ALTERNATIVE REVERSE OSMOSIS TO PURIFY LACTIC ACID FROM A FERMENTATION BROTH

Article Highlights
- Reverse osmosis was used to recover and purify lactic acid from fermentation broth
- Cation rejection occurred at the first BWRO unit, where lactic acid was separated
- Concentration of lactic acid occurred at the second SWRO unit
- Sufficiently high lactate purity and recovery was obtained from two-stage RO units
- Donnan potential, pH and operating pressure-controlled ion rejection

Abstract
Brackish water reverse osmosis (BWRO) and seawater reverse osmosis (SWRO) membranes were used in a two-stage reverse osmosis (RO) unit to recover, pre-purify, and pre-concentrate lactic acid. Calcium lactate, sodium lactate, and ammonium lactate were used as model feed solutions. The operating pressure showed a pronounced effect on lactate passage through the first BWRO unit, and the Donnan exclusion effect and hydrogen bonding were responsible for cation rejection. Calcium ions were rejected at the BWRO unit because of low diffusion rate and charge interaction at the surface. However, monovalent ions formed hydrogen bonds with the carbonyl group of the membrane that allowed passage across the membrane. The second SWRO unit was for pre-concentrating lactic acid. A high lactate purity of 99.2% with a total recovery of 50.5% was acquired from calcium lactate feed solution. Lower purity with higher lactate recovery was obtained when the feed solution was sodium lactate and ammonium lactate. When the actual fermentation broth was used in the two-stage RO unit, a slightly lower recovery and purity of lactic acid were obtained. It was claimed that the total ions present in the fermentation broth were responsible for the low efficiency of the two-stage RO unit.

Keywords: lactic acid; fermentation broth; reverse osmosis; Donnan exclusion effect; ionic strength.

Owing to its chiral structure, consisting of both carboxyl and hydroxyl groups, lactic acid has been extensively used in the food, cosmetic, and pharmaceutical industries. To date, the market demand for lactic acid has increased considerably because of the global trend toward environmental protection that accelerated the supply of biodegradable plastics. Currently, bacterial fermentation is widely used in industrial lactic acid production. During production, the pH is typically decreased as the acid is produced, and the proper operating pH must be maintained at 5.5-6.5 [1]. pH control is carried out by the addition of bases such as calcium bases (CaCO₃ and Ca(OH)₂), NaOH and NH₄OH [2,3]. This generates 2 lactate species (free lactic acid and lactate salt) in the fer-
In the recovery and purification of lactic acid from the fermentation broth, many major process steps are involved: e.g., primary recovery, product purification and finishing processes [4]. The first step in primary recovery units involves cell separation by centrifugation or microfiltration [4-7]. Several downstream techniques have been used for recovering and separating lactic acid, including reactive distillation, reactive extraction, adsorption (ion exchange), and membrane separation (electrodialysis) [8]. Both distillation and electrodialysis have high energy consumption in a large-scale operation because of the low volatility of lactic acid and high electricity loading, respectively [9]. When charged compounds such as amino acids and other organic acids are present, the separation efficiency of electrodialysis decreases as a result of membrane fouling. Electrodialysis fails to reject divalent ions such as calcium and magnesium; therefore, it is not suitable for recovering lactic acid from the conventional calcium base fermentation process where lactate species are present in the form of calcium lactate and lactic acid [10,11]. In reactive extraction, toxicity of the solvent and final product contamination are usually of concern [12]. Using ion exchangers to recover lactic acid requires large amounts of chemicals, enzymes and process water during the resin regeneration and washing steps. This eventually generates a large effluent loading in wastewater treatment. In addition, precautions should be taken in the pretreatment steps before the feed enters the ion exchangers so as to avoid fouling and resin deterioration [13]. Purified lactic acid then enters the evaporator where water is removed, resulting in concentrated lactic acid as the finished product [14-16]. As previously mentioned, process integration is necessary in order to achieve good recovery and purification performance as well as cost effectiveness.

