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Lactic Acid Recovery from Model Solutions and Fermentation Broth by Electrodialysis

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The effect of current density, initial concentration of diluting solution on lactic acid (LA) recovery by electrodialysis from both model solutions and real fermentation broth was investigated. In model solutions, LA recovery efficiency was 93–96% and it was independent of both, current density and initial concentration of diluting solution. The recovery rates at 33, 66 and 100 $A \cdot m^{-2}$ were 76, 175 and 272 $g \cdot m^{-2} \cdot h^{-1}$ respectively and, clearly were linearly depended on current density. In the first stage of the process, both model solution and real fermentation broth showed similarities in the dynamic of LA recovery and water flux. With real fermentation broth, at current density of 100 $A \cdot m^{-2}$ and 41.8 *g/l* initial lactate concentration of diluting solution, the LA recovery rate and the water flux were 279.5 and 679 $g \cdot m^{-2} \cdot h^{-1}$, respectively. However, the recovery efficiency was decreased from a 93–96% range to 71%, compared to that in model solutions. The reason probably was that anion–exchange membrane was fouled by protein from broth.

Keywords: lactic acid recovery, electrodialysis, ion exchange membrane, model solution, fermentation broth.

INTRODUCTION

Lactic acid fermentation (LAF) is one of the most important microbial processes because it is used widely in numerous fields of application as food, pharmaceutical industries and because it has great potential in the manufacture of biodegradable polymers and films (Datta *et al.*, 1995).

LA can be separated from the fermentation broth by dialysis (Pörtner and Märkl, 1998), ion–exchange resin (Srivastava *et al.*, 1992; Vaccari *et al.*, 1993) and extraction (Honda *et al.*, 1995; Von Frieling and Schügerl, 1999). Separation by dialysis has the disadvantage of requiring dilute product stream to effect reasonable mass transfer rate aside from the need to convert LA into salt prior to removal (Pörtner and Märkl, 1998). Extraction needs solvents, many of which were found toxic to both acid–producing

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** Department of Food Biotechnology, Hanoi University of Technology, 1 Dai Co Viet Street, Hanoi, Vietnam

*** Corresponding author (sonomoto@agr.kyushu–u.ac.jp) microorganisms and environment (Hongo *et al.*, 1986; Von Frieling and Schügerl, 1999)

The main disadvantage is that fermentation derived sodium lactate, which was formed during the fermentation through the addition of NaOH to maintain the pH of fermentation broth stable is difficult and expensive to purify (Boniardi *et al.*, 1997). Much of the cost of LA production has been associated with the recovery and purification processes. In the past, efficient and economical technologies for recovery and purification of LA from crude fermentation broth had been the key technology impediments and main process cost centers (Datta *et al.*, 1995). Among the other processes, electrodialysis is promising for the downstream processing in LAF.

Recently, electrodialysis was considered as a desalination process to be applied to such solution as brackish water and seawater (Jain *et al.*, 1985). In particular, electrodialysis can be used as alternative to the downstream processes to recover pollutants from industrial effluent (Andrés *et al.*, 1994). In biotechnology, electrodialysis was used to remove continuously not only lactic (Hongo *et al.*, 1986; Ishizaki *et al.*, 1990; Yao and Toda, 1990), but also acetic (Nomura *et al.*, 1988), propionic (Voyabal *et al.*, 1993) and itaconic (Moresi and Sappino 2000) acids from fermentation broth, in order to enhance microbial acidic productivity and make the final processing easier. In these systems, LA can be recovered either as an acid (Hongo *et al.*, 1986; Yao and Toda, 1990) or as a lactate salt (Vonktaveesuk *et al.* 1994) diffusing through ion–exchange membranes under supplied direct current.

In our laboratory, L–(+)– LA has been produced from sago starch hydrolyzed in an environmentally sound bioprocess with high efficiency. Thus, an environmentally friendly downstream process, such as electrodialytic application, for recovery and purification of LA produced is needed. In this study, the recovery of LA from model solution and fermentation broth by electrodialysis was discussed.

MATERIALS AND METHODS

Microorganisms and culture conditions

Microorganism. *Lactococcus lactis* IO–1 was used throughout this study.

