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An environment-friendly multi-step membrane-based system for succinic acid recovery from the fermentation broth

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ABSTRACT

A novel environment-friendly process of recovery of succinic acid from the actual post-fermentation broth (remained after bioconversion of glycerol) in an integrated system consisting of: ultrafiltration (UF modulus equipped with two tubular ceramic membranes), two steps of ion exchange (two IE columns with a sodium IE resin), nanofiltration (NF modulus with a polymeric flat sheet membrane), and two steps of bipolar membrane electrodialysis (10-chamber large-scale EDBM with a stack consisting of 10 bipolar membranes, 10 anion-exchange, and 1 cation-exchange membranes) was investigated. As the first step the preclarification process of the actual post-fermentation solution carried out by UF was needed in order to remove high molecular contaminants such as biomass, proteins as well as cells. In turn, in the IE process the effective removal of Mg^{2+} and Ca^{2+} salts was obtained that allowed to significantly reduce the scaling process of the membrane in the next separation step which was the NF process. NF was efficiently employed to concentration of succinates and partial removal of other compounds, such as: monocarboxylic acids, glycerol, and lactose. The final two steps of EDBM were allowed to obtain high degree of desalination and high purity of succinic acid. By summing up the obtained results, it can be concluded that using the proposed integrated system was possible to effectively purify and concentrate the succinic acid from the actual post-fermentation broth. The final concentrate obtained after the second stage of the EDBM process contained over 18 g/L of succinic acid contaminated with only a small amount of glycerol (0.3 g/L). Furthermore, the use of the EDBM in the proposed integrated system allowed to eliminate the acidification of broth, which usually generated a considerable amount of wastes. Moreover, it should be stressed that the proposed six steps membrane-based process for the recovery of succinic acid from the actual post-fermentation broth is a totally waste-free technology.

Keywords: Succinic acid; Fermentation broth; Ultrafiltration; Nanofiltration; Bipolar membrane electrodialysis

1. Introduction

It is well known that the microbial methods for production of organic acids can be an alternative to the classical methods of chemical synthesis, which are usually multi-step, repeatedly inefficient, and generate significant amounts of wastes [1–3]. Furthermore, the microbial conversion methods allow for utilization of waste materials including unpurified glycerol phase, a major waste in biodiesel industry [4]. Succinic acid ($C_4H_6O_4$) is one of the most important platform chemicals used in food industry, pharmaceutical industry, and as a precursor of many chemicals, such as 1,4-butanediol and tetrahydrofuran. Succinic acid can be produced by microbial conversion of glycerol using engineered *Escherichia coli* species as reported by Ahn et al. [5]. Moreover, Lee et al. [6] have suggested that *Anaerobiospirillum succiniciproducens* can efficiently convert glycerol to succinic acid with a final succinate concentration

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equal to 19 g/L. Similarly, Vlysidis et al. [7] and Gao et al. [8] have indicated that crude glycerol obtained as a by-product in the biodiesel production can be used as the main carbon source by *Actinobacillus succinogenes* and engineered yeast *Yarrowia lipolytica*, respectively. Furthermore, as has been pointed out by Sadhukhan et al. [9], crude and purified glycerol obtained in the process of biorefining *Crotalaria juncea* can be used in the microbial conversion to the succinic acid with high yield equal to 23.31 g/L. However, the presence of by-products in the actual post-fermentation broth, such as mono- and dicarboxylic acids, nonionic compounds, and a significant amount of inorganic salts, means that design and implementation of an effective and environmentally safe method of separation, have a decisive impact on the biotechnological methods for the production of organic acids on the industrial scale [10]. So far many methods of the separation of low molecular weight organic compounds from the actual post-fermentation broth have been proposed, including crystallization, precipitation, adsorption, extraction, or membrane techniques [11–15]. The membrane-based methods are particularly promising for development of efficient and environment-friendly technologies, and it includes pressure-driven techniques (microfiltration, ultrafiltration (UF), nanofiltration (NF), and forward osmosis(FO)) and electrical-driven membrane methods (electrodialysis (ED) and bipolar membrane electrodialysis (EDBM)) [16–20]. However, the actual post-fermentation broths resulting of microbial conversion of carbon biosubstrates are complex mixtures and their treatment and effective separation of the desired product usually requires the use of two or more separation techniques arranged in integrated systems. Wu et al. [21] presented a method for obtaining 1,3-propanediol with simultaneous recovery of succinic acid using an integrated system consisting of UF and EDBM. Similarly, Sosa et al. [22] demonstrated the novel three-step integrated membrane system consisting of ED, NF, and Donnan dialysis to separate succinic acid from the fermentation broth left after the bioconversion of carob. In addition, many literature reports and our own previous research indicate that integrated systems can be effectively used for the recovery of other organic acids, especially lactic acid [23] and fumaric acid [24]. The aim of this study is to present and characterize an integrated system consisting of six stages such as: UF, two steps of ion exchange (IE_I) and (IE_{II}), NF, and two steps of bipolar membrane electrodialysis ($EDBM_I$) and ($EDBM_{II}$) designed for the process of recovery of succinic acid from the actual post-fermentation broth (which remained after bioconversion of glycerol).

2. Material and methods

2.1. Materials

The actual post-fermentation broth after bioconversion (with *Enterobacter* sp.) of glycerol (Rafineria Trzebinia S.A. PKN Orlen, Poland) to succinic acid (with pH = 8.5 and the composition shown in Table 1) was delivered from the Poznan University of Life Sciences, Poland. The sodium succinate solutions used during EDBM processes were prepared by adding NaOH (Sigma-Aldrich, Poland) to water solutions of succinic acid until pH = 8.5.

