# Batch electrodialysis of lactic acid obtained from *LAB* fermentation

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The aim of this work was to develop the method of lactic acid (LA) separation from fermented whey. CMI-7000 Cation Exchange Membrane and AMI-7001 Anion Exchange Membrane were employed in electrodialysis process. Experiments showed that the selected membranes separated organic acids effectively (including LA) from other organic ingredients present in medium. Selecting an appropriate volume of a receiving chamber could lead to LA concentration. Moreover, membrane fouling during separation was investigated. This phenomenon is negligible which is the main advantage of this process. As it was shown during batch processes, with the voltage increase, the rate of electrodialysis increases as well. It prompts to a reduction of residence time in electrodialyzer during a continuous separation.

**Keywords:** lactic acid, membrane separation, *Lactobacillus rhamnosus*, whey, lactose.

## INTRODUCTION

Lactic acid (LA) is one of the most common organic acids that is used widely in biotechnological and chemical technologies. For instance, in the food industry it is widely used as an acidulant, a preservative and also as an emulgator[1]. In 2016 the yearly world production reached $1.077 \cdot 10^6$ tons and an annual growth rate 14.2% is expected[2].

Beneficial properties of LA for skin condition have been applied in cosmetics production. It is common used as an acidity regulator[3] in cosmetics formulation, but could be also used as active ingredients too. W. P. Smith[4] treated patients' skin with 12% w/v lactic acid and this resulted in increased epidermal firmness and reduction of wrinkles amounts. LA after polymerization as polylactide is a great alternative to botox[5].

A large application of LA comes with its consumption for the synthesis of the polyester PLA (polylactic acid or polylactide) – a biodegradable polymer. It is extensively used in tissue engineering and in drug delivery systems[6]. The global production capacity for PLA increases rapidly every year and the capacity of 800.000 tons should be established in 2020[7] (http://news.bio-based.eu). Hence, the lactic acid requirement is estimated at an annual growth of 5–8% per year[8, 9].

Not only PLA production consumes LA. This organic acid may be converted into a wide range of useful products or semi - products such as acrylic acid, propylene glycol, 2,3-pentanedione, acetaldehyde, pyruvic acid[1].

From the economical point of view and because of its enormous world consumption, new sources (especially natural – derived from food industry) used in lactic acid production are sought. It is quite obvious, that a fermentation process is preferable than a chemical synthesis[10]. The fermentation is performed by lactic acid bacteria – LAB[11] that are able to convert simple carbohydrates such as glucose, galactose and lactose (derived from whey) to LA. LA production from whey is certainly an eco-friendly process and gives surprising fermentable capability[12].

A product of microbiological fermentation is contaminated by unreacted substrate, residual of nutrient solution or – what is the most problematic – propagated bacterial cells. In effect, a purification of this stream is complicated and difficult. Membrane reactor is increasingly popular process solution to produce organic acids[13, 14]. Its application leads to cells and heterogenic pollution separation. Much more complicated are the further steps. In fermentation broth there are huge amounts of impurities such as unreacted sugars, residue of proteins, or other organic acids[12]. The most selectively method to separate these compounds is an ion exchange chromatography[14]. Gonzales et al.[16] used two types of ion exchangers – Lewatit S2568H and Lewatit S3428 and obtained a final purity of lactic acid higher than 99%. There is huge amount of anion and cation exchange chromatography applied: Reillex 425[17], IRA-420[18] or MWA–1 and AG3-X4[19]. The ion exchange chromatography was an effective process in terms of the product quality, but it gave the product recovery in an unfavorable quantity approach.

LA can be also extracted by n-butanol. The advantage of this process is high selectivity of lactic, however factors such as pH, mixing time, initial concentration of lactic acid, and volume ratio between the organic and the aqueous phase affect the extraction yield[20]. An integrated process including filtration, activated carbon treatment and evaporation should be also mentioned[20]. This method gives 85% concentration of LA. Another integrated process of lactic acid recovery and purification process was esterification with distillation. Purified LA in extraction process with n-butanol was mixed with methanol (in molar ratio 1:1.5) and small amount of sulfuric acid. The mixture was directed to fractionating column. LA was a bottom fraction[22]. However, described process requires a lot of energy, therefore is not profitable.