Membrane separation provides advantages of low energy consumption and low toxicity. Nevertheless, a single membrane unit cannot fulfill lactic acid recovery, purification, and concentration of lactic acid [7]. Typically, nanofiltration is used prior to the product finishing step (evaporation in case of lactic acid recovery and purification). Nanofiltration is applied for the removal of trace ions and small, neutral molecules from free lactic acid solution [14]. On the other hand, reverse osmosis (RO) is generally applied for the removal of water in previously published literature [7]. In this study, a two-stage RO membrane-based process was developed for recovering, purifying, and concentrating lactic acid from the model solution and the cell-free fermentation broth by applying the appropriate pressure higher than the osmotic pressure of the species of interest. From the principle of the RO process, solute transport occurs by diffusion through the membrane depending on molecular size and charge [17]. With smaller pore sizes than the nanofiltration membrane, the RO membrane provides high efficiency in the rejection of monovalent ions [18]. It was also reported that some trace organic compounds, such as neutral molecules smaller than the molecular weight cut-off of the membrane, leaked and passed through the membrane [19]. By applying an operating pressure sufficiently higher than the osmotic pressure of the molecules, such molecules can pass through the RO membrane. At the proper pH, free lactic acid passed across the first RO membrane to the permeate side whilst the cation salts remained in the retentate. The second RO unit was for pre-concentrating lactic acid solution. Compared with the other techniques mentioned previously, RO filtration did not require chemicals, enzymes and process water during the operation. The low volumetric rate of the exit stream from this two-stage RO unit resulted in size reduction of the evaporator and lower capital and operating expenditures.

**MATERIALS AND METHODS**

**Chemicals**

Calcium lactate (CaLAC), sodium lactate (NaLAC), and ammonium lactate (NH₄LAC), purchased from Sigma-Aldrich, were used in this study as model solutions. These chemicals were dissolved directly in deionized water to the specified concentration (5 g/L lactic acid equivalent). This equivalent mass concentration of lactic acid resulted in different pH values of the solution, e.g., 4, 4, and 9 for CaLAC, NaLAC, and NH₄LAC solutions, respectively. To obtain the specific tested pH at 4 and 6, the pH of the model solution was adjusted by 5 M NaOH or 1 M H₂SO₄. The concentrations of the lactate species at equilibrium (both free lactic acid and its salts) were dependent on the pH. The following stoichiometry describes the presence of lactate species mimicking those that appear in the fermentation processes.

When CaCO₃ was used for pH control during the fermentation, both lactic acid and calcium lactate were present in the solution:

\[2\text{CH}_2\text{CHOHCOOH} + \text{CaCO}_3 \rightleftharpoons (\text{CH}_2\text{CHOHCOO})_2\text{Ca} + \text{H}_2\text{O} + \text{CO}_2\]
When NaOH was used for pH control during the fermentation, both lactic acid and sodium lactate were present in the solution:
\[ \text{H}_3\text{CHOHCOOH} + \text{NaOH} \rightleftharpoons (\text{CH}_3\text{CHOHCOO})\text{Na} + \text{H}_2\text{O} \]

When NH₄OH was used for pH control during the fermentation, both lactic acid and ammonium lactate were present in the solution:
\[ \text{CH}_3\text{CHOHCOOH} + \text{NH}_4\text{OH} \rightleftharpoons (\text{CH}_3\text{CHOHCOO})\text{NH}_4 + \text{H}_2\text{O} \]

**Fermentation broth preparation**

Lactate fermentation broth was prepared from the cultivation of *Bacillus coagulans* BC-013 in a 5 L stirred fermenter. An active 24 h glucose-yeast extract-peptone slant was used to prepare the bacterial suspension. The bacterial suspension (1% inoculum size) was inoculated in a preculture flask containing the preculture medium. The preculture medium contained (per liter) 10 g glucose, 15 g yeast extract, 4 g NH₄Cl, 0.5 g KH₂PO₄, 0.5 g K₂HPO₄, 5 g CaCO₃ and 20 mL salt solution. The compositions of the salt solution consisted of (per 10 mL) 400 mg MgSO₄·7H₂O, 20 mg MnSO₄·5H₂O, 20 mg FeSO₄·7H₂O and 20 mg NaCl. The preculture flask was incubated at 50 °C, 200 rpm for 3 h. After that, the preculture flask was transferred into the 5 L stirred fermenter containing 2.5 L sterile preculture medium at 10% inoculum size. The fermenter was operated at 50 °C and agitated at 300 rpm with 1 vvm air. After 3 h, 0.5 L of the fermentation medium containing (per L) 720 g glucose was added into the fermenter. Aeration was then stopped. Three different bases, *i.e.*, CaCO₃, NaOH, and NH₄OH, were used for pH control at 6.5. As a result, 3 different lactate salts, *i.e.*, CaLAC, NaLAC, and NH₄LAC, were obtained in the fermentation. Fermentation was continued for 48 h until glucose depletion. Next, the fermentation broth was harvested.

Cell biomass and soluble, neutral macromolecules such as proteins, sugars, etc. were removed from the fermentation broth by microfiltration and ultrafiltration. The cell-free broth obtained was later used in the two-stage RO unit.

**Designing and setting up the RO unit**

An in-house RO unit was constructed by a local Thai company (Icrotech Co., Ltd.) for use in this study. The apparatus set-up is illustrated in Figure 1.