Table 1
Detailed composition of the actual post-fermentation broth

Component	Abbreviation	*C* (g/L)
Succinic acid	SA	11.4
Acetic acid	AA	7.1
Formic acid	FA	4.7
Lactic acid	LA	4.1
Glycerol	Glyc	34.1
Lactose	Lact	16.1
Mg^{2+} and Ca^{2+}	–	12.4

2.2. UF experiment

UF was carried out using a UF setup equipped with two (left and right) commercially available three-channel tubular ceramic membranes: Céram INSIDE® (TAMI, France) with cut-off 15 kDa and the effective surface area of each membrane equal to 0.0042 m^2. The used membranes were made of titanium oxide and zirconium oxide. The preclarification process of the actual post-fermentation solution was carried out by UF at TMP = 0.4 MPa and T = 25°C ± 2°C. During UF 10 L of the post-fermentation solution was pumped from the feed vessel through the UF membrane modules, and during the whole process the retentate was circulated in a closed loop at the volumetric flow rate of 300 L/h. The separation process was performed for 16 h and was repeated three times. After each experiment, the ceramic membrane was cleaned to recover its initial permeability according to the following procedure: (1) water cleaning, t = 5 min, T = 50°C; (2) 5% sodium hydroxide solution cleaning, t = 60 min, T = 60°C; (3) water cleaning, t = 5 min, T = 50°C; (4) 3% nitric acid solution cleaning, t = 10 min, T = 60°C; and (5) water cleaning to pH ≈ 5–6. In addition, before the UF process, the initial permeate flux of water was checked.

2.3. IE experiment

IE processes (IE_I and IE_{II}) were carried out using two identical columns (length 800 mm, diameter 40 mm) packed with a monodispersed strongly acidic resins (S1567 Na^+) of polystyrene–divinylbenzene matrix and the grain size of 0.6 mm (Lewatit, Netherlands). The volume of each column and the cation exchange capacity were equal to 1 L and 1.5 mol/L, respectively. In each experiment a portion of 0.5 L of permeate solution obtained after UF and 0.5 L of retentate solution obtained after NF were fed to the column IE_I and IE_{II}, respectively, collected and analyzed. After each experiment, the IE resin was regenerated using 1 L of cleaning solution consisting of 5 g/L sodium succinate and 100 g/L of sodium chloride.

2.4. NF experiment

The study of NF was carried out using an NF setup SEPA Osmonics (GE Osmonics, USA) with polymeric flat sheet membrane DK (made of polyamide (active layer) and polysulfone), which was supplied by GE Osmonics, USA. The parameters characterizing the membrane used in this study

are: cut-off 150–300 Da, the effective surface area 0.0155 m^2, pH range: 0–14. The NF process of solution obtained after the IE_1 was carried out using the NF unit operated at TMP = 1.6 MPa, T = 25 °C ± 2°C. During the NF process 6 L of solution was pumped from the feed vessel through the NF membrane module, and during the whole process the retentate was circulated in a closed loop at the volume flow rate of 160 L/h, while the permeate was collected. Each of the separation processes was carried out for 18 h. The NF processes were repeated three times, each time using a new portion of the feed solution. During the NF processes the pH value and temperature of the feed solution were controlled. The samples of retentate and permeate fluxes were collected at regular time intervals and analyzed. Each time, after NF processes, the NF unit was cleaned to recover its initial permeability according to the following procedure: (1) washing with water, t = 3 h, T = 25°C; (2) change of the water used for washing; and (3) washing with water, t = 1 h, T = 25°C.

2.5. EDBM experiment

The bipolar membrane electrodialysis was carried out using a 10-chamber large-scale EDBM with a stack consisting of 10 bipolar (PC 200bip) membranes, 10 anion-exchange (PC 200D), and 1 cation-exchange (PC-SK) membranes separating the electrolyte solution (0.3 M H_2SO_4) produced by PCCell GmbH, Germany, placed between cathode and anode made of steel 316 and iridium-plated titanium, respectively (Fig. 1). The spacing between the membranes was 0.5 mm and the effective surface area of each membrane was equal to 0.0207 m^2. Moreover, the membrane stack was connected to DC sources, while the 10-chamber large-scale EDBM setup was connected to a flow pump (Verder, Poland), DC power supply (NDN), and a multifunction meter (Elmetron, Poland) measuring pH, temperature, and conductivity of treated solutions. In each chamber the diluate and the concentrate solutions were circulated at the flow rate of 100 L/h, which was measured by a flow meter. The processes were carried out at 25°C ± 2°C and under a constant electric field of current density equal to 120 A/m^2. Each time during the EDBM processes the following parameters: pH-value, conductivity, and temperature of the diluate and the concentrate solutions were controlled. In each experiment 1.5 L of the diluate and the concentrate solutions (1.6 g/L of succinates) were fed into the respective chambers. The use of succinate solution allowed for the reduction of electric resistance at the EDBM stack at the beginning of the process. Additionally, the concentration of the succinates equal to 1.6 g/L was the lowest one at which the highest efficiency of EDBM was obtained. Samples of diluate and concentrate were collected at regular time intervals and analyzed. Moreover, the voltage changes as a function of time were recorded to evaluate the electric resistance of the stack. The EDBM experiments were conducted for 3 h and each of them was repeated three times. During the EDBM processes, when a constant electric field was applied, succinate ions present in diluate solution were transported across the anion exchange membrane (AM) to the concentrate chamber. At the same time, succinate ions were converted into succinic acid form in the concentrate chamber with the protons produced during water dissociation by the bipolar membrane. Each time, after EDBM processes, the EDBM unit was cleaned according to the following procedure: (1) washing with water, t = 3 h, T = 25°C; (2) change of the water used for washing; and 3) washing with water, t = 0.5 h, T = 25°C.

