, it was found that erythritol permeated through the membrane while the citric acid in the form of its sodium salt was rejected. A nanofiltration process may therefore be proposed as one of the purification steps of the fermentation broth.

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