This work tries to outline the promising field of LA purification by electrodialysis (ED). This approach is intensively developing nowadays. Membrane processes are cheaper, more efficient than other purifying processes (e.g. chromatography or extraction) and works usually in continuous system, what make them valuable from industrial point of view. These are advantages and ED as a type of membrane process is characterized by the same.

ED membranes are mechanically and chemically stable in different electrolyte solutions[23], so in effect they may be used for a long time, especially when it comes to

continues system processes. Additionally, regeneration is quite easy and cheap (using NaOH, HCl or NaCl)

Many membrane systems have been already described but this membrane technique is constantly studied. There are many various types of this process: desalting electrodialysis with membranes stacked alternately or water splitting electrodialysis with bipolar membrane, one stage system or multi – stage system etc.[24, 25]

This type of separation process is widely used to the separation and purification of organic acids[26], besides lactic acid, also citric acid[27]. Habova et al.[28] received a final concentration of LA (derived from a fermentation broth) 151 g L$^{-1}$. Boyaval et al.[29] coupled electrodialysis unit with continuous fermentation and achieved 85 g L$^{-1}$ concentration in outlet stream. Kim and Moon[25] employed one-stage ED process, which lead to a high volumetric productivity – 72 g L$^{-1}$ h$^{-1}$, that in comparison to a multi – stage separation leads to a decrease in capital and operating costs. All of described above processes refer to continuous process.

ED is very specific type of membrane process, because as an one is a based on electric potential difference. Authors rarely investigate the problem of transport through the membrane, which is undoubtedly very important from a process design point of view. They had pretested results (often very satisfactory) of separation made on industry or laboratory equipment, but do not id not mention about mechanism.

In presented work, one – step batch electrodialysis process was tested. An investigation a process using a batch system could provide some information about mechanism of separation, what is a quite difficult in continues system case. CMI-7000 Cation Exchange Membrane and AMI-7001 Anion Exchange Membrane from Membranes International, USA, (which have not employed to LA separation yet) were tested. These membranes should have a great potential in LA purification[30]. Separated fermented broth derived from membrane bioreactor with *Lactobacillus rhamnosus* while post-production whey was the carbon and energy source. The main task was to separate LA from another small organic compounds like lactose or peptides occurring in broth[31].

## MATERIALS AND METHODS

### Materials

Medium, which was purified, derived from membrane bioreactor with *Lactobacillus rhamnosus* strain immobilized. In outlet stream there was microfiltration membrane (pore diameter – 0.40 μm), so it leads to preliminary purification of liquid from the largest structures and bacteria. Inlet stream of bioreactor consist of whey diluted by the medium responded to the MRS[32]: (in g · L$^{-1}$) $CH_3COONa$ (5), Tween (1), $K_2HPO_4$ (2), Triamonium citrate (2), $MgSO_4 · 7H_2O$ (0.2), $MnSO_4 · 4H_2O$ (0.05). The whole process is precisely described in previous work[27].

Concentration of LA in outlet stream (simultaneously in electrodialyzed solution) was approximately 7.2 g · L$^{-1}$ (was not constant because came from microbiological process). It contains small amount of unreacted lactose (1.4 g · L$^{-1}$), proteins (0.5 g · L$^{-1}$) and other (in insig-

nificant concentration) organic acids – especially citric and acetic acid. A presence of these acids generated a pH value about 4.0.

In order to estimate a concentration of lactic acid salt (during process outside acid there is formed its salt too) 1mL medium was mixed with 1M $H_2SO_4$ (Poch, Poland, CAS: 7664-93-9) and then lactic acid concentration was measured with HPLC. The difference between based on HPLC obtained value, and concentration (measured in medium without sulfuric acid), is approximate salt content.