2.6. Analytical methods

The contents of mono-, dicarboxylic acids, glycerol, lactose, and their salts in individual solutions were determined using a high-performance liquid chromatography using Agilent 1100 Series (Germany) equipped with an auto

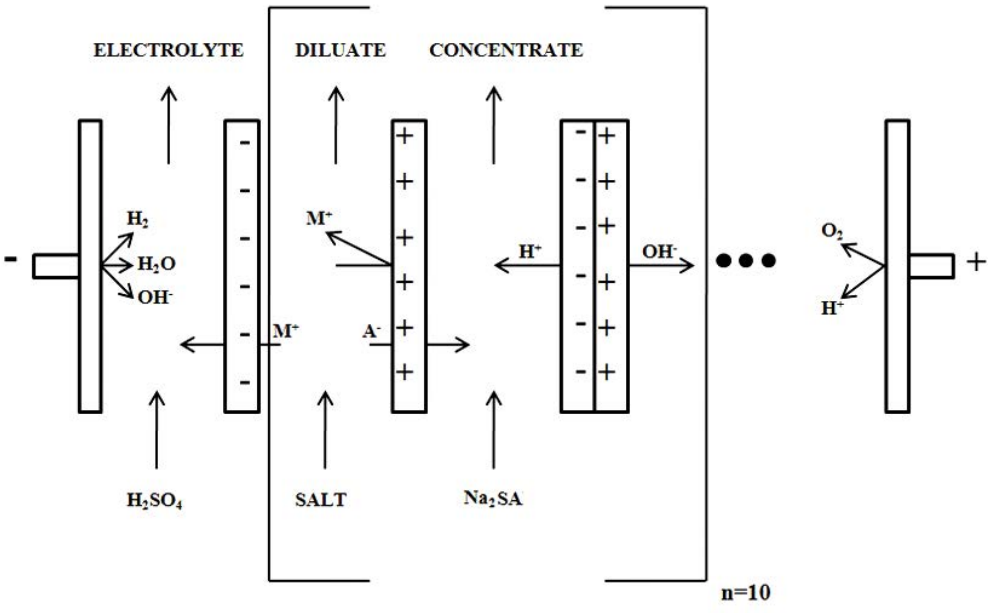

Fig. 1. The scheme of EDBM stack configuration.

sampler, interface (HP 35900), RI Detector (HP 1047A), pump (HP1050), and separating column Rezex ROA-Organic Acid H^+ (8%), Phenomenex®. The eluent of 2.5 mM H_2SO_4 (Sigma-Aldrich, Poland) solution was supplied continuously at the rate of 0.9 mL/min. The column and the input to the detector were operated at T = 40°C, P = 0.56 MPa. All samples were acidified to pH ≤2 by addition of 0.1 mL 25% H_2SO_4 to 1 mL of sample before analysis.

In order to determine the concentration of magnesium(II) and calcium(II) ions present in the analyzed solutions, the titration of solution samples (10 mL) with an ethylenediaminetetraacetic acid (EDTA) solution (14.6 g/L), in the presence of eriochrome black as an indicator, was carried out and each titration procedure was repeated three to five times. The total concentration of magnesium(II) and calcium(II) ions in UF permeate and NF retentate solutions was calculated from the following equation:

$$n_{Mg^{2+}} + n_{Ca^{2+}} = \frac{V_{EDTA} \cdot C_{EDTA}}{V_s} \quad (1)$$

where $n_{Mg^{2+}} + n_{Ca^{2+}}$ is the total concentration of Mg^{2+} and Ca^{2+} (g/L); V_{EDTA} is the volume of using EDTA solution (mL); C_{EDTA} is the concentration of EDTA solution (g/L); and V_s is the volume of analyzed sample (mL).

3. Calculations

The degree of dissociation of monoprotic acid was calculated as follows:

$$\alpha_1 = \frac{\left[A^-\right]}{\left[HA\right]\left[A^-\right]} = \frac{\left[A^-\right]}{C_{HA}} \quad (2)$$

where C_{HA} = [HA] + [A^-] is the total concentration of weak acid.

The fraction of the nondissociated acid was calculated as follows:

$$\alpha_0 = \frac{\left[HA\right]}{\left[HA\right]\left[A^-\right]} = \frac{\left[HA\right]}{C_{HA}} \quad (3)$$

and moreover the acid dissociation constant was calculated on the basis of the following equation:

$$K_a = \left[H^+\right]\frac{\alpha_1}{\alpha_0} \quad (4)$$

$$\log\frac{\alpha_1}{\alpha_0} = pH - pK_a \quad (5)$$

where K_a is the dissociation constant.

The average flux of the solution was calculated as follows:

$$J = \frac{V}{t \cdot A} \quad (6)$$

where J is the flux of the solution (L/h m^2); V is the volume of the permeate solution (L); t is the time (h); and A is the membrane area (m^2).

The rejection rate obtained after NF process was calculated as follows:

$$R = \left(1 - \frac{C_p}{C_f}\right) \times 100\% \quad (7)$$

where R is the rejection rate (%); C_p is the concentration of a component in a permeate solution (g/L); and C_f is the concentration of a component in a feed solution (g/L).

