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Separation of carboxylic acids from post-fermentation broth obtained in bioconversion of waste raw materials using multi-stage membrane systems. A mini review

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ABSTRACT

Biotechnology offers attractive options for syntheses of new chemicals because of mild conditions of the processes and the possibility of using a wide range of relatively inexpensive, renewable materials often waste products. However, the major limitation of bioprocesses are the problems associated with the separation of the main bioconversion product, for example, carboxylic acids, from a very complex fermentation broth left after biosynthesis. The paper presents a brief review of literature data and the results of our study on the use of integrated membrane-based systems, mainly utilizing pressure and current membrane techniques, for purification and concentration of carboxylic acids (fumaric, succinic, lactic, and gluconic) from the multi-component broth which always remains after bioconversion of waste biomass (as a carbon source).

Keywords: Bioconversion; Fermentation broth; Carboxylic acids; Membrane separation; Ceramic membranes

1. Introduction

In the era of striving for sustainable development, protection of the natural environment is one of the most important international policy priorities, especially in developed countries. The problem of progressive environment degradation is caused primarily by large amounts of man-made waste. Both municipal and industrial waste is usually very difficult to dispose of or manage, not to mention the possibility of its economic use. In recent years, there has been a clear increase in the interest in the possibility of transformation of waste materials in biotechnological methods, which include the group of processes called white biotechnology. On the other hand, production based on petroleum products is increasingly replaced by bioprocesses [1–3].

The essence of these processes, often referred to as the industrial production of the future, is the use of microorganisms, such as bacteria, mold fungi, yeast, and the enzymes they produce, for the effective conversion of industrial waste, by-products, or various types of post-production residues from, for example, food industry, to the desired chemicals, materials and medicines [4–6]. This approach is of great interest because industrial processes based on biotechnology are both more environmentally friendly, safe, and less costly than traditional methods of chemical synthesis [7]. Biological processes are energy-efficient and usually run at low temperatures, which reduces fossil fuel consumption and carbon dioxide emissions. However, it should be remembered that the final product of any biosynthesis process is a multi-component mixture [8,9]. The composition of the post-cultivation mixture is always very complex, because of the metabolic characteristics of microorganisms, and a number of by-products very often with physicochemical properties similar to those of the main product, are formed [10]. All post-fermentation

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mixtures contain, in addition to the main bioconversion metabolites: the following four groups of components: biomass, unreacted substrates, inorganic salts (introduced into the process as components of the culture medium for microorganisms), and by-products formed during fermentation [11]. Therefore, the possibility of industrial implementation of biotechnological production of desired organic substances (e.g., carboxylic acids) from renewable raw materials (such as glycerol, molasses, starch, or vegetable oils) requires the development of an effective method of isolating the main biosynthesis product from the resulting broth [12]. It should be emphasized that the separation of organic acids obtained in the course of the processes catalyzed by means of microorganisms is a multi-stage and complex process, usually requiring individual solutions for a given broth. For this reason, the costs of separating bio-products are usually very high and determine the economic efficiency of the entire technology [13]. Another important factor determining the costs of separation of bio-products is their content in the post-cultivation mixture. It is assumed that when the product content in the mixture is above 30 kg/m^3, the separation costs represent only about 20% of the overall costs of the process. If the content of the bio-product is of the order of a few grams per liter, then the separation costs are above 50%, while when the product concentration drops below 1%, these costs increase up to 90% [14].

Separating the desired metabolite from the multi-component fermentation broth requires not only large expenditures but also the use of multi-stage separation systems [15]. Economic considerations play an important role in the selection of techniques for separating and concentrating the components of broth, although ecological aspects are equally or even more important. Quite so for ecological and pro-social reasons, the traditional separation techniques, that is, precipitation, crystallization, distillation, ion exchange, or adsorption, are not very attractive because they usually require the consumption of large amounts of organic solvents, and additionally, generate onerous waste as well as consume significant amounts of energy [16]. High expectations are associated with the possibility of using membrane separation techniques, commonly recognized as processes almost completely free of waste and not requiring the use of any additional chemical components. The membrane processes fully meet the requirements of environmentally friendly technologies [15].

This paper presents several examples of multi-stage membrane separation systems for the separation and concentration of low-molecular bio-based carboxylic acids (lactic, fumaric, succinic, and gluconic) from the post-fermentation broths obtained in the fermentation processes of waste of renewable raw materials. In this mini-review, we also present the results of our research in this field.

2. Production of bio-based carboxylic acid

Low molecular weight carboxylic acids are a large group of valuable metabolites obtained through the biotechnological conversion of biomass. In view of the wide application possibilities in the chemical, food, pharmaceutical, and agricultural industries, the production of bio-based carboxylic acids is nowadays the subject of interest of many research groups around the world. Table 1 summarizes several examples of carboxylic acid production, obtained in the waste bioconversion processes of biomass.

2.1. Lactic acid

Lactic acid (LA) is a typical example of monocarboxylic acid produced in the bioconversion process, which is used in food, pharmaceutical, and agricultural industry [32]. In addition, LA can be used to synthesize a bio-polymer, that is, poly(lactic acid) (PLA) [33]. It is known that LA occurs in the form of two optical isomers: L-lactic (L-LA) (Fig. 1a) and D-lactic (D-LA) (Fig. 1b) acid.