Two RO membrane filtration units were subsequently connected with auxiliary instruments, including boost pumps, pressure gauges, flowmeters for the feed, concentrate and permeate, valves and storage vessels. Negatively charged brackish water RO (BWRO) elements (DOW FILMTEC™ BW60-1812-75) were installed at the first RO unit for recovering lactate from the model solution while allowing some salts to pass through the membrane at a rejection percentage of 97-99% (Table 1). In the second RO unit, positively charged seawater RO (SWRO) elements (DOW FILMTEC™ SW30-2521) were installed for pre-concentrating recovered lactate obtained from the first RO unit where water was expelled. The rejection percentage was higher than 99.4% (Table 1). As a result, lactate was passed through the BWRO unit to the permeate side and later entered the SWRO unit. Lactic acid was then concentrated in the following SWRO unit and remained in the retentate.

Batch operation was used in the first RO unit where the feed solution entered the unit and the permeate discharged from the first unit to enter the second RO unit later on. In the second RO unit, the retentate was recycled so that most of the remaining water in the retentate was discharged into the permeate. The maximum operating pressure for the BWRO and SWRO units was set at 6 and 15 bar, respectively, owing to the pressure limit of apparatus housing, pumps, and piping systems.

![Figure 1. Schematic diagram of the in-house RO unit for recovery and purification of lactic acid.](image-url)
Determining the operating conditions

To utilize the principle of RO in the recovery of lactic acid, osmotic pressure was introduced as a key factor determining the process conditions. The operating pressure was adjusted over the osmotic pressure of lactic acid so that lactic acid could pass through the BWRO membrane to the permeate, with other fermentation impurities remaining in the retentate. Eq. (1) expresses osmotic pressure as a function of molarity and temperature:

$$\pi = iMRT$$

where \(\pi\) is the osmotic pressure (bar), \(i\) is the dimensionless van't Hoff factor, \(M\) is the molar concentration of the solution/species of interest (in this case, lactic acid and its salts), \(R\) is the gas constant (0.082 L-bar/(K·mol)) and \(T\) is the temperature in K.

From Eq. (1), the osmotic pressures of the 3 model solutions, \(e.g.,\) CaLAC, NaLAC, and NH₄LAC with 5 g/L lactate equivalent to be studied are 2.12 bar, 2.82 and 2.81 bar, respectively. The osmotic pressure of lactic acid solution at 5 g/L is 1.41 bar.

The effects of pH and operating pressure on lactate separation efficiency at the BWRO unit were investigated. The lactate model solution (2 L) at 5 g/L LAC equivalent was adjusted to the tested pH values of 4 and 6 using either NaOH or H₂SO₄. The operating pressure was varied at 4 and 6 bar. Further increasing the concentration of the lactate model solution to higher than 5 g/L LAC equivalent resulted in higher osmotic pressure that exceeded the maximum pressure threshold in the BWRO apparatus (7 bar) and consequently led to reduced mass flux and separation efficiency. Therefore, the tested concentration of the model solution was limited at 5 g/L LAC equivalent.

The model solution (2 L) in the feed tank was fed into the first BWRO unit where free lactic acid was supposed to be separated from other impurity species, including Ca²⁺, Na⁺, NH₄⁺ and SO₄²⁻ (in case the model solution was adjusted to the desired pH by H₂SO₄). The operating temperature was set at 30 °C. The apparatus was run until the collected volume of the permeate of 1.6 L was obtained. Samples (20 mL) were periodically collected from both permeate and retentate for analyses of free lactic acid concentration and ion species.

The permeate that left the BWRO unit and collected in the SWRO feed tank (1.6 L) was passed through the SWRO unit where water separation occurred, which resulted in lactate concentration in the retentate. The operating temperature was also set at 30 °C. The effects of pH and pressure on separation efficiency were determined. The tested pressure was set at 13 and 15 bar. At the first 5 min of operation, the retentate was recycled into the SWRO feed tank. After the recycling was stopped, the operation was continued until the permeate flux became zero. Samples (20 mL) were periodically collected for analyses of lactic acid and all the major remaining impurities.

**Sample analyses**

During the runs, samples from the permeate and retentate obtained in each RO unit were collected periodically for determining discharge volume, measuring pH and analyzing substances that remained. For ion analyses, the collected samples were analyzed for concentrations of lactic acid and major impurity ions, including Ca²⁺, Na⁺, N (representing NH₄⁺), SO₄²⁻, Cl⁻, P and Mg²⁺.

L-lactate ion in the sample was analyzed with a glucose–lactate analyzer (YSI2700, Yellow Spring Instruments Inc.) within the detection range of 0-2.67 g/L. The sample size of 25 μL was automatically injected into the reaction chamber where the enzymatic reaction occurred. The reading of L-lactate concentration was explained by the action of L-lactate oxidase immobilized at the membrane sensor.