The desalination degree of succinic acid obtained during EDBM was calculated as follows:

$$\eta_{des} = \left(1 - \frac{C^t_{dil}}{C^0_{dil}}\right) \times 100\% \quad (8)$$

where η_{des} is the desalination degree (%); C^t_{dil} is the concentration of succinic acid in diluate chamber after time t (g/L); C^0_{dil} is the initial concentration of succinic acid in diluate chamber (g/L).

The average amount of energy consumed for 1 kg of succinic acid production was determined using the equation:

$$E_C = \frac{U \cdot I \cdot t}{m} \quad (9)$$

where E_C is the energy consumption needed to produce 1 kg of succinic acid (kWh/kg); U is the voltage (V); I is the current (A); m is the mass of the final product (kg); and t is the time (h).

The average value of the current efficiency was calculated on the basis of the following equation:

$$C_E = \frac{F \cdot z \cdot V \cdot \Delta C_{dil}}{n \cdot I \cdot \Delta t} \times 100\% \quad (10)$$

where C_E is the current efficiency (%); F is the Faraday's constant (96,485) (C/mol); I is the current (A); z is the valence of ions; V is the diluate volume (L); ΔC_{dil} is the change of succinates concentration in diluate chamber (mol/L); n is the number of cells; and Δt is the time (s).

4. Results and discussion

4.1. Integrated system

The integrated system we propose (Fig. 2) consists of six stages such as: (1) UF, (2) IE_I, (3) NF, (4) IE_{II} and (5) and (6) two steps of the bipolar membrane electrodialysis ($EDBM_I$) and ($EDBM_{II}$). It was designed for the recovery of succinic acid from the actual post-fermentation broth left after bioconversion of glycerol. At first, the UF process was performed as a pre-treatment step to remove the residues of biological materials from the actual post-fermentation broth. At the second stage, the clarified permeate solution obtained in UF was passed through the ion-exchange column filled with resin(I). In the next step the column effluent, deprived of a large part of magnesium(II) and calcium(II) ions was used as a feed in the NF process and then directed to the second IE column with resin(II). Finally, the two steps of bipolar membrane electrodialysis ($EDBM_I$) and ($EDBM_{II}$) were carried out.

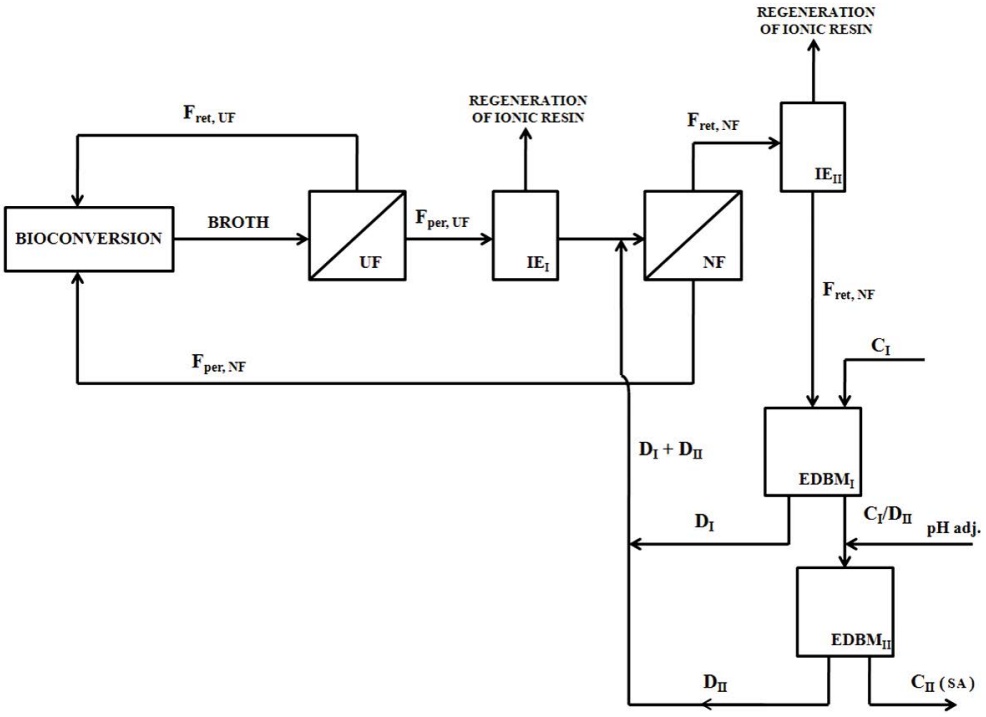

Fig. 2. The diagram of the multistage purification of succinic acid from actual post-fermentation broth after bioconversion of glycerol.

4.2. UF of the actual post-fermentation broth

The process of UF of the actual post-fermentation broth left after bioconversion of glycerol was performed in order to remove high molecular contaminants present in the feed solution such as: biomass, proteins, or cells. As a result of the UF process, the feed solution (10 L) was divided into permeate stream ($F_{per,UF}$) and retentate stream ($F_{ret,UF}$) with volumes equal to 6 and 4 L, respectively. It should be mentioned that before the UF of actual post-fermentation broth, the maximum efficiency of the used membrane (which was proportional to the applied pressure difference [25]) was determined using pure water. Changes in the flux of water and the actual post-fermentation broth during UF processes are illustrated in Fig. 3.