Of key importance is that the use of microbiological production methods allows utilization of waste fractions and obtaining optically pure form L- or D-LA, in contrast to the standard synthetic method of LA production in which the main product is a racemic mixture of DL-LA [33]. It is worth emphasizing that the global market of LA production is mainly based on the fermentation process (over than 90%) [34]. As reported in literature, many species of bacteria and yeasts such as *Bacillus coagulans*, *Bacillus stearothermophilus*, *Escherichia coli*, *Lactobacillus plantarum*, *Saccharomyces*, *Candida*, and others are capable of biosynthesizing LA, using different waste carbon sources, with high efficiency [35–37]. For example, Tang et al. [38] have presented an effective method of fermentation of food waste with indigenous microbiota. The cited authors indicate that in the used process conditions, that is, T = 37°C and pH 6 it is possible to obtain LA with a quite good yield, equal to 0.46 g/g-TS and productivity (278.1 mg/L h). Similarly, as reported by Ahring et al. [39] *B. coagulans* (strain AD) can be used for the continuous fermentation of clarified corn stover hydrolysate. Moreover, the above-cited authors indicated that under optimal conditions, the LA could be produced with a maximum productivity of 3.69 g/L/h and yield of 0.95 g/g biomass sugars. Karp et al. [40] have also proposed the bioconversion of soybean vinasse as the main substrate to L-LA using *Lactobacillus agilis* LPB 56. L-LA can also be produced from glycerol, which is a by-product in the biodiesel production process, as reported in [41–43]. Many literature reports indicate that membrane-based processes such as ultrafiltration (UF), nanofiltration (NF), electrodialysis (ED), and electrodialysis with the bipolar membrane (EDBM) can be effectively used in the processes of LA recovery from fermentation broth [44–47]. However, in order to limit the fouling phenomenon and to obtain LA of high quality, it is necessary to use several separation techniques combined in the integrated system. Mai et al. [17] developed an effective method of separation of D-LA from cassava starch fermentation. In the first stage, the pre-treatment of the actual post-fermentation broth using spiral-wound microfiltration (0.1 μm, Synder's Filtration, USA) and nanofiltration DK4040F1021 (GE Osmonics, DK series, USA) was carried out. The cited authors indicate that the applied pressure-driven membrane processes allow the removal of bacterial cells, protein, and color compounds. Furthermore, in the NF process, it is possible to observe high retention of multivalent inorganic ions based on two mechanisms of exclusion (size exclusion and electrostatic interactions). Then, the influence of pervaporation

Table 1
Downstream processes based on membrane technology of organic acid separation from fermentation broth

Carbon sources	Main product	Main purification steps	Main results	Reference
Cassava starch	D-lactic acid	Microfiltration – nanofiltration – pervaporation assisted esterification	High optical purity for poly(D-lactide) production	[17]
Coffee residues (mucilage)	L-lactic acid	Filtration – electrodialysis – ion exchange chromatography – distillation	930 g/L L-lactic acid with 99.8% optical purity	[18]
Not shown	Lactic acid	Ultrafiltration – nanofiltration – ion exchange – vacuum assisted evaporation	Lactic acid with purity >99.5%	[19]
Not shown	Lactic acid	Ultrafiltration – nanofiltration – bipolar membrane electrodialysis	Conversion of 95% of sodium lactate to lactic acid with NaOH recovery	[20]
Food waste	Lactic acid	Centrifugation – nanofiltration – bipolar membrane electrodialysis	High purity lactic acid with removal of 90% of mineral ions and 84% of glucose	[21]
Saccharose	Lactic acid	Microfiltration – chromatography-monopolar electrodialysis – bipolar membrane electrodialysis	Complete separation of lactic acid and salt-ions with yield of LA more than 90%	[22]
Sweet sorghum juice	L-Lactic acid	Filtration (coarse and ultra-) – monopolar and bipolar membrane electrodialysis – vacuum distillation	High concentrated lactic acid solution (905.8 g/L) with optical purity of 98.9%	[23]
Municipal solid waste (OFMSW)	L-Lactic acid	Microfiltration – nanofiltration – softening – monopolar electrodialysis – bipolar electrodialysis – decolorization – anion exchange – cation exchange – distillation	L-LA with high purity 98.7% and recovery rate 51.5%	[24]
Carob pod extract	Succinic acid	Electrodialysis – nanofiltration – Donnan dialysis	High retention of succinates (above 90%)	[25]
Glucose	Succinic acid	Nanofiltration – vapor permeation assisted esterification	98% recovery yield of SA with 80% protein removal after NF step	[26]
Glucose	Succinic acid	Microfiltration – nanofiltration assisted with crystallization	High purity succinic acid crystal (99.18%)	[27]
Raw glycerol	Succinic acid	Ultrafiltration – bipolar membrane electrodialysis – reactive extraction	Removed of succinic acid with more than 90% efficiency after three-step reactive extraction	[28]
Raw glycerol	Succinic acid	Ultrafiltration – nanofiltration – bipolar membrane electrodialysis	High purity succinic acid solution (18 g/L) with small amount of glycerol (0.3 g/L)	[15]
Raw glycerol	Fumaric acid	Nanofiltration – bipolar membrane electrodialysis	53 % of desalination of fumarate and conversion of them to fumaric acid form	[29]
Raw glycerol	Fumaric acid	Nanofiltration – bipolar membrane electrodialysis – reactive extraction	High concentration of fumarate 80%–98% yield, 90% efficiency after three-step reactive extraction	[30]
Hydrolyzed sugarcane juice	Gluconic acid	Microfiltration – two stage nanofiltration	High concentrated gluconic acid up to 540 g/L	[31]

dehydration on the esterification reaction between D-LA and ethanol was investigated. In this case, the use of the hydrophilic membrane increases the efficiency of esterification (93% of LA conversion) due to higher water removal (95%) from the mixture. Finally, high purity D-LA can be obtained after distillation and hydrolysis with deionized water. In the study of Neu et al. [18] the mucilage, a residue from coffee production was used as the main carbon source in the process of fermentation to give L-LA. These authors proposed the membrane separation process in which 45 L of crude fermentation broth with a concentration of LA (sodium lactate form) equal to 43.4 g/L was used. The application of membrane technology was motivated by high environmental impact and the operating costs of classical methods of precipitation, which generate a large amount of waste. In the membrane process, the following main stages were carried out: (i) microfiltration (removal of bacteria cells), (ii) nanofiltration (removal of remaining

