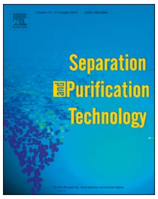
Separation and Purification Technology 192 (2018) 360–368

Contents lists available at ScienceDirect

Separation and Purification Technology

journal homepage: www.elsevier.com/locate/seppur



Removal of succinic acid from fermentation broth by multistage process (membrane separation and reactive extraction)

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ARTICLE INFO

Keywords:
Succinic acid
Fermentation broth
Ultrafiltration
Bipolar membrane electrodialysis
Reactive extraction

ABSTRACT

An environmentally friendly process for separation of succinic acid from the model solutions as well as the actual post-fermentation broth left after bioconversion of raw glycerol (which is generated in a large amount as a by-product during the production of biodiesel) was studied. An integrated system to realize this process was proposed which consisted of: ultrafiltration (UF), bipolar membrane electrodialysis (EDBM) and 3-step reactive extraction (RE) with commercial solvating extractants.

Pre-clarification process carried out by UF allowed the removal of high molecular contaminants present in the feed solution, such as: biomass, proteins as well as bacteria cells. Significant reduction in permeate flux during the process was observed due to fouling of ceramic UF membranes. However, the fouling layer was effectively removed by applying hydraulic and chemical cleaning baths.

Application of the EDBM process in the proposed integrated system allowed elimination of acidification of broth which usually generates a considerable amount of wastes. The succinic acid, present in the aqueous stream after EDBM was removed in a three-step reactive extraction at more than 90% efficiency. Extraction can support membrane techniques to separate efficiently carboxylic acids from the post-fermentation broth, however, it is not selective enough to separate succinic acid from other acids present in the broths. The only way to reach selective extraction of succinic acid over acetic, lactic acids and glycerol is to decrease pH to 2 and use Cyanex 923 as an extractant.

1. Introduction

Biodiesel is mainly produced from vegetable oils with an addition of methanol and in the presence of an alkaline catalyst in a biotechnological process, in which the most important step is the transesterification of triglycerides present in vegetable oils. During the production of biodiesel, a large amount of raw glycerol is generated as a by-product (up to 10 wt% of fuel production). One of the ways of disposal of the excessive unpurified glycerol is through the fermentation process. Depending on the bacterial strains used for bioconversion, post-fermentation solutions with different, but always very complex composition are obtained. They contain besides the main product, a number of other compounds, e.g. mono- and multivalent carboxylic acids, low molecular weight neutral organic compounds (unreacted glycerol, lactose) and a large amount of inorganic salts. The use of impure glycerol phase as the main carbonate source for biotechnological synthesis of different organic compounds, has been proposed in several works [1,2]. The fermentation process generates a broth containing the dissociated and non -dissociated forms of metabolites and residual mineral salts. Various unit operations are required for separation, concentration and purification of organic compounds from the fermentation broth. The traditional method for isolating carboxylic acid from fermentation broths is precipitation of the acid salts with $Ca(OH)_2$ or $Ca(CO)_3$, and then acid recovery with sulfuric acid. However, large amount of calcium sulfate formed within this process, which should be managed, outbalances the benefits of this process. Carboxylic acids can be separated also by means of porous solid sorbents characterized with large porous surfaces, such as activated carbons or various resins. However, a drawback of this technique is the limited sorption capacity. Recently also some membrane techniques have been proposed for separation of carboxylic acids, i.e. electrodialysis, ultrafiltration, nanofiltration and reverse osmosis [3,4]. Other techniques proposed for carboxylic acid separation from aqueous solutions are bipolar membrane electrodialysis or liquid-liquid extraction, called also reactive extraction.

The ultrafiltration is a membrane separation technique in which the driving force of the process is the pressure difference across the membrane. The transmembrane pressure range used in the ultrafiltration processes is between 0.3 and 1 MPa. The transport of molecules through

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http://dx.doi.org/10.1016/j.seppur.2017.10.043
Received 23 July 2017; Received in revised form 19 October 2017; Accepted 19 October 2017
Available online 21 October 2017
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the ultrafiltration membrane is based on the sieve mechanism and the size of retained particles defines the (cutoff) limit molecular weight membrane. The ultrafiltration processes applied for clarification of continental water to get drinking water or clarification of industrial wastewater, as well as in dairy industries such for milk dehydration and whey concentration. The pore size of ultrafiltration membranes disqualifies this process for direct separation of low molecular weight organic compounds. However, many literature reports suggest the advantages of applying the ultrafiltration as a pre-treatment step of post-fermentation solutions for removal of suspended solids, turbidity and large microorganisms, as well as dissolved macromolecules, colloids and small bacteria [5]. Juang et al. [6] have used UF to remove the residues of biological material from fermentation broth of *Serratia marcescens* SMDR, while Cho et al. [7] have applied the ultrafiltration as the first step of extracting organic acids (acetic and butyric) to remove micro-organisms and macromolecular compounds from the solution after bioconversion of woodchips. The ultrafiltration process of post-fermentation broth obtained during the production of succinic acid from biomass has also been reported [8,9].