### Equipment

A laboratory equipment to electrodialysis (our construction) had three chambers divided by ion-exchanging membranes (CMI-7000 Cation Exchange Membranes and AMI-7001 Anion Exchange Membranes, Membranes International, USA). Parameters of these membranes

**Table 1.** The technical specifications of the ion-exchanging membranes

|  | AMI-7001 Anion Exchange Membranes | CMI-7000 Cation Exchange Membranes |
|---|---|---|
| Polymer Structure | Gel polystyrene cross-linked with divinylbenzene | Gel polystyrene cross-linked with divinylbenzene |
| Functional Group | Quaternary Ammonium | Sulphonic Acid |
| Electrical Resistance [Ω cm$^{-2}$] 0.5M NaCl | < 40 | < 30 |
| Maximum Current Density [A m$^{-2}$] | < 500 | < 500 |
| Permselectivity [%] 0.1 mol KCl kg$^1$/0.5 mol KCl kg$^{-1}$ | 90 | 94 |
| Total Exchange Capacity [meq g$^{-1}$] | 1.3 ±0.1 | 1.6 ±0.1 |
| Water Permeability [ml min$^{-1}$ cm$^{-2}$) – 0.03 MPa | < 0.19 | < 0.19 |

are presented in Table 1. The area of each membrane was 3.2 cm$^2$.

A cathode was placed in the left chamber (chamber I, V = 30 mL) detached by the cation – exchanging membrane while in the right chamber (chamber III, V = 30 mL) an anode was placed. The electrodes were submerged in distilled water and connected with direct current source (MS Major Science MP – 300V). A middle chamber (chamber II, V = 60 mL) was filed with a post
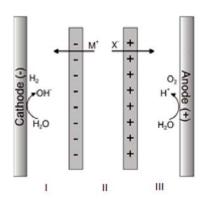


**Figure 1.** Scheme of an electrodialysis set

– culture medium. The scheme of the system described is presented in Fig. 1.

During separation an electrical voltage, current, temperature and the concentration of lactic acid were measured.

Membranes were regenerated by 1% HCl (for the cation exchange membrane) and 1% NaOH (for the anion exchange membrane) poured into an adequately chamber. Additionally, ultrasounds for 5 min were applied.

## Lactic acid analysis

The concentration of lactic acid was analyzed with HPLC (Waters, USA) under isocratic conditions using Synergii 4μ Hydro – RP 80 Å column (Phenomenex, USA).

Samples were first filtered through a 0.22 μm syringe filter and then eluted with 20 mM $K_2HPO_4/H_3PO_4$ at pH 2.9 and 30°C for 10 min. The flow rate was 0.042 L h$^{-1}$ and peak absorbance was monitored at 214 nm. Lactic acid (Sigma – Aldrich, CAS: 79 – 33 – 4), citric acid (Poch, Poland, CAS: 538210118) and acetic acid (Sigma – Aldrich, CAS: 64–19-7) were run as standards. Under these conditions retention time for lactic acid was 6.12 min and concentration was calculated using standard curve:

$$C \ [g \ L^{-1}] = 4.35 \cdot 10^{-6} \cdot A \tag{1}$$

where: A – area under area of peaks [μV·min]

## RESULTS AND DISCUSSION

The separation of LA from a fermented broth was the main aim of this research. A processing stage of LA synthesis in bioreactor is a complex microbiological process, in which very various products can be formed. Moreover, LA salts (sodium or calcium lactate) can be found in fermented broth[33]. Electrodialysis process does not distinguish these ingredients; lactate anions derived from both acid and salts migrate to chamber III. In effect at the equal volume of all chambers we can obtain higher concentration of LA in chamber III than in chamber II in the initial stage.

The experiment for total amount of LA determination (extraction by 1M sulfuric acid) indicated that in our case almost 16% of LA molecules came from salts.

