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Separation of Fermentation Products by Membrane Techniques. Part III. Continuous Isolation of Lactic Acid by Facilitated Membrane Extraction

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ABSTRACT

Facilitated membrane extraction (FME) has been examined for the continuous isolation of lactic acid from its dilute solutions. The technique is intended for continuous fermentation technology, with the pH of a broth regulated by extraction of the acid. Standard dialysis membranes and a conventional diffusion dialyzer were the equipment used for carrying out the separation. By FME, slow diffusional transport across a membrane is accelerated by the neutralization of an acid in the receiving side of a dialyzer. By using this technique the fluxes of lactic acid were found to be 0.7–1.3 mol·m^{-2}·h^{-1} (pH 11.7–12.8) against 0.013–0.015 mol·m^{-2}·h^{-1} reported for conventional diffusion dialysis (the same feed, c_{acid} = 0.01 mol·dm^{-3}). The accumulation of lactate in a permeate established experimentally was 0.5–0.6 mol·dm^{-3}. The concentration of lactic acid left in a feed after extraction was 10^{-3}–10^{-4} mol·dm^{-3}. FME integrated with electrodialysis seems to offer an effective separation system for continuous fermentation technology.

INTRODUCTION

In a conventional technology of lactic acid production (LA) by batch fermentation, the broth is continuously or periodically treated with alkali to keep the acidity optimal for living cells, i.e., at pH 5 to 6. The need for neutralization results from the toxicity of the partly dissociated acid molecules for bacteria.

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Cell growth for *Lactobacillus delbruckii* becomes inhibited at a free lactic acid concentration as low as 1.3 mM (1). Yet, lactates (calcium, sodium, ammonium) also inhibit fermentation at higher concentrations. Thus, continuous separation of the product seems to be a solution to the problem. In this respect, the recovery of lactate by membrane ultrafiltration integrated with electrodialysis for the conversion of lactate to lactic acid has been recommended and well described (2–9).

A different approach is required for continuous fermentation processed without pH adjustment. In this technology the removal of lactic acid, instead of neutralization, will be the method for keeping the pH of the broth admissible for bacteria. The technique designed for isolating the acid in situ should be effective in a low or very low acid concentration. Considering the complex content of the broth containing viable cells, untransformed carbohydrates, nutrients, and low molecular weight inorganic supplements, it would be desirable to have the technique selective for isolating the acid only, leaving all the other compounds in a retentate.

In this paper we present facilitated membrane extraction (FME) for the continuous isolation of lactic acid in situ. Despite "extraction" in the name of the technique, neither a liquid phase nor a soluble carrier is involved. In practice, the term denotes the transport of lactic acid through a diffusion dialysis membrane to a permeate of controlled alkalinity. On the molecular level, this is the diffusional permeation of lactic acid across a membrane combined with a fast irreversible reaction of a permeant on the receiving side of a dialyzer.

The diversity of membrane diffusion-reaction systems has been classified by Selegny (13). The theory for FME with ion-exchange membranes as the separating barriers has been worked out by Langevin et al. (14). The authors proved that the velocity of diffusional permeation facilitated by reaction could be 80–200 times that of passive diffusion.

A membrane system named "neutralization diffusion" was described by Bleha (10). He used this technique for the desalination of water by double neutralization of the cations and anions removed. Another system known as "Poźniak dialysis" has been published in Ref. 11. This technique was intended for the deacidification or dealkalization of industrial wastes.

In a preceding paper (12) we presented the results for the separation of lactic acid (LA) from sodium lactate (NaLA) as carried out with a solution containing both solutes in concentrations like those in a broth produced by batch fermentation. The diffusion dialysis membranes Neosepta AFN and Selemion DSV were used for the separation. The separation factor found was ~20 for the Selemion DSV membrane and ~30 for the Neosepta AFN membrane. Despite satisfying results obtained for the separation, the rate of lactic acid permeation, particularly from a solution of low concentration, was

found to be only 0.013–0.015 mol·m^{-2}·h^{-1}, which is much below the level of technical interest.

Here we present a solution to the problem.