One of the most important issues concerning all pressure-driven membrane techniques applied in industrial scale is membrane fouling phenomenon, which is mainly caused by the deposition of organic and inorganic substances on the membrane surface and in its pores. A significant reduction in the permeate flux observed during UF process (caused by deposition of particles and molecules on membrane) is one of the major factors limiting the wider application of ultrafiltration process in industry [10]. The formation of biomass cake layer on the membrane surface in time of pre-treatment of post-fermentation broths could have negative influence not only on the filtration yield, but also on the retention of components present in the working solutions. The way of formation of the fouling layer on membrane surface depends on a number of factors, e.g. the structure of retained particles and molecules (the size, shape, affinity for the membrane surface) as well as the morphology of membrane surface (porosity) and the nature of the membrane material (hydrophilicity) or processing conditions (TMP, T, Q_f) of ultrafiltration process [11,12].

Due to the presence of a significant number of byproducts in the actual post-fermentation solutions, such residues as the unreacted glycerol, lactic, formic or acetic acids, magnesium(II) and calcium(II) inorganic salts, and a large amount of lactose or ethanol, it is problematic (or sometimes even impossible) to isolate final product with high purity and good yield using a one-step separation technique. Therefore, the integrated separation processes including pre-treatment, organic acid separation from fermentation broth and conversion of carboxylic salts to organic acid, has attracted lots of attention [13,14].

Electrodialysis process is an example of membrane separation techniques in which the transfer of ions through the ion-selective membranes followed under the influence of an applied electric potential difference. The process uses an electrical driving force to transfer ions from the diluate chamber through the cathode (positively charged ions) and anode (negatively charged ions) to a concentrate chamber, creating a more concentrated stream.

The bipolar membranes (BP) consist of two membranes: the anion exchange and cation exchange, separated by a thin water layer of a thickness of about 2 nm [15]. Under the influence of a direct current (DC) voltage, in the catalytic space of the bipolar membrane the water molecules are split to hydrogen and hydroxyl ions [16]. Although the EDBM process has successfully been applied in various areas of technology, e.g. production of food or production of useful chemical compounds from industrial wastewater, such as ammonia [17] or the desalination of industrial saline stream [18], the number of applications of EDBM is continuously increasing and nowadays it is more often applied for environment friendly technologies, e.g. the production of organic acids from the solutions after bioconversion processes [19]. Several studies have indicated that EDBM is available process for producing organic acids, such as acetic, oxalic and citric [16] lactic acid [20] or succinic [21].

Such acids as lactic, propionic, succinic, maleic, butyric, oxalic or acetic can be efficiently transported from the aqueous to the organic phase by organophosphorus solvating extractants [22–24], aliphatic amines [22,25–28], quaternary ammonium [22,29] or phosphonium salts [30–32]. Also an integrated system of nanofiltration, bipolar membrane electrodialysis and reactive extraction has been recently proposed by our team to successfully remove fumaric acid from the fermentation broth [33].

In this investigation, an integrated system consisting of three stages: ultrafiltration (UF), bipolar membrane electrodialysis (EDBM) and reactive extraction (RE) was proposed for separation of succinic acid from the fermentation broth left after bioconversion of glycerol.

2. Material and methods

2.1. Materials

For the investigation of separation and concentration of succinic acid from model solutions five organic compounds were used: succinic acid (Suc) (Sigma-Aldrich, Poland), glycerol (Glyc) (*POCH S.A, Poland*), formic acid (Form) (POCH S.A, Poland), acetic acid (Ac) (POCH S.A, Poland), lactic acid (Lact) (Sigma-Aldrich, Poland). Two synthetic water solutions were subjected to separation by bipolar membrane electrodialysis: (1) 2-component solution, with succinic acid and glycerol and (2) 5-component solution, containing glycerol and 4 carboxylic acids: succinic, formic, acetic and lactic (the composition of 5-component model solution was similar to the permeate solution after ultrafiltration of actual post-fermentation broth). Model solutions were prepared by dilution with deionized water of a conductivity not-exceeding 3 μS/cm. The pH of these solutions was adjusted to 8.5 by adding an appropriate amount of sodium hydroxide (Sigma-Aldrich, Poland).

The actual post-fermentation broth (pH = 8.5) left after bioconversion of glycerol to succinic acid (preliminary centrifugated to remove biomass) was delivered from the Poznan University of Life Sciences. The composition of the model solutions and the actual post-fermentation broth are shown in Table 1.

Solvating (mixture of trialkylphosphine oxides - Cyanex 923) or basic (trioctylamine - TOA) extractants were used as received to prepare 0.1 or 0.4 mol/dm^3 organic solutions of extractant in octanol or in low-aromatic kerosene Exxsol D 220/230 (aromatic content: 0.05 wt%, distillation range 222–234 °C, Exxon Mobil Chemical, Germany).

2.2. Ultrafiltration (UF)

UF process was carried out on a laboratory ultrafiltration unit, which was equipped with two (left – l_m and right – r_m) commercially available tubular ceramic membranes: Céram INSIDE® (TAMI, France). Technical parameters of the membranes applied in this study are given in Table 2. The volume of the feed tank amounted to 10 dm^3.

Both membranes before UF process were conditioned until a constant water flux was established. The flux of deionized water is called the "initial water flux" (J^i_{lm}/J^i_{rm}). Pre-clarification of the post-fermentation solution by ultrafiltration was carried out under the following

Table 1
Composition of the model solutions and the actual post-fermentation broth after bioconversion of glycerol to succinic acid.

Solution	Concentration, C^0, g/dm^3				
	Suc	Glyc	Form	Ac	Lact
2-component model solution	23.3	15.2	–	–	–
5-component model solution/actual post-fermentation broth	23.3	15.2	9.9	8.4	6.4

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