An atomic absorption spectrophotometer was used to determine the metal concentration, including Ca²⁺ and Na⁺, in the sample. The sample was prepared by dilution with 5 vol.% HNO₃ solution. An air flame of 13.60 L/min along with an acetylene flame of 2 L/min was used for metal atomization of the sample.
before reading the atomic absorption with the atomic absorption spectrophotometer (AA280FS, Varian, Inc.). Aqueous standard solutions were prepared by dilution to appropriate concentrations (2, 4, 6, and 8 mg/L for Ca²⁺, and 5, 10, 20 and 30 mg/L for Na⁺). The concentration of Ca²⁺ and Na⁺ in the sample was calculated by comparing the spectra with the standard calibration curves.

Nitrogen content was determined by the total Kjeldahl nitrogen technique. Nitrogen in the sample was first converted to NH₃ by metal-catalyzed acid digestion. The resulting NH₃ was separated from the sample by distillation. The released NH₃ was captured in a diluted H₂SO₄ solution. The result represented organic nitrogen after digestion and distillation in the sample. The digestion reagent (catalyst) was prepared by mixing 134 g K₂SO₄ and 7.3 g CuSO₄ in 134 mL concentrated H₂SO₄. After that, the volume was made up to 1 L. Digestion reagent (50 mL) was added into the sample, and digestion proceeded for 30 min (Buchi, K499). Later, 50 mL boric acid was added into the reaction mixture as the absorbent solution during NH₃ distillation (Buchi, K350). Finally, NH₃ was determined by titration with a standard solution (Buchi, K350) [20].

Chloride was analyzed by the potentiometric method. The solubilized chloride ion in the sample was measured by a chloride ion-selective electrode during titration (Orion 720A, Labx Inc.). The sample was mixed with concentrated HNO₃ before dilution to the proper concentration. Titration was performed with a standard AgNO₃ solution as the reference.

Phosphorus was determined by the total phosphorus method using persulfate digestion. The sample (50 mL) was mixed with 11 N H₂SO₄ (1 mL). Next, dissolved and particle phosphorus in the sample was digested with (NH₄)₂S₂O₈ (0.4 g) to convert phosphorus into orthophosphate (mixed and boiled to obtain a final volume of 40 mL). The orthophosphate concentration was measured by a spectrophotometer (Nova Spec 2, Pharmacia Biotech, Inc.) using a standard calibration curve. The calibration curve was prepared from a standard phosphorus solution (0.3–1.2 mg P/L).

Sulfate ion in the sample was determined by the turbidimetric method. Sulfate ion present in the sample was converted into a BaSO₄ suspension under controlled conditions. The sample (80 mL) was mixed with 20 mL buffer solution containing (per liter) 30 g MgCl₂·6H₂O, 5 g CH₃COONa·3H₂O, 1 g KNO₃ and 20 mL acetic acid (99%). Then, BaCl₂ was added into the reaction mixture to obtain BaSO₄ precipitate. The turbidity was measured by a spectrophotometer (2100P, HACH). The concentration was determined using the calibration curve of the standard sulfate solution [20].

**Investigating the performance of the two-stage RO unit**

The performance of the two-stage RO unit was evaluated using 6 criteria: mass flux of lactic acid, lactic acid separation, ion separation, lactic acid recovery, overall recovery, and purity.

The mass flux of lactic acid ($J_{LA}$) at the BWRO unit was calculated by the following equation:

$$J_{LA} = \frac{m_{LA,BWP}}{At}$$  \hspace{1cm} (2)

where $m_{LA,BWP}$ is the lactic acid mass (g) passing through the membrane, A is effective membrane surface area (m²), and t is time (h).

The efficiency of the BWRO unit to separate lactic acid from other ions can be explained by lactic acid separation ($S_{LA}$) in percentage defined by Eq. (3):

$$S_{LA} = \frac{100m_{LA,BWP}}{F_{LA}}$$  \hspace{1cm} (3)

where $m_{LA,BWP}$ is the lactic acid mass (g) passing through the BWRO unit and $F_{LA}$ is the initial mass of lactic acid present in the feed solution (g).

The ion (i) leakage at the BWRO unit can be described by the separation percentage ($S_{i}$), as seen in Eq. (4):

$$S_{i} = \frac{100m_{i,BWP}}{F_{i}}$$  \hspace{1cm} (4)

where $m_{i,BWP}$ is the mass of ion i (g) moving through the BWRO unit and $F_{i}$ is the initial mass of the ion (g) present in the feed solution.