EXPERIMENTAL

Membranes

Two diffusion dialysis anion-exchange membranes, Neosepta AFN-7 by Tokuyama Co., Japan and Selemion DSV by Asahi Glass Co., Japan, were used for our experiments. The characteristics of the membranes were published in Ref. 12. Both membranes contain quaternary ammonium functional groups, with some amount of weak base groups in Neosepta. The total capacity and water content of membranes used were:

Neosepta AFN-7: 3.24 mmol/g, 0.527 g/g

Selemion DSV: 2.29 mmol/g, 0.359 g/g (standard Cl^- form)

The high capacity and swelling make the Neosepta membrane easily permeable for diffusing solutes.

Experimental Setup

The laboratory diffusion dialyzer and the experimental setup (Fig. 1) were described in detail in Part I (12). For carrying out the FME, the pH controller

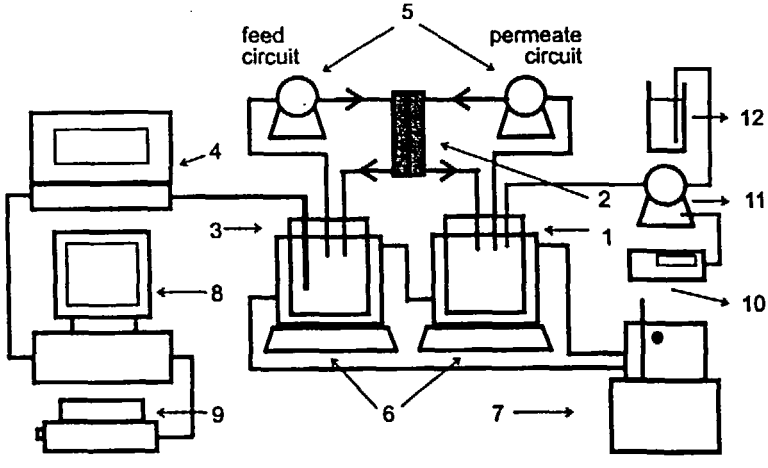

FIG. 1 The experimental arrangement for facilitated membrane extraction: (1) permeate tank, (2) diffusion dialyzer, (3) feed tank, (4) conductivity meter, (5) pump, (6) magnetic stirrers, (7) thermostat, (8) computer, (9) printer, (10) pH controller, (11) metering pump, (12) base tank.

(Cole-Parmer, USA) was incorporated into the loop of the receiving side of a dialyzer to maintain a constant alkalinity of a permeate. The concentration of the injected sodium hydroxide was ~1.5 mol·dm^{-3}.

The membrane extraction was carried out with a solution of lactic acid with a concentration of 0.01 mol·dm^{-3} (POCh, Gliwice, Poland), and the pH of the permeate side of the dialyzer was 11.7–12.8. Final experiments aimed at testing the technique for the continuous isolation of lactic acid were performed with real broth from "Akwawit," Leszno, Poland.

Analysis

The concentration of lactic acid in a feed was measured by conductometry (Elmetron CX-721, Zabrze-Mikulczyce, Poland). The concentration of lactate in the alkalized permeate was determined by capillary electrophoresis (EA-100, Labeco, Slovakia).

RESULTS AND DISCUSSION

Facilitated Membrane Extraction of Lactic Acid

Facilitated membrane extraction was tested as a technique for the isolation of lactic acid from fermenting broths, i.e., from media with a low concentration of the acid (Table 1).

In the experiments presented here, the starting molarity of lactic acid was 0.01 mol·dm^{-3}. At that molarity the diffusional flux of lactic acid to water was found to be 0.015 mol·m^{-2}·h^{-1} with the Neosepta AFN-7 membrane and 0.013 mol·m^{-2}·h^{-1} with the Selemion DSV membrane (12).

It is known that slow, downhill transport can be accelerated by complexation, precipitation, or neutralization of a diffusant on the permeate side of a membrane, and that the reaction enables accumulation of the diffusant over its concentration in a feed. Here, neutralization was the reaction used for facilitating acid permeation.