Culture conditions. Culture conditions were carried out as described previously (Ishizaki and Ohta, 1989).

Sago starch hydrolysis. Sago starch hydrolysis was carried out as described previously (Nolasco–Hipolito *et al.*, 2000).

Fermentation. After 3 h of incubation, 40 ml of prepared preculture was aseptically inoculated with 360 ml of main medium in a 1*l*–jar fermentor. The main medium contained (g/*l*): polypeptone (Nihon Seiyaku, Tokyo, Japan), 10; yeast extract (Difco Laboratories, Detroit, MI, USA), 10; NaCl, 5; and glucose from hydrolyzed sago starch, 40.

Analysis. Glucose and lactate concentrations were determined by HPLC (LC–10AD, RID–6A Refractive Index Detector, Shimadzu, Kyoto, Japan) using an Aminex HPX–87 H column (Bio–Rad Laboratories, CA, USA) at 50 °C and 0.6 ml/min flow rate of 5 mM H_2SO_4 as a mobile phase. Cell density was measured in terms of optical density, at 562 nm, in a spectrophotometer (UVIDEC–320 Spectrophotometer, Jasco Co., Tokyo, Japan) and converted to dry cell weight (DCW) from calibration curve.

Apparatus for electrodialysis

Experimental set–up for electrodialysis system is shown in Figure 1; which consists in a five–compartments electrodialyzer: cathode, anode, concentrated and two diluted compartments. Each compartment was separated by cation and anion exchange membranes. The electrodialyzer was provided two anion exchange (Neosepta AM1) membranes and two cation exchange (Neosepta CM1) membranes (both from Tokuyama Co., Tokyo, Japan). The cathode and anode were made of platinum. The process flow sheet is shown in Figure 1 with four independent streams: the electrode solution was supplied by the same stream to both cathode compartment and anode compartment, while three other solutions were recirculated independently in concentrated compartment and two diluted compartments. Starting from the reservoirs, each solution was pumped through the compartment and then returned to the respective reservoir until the electrodialysis finished.

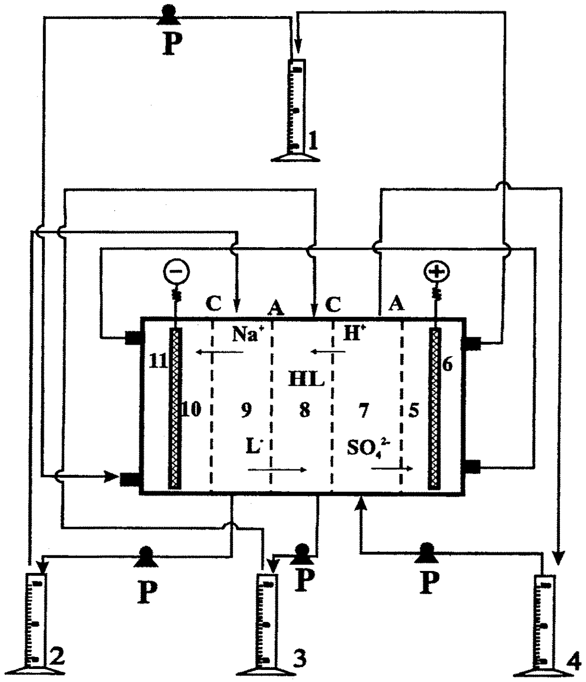

Fig. 1. Schematic diagram of the electrodialyzer. L–: lactate ion; HL: lactic acid; C: cation–exchange membrane; A: anion–exchange membrane; P: pump; (–): cathode; (+): anode. 1: 3% Na_2SO_4 solution; 2: sodium lactate solution; 3: concentrated solution; 4: 0.2 M H_2SO_4; 5: anode compartment; 6: anode; 7&9: diluted compartments; 8: concentrated compartment; 10: cathode compartment; 11: cathode.

To determine the volume variations generated during the electrodialysis process, reservoirs with scale resolution ±2 ml accuracy were used. Direct current was supplied by a power unit (NISC Regulated DC Power Supply, Stabilizer Industry, Osaka, Japan). This power unit employed can be set independently either the voltage in the range of 0–35 V, or the current in the range of 0–2 A. All experiments were carried out in constant current values and would be stopped when the voltages reached 35 V. In all experiment runs, the initial volume of all solution were 500 ml.