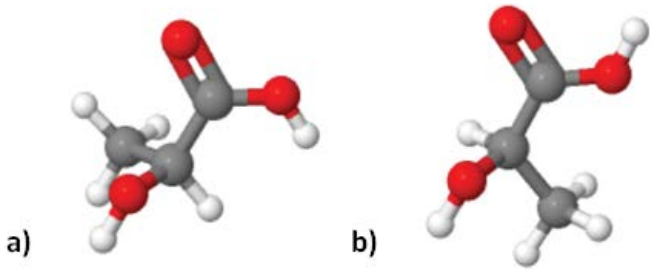

Fig. 1. Chemical structure of L-LA (a) and D-LA (b) acid.

sugars and proteins), and (iii) mono- and bipolar electrodialysis (separation of the salt ions and conversion of sodium lactate salt to the acid form). In addition, as the final purification of acid stream, decolorization, cation-exchange chromatography, and vacuum evaporation were applied. In consequence, 0.8 L of a LA solution in a concentration equal to 930 g/L of high optical purity (99.8%) was obtained. Lee et al. [19] have also proposed the separation of high purity LA (>99.5%) from fermentation broth using an integrated membrane system. Pressure-driven membrane processes such as ultrafiltration and nanofiltration as well as ion-exchange (IEX) and vacuum-assisted evaporation were used in this study. A general scheme of the integrated membrane system proposed by Lee et al. [19] is presented in Fig. 2.

Wang et al. [20] have also reported an integrated membrane system in which the pressure-driven (UF, NF) and electrically-driven techniques (EDBM) were combined. These authors claim that the use of a ceramic membrane with a pore size of 50 nm allows reaching a high flux (192 L/m^2 h) and simultaneously high removal of cells (99.3%). Moreover, the high retention of multivalent ions, for example, Ca^{2+} (87.7%) and Mg^{2+} (95%) and proteins (98.95%) can be achieved in the NF process under operating transmembrane pressure (TMP) 2.0 MPa. Subsequently, the electrodialysis with a bipolar membrane using two-compartment EDBM stack with the membrane configuration of bipolar membrane (BPM) – anion exchange membrane (AEM) – bipolar membrane (BPM) was investigated. Wang et al. have also suggested that the use of EDBM process under constant current density equal to 400 A/m^2 enables conversion of sodium lactate (95%) to LA with simultaneous recovery of sodium hydroxide and the energy consumption equal to 1.05 kWh/kg. A two-step separation procedure: nanofiltration – bipolar membrane electrodialysis has been used by Kim et al. [21] for separation of LA from food waste fermentation broth after centrifugation (removal of solid materials). Next, the nanofiltration under TMP in the range 50–300 psi (0.35–2.01 MPa) was investigated. The increase in retention of lactate (0.16–0.28) and Na^+ (0.13–0.33), K^+ (0.13–0.35), Mg^{2+} (0.67–0.83), and Ca^{2+} (0.33–0.83) with increasing TMP was observed. Moreover, electrodialysis with a bipolar membrane was found to remove 90% of monovalent ions and in this process the conversion of lactate to LA form without the loss of acid took place. The mono- and bipolar membrane electrodialysis were used in the system developed by Pleissner et al. [22]. The monopolar electrodialysis was used to remove sodium hydroxide and hydrochloric acid from pre-treated fermentation broth (microfiltration and ion chromatography). In this step, two individual fractions, that is, high-concentrated salt solution and low-concentrated diluate solution were obtained, respectively. On the other hand, electrodialysis with a bipolar membrane can be used for simultaneous separation of cations and anions and their conversion to NaOH 1.1 mol/L and HCl 0.8 mol/L, respectively. It is also important that the solutions of the base and acid obtained in the EDBM process can be used in fermentation as well as in pre-purification processes. Olszewska-Widdrat et al. [23] have described recent studies on the bio-production of L-LA and the recovery of this metabolite from the fermentation broth. In the first part of this study, these authors proposed sweet sorghum juice as the main feedstock in L-LA production while indicating its wide application in bio-production processes of bioethanol, two-stage

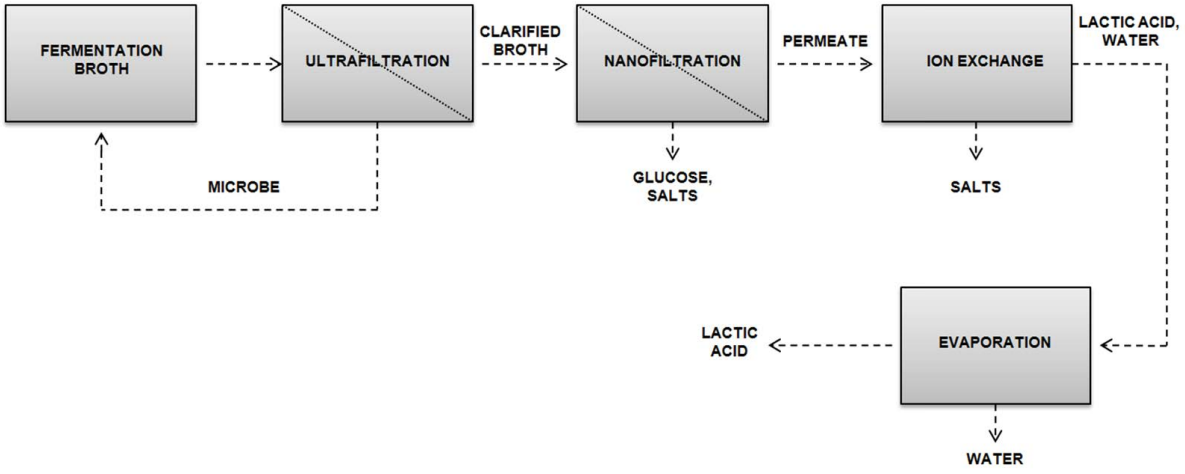

Fig. 2. General scheme of integrated membrane system used in the recovery of LA from fermentation broth (ultrafiltration – nanofiltration – ion exchange – evaporation) based on Lee et al. [19].