The pH effect on the rate of extraction of LA is seen in Figs. 2 and 3(a). In Figs. 2 and 3(b) the fluxes of lactic acid are drawn against the concentration

TABLE 1
Concentration of Free Lactic Acid at pH 5–6 in a Solution of Total Sodium Lactate and Lactic Acid Concentration 0.2 mol·dm^{-3}

pH	Concentration of LA (mol·dm^{-3})
5.0	0.0145
5.5	0.0045
6.0	0.0014

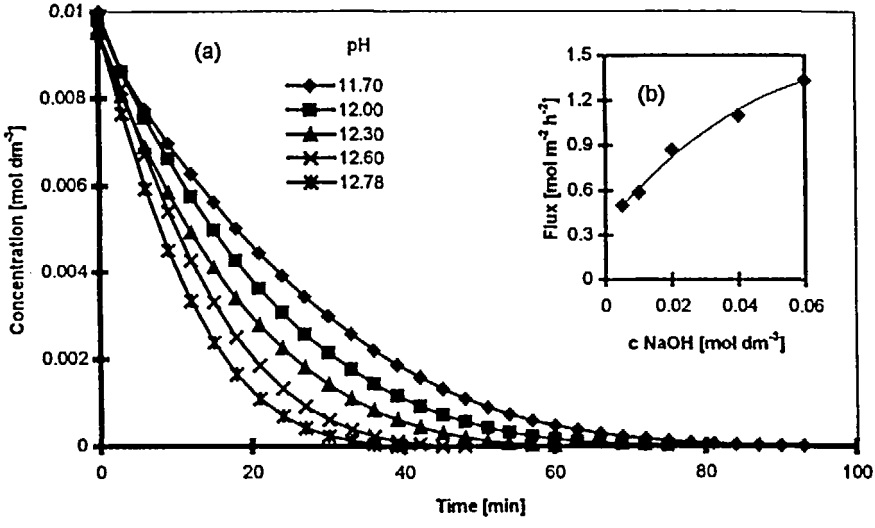

FIG. 2 Effect of alkalinity of the permeate side in a dialyzer on the rate of extraction of lactic acid with the Neosepta AFN-7 membrane: (a) kinetics of extraction, (b) effect of alkalinity on the fluxes.

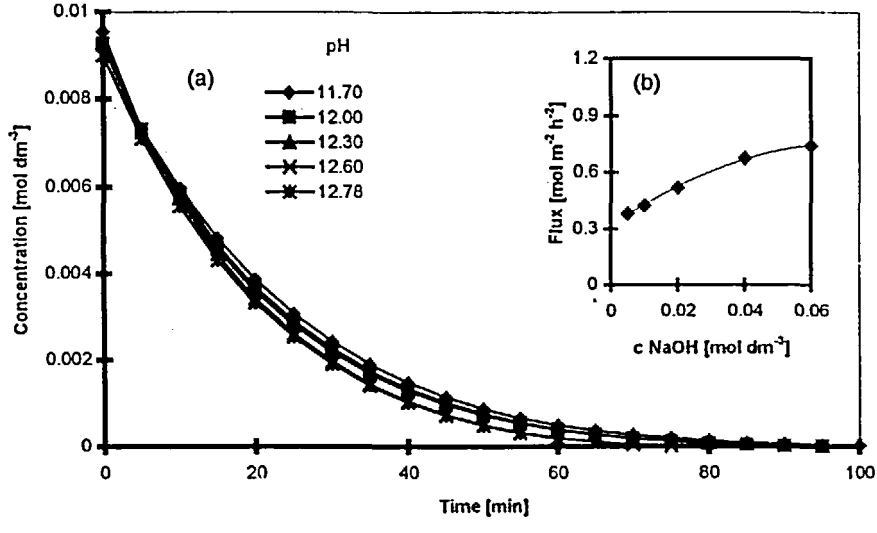

FIG. 3 Effect of alkalinity of the permeate side in a dialyzer on the rate of extraction of lactic acid with the Selemion DSV membrane: (a) kinetics of extraction, (b) effect of alkalinity on the fluxes.