Solutions of sodium lactate, 0.2 M Na_2SO_4 and 0.2 M H_2SO_4 were used. The former and the latter were recirculated in two dilute compartments respectively while solution of Na_2SO_4 was supplied for both cathode and anode compartment. Water was used for recirculation in concentrated compartment. Initial volumes of all solution were 500 ml, except in the case of concentrated compartment, the initial volume of water was just enough to fill up the compartment. Before running every experiment, membranes were cleaned in situ by recycling a 0.05 M HCl solution during 1 h, followed by a 0.05 M NaOH solution and then HCl again. Finally, the system was rinsed by deionized water.

RESULTS AND DISCUSSION

Effect of current density

The current density plays an important role in electrochemical process, where current is the driven force for the transport of ionic species. In the separation of electrochemical process such as electrodialysis, it was found that the transport of ionic species is decided not only by both, properties of that species and type of membranes, but also by current density (Moon *et al.*, 1993). Experiments were carried out by using the same 40 *g/l* initial concentration of sodium lactate. During electrodialysis process, the concentration of sodium lactate and H_2SO_4 in diluting compartments decreased, and the electric resistance in the electrodialyzer unit increased. Therefore, the direct current supplier had to increase automatically the voltage applied to electrodes up to value of 35 V to maintain the current density constant. This operation was called the constant–current mode (Lee *et al.*, 1998). After reaching the maximum value of 35 V the current began to decrease. All experiments were practically stopped at that moment to avoid damage membranes by overheat phenomenon. Especially, after finish the constant current mode, the LA recovery efficiency was decreased and the LA concentration in the concentrated solution changed very slow (data not shown). For this reason, it had better to operate electrodialysis in the constant–current mode when electrodialyzer would be combined with a fermentation system to remove continuously lactate produced. Figure 2 shows the time course of lactate ion recovery from salt form into acid form. As expected, the lower the current density value applied the longer the process time needed to separate LA. At three different current densities: 33, 66 and 100 $A \cdot m^{-2}$, the LA recovery rates were 76, 175 and 272 $g \cdot m^{-2} \cdot h^{-1}$, respectively. The LA recovery was practically finished within 19, 8 and 6 h, respectively. At the high current density (100 $A \cdot m^{-2}$) the electrodialysis operated in the constant current mode while the residual sodium lactate in diluting compartment was being maintained higher than 5% (in comparison with the initial sodium lactate) or lactate concentration in diluting compartment lower than 5 *g/l* (4.1–4.4 *g/l*). This value of switching concentration was lower than that obtained by Lee,

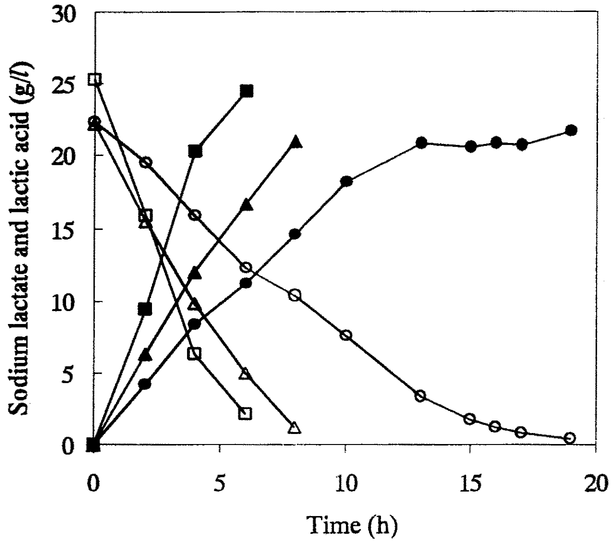

Fig. 2. Time course of the recovery of sodium lactate by electrodialysis containing 40g/*l* of lactate at different current densities; symbols: 33 A·m^{-2} (●, ○), 66 A·m^{-2} (▲, △), 100 A·m^{-2} (■, □). Closed symbols: LA recovered. Open symbols: LA equivalent (salt form).