The efficiency of the SWRO unit to pre-concentrate lactic acid can be represented by lactic acid recovery in percentage ($R_{LA}$) described by Eq. (5):

$$R_{LA} = 100 \frac{C_{LA,SWF} - C_{LA,SWP}}{C_{LA,SWF}}$$  \hspace{1cm} (5)

where $C_{LA,SWF}$ and $C_{LA,SWP}$ are lactic acid concentrations (g/L) present in the feed solution entering the SWRO unit and the permeate leaving the SWRO unit.

The overall recovery ($R_{overall}$) of lactic acid product obtained from the two-stage RO unit can be described by Eq. (6):

$$R_{overall} = 100S_{LA}R_{LA}$$  \hspace{1cm} (6)

where $S_{LA}$ and $R_{LA}$ were defined from Eqs. (3) and (5), respectively.
The purity of lactic acid product \((P_{LA})\) obtained from the two-stage RO unit can be described by the mass ratio of lactic acid and the total ions that remained in the retentate of the SWRO unit (Eq. (7)).

\[
P_{LA} = \frac{100m_{LA,SWR}}{m_{T,SWR}}
\]  

where \(m_{LA,SWR}\) and \(m_{T,SWR}\) represent the mass of lactic acid product and the total mass of ions that remained in the retentate leaving the SWRO unit.

**RESULTS AND DISCUSSION**

**Lactate separation at the BWRO unit**

A feed solution of different lactate salts, including CaLAC, NaLAC, and NH\(_4\)LAC, was prepared at an equivalent lactate concentration of 5 g/L. To obtain the specific tested pH at 4 and 6, NaOH and H\(_2\)SO\(_4\) were added into the solution to adjust to the desired tested pH, which eventually resulted in changes in the molar concentration of chemical ions present in the model solutions (Table 2).

The obtained model solutions were then transferred into the feed tank to be pumped into the BWRO unit at different operating pressures (4 and 6 bar) where lactic acid presumably passed through, whereas the other cations remained in the retentate. Figure 2 shows the lactate mass flux passing through the BWRO membrane. The lactate mass flux increased with increasing operating pressure. It appears that the pH strongly influenced lactate transport through the BWRO membrane for CaLAC and NaLAC. On the other hand, pH showed less effect on lactate transport when compared with the operating pressure for NH\(_4\)LAC.

The separation efficiency of the BWRO unit is expressed by Eqs. (3) and (4) and displayed in Figure 3. It was observed that the operating pressure exhibited a strong effect on lactate separation in the BWRO unit. High lactate separation efficiency (% lactate passage) was obtained from all 3 model solutions at 6 bar regardless of changes in pH compared with the runs using an operating pressure of 4 bar. It was suggested that increasing the operating pressure from 4 to 6 bar improved lactate separation owing to the larger difference in operating pressure and the osmotic pressure of lactic acid generating a larger driving force across the membrane, which eventually resulted in a higher diffusion rate (high lactate flux as seen in Figure 2). Operating pressures higher than the osmo-

<table>
<thead>
<tr>
<th>Species content, mol/L</th>
<th>pH 4</th>
<th>pH 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>CaLAC</td>
<td>NaLAC</td>
<td>NH(_4)LAC</td>
</tr>
<tr>
<td>Total LAC</td>
<td>0.056</td>
<td>0.056</td>
</tr>
<tr>
<td>Free LA (cal)</td>
<td>0.024</td>
<td>0.025</td>
</tr>
<tr>
<td>LAC(^{−}) (cal)</td>
<td>0.032</td>
<td>0.031</td>
</tr>
<tr>
<td>Ca(^{2+})</td>
<td>0.016</td>
<td>-</td>
</tr>
<tr>
<td>Na(^+)</td>
<td>-</td>
<td>0.031</td>
</tr>
<tr>
<td>NH(_4)(^+)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>SO(_4)(^{2−})</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>H(_2)O(^+)</td>
<td>10(^{−4})</td>
<td>10(^{−4})</td>
</tr>
<tr>
<td>OH(^−)</td>
<td>10(^{−10})</td>
<td>10(^{−10})</td>
</tr>
<tr>
<td>Total ions</td>
<td>0.0721</td>
<td>0.0821</td>
</tr>
</tbody>
</table>

**Figure 2.** Lactate flux at the permeate of the BWRO unit operated at 30 °C. CaLAC, NaLAC and NH\(_4\)LAC containing the initial lactate equivalent of 5 g/L at different pH values were passed through the BWRO unit at different operating pressures.
tic pressure of the solution resulted in an increasing mass flux throughout the membrane and thus the separation efficiency [21]. It should be noted that the osmotic pressure of the solution was increased with increasing concentration of the feed solution [22]. Therefore, operation at a certain pressure with varied feed concentrations resulted in a different permeate flux, and eventually lactate separation efficiency, in the 3 model solutions studied.