ethanol-methane as well as bio-butanol. The experimental results of fermentation process with thermophilic *B. coagulans* indicate that in both, laboratory and pilot-scale process (50 L) it is possible to obtain LA in high concentration, with high yield and productivity (78.75 g/L, 0.78 g/g, and 1.77 g/L h, in lab-scale) (73 g/L, 0.70 g/g, and 1.47 g/L h, in pilot-scale). Next, the environmentally friendly downstream process of separation and purification was carried out. The LA separation process proposed consisted of unit processes such as coarse ultrafiltration (removal of bigger particles and biomass), monopolar electrodialysis (concentration of sodium lactate), and electrodialysis with bipolar membrane (conversion of lactate to acidic form). Besides, in order to reduce the fouling of IEX membranes in ED processes, as well as to obtain a product of high purity, the cited authors also used assistant processes including softening (removal of Ca^{2+} and Mg^{2+}), cation and anion exchange, decolorization, and vacuum distillation. The separation and purification procedures allowed obtaining highly concentrated LA solution (905 g/L) with an optical purity of 98.9%. Besides, López-Gónzalez et al. [24] have demonstrated the method of production of LA of high enantiomeric purity from municipal solid waste (OFMSW). These authors indicate that the high optical purity of LA (the number of impurities should not exceed 0.05 mol %) is necessary for the production of PLA. That is why the proposed method is multi-stage and consists of such processes as microfiltration – nanofiltration – softening – monopolar electrodialysis – bipolar electrodialysis – decolorization – anion exchange – cation exchange (CEM) – distillation. In their study, the high purity L-LA (98.7%) with a simultaneous recovery rate equal to 51.5% was obtained. It should be noted that the proposed membrane-based method for recovery of LA is an eco-friendly alternative to the two-step process based on $Ca(OH)_2$ precipitation and H_2SO_4 acidification.

2.2. Succinic acid

Succinic acid (Fig. 3) (SA) is a dicarboxylic acid of the general formula $C_4H_6O_4$, which is widely used in chemical industry, mainly in production of biodegradable polymers, polyester resins, in medicine for the treatment of insulin-dependent diabetes, in pharmaceutical and cosmetic industries and as agricultural additive [48,49]. Besides, SA can be used as a precursor of many useful chemicals, for example, adipic acid, 1,4-butanediol, tetrahydrofuran, and others [50–52]. Therefore, SA is one of the twelve most essential bio-products obtained in the fermentation process. Not without significance is the fact that SA can be used in the production of biodegradable polymers, including poly(butylene succinate) (PBS) and poly(ethylene succinate) (PES) [52–54]. At present, most of the commercially available SA is produced by the catalytic hydrogenation of maleic anhydride, which is a result of the conversion of butane by its oxidation using catalysts (vanadium and phosphorus oxides) [55]. Admittedly, SA production using substrates of petrochemical origin is much cheaper than via biotechnological methods. Nevertheless, the gas used for its production is a non-renewable resource, and its further operation generates an increase in the costs of the entire process [56].

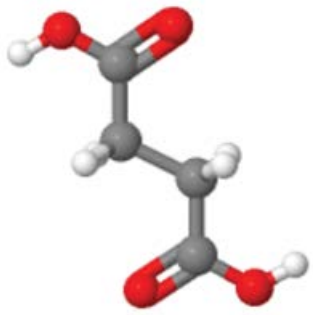

Fig. 3. Chemical structure of SA.

Many literature reports point to the possibility of managing waste, including molasses and waste glycerol fraction, which constitute a carbon source for many microorganisms, such as *Actinobaccilus succinogenes*, *Mannheimia succiniciproducens*, and *Anaerobiospirillum succiniproducens* as well as Recombinant *E. coli* [56–59]. Thuy et al. [60] have used *Actinobacillus succinogenes* ATCC55618 in the fermentation process of fresh cassava root as the ideal (low and high starch content) carbon source. Under optimal process conditions, high concentration of SA 151 g/L with yield and productivity of 1.51 g SA/g glucose and 3.22 g/L/h can be achieved by this method. On the other hand, bio-production of SA with crude and purified glycerol (a by-product of transesterification of *Crotalaria juncea* oil) using *E. coli*

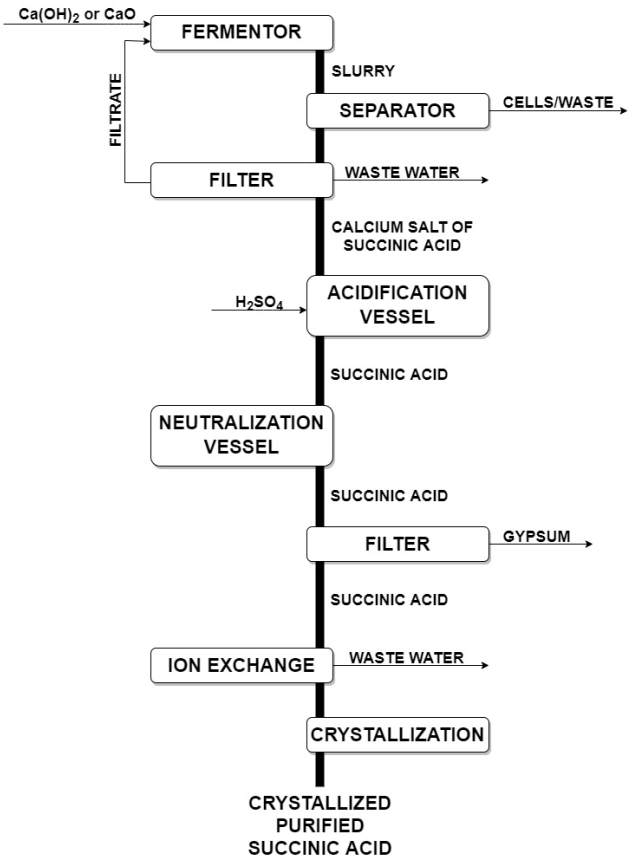

Fig. 4. Pathway of recovery of SA from fermentation broth using classical separation methods [51].