et al. (1998) when they finished the constant–current mode at switching concentration of lactate in diluted compartment was being remained about 10% (lactate concentration was about 11 g/*l*), even they used low current densities, in a range of 35–45 A·m^{-2}. The results obtained showed that the lower the current densities, the lower the values of switching concentration. At current densities of 66 A·m^{-2} and 33 A·m^{-2}, the final lactate concentration in diluting compartment was 2.4 and 0.8 g/*l*, respectively. During the constant–current mode, the LA recovery rate was almost linearly dependent on current density. After that, recovery rate decreased because the electric resistance increased due to the removing of ions in diluting compartments. From this result, it can be expected to maintain the lactate concentration in fermentation broth at a level low if the electrodialyzer is installed so that its capability of converting lactate ion is in agreement with the lactic acid production rate in the fermentation system. However, the recovery efficiency of LA did not depend on current density. It was found that there was no influence of the current density on the recovery efficiency, in the case of the same initial lactate concentration. Approximately 93–96% of recovery efficiency was obtained in all three different current densities.

During the transport of ionic species, some hydration water molecules bound to ions also diffused with ions through membranes. This phenomenon was called electro–osmosis and generates an increase in the volume of the concentrated solution, and a simultaneous decrease in that of the diluting solutions. Figure 3 shows the volume

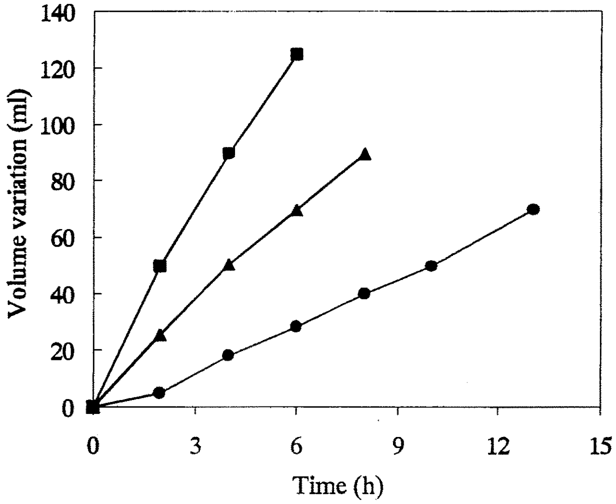

Fig. 3. Volume variation of concentrated solution as a function of time at different current densities; symbols: (●), 33 A·m^{-2}; (▲), 66 A·m^{-2}; (■), 100 A·m^{-2}.

variations in the concentrated solution against the time. After the first hour of the operation, it seems that the volume variations in the concentrated solution increased proportionally with the time and depend linearly on current density. At the beginning of the electrodialysis, the water flux⁽¹⁾ was slow because the concentration of LA in the concentrated compartment was still very low and this caused the limitation of the ion transportation. The increase in current density resulted in an increase the water flux. At three different current densities: 33, 66 and 100 A·m^{-2}, the water fluxes were 235, 458 and 667 g·m^{-2}·h^{-1}, respectively. However, these water fluxes would limit the maximum LA concentration achievable. From the results obtained in water flux and LA recovery rate, it is possible to estimate the maximum LA concentration achievable to be about 407 g/*l*. This result was similar to that obtained by Boniardi, *et al.* (1997).

It can be seen from Figure 4 that the volume variation trend in the sodium lactate solution was similar to that in the concentrated solution. Higher current density caused the higher volume variation of sodium lactate solution. At three different current densities: 33, 66 and 100 A·m^{-2}, the volume variations of sodium lactate solution were 359, 750 and 1042 g·m^{-2}·h^{-1}, respectively. However, at the same current density, the volume variation of sodium lactate solution was from 1.42 to 1.64 times greater than that of the concentrated solution.