From Figure 3, pH was found to be responsible for lactate separation in the BWRO unit. This was related to the amount of total ions present in the feed solution, which played a role in mass transport across the membrane (Table 2). Liew et al. claimed that as the ionic strength of the feed solution increased, the permeate flux decreased as a result of increases in osmotic pressure and viscosity [23]. It was observed that the higher total ion concentration lowered the lactate flux (Figures 2 and 3). At the same operating pressure, higher permeate flux resulting in significantly higher lactate separation at the BWRO unit was achieved at a lower pH with a rapid diffusion rate (pH 4 compared with pH 6) in the case of CaLAC and NaLAC feed solutions, when the total ion concentration of the feed solution was lower (Table 2). On the other hand, in the case of NH4LAC, slightly increasing permeate flux and lactate separation were obtained at pH 6. This was presumably due to the slight change in total ion concentration, resulting in a similar ionic strength between the 2 pH values studied. The findings in this work confirmed that the pH and the total ion concentration of the feed solution played a role in controlling permeate flux, and thus separation efficiency, at the BWRO unit.

**Ion rejection by the BWRO membrane**

As previously mentioned, the separation of lactate from other ions was expected at the BWRO unit. Nonetheless, not only lactate species but also calcium, sodium and ammonium ions could pass through the BWRO membrane (Figure 3). Several interaction mechanisms of salt passage through the membrane have been investigated, including convection, diffusion, and charge repulsion. It was claimed that both membrane charge and feed ionic strength played a significant role in salt rejection [24]. When a typical feed solution interacted at the surface of the negatively charged membrane, the ion shift was generated at the boundary between the membrane and the solution, resulting in an electrical potential known as the Donnan exclusion effect [22]. In the case of uncharged solutes such as undissociated lactic acid, solution transport mainly occurred through diffusion and convection. The larger the difference between the oper-
ating pressure and the osmotic pressure, the larger the percentage of undissociated lactic acid that passed through the BWRO membrane. When lactic acid species were present in the dissociated form at an operating pH higher than the pKa value (3.86), the Donnan exclusion effect governed the transport of ion species through the BWRO membrane [25]. Thus, higher lactate rejection was observed in all 3 model solutions at pH 6 owing to a larger electric repulsive force by the negatively charged surface (Figure 3).

Considering the passage of Ca\(^{2+}\), Na\(^{+}\), NH\(_4\)\(^{+}\), and H\(^{+}\) through the BWRO membrane, these cations typically bind at the membrane surface. The higher the pH, the more the dissociated lactate and the more the negatively charged membrane brings larger cations to the membrane surface [21]. It was suggested that the larger ions had lower diffusion rates and thus were expected to have lower concentrations in the permeate. Size controlled ion diffusion, and the ability of ions to form hydrogen bonds with the carbonyl group of the polyamide membrane facilitated the passage of such ions [22,23]. In addition, Tu et al. and Zaidi et al. confirmed that salt rejection by the BW30 membrane was dominated mostly by size exclusion [26,27]. Thus, in our work, most of Ca\(^{2+}\) were retained whereas Na\(^{+}\) and NH\(_4\)\(^{+}\) apparently passed through the BWRO membrane.

**Water permeability and solute rejection at the SWRO unit**

Lactate concentration was determined in the SWRO unit under different operating pressures. After passing through the BWRO unit, the model solution was passed through and recirculated in the SWRO unit for 5 min. The sample was collected for analyses of lactate in both the retentate and permeate. The performance of the SWRO unit in terms of lactate recovery and water permeation is shown in Table 3.

![Image](186)

Slightly increasing the pressure from 13 to 15 bar did not result in significant changes in SWRO performance. A slight increase in lactate concentration at the retentate with a higher water permeable flux was obtained at 15 bar. From Table 3, it is apparent that a relatively high rejection percentage of 95-99% was obtained at the SWRO unit operated at 13 bar. Therefore, running SWRO at this pressure not only gave an increasing concentration of lactic acid product at the retentate, but the operating cost could also be reduced [28]. Although the operating pressure used in this study did not show a significant effect on lactate recovery owing to the limited applied pressure to the apparatus up to 15 bar, it is believed that with higher operating pressure, higher lactate rejection rate and water flux should have been obtained, eventually resulting in increasing concentration of lactic acid product at the retentate [28].