The results shown in Fig. 3 indicate that the maximum flux value, obtained for deionized water permeating through the clean membrane, was constant during the whole time of UF and equal to 830 L/h m^2. Furthermore, the permeate flux during UF process of the actual post-fermentation broth decreased rapidly from 271 to 114 L/h m^2, which was a consequence of the concentration polarization and fouling/biofouling phenomena. As widely known, the clarification of the actual post-fermentation broth is associated with deposition of contaminants, formation of a cake layer on the membrane surface as well as blocking of the membrane pores [26]. The observed decline of permeate flux leads to an increase in time and cost of the process and a decrease in its effectiveness. However, taking into account the results obtained in preliminary experiments as well as in many literature reports [23,27], the pre-treatment of the actual post-fermentation broth using UF process is implicitly necessary to perform the next steps in the integrated system.

4.3. Removal of multivalent ions by IE method

The permeate solution obtained after UF ($F_{per,UF}$) was directed into the IE_I in order to remove multivalent ions, especially magnesium(II) and calcium(II) ions. The inorganic salts (the residue of culture medium) present in the actual post-fermentation broth can be particularly dangerous for membranes used in the NF process as well as in the EDBM, as they lead to scaling phenomenon on the surface of the membranes [28,29]. During the EDBM process, as a result of pH change of the solutions in diluate and concentrate chambers, magnesium(II) and calcium(II) ions may precipitate due to the interaction with OH^- ions (produced from the water split by bipolar membrane or as

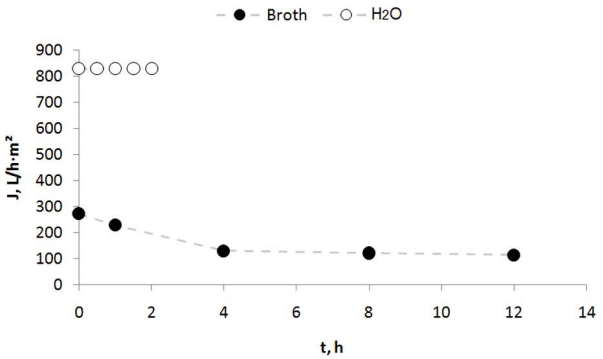

Fig. 3. Comparison of water and the permeate fluxes obtained during the UF of actual post-fermentation broth, TMP = 0.4 MPa, T = 25°C ± 2°C.

products of water reduction which occurs at the cathode) as follows [30]:

$$Mg^{2+}_{(aqueous)} + 2OH^{-}_{(aqueous)} \longleftrightarrow Mg(OH)_{2(solid)} \quad (11)$$

$$Ca^{2+}_{(aqueous)} + 2OH^{-}_{(aqueous)} \longleftrightarrow Ca(OH)_{2(solid)} \quad (12)$$

On the other hand, the residues of scaling ions present in a separated solution leaving the IE_I, might be rejected in the NF process, that is, the next step of the proposed multistage integrated system (Fig. 2), but it leads to decline in process efficiency and is an obstacle for purification of the final product. Therefore, we decided that, similarly as the permeate after UF, the retentate after NF process should be additionally treated in the IE_{II} process. The total concentration of magnesium(II) and calcium(II) ions determined in the solutions before and after the IE processes is shown in Table 2. On the basis of these results it can be concluded that approximately 90% of Mg^{2+} and Ca^{2+} ions were removed from UF permeate solution in IE_I process, that is, to the value of 1.7 g/L. Furthermore, the concentrated scaling ions present in the retentate solution obtained after NF were successfully removed in IE_{II} process so that their content decreased from 6.5 to 0.3 g/L, it means with the effectiveness equal to 95%. The removal of a significant amount of inorganic ions from the separated solutions is especially important as it allows for the effective reduction of the negative impact of the scaling phenomenon and improves the efficiency of succinic acid recovery in the next stages of its separation from the actual post-fermentation broth. Similarly, other authors have indicated on the need for the removal of magnesium(II) and calcium(II) ions present in streams directed to the module with bipolar membranes in integrated systems such as: reverse osmosis, monopolar ED, and EDBM [31].

4.4. NF process

The next objective of our study was to concentrate the succinic acid from the solution obtained after the IE_I and partial desalination by NF. As well known, the NF can be used as an effective method for both, separation and concentration of organic acid salts present in the actual post-fermentation broth as well as for partial removal of nondissociated compounds such as: glycerol, ethanol as well as lactose [32,33]. As a result of the NF process, the feed solution (6 L) was divided into the permeate stream ($F_{per,NF}$) and retentate stream ($F_{ret,NF}$) (enriched in the succinate ions) with volumes equal to 4.5 and 1.5 L, respectively. The pH of the feed solution was equal to 8.5. As it has been reported by other authors, the pH value of the feed solution in NF processes is very important for rejection of weak organic acids due to the electrostatic interactions between the charged ionic forms present in the basic medium and the charged membrane [32,34]. Fig. 4 shows the relation between the degree of dissociation of solution of succinic acid and pH determined on the basis of Eqs. (1)–(4). As one can see, the maximum degree of dissociation of succinic acid can be achieved in solutions of pH higher than 8. In addition, the analysis of changes in the zeta potential of the membrane surface shows that in the solutions of pH higher than 5 the surface of the membrane used has a negative charge [35]. Therefore, the maintenance of a constant pH value higher than 8 of the separated solution was justified. Table 3 shows the retention of components of the feed solution and their concentrations in the solutions after NF (i.e., permeate and retentate). As shown in Table 3, the obtained rejection of succinic acid was high and equal to 92%. Additionally, the rejection rates obtained for other carboxylic acids present in the separated solution were equal to –33.8%, –34%, and –34.2% for acetic, formic, and lactic acid, respectively. Thus, the concentration of other carboxylic acids in the retentate flux obtained after the NF process was below the limit of quantification. This observed effect results of the charges of acid molecules (−2 for succinate and −1 for other acids) and their sizes decrease in the order: succinate > lactate > acetate > formate. On the other hand, negative rejection determined for acetic, formic, and lactic acid could be explained by the pumping effect as reported by Kang and Chang [15]. They have indicated that the facilitated transport and negative rejection of monovalent anions can be achieved in the presence of divalent ions which can be strongly repulsed by the membrane surface simultaneously pushing the monovalent anions toward it. Similarly, a high rejection of succinic acid and the negative rejection of other monovalent acids observed for commercial NF membranes such as: NF270, NF-DK, and NF-DL have been described in Ref. [22].