TABLE 2
Effect of the pH of a Permeate Side on Transport Rate of LA

	Flux of LA ($mol \cdot m^{-2} \cdot h^{-1}$)	
	To water	To alkalized permeate; pH 12.8
Neosepta AFN-7	0.015	1.3
Selemion DSV	0.013	0.8

of sodium hydroxide in the receiver. The figures present the results with the Neosepta AFN-7 and Selemion DSV membranes. A drastic shortening of the extraction time is seen for both membranes.

Also common for both membranes is the residual acid concentration in the feed. Beginning from 0.01 $mol \cdot dm^{-3}$, the concentration of LA at the end of the experiment was as low as $3–4 \times 10^{-4}$ $mol \cdot dm^{-3}$.

The fluxes of lactic acid and the effect of pH on the rates of acid permeation differ for the two membranes (Table 2). For the Selemion DSV membrane, the fluxes are not affected much by the pH of the permeate. On the other hand, the pH-dependent transport rate of the Neosepta AFN-7 membrane makes it possible to adjust the alkalinity of the receiver to the rate of fermentation, so the alkalinity of the permeate could be one more variable for controlling the system. A different effect of pH on the rate of acid transport can be ascribed to the differences in the membrane capacities and the water content. The high capacity and high water content of Neosepta AFN-7 cause the internal membrane phase of this membrane to be easily permeable. It is possible, however, that calculations based on the theory of membrane extraction will add to our present understanding of the phenomena.

Backdiffusion of Lactate

After some time, an increasing concentration of lactate in a permeate becomes a driving force for two effects: backdiffusion and osmosis, both of which limit the molarity of a product. Experiments performed with single permeant solutions (12) proved that the fluxes of lactate are always much lower than those of the acid. In the medium concentration range, the fluxes of sodium lactate are 20 to 30 times lower than the fluxes of lactic acid. Thus, despite the common effect of backdiffusion, the rate of lactate permeation back to a feed will be rather slow.

Osmotic Limitation of the Concentration of Lactate

A low resistance of a membrane against the transport of water has been documented (15, 16). Here, the osmotic fluxes were measured using a system

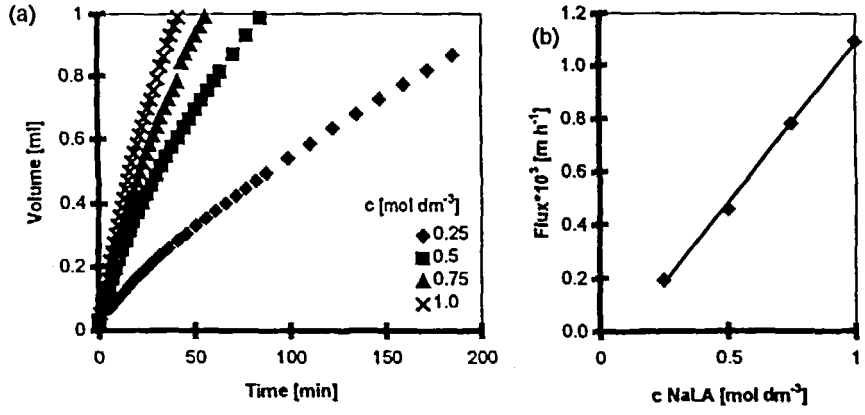

FIG. 4 Osmotic flux of water toward sodium lactate with Neosepta AFN-7 membrane: (a) volume of permeating water versus time, (b) flux of water against molarity of NaLA.

with a membrane separating pure water and sodium lactate of molarities 0.25 to 1.0. Figures 4 and 5(a) display the volume of the permeating water against time, and Figs. 4 and 5(b) the fluxes of water versus the concentration of sodium lactate. For both membranes the water fluxes, which range from a few up to several dozens of moles per square meter per hour, greatly exceed