**Table 3. Effect of operating pressure on lactate recovery at the SWRO unit**

<table>
<thead>
<tr>
<th>Starting feed</th>
<th>CaLAC</th>
<th>NaLAC</th>
<th>NH(_4)LAC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At 13 bar</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactate at permeate, g/L</td>
<td>0.05</td>
<td>0.20</td>
<td>0.09</td>
</tr>
<tr>
<td>Lactate at retentate, g/L</td>
<td>6.45</td>
<td>9.68</td>
<td>7.97</td>
</tr>
<tr>
<td>Lactate rejection, %</td>
<td>98</td>
<td>95</td>
<td>99</td>
</tr>
<tr>
<td>Cation rejection, %</td>
<td>99</td>
<td>99</td>
<td>99</td>
</tr>
<tr>
<td>Water flux, L/(m(^2) h)</td>
<td>11.4</td>
<td>10.8</td>
<td>8.4</td>
</tr>
<tr>
<td>At 15 bar</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactate at permeate, g/L</td>
<td>0.07</td>
<td>0.22</td>
<td>0.11</td>
</tr>
<tr>
<td>Lactate at retentate, g/L</td>
<td>7.47</td>
<td>11.50</td>
<td>9.54</td>
</tr>
<tr>
<td>Lactate rejection, %</td>
<td>98</td>
<td>95</td>
<td>100</td>
</tr>
<tr>
<td>Cation rejection, %</td>
<td>99</td>
<td>99</td>
<td>90</td>
</tr>
<tr>
<td>Water flux, L/(m(^2) h)</td>
<td>13.2</td>
<td>12.0</td>
<td>10.8</td>
</tr>
</tbody>
</table>

A typical RO operation involved the removal of inorganic and organic salts from the aqueous solution [29]. The SWRO membrane used in this work was the positively charged membrane containing free amine groups; therefore, high cation rejection was expected, especially at the lower pH (pH 4) when the feed solution was more protonated and lactic acid was present more in the undissociated form. Similar to observations in the BWRO unit, evidence of high rejection percentages of both cations (Ca\(^{2+}\), Na\(^{+}\), and NH\(_4\)\(^{+}\)) and lactate ions confirmed that the SWRO unit was successfully utilized to concentrate lactate at the retentate by expelling water through the membrane (Table 3).

**Total mass balance and efficiency of lactate recovery at the two-stage RO units**

Figure 4 presents the total mass balance over the two-stage RO units. The first BWRO unit was considered the key operating unit where lactate ions were separated from the cations, and the second SWRO unit was for concentrating the product remaining in the retentate.

From the three model solutions studied, it was found that more than 50% of lactic acid from the feed
stream was recovered from the two-stage RO units (Table 4). Compared with the other 2 feed solutions, when the feed stream was CaLAC, a lactic acid purity of 99.2% was obtained. Nonetheless, the total recovery seemed to be slightly low (50.5%). It should also be noted that the highest lactic acid purity was obtained with the lowest recovery percentage.

In addition, the efficiency of the two-stage RO unit was tested with the actual lactic acid fermentation broth. The fermentation broths, including CaLAC broth, NaLAC broth, and NH₄LAC broth, primarily passed through the microfiltration and ultrafiltration units where cells and proteins were separated. The solutions were then diluted to obtain the equivalent concentration of lactic acid of 5 g/L before entering the RO units operated at optimized conditions determined before allowing lactic acid recovery and purification. Table 5 presents the efficiency of the two-stage RO units constructed in this study on lactic acid separation and purification from the actual ferment-

Table 4. Overall recovery and purity of lactic acid from the 3 different model solutions after passing through the two-stage RO units operated at optimized conditions

<table>
<thead>
<tr>
<th>Feed solution</th>
<th>CaLAC</th>
<th>NaLAC</th>
<th>NH₄LAC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Operating conditions</td>
<td>pH 4, 6 bar at BWRO</td>
<td>pH 4, 6 bar at BWRO</td>
<td>pH 6, 6 bar at BWRO</td>
</tr>
<tr>
<td></td>
<td>pH 4.6, 15 bar at SWRO</td>
<td>pH 4.5, 15 bar at SWRO</td>
<td>pH 5.9, 15 bar at SWRO</td>
</tr>
<tr>
<td>Total lactic acid recovery</td>
<td>50.5%</td>
<td>66.4%</td>
<td>70.3%</td>
</tr>
<tr>
<td>Purity</td>
<td>99.2%</td>
<td>89.9%</td>
<td>89.7%</td>
</tr>
</tbody>
</table>
ation broths. Compared with the model solutions, the overall lactate recovery was similar whereas the purity was lower.

Various ions present in the actual fermentation broth were claimed to be responsible for the lower purity (Figure 5). The amount of total ions present in the feed fermentation broth was higher than that of the model solution; therefore, the Donnan exclusion effect was lowered, resulting in increasing ion passage across the BWRO membrane [24].