Furthermore, as shown in Table 3, a high rejection of divalent calcium(II) and magnesium(II) ions present in the feed, which remained in the feed solution after IE_I, was also achieved. As reported in Ref. [34] the high rejection of calcium(II) and magnesium(II) ions can be explained by the size exclusion effect (high Stokes radii equal to 0.33 and 0.35 nm

Table 2
Total concentration of Mg^{2+} and Ca^{2+} ions in the solutions before and after the step of purification on the ion exchange I (IE_I) and the ion exchange II (IE_{II})

	Total concentration of Mg^{2+} and Ca^{2+} (g/L)	
	IE_I	IE_{II}
Before	12.4	6.5
After	1.7	0.3

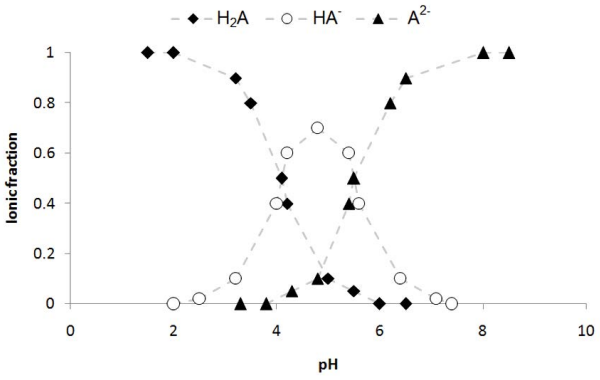

Fig. 4. Degree of succinic acid dissociation as a function of pH value.

Table 3
The retention of the feed components and their concentrations in nanofiltration streams, TMP = 1.6 MPa, pH = 8.5, T = 25°C ± 2°C

Component	$C_{feed\ NF}$ (g/L)	$C_{ret.\ NF}$ (g/L)	$C_{per.\ NF}$ (g/L)	R (%)
Succinic acid	11.4	43.0	0.9	92.0
Acetic acid	7.1	0	9.5	−33.8
Formic acid	4.7	0	6.3	−34.0
Lactic acid	4.1	0	5.5	−34.2
Glycerol	34.1	42	31.5	7.6
Lactose	16.1	26.0	12.7	20.5
Mg^{2+} and Ca^{2+}	1.7	6.5	0.1	94.1

for Ca^{2+} and Mg^{2+}, respectively), adsorption phenomena as well as the ability to create the divalent cation complexes with the negatively charged surface of the NF membrane. At the same time the rejection rates obtained for other components present in the separated solution, such as: glycerol and lactose, were equal to 7.6% and 20.5%, respectively. As well known, the transport of neutral components in the nondissociated form present in the feed solution is a consequence of the sieving effect (size-based exclusion) according to which the molecules with sizes larger than the pore size are rejected by the membrane [15,33,36]. Obviously, in this case the rejection of glycerol is much lower than the rejection of lactose which is related to the Stokes radii of their molecules, equal to 0.19 and 0.471 nm, respectively [37,38]. In the consequence, the NF allowed for obtaining a concentrated solution of the composition is shown in Table 3, which was purified in the next stages. However, due to the presence of a certain amount of calcium(II) and magnesium(II) ions, the retentate solution obtained after NF, before the process of EDBM, was subjected to IE_{II} (see Section 4.3).

Fig. 5 shows changes in the permeate flux during the NF process. It could be noted that the permeate flux rapidly declined during the process, which was typical for the separation of organic acids from the actual post-fermentation broth [23]. The decline in the permeate flux during NF, similarly as in the UF process, was the consequence of the fouling phenomenon. In this case, the main reason for the fouling layer formation was the presence of positively

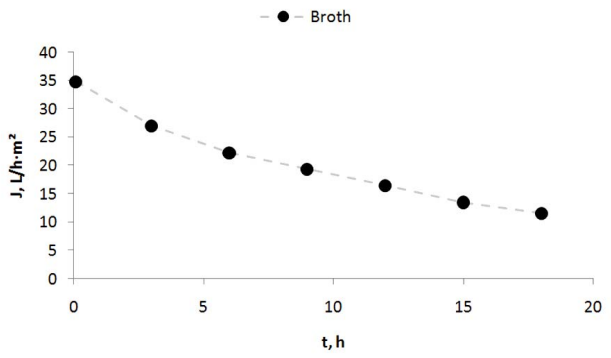

Fig. 5. Changes in the permeate flux during NF process, TMP = 1.6 MPa, T = 25°C ± 2°C.

charged cations, which could be more readily adsorbed on the membrane surface than the divalent and monovalent anions. As reported in Ref. [39] during the NF of humic acid, the magnesium(II) and calcium(II) ions can act as a bridge between the negatively charged acid molecules as well as their negatively charged carboxyl groups and the membrane surface. Additionally, the NF efficiency could be limited by the polarization concentration phenomena and by the increase in the osmotic pressure difference at a higher solute concentration [40–42]. Moreover, as described in our previous work [24], the efficiency of the process can also be influenced by such factors as: temperature, filtration geometry, operating pressure as well as membrane characterization (i.e., surface roughness, zeta potential, and hydrophobicity).