(ATCC 8739) has been reported by Sadhukhan et al. [61]. Gao et al. have also used crude glycerol (by-product in biodiesel industry) without a pre-treatment procedure. In this case, the use of engineered yeast *Yarrowia lipolytica* PGC01003 allowed obtaining SA in concentration of 43 g/L, while pointing out the possibility of getting a high concentration of SA (160 g/L) using the fed-batch fermentation strategy. Moreover, the possibility of sufficient production of SA from low-cost sucrose and sugarcane molasses was presented in a study described in Gao et al. [62]. The highest concentration equal to 62 g/L was obtained in a simple-batch fermentation using metabolically engineered *E. coli* KJ122-pKJSUC-24T.

The composition of the fermentation broth, the initial concentration of SA, physicochemical properties as well as the form of occurrence (calcium succinate, ammonium succinate, and sodium succinate) determine the strategy of its recovery. The classical industrial methods of separation of SA from fermentation broth based on precipitation with calcium hydroxide or oxide usually consist of many operation steps (Fig. 4) [51]. Moreover, separation strategy based on precipitation has several disadvantages, such as the necessity of using mineral acids (acidification stage) as well as the formation of a large amount of solid waste (calcium sulfates).

Sosa et al. [25] have investigated the membrane-based strategy of separation of SA from carob fermentation broth. The environmental friendly electrodialysis unit equipped with classical ED configuration CEM–AEM was used in the first step of separation in order to concentrate and partly desalinate fermentation broth. In the second stage, the concentrated solution obtained after the ED process, enriched with succinate ions, was concentrated in the NF process using commercial nanofiltration membranes such as NF270, NF-DK, and NF-DL. In all NF processes carried out using the selected membranes, high retention of succinates (above 90%) was observed. The last stage of the proposed integrated system included the Donnan dialysis process carried out to convert the concentrated succinate salt to the acid form. The integrated system consisting of ceramic nanofiltration and vapor permeation (VP) was applied and investigated by Lubsungneon et al. [26]. Firstly, the crude fermentation broth obtained using *A. succinogenes* ATCC 55618 was pre-purified by centrifugation and microfiltration. Then, the permeate after MF was used in the NF process with a mono-channel ceramic membrane (450 Da) in order to remove proteins, color components, and macromolecular contaminants larger than molecular weight cut-off (MWCO). Finally, VP-assisted esterification of fermentation broth (after NF step) and ethanol was carried out to increase the productivity of diethyl succinate. Moreover, the cited authors indicate that the high purity SA can be obtained after distillation and hydrolysis with deionized water. In turn, Thuy and Boontawan [27] have proposed a process of separation of high-purity SA from the fermentation broth in a multi-stage system: microfiltration–nanofiltration supported by the crystallization process. In the first stage, the actual post-fermentation broth (formed in the microbiological process of glucose conversion using *A. succinogenes* ATCC 55618) was subjected to a cross-flow MF purification operation (with simultaneous analysis of the fouling mechanism of the MF membranes used) in order to remove bacterial cell debris. During NF with spiral-wound membrane (GE, USA) processed proteins (95.3%), macromolecules, and especially multivalent ions (SO_4^{2-}, Mg^{2+}, and PO_4^{3-}) were removed. Besides, the authors conducted a dia-nanofiltration (DNF) process to recover the SA present in the concentrated

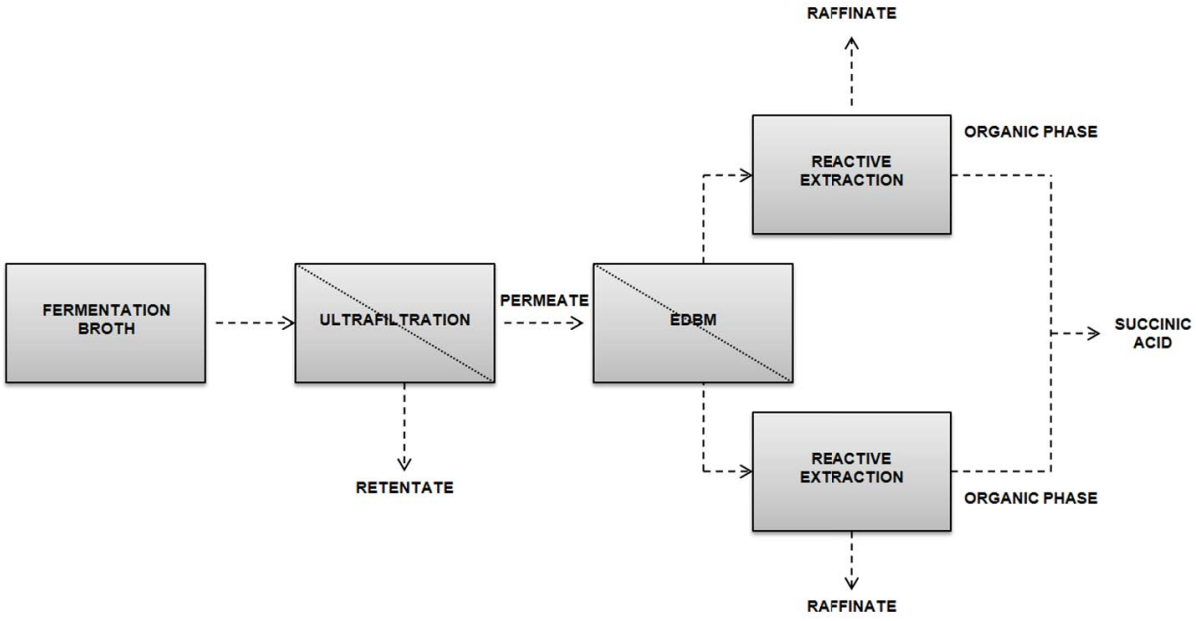

Fig. 5. General scheme of an integrated membrane system used in the recovery of SA from fermentation broth (ultrafiltration – EDBM – solvent extraction) based on Prochaska et al. [28].