## Composition of separated medium

Electrodialysis is a specific membrane technique where a driving force is an electric potential difference. Different ions with the same sign migrate throughout the membrane in the same direction. Thus, the presence of other acids (other than LA) can be observed in chamber III. They came from whey, which was used as a medium in bioreactor. *L. rhamnosus* is a homofermentative L-(+)-lactic acid producer[34]; due to its metabolism only LA is formed. Additionally, *L. rhamnosus* can used a citric acid as well as lactose[35], as a carbon source. Therefore, the concentration of this acid decreases during fermentation process (Fig. 2).

## Profile of product concentration in chamber    III

At the beginning, the middle (II) chamber was filled with the fermented broth, microfiltrated previously. Average LA concentration in this solution was 7.2 g
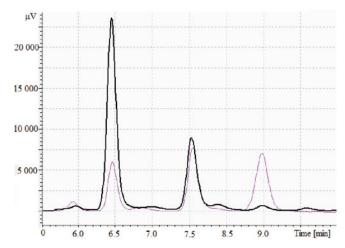


**Figure 2.** Organic acids present in whey (thin, pink line) and after fermentation process (thick black line): 1$^{st}$ peak – lactic acid (retention time – 6.4 Min), 2$^{nd}$ peak – acetic acid (retention time – 7.5 Min ), 3$^{rd}$ peak – citric acid (retention time – 8.5 min)

L$^{-1}$. Then the LA molecules, and also other organic acid molecules migrated to the right chamber using the appropriate voltage – from 25 to 100 [V].

At each voltage the concentration profiles in chamber III were similar, but the process rate increased with a voltage increase (Fig. 3). After 5 hours of separation process, the concentration of LA was approximately 3.6 g L$^{-1}$ (it estimated for 25% initial mass of LA). The faster this condition was achieved the higher voltage was
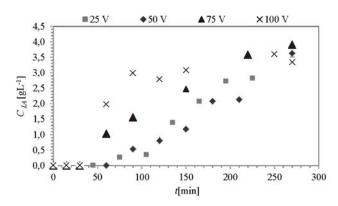


**Figure 3.** LA concentration in the right (III) chamber in time of electrodialysis. The initial concentration in the middle (feed) chamber (II) C0,II_25V = 7.42 g L$^{-1}$; C0, II_50V = 7.43 g L$^{-1}$; C0,II_75V = 7.25 g L$^{-1}$; C0 II_100V = 7.48 g L$^{-1}$

applied. As expected, ions migrated after this time, but with lower intensity.

During electrodialysis, the concentration of acids in the middle chamber decreased (Fig. 4).

## Profile of resistance and temperature in the system

Electrical resistance and temperature were monitored during the whole electrodialysis process. In each chamber temperature was the same. For the first 20 minutes electrical resistance of the system decreased quite rapidly and at the same time temperature increased for each applied voltage (Figure 5A and B). At the beginning of the process the resistance of system is very high and the reason of this is a presence of distilled water in I and
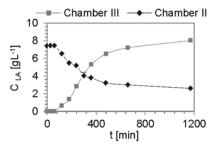
**Figure 4.** The change of LA concentration during electrodialysis carried out at 25 V

III chamber (of course, an introduction of some ionic solution could decrease this phenomenon, but it could lead to product contamination and that idea was abandoned), so in effect the significant rise of temperature takes place.

After 1.5 hour of the electrodialysis process, temperature was quite stable and LA concentration and resistance increased modestly. These two last parameters are mutually bonded with each other. The higher acids concentration in liquid in chamber III resulted their lower concentration in liquid in chamber II. It leads to the situation, in which there are less and less energy conveyors and the middle chamber becomes a source of resistance.

A relative change of temperature associated with a given change in resistance for semiconductor (mentioned scheme is certainly one of them) is described by equation:

$$R_t = R_0 \cdot e^{\frac{\alpha}{\Delta T}} \quad [1]$$

where: $R_t$ – resistance in given time, $R_0$ – resistance at the begging, $\Delta T$ – the difference between T and $T_0$, $\alpha$ – temperature coefficient.