4.5. Two-step EDBM of NF retentate

The final step of the proposed multistage integrated system is the separation and concentration of succinic acid from the actual post-fermentation broth after pre-treatment (UF, IE_I, NF, and IE_{II}) by a two-stage EDBM. Many literature reports have pointed the possibility of using EDBM for separation and production of concentrated organic acids from their aqueous solutions [19,43–45]. It is important that the environment-friendly EDBM in the proposed multistage integrated system allows for the elimination of the acidification step, which can generate considerable amount of wastes [46]. Additionally, the use of a cell configuration of AM–BM enables separation of succinates from nonionic compounds present in the separated solution. In the first stage of bipolar membrane electrodialysis ($EDBM_I$) 1.5 L of retentate after NF ($F_{ret,NF}$) was used as a diluate solution. In the second step of bipolar membrane electrodialysis ($EDBM_{II}$) the concentrate solution obtained in $EDBM_I$ (after adjusting its pH to 8.5 (C_I/D_{II})) was used as a diluate solution. Each time the concentrate chamber contained succinates solutions in the concentration of 1.6 g/L. All EDBM processes were performed at a constant current density 120 A/m² in 180 min. The compositions of solutions before and after $EDBM_I$ and $EDBM_{II}$ are shown in Table 4. It can be seen that after 180 min of the $EDBM_I$ process the concentration of succinic acid reached 24.2 g/L. The achieved degree of desalination, the current efficiency, and the energy consumption obtained after 180 min of $EDBM_I$ process were equal to 56%, 22%, and 3.87 kWh/kg, respectively. However, the diffusive transport of glycerol and lactose was also observed, which was a consequence of their high concentration in the separated solution. Therefore, the additional second step of EDBM was carried out in order to obtain a higher purity of the final product. As shown in Table 4, the second stage of EDBM allowed for obtaining succinic acid at a concentration of

Table 4
Composition of solutions before and after $EDBM_I$ and $EDBM_{II}$ processes

Component	$F_{ret,NF}$ (g/L)	C_I/D_{II} (g/L)	C_{II} (g/L)
Succinic acid	43	24.2	18.3
Glycerol	42	4.6	0.3
Lactose	26	1.6	0

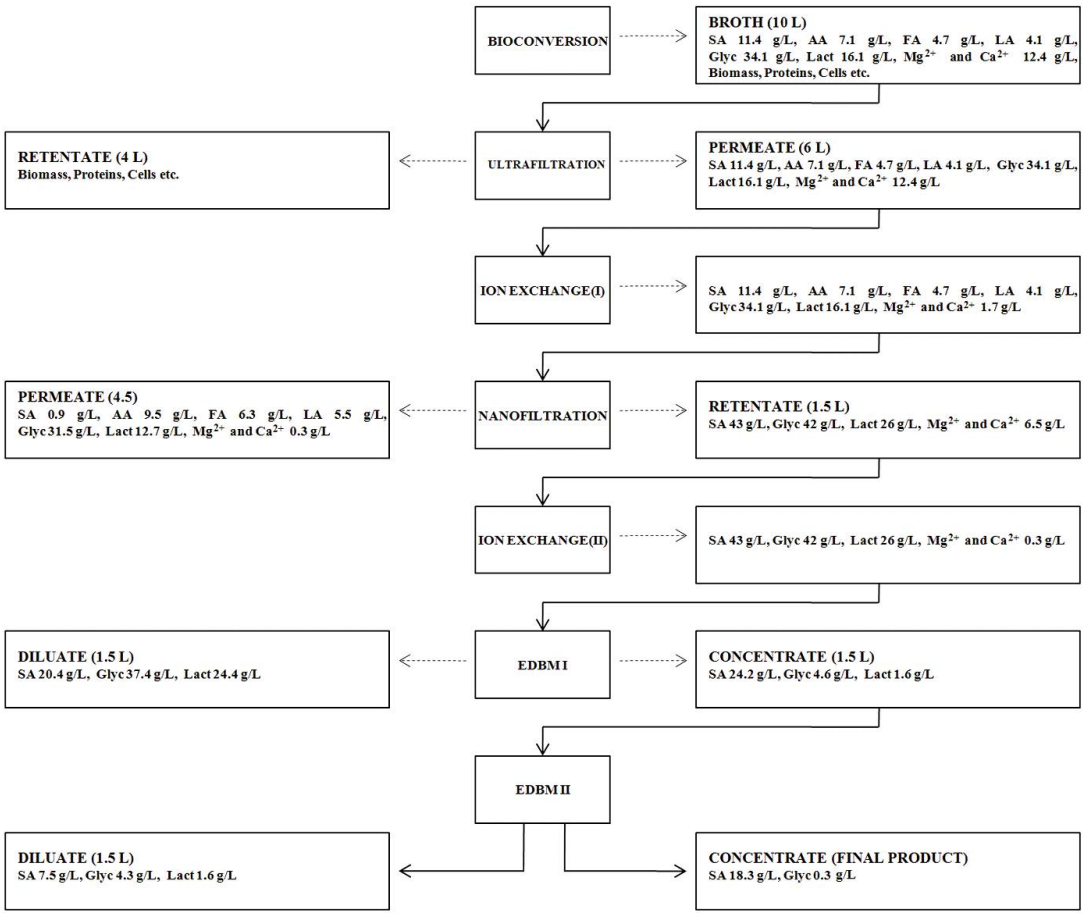

Fig. 6. The block diagram presenting the steps of the proposed integrated system including streams characterization.