From the findings in this study, it can be presumably concluded that the membrane-based process to recover and purify lactic acid from the fermentation broth has 2 major advantages. The first one is that no pretreatment is required for the cell-free fermentation broth before entering the two-stage RO unit to recover, purify, and concentrate lactic acid. In general, pretreatment of the cell-free fermentation broth by acidification using $\text{H}_2\text{SO}_4$ is necessary for lactate recovery by the typical ion exchange resin-based process. Furthermore, using the typical ion exchanger to separate lactic acid from the fermentation broth requires 3 main steps including feed stream loading (adsorption), washing (to remove unbound solution from the resins), and lactic acid elution by proper eluant (desorption) [8]. This resulted in the increasing consumption of chemicals, wastewater treatment, and eventually dilution of the fermentation broth after acidification. Secondly, without pretreatment of the cell-free fermentation broth and applying the two-stage RO unit for lactic acid recovery, the volume of cell-free fermentation broth remained unchanged. Therefore, the downstream equipment sizing can be smaller compared with the typical downstream process using ion exchange resins. Although the broth had to be diluted to 5 g/L before entering the two-stage RO unit, the performance of this unit to recover, pre-purify, and pre-concentrate lactic acid was evident. This strongly indicated the beneficial outcome of this process, especially when we could operate without the pressure limit as experienced in this work with our in-house apparatus.

It is suggested that lactic acid loss in the retentate at the BWRO unit can be reduced by stream recycling at the feed tank. In addition, to increase the purity of lactic acid product, the retentate from the SWRO unit is recommended to pass through another membrane unit, i.e., a nanofiltration unit, to eliminate the remaining trace ions. By selecting the proper membrane, it is believed that the purity of lactic acid product can be increased [30]. From the findings of this study, it can be claimed that the two-stage RO membrane process has for the first time been reported as a simple operation but effective in recovering and purifying lactic acid from the fermentation broth.

### Table 5. Performance of the two-stage RO unit to recover and purify lactic acid from the fermentation broth

<table>
<thead>
<tr>
<th>Feed solution</th>
<th>CaLAC broth</th>
<th>NaLAC broth</th>
<th>NH4LAC broth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Operating conditions</td>
<td>pH 6, 6 bar at BWRO</td>
<td>pH 6, 6 bar at BWRO</td>
<td>pH 6, 6 bar at BWRO</td>
</tr>
<tr>
<td></td>
<td>pH 5.39, 15 bar at SWRO</td>
<td>pH 5.81, 15 bar at SWRO</td>
<td>pH 5.96, 15 bar at SWRO</td>
</tr>
<tr>
<td>Lactate passage at BWRO</td>
<td>54.2%</td>
<td>66.9%</td>
<td>72.0%</td>
</tr>
<tr>
<td>Lactate rejection at SWRO</td>
<td>100%</td>
<td>97.4%</td>
<td>99.6%</td>
</tr>
<tr>
<td>Total lactic acid recovery</td>
<td>54.2%</td>
<td>65.2%</td>
<td>71.7%</td>
</tr>
<tr>
<td>Purity</td>
<td>86.1%</td>
<td>73.3%</td>
<td>74.7%</td>
</tr>
</tbody>
</table>

Figure 5. Percentage of ion leakage into the permeate. The different cell-free lactate broth solutions, including CaLAC broth, NaLAC broth and NH4LAC broth, entered the BWRO unit operated under the optimized conditions previously determined.
CONCLUSIONS

The in-house two-stage RO unit constructed in this study was used to recover and purify lactic acid. The acceptable amount of free lactic acid recovered with sufficiently high purity was obtained under optimized operating conditions. This unit was applicable to different fermentation broth solutions, including calcium lactate, sodium lactate and ammonium lactate. Although the operating pressure was set at a higher value than the lactate osmotic pressure, lactate rejection was still observed at the BWRO unit where most of the lactate was expected to pass through the membrane while other ions remained in the retentate. It was found that the total ion concentration of the feed solution and the operating pH both played a crucial role in controlling ion leakage across the membrane, thereby controlling both lactate recovery and purity of this RO membrane system. From the results obtained in this study, it is suggested that by coupling this two-stage RO unit with the upstream fermentation and primary cell and protein separation (microfiltration and ultrafiltration) units, the simple design of continuous fermentation and lactic acid recovery to achieve high productivity in long-term operations is feasible.

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REFERENCES

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NAUČNI RAD

ALTERNATIVNA REVERZNA OSMOZA ZA PREČIŠĆAVANJE MLEČNE KISELINE IZ FERMENTACIONE KOMINE


Ključne reči: mlečna kiselina; fermentaciona komin; reversna osmoza; Donnan ekskluzioni efekat; jonska jačina.