solution. It has been shown that the degree of protein and magnesium sulfate removal using the NF process was high and equal to 96% and 98%, respectively. The bio-based SA with very high purity (99.18%) was successfully obtained after crystallization to solid form. In 2018, we proposed a three-stage separation system to be used in the process of SA removal from fermentation broth left after bioconversion of raw glycerol [28]. In this study, membrane techniques (ultrafiltration and EDBM) were supported with reactive extraction (RE) with commercial solvating extractants (TOA and Cyanex 923), as shown in Fig. 5. On the one hand, membrane processes allow pre-clarification (UF) and electro-acidification (EDBM) of fermentation broth. On the other, the removal of more than 90% of SA permitted the use of three-step reactive extraction. Moreover, another part of our research has shown that the SA obtained from bioconversion of waste glycerol can also be recovered using a 6-step integrated system consisting of ultrafiltration – ion-exchange I (IE) – nanofiltration – ion-exchange II (IE) and two-step EDBM as shown in Fig. 6 [15].

2.3. Fumaric and gluconic acid

Fumaric acid (FA) (Fig. 7) also known as 2-butenedioic acid and trans-butenedioic acid is a four-carbon dicarboxylic acid participating in the tricarboxylic acid cycle (TCA) [63]. Due to its chemical structure (two carboxylic groups and two double bonds), FA is a crucial component in chemical synthesis as well as the production of synthetic resins and biodegradable polymers [63–65]. For this reasons, the global market of FA has been increasing each year from 240,000 t (2014) to 350,000 t (projected market in 2020) as reported by Das et al. [66].

Undoubtedly, the microbiological conversion of waste streams can be an alternative to the chemical synthesis of FA via isomerization of maleic acid, which is produced from maleic anhydride [63]. A particularly important role in the biotechnological production of FA from waste carbon sources play the fungi of *Rhizopus arrhizus* [67–69]. Papadaki et al. [68] have described in detail the application of *R. arrhizus* NRRL 2582 in the microbial production of FA from soybean cake (very high polarity (VHP) sugar from sugarcane mills). A high concentration of FA equal to 40 g/L was obtained in this study. Wang et al. [70] have also presented the biosynthesis of FA with *Rhizopus oryzae*. In this study, wheat bran via dilute sulfuric acid hydrolysis (100°C for 30 min) was used as the primary feedstock. The experimental results obtained by these authors indicate that under optimal process conditions, the production of FA equal to 20.2 g/L can be obtained. On the other hand, Zhou et al. [71] have proposed the practical method of bio-production of FA from crude glycerol. In this study, they indicated that the highest concentration of FA (22.81%) could be achieved during co-fermentation of two carbon sources, that is, crude glycerol and glucose with the same initial concentration of both reagents equal to 40 g/L.

The effective and economically justified recovery of FA from fermentation broth is significantly hindered due to its low solubility in aqueous solutions. Two FA separation pathways based (depending on the type of neutralizing agent used) on classical methods are presented in Fig. 8 [72]. On the one hand, the application of classical methods is much cheaper than the processes based on the membrane, which can be justified from the economic point of view. On

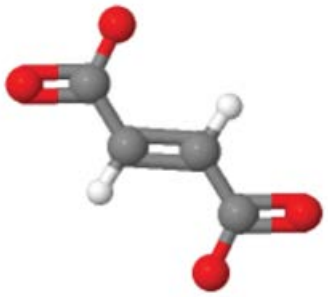

Fig. 7. Chemical structure of FA.

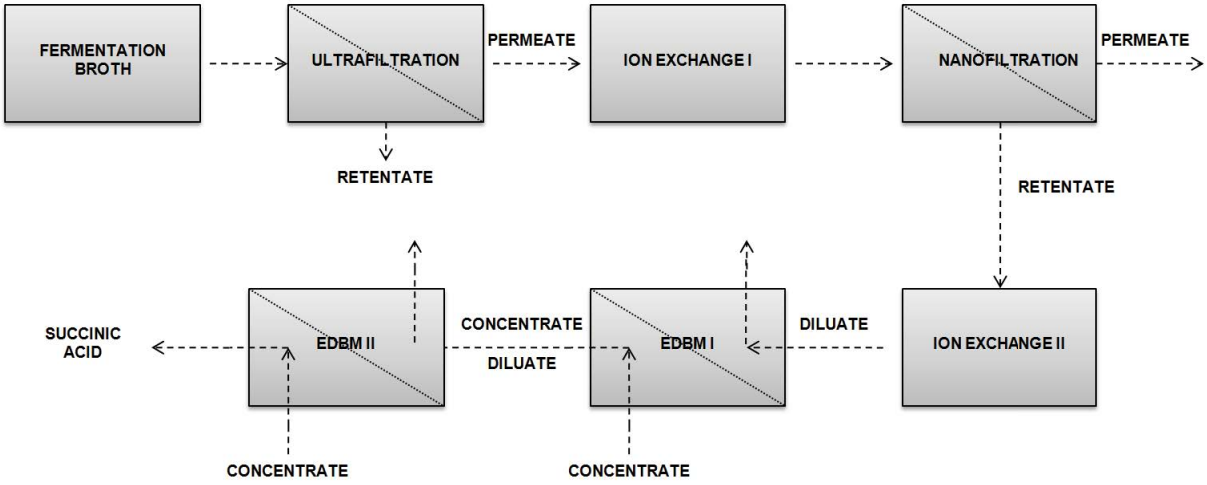

Fig. 6. General scheme of integrated membrane system used in the recovery of SA from fermentation broth (ultrafiltration – ion exchange (I) – nanofiltration – ion exchange (II) –EDBM I and EDBM II) based on Antczak et al. [15].