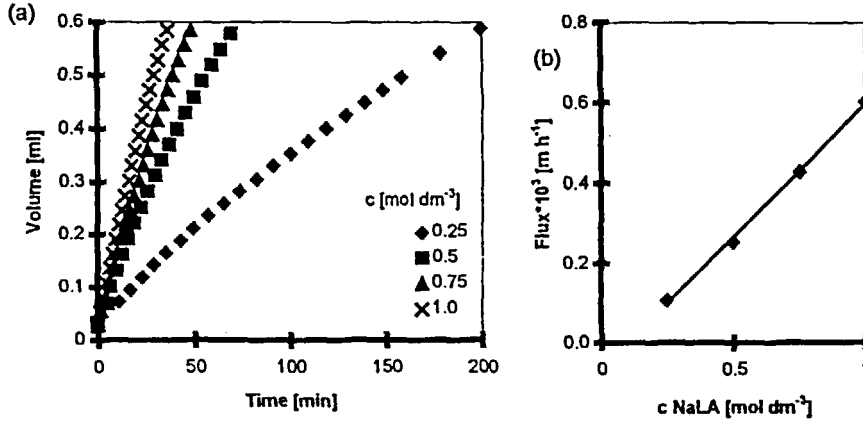

FIG. 5 Osmotic flux of water toward sodium lactate with Selemion DSV membrane: (a) volume of permeating water versus time, (b) flux of water against molarity of NaLA.

the lactic acid fluxes. For the Neosepta AFN-7 membrane the effect is rather drastic; for the Selemion DSV membrane the osmotic flow is not as high, but it is still quite significant.

We measured the osmotic fluxes for the most unfavorable system by having pure water on one side of the membrane. For a broth (instead of water) containing a low molecular weight substrate and inorganic ingredients, the differences in the chemical potential of water on the opposite membrane side will be lower, thus lowering the flux of water. Nonetheless, osmotic dilution of lactate in a receiver will occur. The conclusion drawn from the results in Figs. 4 and 5 is that the dilution of lactate by the osmotic transport of water will cause, after some time, any further operation of the system to be ineffective.

To find the limit of the concentration of lactate in a receiver (c_{max}), we approached the problem semiempirically. Starting from the equation for the maximum concentration of lactate written as a limit:

$$dc/dt = 0 \tag{1}$$

and accounting for the effects produced by both fluxes to the concentration of lactate, that is, the fluxes of acid ($J_a = dn/dt$) and water ($J_w = dV/dt$), by differentiating Eq. (1) we got the following equations:

$$\frac{dc}{dt} = \frac{d(n/V)}{dt} = \frac{VJ_a - nJ_w}{V^2} \tag{2}$$

and

$$\frac{n}{V} = \frac{J_a}{J_w} \tag{3}$$

where n = moles of lactate and V = volume of a permeate.

By taking the experimental results for the osmotic fluxes (Figs. 4 and 5) and limiting computations to the linear relation among the variables, it was possible to find the constant a in the relation between water flux and the concentration, written as $J_w = ac$. Thus, the final form for the equation for c_{max} is

$$c_{max} = \sqrt{J_a/a} \tag{4}$$

For the Neosepta AFN-7 membrane with $a = 1.1\ m^4 \cdot mol^{-1} \cdot h^{-1}$ and $J_a = 1.3\ mol \cdot m^{-2} \cdot h^{-1}$, the maximum concentration of lactate amounts to $1.08\ mol \cdot dm^{-3}$. For the Selemion DSV membrane the respective figures are $a = 0.6\ m^4 \cdot mol^{-1} \cdot h^{-1}$, $J_a = 0.8\ mol \cdot m^{-2} \cdot h^{-1}$, and $c_{max} = 1.15\ mol \cdot dm^{-3}$. Figure 6 presents the curve computed for the accumulation of lactate in a permeate versus time. This is certainly the simplified solution. A more precise solution for the system with the fluxes (vector) and the reaction (scalar) is rather