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(1) Water flux: Average of the absolute volume variation due to water transfer into concentrated compartment, gram per unit cell pair surface area and time (g·m^{-2}·h^{-1})

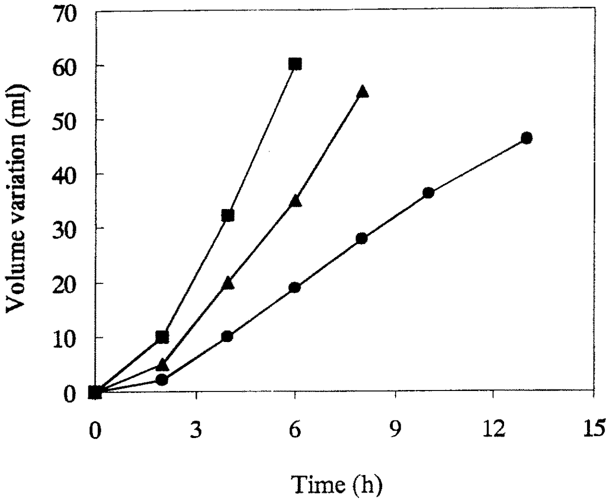

Fig. 4. Volume variation of sodium lactate solution as a function of time at different current densities; symbols: (●), 33 A·m^{-2}; (▲), 66 A·m^{-2}; (■), 100 A·m^{-2}.

Effect of initial concentration of sodium lactate on the lactic acid recovery

The influence of the initial concentration of sodium lactate was investigated in three different values: 40, 60 and 86.4 *g/l*. The main reason for this choice was the final lactate concentrations in most of fermentation systems reported so far are lower than 90 *g/l* (Hofvendahl and Hahn–Hägerdal 2000). The effect of the initial sodium lactate concentration on the LA recovery is shown in Figure 5. The data obtained showed that there was no influence of the initial concentration of sodium lactate. It can be seen from this figure that all experimental points representing the LA recovered lay almost around a straight line and all lines representing sodium lactate converted are parallel (with the same slope). This means both the LA recovery rate and converted lactate rate did not change. However, higher initial concentration of sodium lactate caused longer time process. In all runs, the LA recovery rates were approximately 93–96%. The volume variation of the concentrated solution was investigated and it was found that the initial concentration of sodium lactate had no influence on the water fluxes (data not shown).

Electrodialysis of fermentation broth

The electrodialysis of real fermentation broth was conducted to compare with the observations made using above model solutions. The results obtained by using electrodialysis to recover LA from real fermentation broth are reported in Table 1 and shown in Figure 6. During the three first hours, the lactate recovery rate was almost maintained the same value comparing with that obtained in model solution. However, after that the recovery rate was significantly decreased and the overall recovery efficiency fell from a 93–96% range, observed in model solutions, to 71% (Table 1). The process time in the

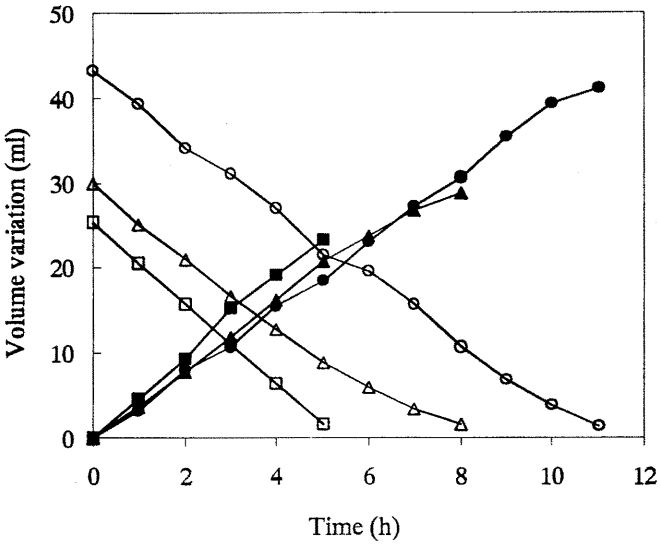

Fig. 5. Time course of the recovery of sodium lactate by electrodialysis at the same current density (100 A·m^{-2}); symbols: (●, ○), initial concentration of 86.4 g/*l*; (▲, △), initial concentration of 60 g/*l*; (■, □), initial concentration of 40 g/*l*. Closed symbols: LA recovered. Open symbols: LA equivalent (salt form).

constant–current mode was 6 h, similar to that observed in model solution, but the electrodialysis was only operated in the constant–current mode until the sodium lactate concentration in diluting compartment reached the level of 13 g/*l*. The reason could be that the presence of complex compounds in the broth affecting the electrodialysis. It was found that some electrical charge compounds (e.g. proteins), adhered to anion–exchange membrane. This would limit the transport of lactate ion through the membrane. The water flux of 679 g·m^{-2}·h^{-1}, was not significantly different from the value of 667 g·m^{-2}·h^{-1}, obtained in model solution. The LA recovery was practically finished within 6 h.