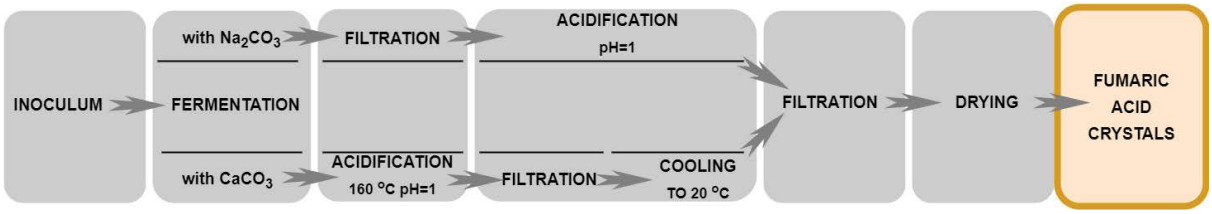

Fig. 8. Pathways of recovery of FA from fermentation broth using classical separation method (based on Martin-Dominguez et al. [72]).

the other hand, the classical method requires a step of acidification using a large amount of H_2SO_4. Therefore, in paper [29] published in 2014, we showed the potential application of membrane technology including nanofiltration and electrodialysis with a bipolar membrane in order to recover FA from fermentation broth (left after bioconversion of glycerol). On the basis of the presented experimental results, it can be concluded that the NF process (ceramic membrane 450 Da) can be successfully used to concentrate FA, especially when alkaline solutions are used. Next, the EDBM process under constant current density equal to 50 A/m^2 (of retentate obtained after NF) was used to recover of 53% of FA with simultaneous electro-acidification in the time of 3 h. Furthermore, a combination of membrane techniques with reactive extraction was also investigated in a 3-step integrated system NF – EDBM – RE [30]. The general scheme of an integrated membrane system of the type NF – EDBM – RE is shown in Fig. 9. In addition, it can be concluded that similarly as in the case of SA recovery [28], the application of a 3-step reactive extraction with quaternary ammonium chloride (Aliquat 336) or alkylphosphine oxides (Cyanex 923) allows obtaining high yield, between 60% and 98%.

Gluconic acid (GA) ($C_6H_{12}O_7$) (Fig. 10) is another organic compound which can be produced by biotechnological methods [73]. Due to its chemical structure, GA can be classified as acid sugar. Moreover, GA is stereoisomer of 2,3,4,5,6 pentahydroxyhexanoic acid [74]. It is commonly known that the GA is used in chemical, food, and construction industries [75]. What is more, the salts of GA (e.g., calcium, zinc, and ferrous) are metal ion supplements applied in medicine [76]. The above-mentioned practical applications have led to increased interest in gluconic acid. Moreover, its annual production based almost entirely on biotechnological methods is estimated at around 100,000 t/y, as reported in [74]. Different waste feedstock (especially agro-food by-products) including potato waste, strawberry surpluses, and sugarcane molasse can be effectively used as the main carbon source of fermentative production of GA [77–79].

Purane et al. [80] demonstrated a fermentative method of GA production from golden syrup using a mutant strain *Aspergillus niger* NCIM 530. These authors selected

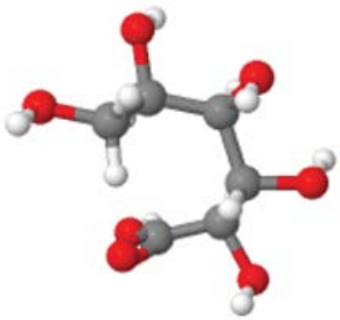

Fig. 10. Chemical structure of D-GA.

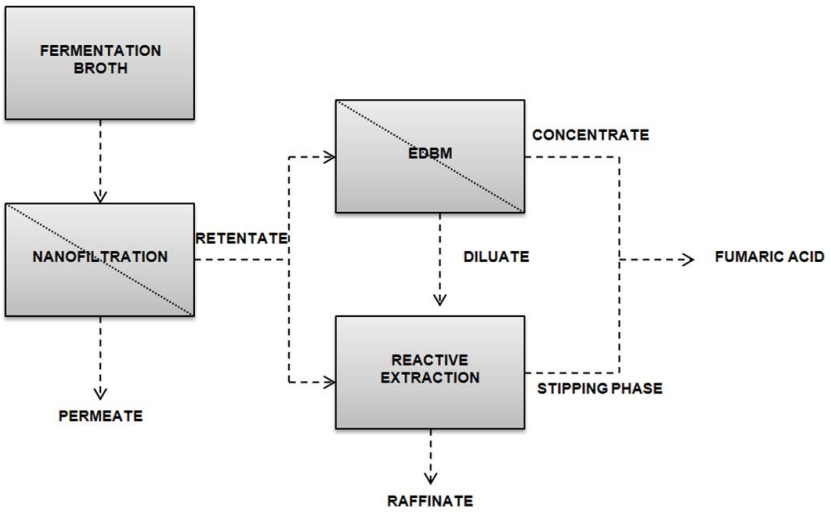

Fig. 9. General scheme of integrated membrane system used in the recovery of FA from fermentation broth (nanofiltration – EDBM – solvent extraction) based on Prochaska et al. [30].

a potential cheap raw material rich in glucose (47%), which is an industrial by-product received in the process of refining sugar cane juice into sugar. Using this technology, in 44 h of the fermentation process in a 50 L semiautomatic stirred-tank fermenter, a high conversion of glucose (86.97%), as well as a high concentration of GA (85.2 g/L), were obtained. Sainz et al. [81] have carried out a study of biotechnological production of D-GA using a broad group of acetic acid bacteria (AAB). It has been found that some microorganisms are capable of selective bioconversion of D-glucose to D-GA, bypassing the D-fructose bioconversion. In addition, these authors have concluded that the *Gluconobacter japonicus* CECT 8443 and *Gluconobacter oxydans* Po5 strains are the most suitable for the process and show a high application potential in industrial conditions, that is, continuous fermentations with pH and oxygen controls. Similarly, the utility of *G. japonicus* CECT 8443 in the microbial synthesis of GA from glucose in strawberry purée has been demonstrated by Cañete-Rodríguez et al. [82].