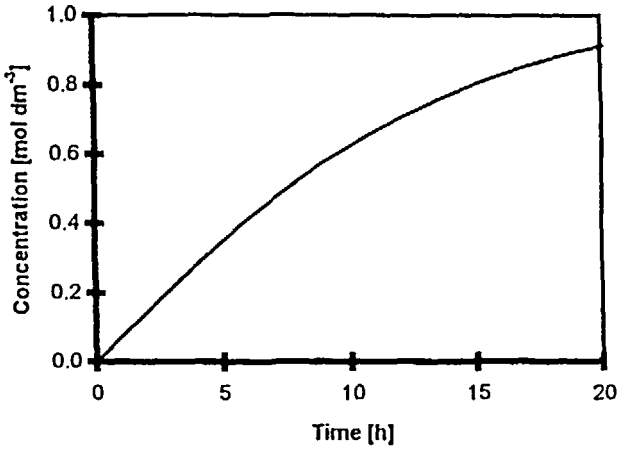

FIG. 6 Accumulation of sodium lactate in the permeate side of a dialyzer. Computed curve (Eq. 2) (assumed alkalinity of a permeate 12.78; $S_{membrane}$ = 50 cm^2).

complex. The limit for the concentration of sodium lactate, found experimentally, was 0.5–0.6 mol·dm^{-3}, whereas the one predicted by computation was ~1 mol·dm^{-3}. The concentration found by computations may roughly be that one expected in a system with a permeate in contact with real broth instead of with water.

Continuous Isolation of Lactic Acid from a Broth

In order to find a technique for the continuous isolation of lactic acid from a broth, more experiments were performed. A full content fermenting broth was obtained from "Akwawit," Leszno, Poland.

After sedimentation of the largest particles, the broth was diluted and allowed to circulate in the loop of a dialyzer as a feed (the full content of the broth is confidential). For the entire time of operation the concentration of the acid in the broth was kept constant by continuous completion of the acid, thus simulating production of the acid by bacteria. The flux of lactic acid and the effect of fouling of the membrane surface were controlled. At a lactic acid content in the broth of about 0.01 mol·dm^{-3} (intentionally low) and a permeate alkalinity of pH 13, the flux of lactic acid was 0.7 mol·m^{-2}·h^{-1} for a long operation time.

To check for membrane surface blocking by bacteria or by particles charged oppositely to the polymer, a sample of membrane was placed overnight into full content broth. Then the membrane was washed out gently with a small volume of distilled water and checked for the presence of any sticking mate-

rial. The membrane surface appeared clear and did not look as if any adsorption had occurred. This experiment examined the response of bacteria to the chemistry of the membrane.

After about 30 hours of continuous extraction a white precipitate, soluble in acid, appeared. The precipitate was calcium carbonate used at the factory for adjusting the pH of the broth. The use of sodium hydroxide instead of calcium carbonate would eliminate the precipitate.

Both experiments should be treated as preliminary. More experiments should be performed with a membrane contacting with fermenting broth for a detailed examination of fouling.

CONCLUSIONS

- Facilitated membrane extraction (FME) was proven to be effective for the continuous isolation of lactic acid from dilute solutions characteristic of a fermenting broth. The diffusion dialysis membranes used for the separation are impermeable to high molecular weight components, whereas the rates of permeation of low molecular weight organic and inorganic salts are much below the rate of the acid. By using this technique, continuous treatment of the broth with alkalies becomes possible.
- A diffusion dialyzer is the proper equipment for carrying out FME.
- The flux of the acid extracted in a permeate at pH 11.7–12.8 was found to range from 0.4 up to 1.3 $mol \cdot m^{-2} \cdot h^{-1}$. Yet, high alkalinity on the receiving side of a dialyzer should be avoided.
- The concentration of sodium lactate in a permeate is limited by an osmotic flow of water. However, getting the concentration up to 1.0 $mol \cdot dm^{-3}$ seems possible. The concentration of lactic acid left in a feed could be as low as 10^{-3}–10^{-4} $mol \cdot dm^{-3}$.
- No energy is consumed for separation by FME except the energy for pumping. By integrating a dialyzer with the electrodialysis unit (for the backconversion of lactate to lactic acid), the sodium hydroxide solution formed during electrodialysis in the cathode compartment can be used for the alkalization of a permeate in the dialyzer. In such an operation, sodium hydroxide will be circulating in the Dd-ED loop, thus enhancing the economy of the system.

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