18.3 g/L with a high degree of desalination equal to 78%, while the concentration of glycerol in the succinic acid concentrated stream was minimal and the lactose was not at all present.

The detailed description of each step of the multistage integrated system proposed for separation and concentration of succinic acid from the actual post-fermentation broth left after bioconversion of glycerol (after pre-treatment) is given in the block diagram presented in Fig. 6. As one can see, the proposed system is completely waste-free and with no need of using any auxiliary components. Thus, it meets the assumptions of environment-friendly technologies.

5. Conclusions

The novel and environment-friendly six-step integrated system was used for separation and concentration of succinic acid from the actual post-fermentation broth left after bioconversion of glycerol. Preclarification process carried out by UF allowed for the removal of high molecular contaminants present in the feed solution, such as: biomass, proteins as well as cells. Subsequently, the effective removal of Mg^{2+} and Ca^{2+} salts was obtained in the IE process. NF was efficiently employed for concentration of succinates (92% of rejection) and partial removal of other compounds, such as: monocarboxylic acids, glycerol, and lactose. The final two steps of EDBM were allowed for obtaining a high degree of desalination and a high purity of succinic acid. The final concentrate obtained after the second stage of the EDBM process contained over 18 g/L of succinic acid contaminated only with a small amount of glycerol (0.3 g/L). Moreover, the application of the EDBM was allowed for the avoidance of an acidification of the pre-treated broth, which usually generated considerable amount of wastes. Finally, it can be concluded that the proposed concept can be the alternative for bio-succinic acid downstream processing. Moreover, it should be emphasized that the proposed system is completely waste-free, because the solutions (NaOH and HNO_3) obtained after washing the modules as well as the waste from the regeneration process of the IE resin can be recirculated to the bioconversion process. Thus, the proposed six-step membrane-based process of recovery of succinic acid, as a completely waste-free and deprived of the need of using any auxiliary component, fully satisfies the assumptions of environment-friendly technologies.

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Symbols

Stages

EDBM	—	Bipolar membrane electrodialysis
$EDBM_I$	—	First stage of bipolar membrane electrodialysis
$EDBM_{II}$	—	Second stage of bipolar membrane electrodialysis
IE_I	—	Ion exchange(I)
IE_{II}	—	Ion exchange(II)
NF	—	Nanofiltration
UF	—	Ultrafiltration

Materials

AA	—	Acetic acid
FA	—	Formic acid
Glyc	—	Glycerol
LA	—	Lactic acid
Lact	—	Lactose
SA	—	Succinic acid

Streams

C_I	—	Concentrate solution before $EDBM_I$
C_{II}	—	Concentrate solution obtained after $EDBM_{II}$
C_I/D_{II}	—	Concentrate solution obtained after $EDBM_I$/diluate solution before $EDBM_{II}$
D_I	—	Diluate solution obtained after $EDBM_I$
D_{II}	—	Diluate solution obtained after $EDBM_{II}$
$D_I + D_{II}$	—	Combine stream of diluate solutions
$F_{per,NF}$	—	Stream of nanofiltration permeate
$F_{ret,NF}$	—	Stream of nanofiltration retentate
$F_{per,UF}$	—	Stream of ultrafiltration permeate
$F_{ret,UF}$	—	Stream of ultrafiltration retentate

Symbols

A	—	Membrane separation area, m^2
α	—	Degree of dissociation of monoprotic acid
C^t_{dil}	—	Concentration of succinic acid in diluate chamber after time t, g/L
C^0_{dil}	—	Initial concentration of succinic acid in diluate chamber, g/L
ΔC_{dil}	—	Change of succinates concentration in diluate chamber, mol/L
C_p	—	Concentration of component in permeate solution, g/L
C_f	—	Concentration of component in feed solution, g/L
C_{EDTA}	—	Concentration of EDTA solution, g/L
$C_{feed\ NF}$	—	Concentration of compounds in feed solution before NF, g/L
$C_{per.\ NF}$	—	Concentration of compounds in permeate solution after NF, g/L
$C_{ret.\ NF}$	—	Concentration of compounds in retentate solution after NF, g/L
C_E	—	Current efficiency, %
E_C	—	Energy consumption needed to produce 1 kg of succinic acid, kWh/kg
F	—	Faraday's constant (96,485), C/mol
I	—	Current, A
J	—	Flux of a solution, L/h m^2
K_a	—	Dissociation constant
m	—	Mass of the final product, g
n	—	Number of cells
η_{des}	—	Desalination degree, %
$n_{Mg^{2+}} + n_{Ca^{2+}}$	—	Total concentration of Mg^{2+} and Ca^{2+},g/L
R	—	Rejection rate, %
t	—	Time, h
Δt	—	Time, s
T	—	Temperature, ºC
TMP	—	Transmembrane pressure, MPa
U	—	Voltage drop on the EDBM stack, V
V	—	Volume of permeate solution, L
V_{EDTA}	—	Volume of EDTA solution used for titration, mL
V_s	—	Volume of analyzed samples, mL
z	—	Valence of ions

Membranes

AM	—	Anion exchange membrane
CM	—	Cation exchange membrane
BP	—	Bipolar membrane

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