Unlike many previous electrodialysis units, in this electrodialysis system LA was formed by indirect reaction between sodium lactate and H_2SO_4. Vonktaveesuk *et al.* (1994) studied fermentation system using electrodialysis unit to remove lactate from broth, however, the final product was sodium lactate not LA. Earlier, Hongo *et al.* (1986) and Nomura *et al.* (1988) used electrodialysis to control the fermentation and to remove directly LA from broth but it was very difficult to maintain pH stable for long time. Moreover, it was found that the rate of LA production was greatly improved by converting LA produced to sodium lactate and continuously removing this salt from the fermentation broth. In addition, they could not maintain the optimum pH of the broth by the electrodialysis of sodium lactate (Yao and Toda, 1990). The reason could be that the LA production rate overcame conversion capability of the electrodialyzer. Therefore the system reported in this study possesses some advantages over others reported before, because

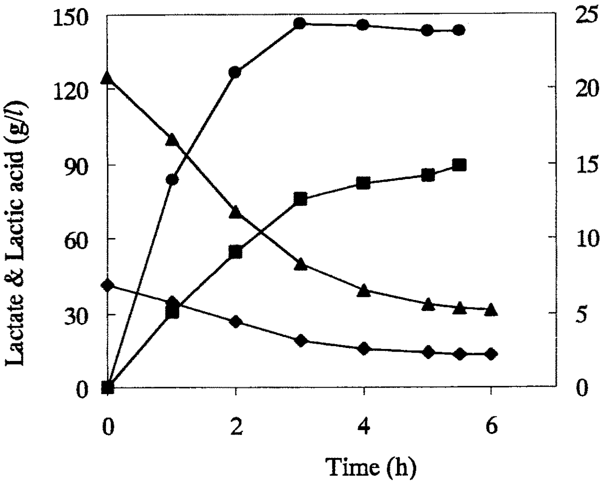

Fig. 6. Time course of the LA recovery from fermentation broth by electrodialysis at current density of 100 A·m^{-2}; 41.8 g/*l* initial lactate concentration in broth. Symbol: (●), LA in the concentrated solution; (■), LA recovered; (▲), Sodium lactate consentration in broth; (◆), residual lactate in the broth.

Table 1. Comparison of LA recovery by electrodialysis at different conditions

Exp.	Current density, (A·m^{-2})	Initial feed conc., (g/*l*)	Switching conc., (g/*l*)	Operating time, (h)	Final LA conc. recovered, (g/*l*)	LA recovery efficiency, %	Water flux, (g·m^{-2}·h^{-1})	LA recovery rate, (g·m^{-2}·h^{-1})
1	100	40	4.3	6	222.6	96.84	667	272
2	100	60	4.1	9	193.7	97.07	–	–
3	100	86.4	4.4	11	210.5	95.28	–	–
4*	100	41.8	13.1	6	146.0	70.89	679	279.5
5	66	40	2.4	8	210.3	94.56	458	175
6	33	40	0.8	19	196.9	96.87	235	76

* Experiment with real fermentation broth. LA=lactic acid; conc.=concentration.

the possibility to obtain directly the final product as LA, would reduce the cost of the downstream process and would greatly enhance the competitiveness of fermentative lactic acid fermentation.

In this study, the capability of converting sodium lactate into LA by using electrodialysis was investigated. From the results obtained, it is possible to design a suitable electrodialyzer so that its capability can correspond to the productivity of fermentation system. Studies to solve the problem of fouling anion–exchange membrane and to completely make a continuous fermentation system with product recovery by electrodialysis are in progress.

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