Pal et al. [31,83] have widely described the integration of the fermentation process of GA and its purification using membrane processes. Intensification of microbial conversion of hydrolyzed sugarcane juice by *G. oxydans* can be achieved by continuous fermentation with simultaneous separation and recycle of cells (microfiltration) and unreacted sugars (nanofiltration) [31]. Moreover, in the proposed hybrid system, a high concentration of GA (44.7 g/L) with yield (0.94 g/g) and productivity (6.5 g/L/h) was observed. The cited authors have also indicated that after microfiltration and two stages of the nanofiltration process it is possible to obtain GA in very high concentration (540 g/L) and purity (97%). It is also important that in comparison to the traditional microbial production and purification method of gluconic acid, the proposed production based on membrane technology requires a smaller number of unit operations, as shown in Fig. 11.

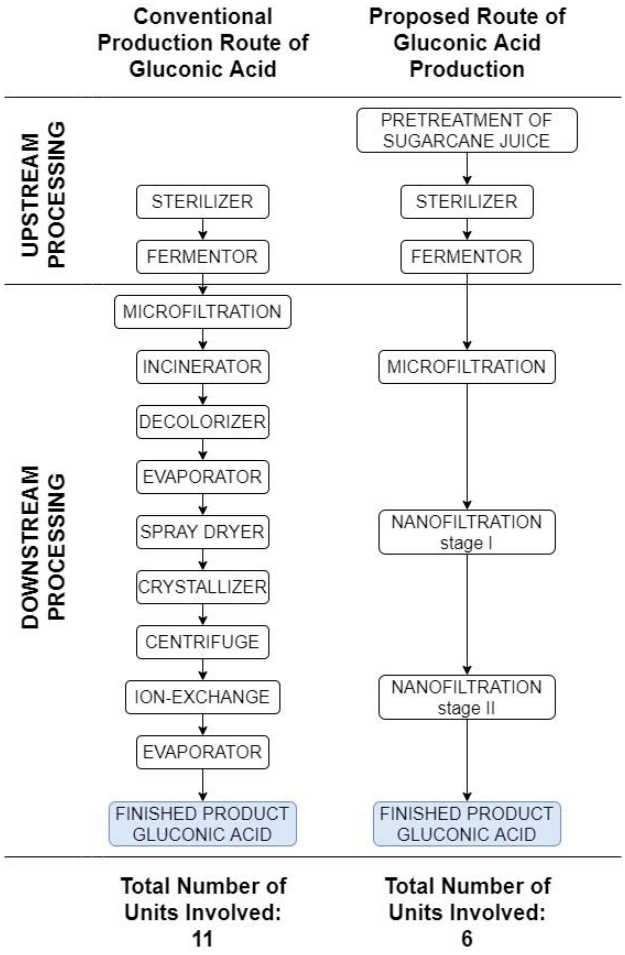

Fig. 11. Comparison of traditional (left) and membrane based (right) separation of GA from fermentation broth on the basis of Pal et al. [31].

3. Conclusions

The natural tendency to improve the quality of life while maintaining biodiversity and richness of natural resources has stimulated increasing interest in the development of biotechnology. The achievements of biotechnology have brought a significant contribution to further intensive development of different industries, like chemical, pharmaceutical, or cosmetics, in line with the principles of sustainable development. Biotechnology gives the opportunity to use unconventional and renewable raw materials to produce many valuable substances. Undoubtedly, the production of organic acids by fermentation has been remarkably developed over the last decade. Due to the reliability of microorganisms as bio-production platforms for organic acids, bioprocesses have become a viable alternative to traditional methods of chemical synthesis.

On the other hand, one should have in the mind, that the downstream process of biologically produced carboxylic acids after bioconversion requires product recovery, concentration, acidification, and purification. It is worth stressing that the separation of main metabolites from the fermentation broths is one of the most problematic steps in biotechnological processes. Moreover, the isolation and purification of bioconversion products contribute the most to the overall cost of the production of bio-based acids obtained by the fermentation process. Therefore, the commercialization of the biotechnological production of bio-based carboxylic acids requires an efficient and selective separation technology. Various traditional methods such as precipitation, crystallization, solvent extraction, and others, can be applied to recover and purify bio-based acids from fermentation broths. However, undoubtedly, the membrane-based separation techniques are an emerging alternative of significant potential. Over the past few years, many different multi-step technologies have been proposed. Hybrid processes combining different types of membrane separation are promising as energy-efficient and economical approaches for bio-based carboxylic acid separation. The need to use both pressure-driven and current-driven membrane techniques should be clearly emphasized. As the preliminary purification of the fermentation broth, the MF/UF processes should be used which allows the removal of biomass residue, macromolecular compounds, and other impurities. The separation of ionic and non-ionic compounds and removal of most inorganic salts is possible in the NF process. Next, using the current-driven membrane processes

such as EDBM the further purification of separated organic acids is possible, and, importantly, the simultaneous conversion of carboxylates to the acid form.

This mini-review presented in the paper covers merely several solutions proposed in the literature for bio-based carboxylic acid separation from fermentation broth with the focus on membrane-based separation. Nevertheless, the analysis of the presented examples shows that further research into the development of a cost-effective hybrid scheme of high productivity and guaranteeing the separation and concentration of bio-based carboxylic acids in an environmentally friendly manner is necessary.

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