Obtained results (profile of the temperature – Fig. 6A) confirmed this theory, because according to it at the beginning of process there is rapidly increase of temperature and after initial time (in this case approximately 1 hour) slowly decline is observed. A mentioned high temperature at the beginning of separation is caused by I and III chamber – there is very low concentration of ions - so these chambers became resistors. As the concentration increase the scheme does not put up such resistance anymore. A further increase of temperature is present because the response of this parameter is delayed.

**Fouling on electrodialysis membranes**

One of the disadvantages of any membrane separation is membrane fouling[36]. During electrodialysis not only acids can block the membrane surface. The post-production fermented broth used as a feed in presented research contained unreacted lactose (approximately at 1.5 g L$^{-1}$) and whey proteins (approximately at 0.2 g L$^{-1}$).

In order to check how the membranes are blocked, the experiment was performed with and without cleaning procedure between successive periodic processes lasting 5 hours. As it is presented in Fig. 7 the process run with the same efficiency on the regenerated membranes as on the membranes using previously by 5 hours. It looks like using this type of membranes, fouling could be
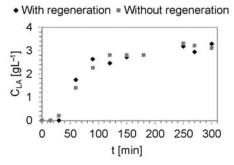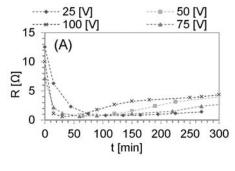


**Figure 7.** The profile of LA concentration in chamber III (the process at 50 V) using the membranes with and without regeneration
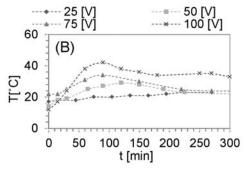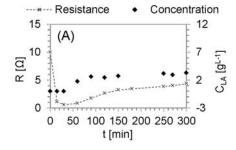


**Figure 5.** Profile of electrical resistance (A) and temperature (B) during electrodialysis separation
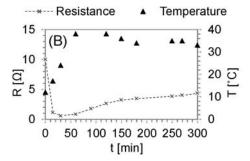


**Figure 6.** An example of the resistance, temperature and LA concentration in chamber III profile in time (electrodialysis at 100 V)

neglected. It is a significant advantage of this type of membrane separation.

## CONCLUSIONS

The aim of the presented work was to recognize the separation process of LA, obtained during whey fermentation. The broth was rich in proteins, peptides, lactose and organic acids. CMI-7000 Cation Exchange Membrane and AMI-7001 Anion Exchange Membrane were selected to the separation of organic acids by electrodialysis. The research was conducted in a batch system.

Taking the obtained data into consideration a few advantages can be indicated:

1) LA is efficiently separated from other organic compounds present in broth. Particularly, the acids transportation while lactose and peptides cannot be transported caused this process to be more interesting than nanofiltration[15].

2) It is possible to concentrate all molecules of LA in the receiving chamber, also the ones obtained from lactates. Final concentration strongly depends on the receiving chamber volume.

3) Membrane fouling does not influence strongly on the process.

One the most important disadvantage of the proposed process is the presence of other acids in the receiving chamber. Their concentration is tightly connected with a broth quality, that strongly depends on other acids concentration in whey and the fermenting culture properties. These acids can be separated using chromatographic techniques[37].

During analysis voltage increase was observed and the rate of electrodialysis increased as well. It suggests a reduction of residence time in electrodialyzer during a continuous separation at the application of the higher values of voltage. The estimated mass stream at the first significant hours was variable, but ranged several mg $\cdot$ L$^{-1}$ $\cdot$ cm$^{-2}$ $\cdot$ s$^{-1}$. The influence of this parameter on ED separation should be carefully examine during the continuous processes, in which a stagnation phase will be omitted.

## ACKNOWLEDGMENTS

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