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# Recovery of lactic acid and other organic acids from food waste ethanol fermentation stillage: Feasibility and effects of substrates

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ABSTRACT

Recycling ethanol fermentation stillage from food waste could help to resolve the pollution problems, but it would lead to the accumulation of lactic acid (LA), which had a negative effect on ethanol fermentation. In this study, bipolar membrane electrodialysis was used to recover LA as well as other fatty acids in the stillage, and the corresponding parameters that affected this process were investigated. Pretreatment via centrifugation and ultrafiltration reduced the turbidity successfully. Experiments on simulated and real stillage showed that organic acids such as formic acid and acetic acid had a slight effect on the separation of LA, but the existence of chloride ions could form strong anion competition with lactate ions. Simulation experiments with sodium chloride reduced the recovery of LA from 98% to 92%. The recovery rate of LA from real stillage could reach 71.2%. After electrodialysis, the stillage can be reused in ethanol fermentation, the fermentation time was shortened to 24 h, and the ethanol yield increased.

## 1. Introduction

For the sustainable development of society, the recycling of waste has become more and more important. Food waste (FW) accounts for a large proportion of municipal solid waste (15%–63%) [1]. It has high organic, volatile solid (85%–95%), and moisture content (75%–85%) and is easily degraded and deteriorated. There are many ways of reusing the waste, such as composting and production of pig feed, biomethane, biodiesel, ethanol, and lactic acid (LA) [2].

The use of FW to produce ethanol can take full advantage of the rich nutrients in FW and obtain fuel. However, a large quantity of stillage will be produced; one way for dealing it is stillage recycle fermentation [3]. This method can reduce pollution and utilize proteins, amino acids, and various metal ions in stillage [4]. In our previous study, LA was determined to be the major accumulated by-product along with stillage recyling. A significant inhibition phenomenon was observed after 5 times recycling. Although the addition of calcium carbonate could alleviate the influence of LA on ethanol fermentation, if we can separate the accumulated LA in the stillage, it will provide better economic benefits.

LA is an important natural organic acid. It has received increasing attention as one of the most important building blocks for the production of polylactic acid [5,6]. At present, the main methods of LA production are microbial fermentation and chemical synthesis. Biological fermentation is inexpensive, utilizes a wide range of raw materials, and produces optically pure L- or D-lactic acid, which make it superior to chemical synthesis. More attention has also been paid to the utilization of renewable carbohydrates as a carbon source for LA fermentation [7,8]. However, the major cost of LA production is due to its downstream separation and purification steps [9]. As LA exists in the form of lactate salt in the fermentation broth, acidification and removal of impurities are required for future separation [10].

The extraction methods of LA include precipitation, solution extraction, adsorption, distillation, and nanofiltration [11]. Electrodialysis has the advantages of diversity, sophisticated functions, and technological compatibility [12,13]. Danner et al. used monopolar electrodialysis to extract LA from grass silages and obtained press extraction yields of 31–96 g of lactate per kilogram of silage dry matter (g LA/kg DM) [14]. Chen used conventional electrodialysis (CED) to remove 80% lactate from acid whey [15]. Lopez-Garzon used bipolar membrane electrodialysis (BMED) to recycle LA and other carboxylic acids in the fermentation broth [16]. BMED is known as an energy-saving process because it can directly convert electrolyte salts into corresponding acids and bases without the addition of any other chemical agents [17]. The main factors affecting electrodialysis are pH, current, velocity, and concentration ratio. In the stillage, a lot of

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organic acids such as lactic acid, formic acid, acetic acid, and propionic acid are found. BMED can conduct simultaneous recovery of various acids, but there are few studies on the effects of various acids under electrodialysis on the extraction of LA. Furthermore, the effects of other components in the stillage from FW ethanol fermentation would also be investigated.

In this study, the ethanol fermentation stillage from FW was used as the raw material to extract LA. It contained a large amount of suspended solids, oils, and fats, and a small amount of residual sugar, protein impurity, and amino acids [18,19]. The ion exchange membranes (IEMs) of electrodialysis have poor pollution resistance; in addition, the presence of various impurities will increase the viscosity of the crude liquor and affect the turbulence level of the solution [20], leading to a decrease in membrane performance and an increase in running costs [21]. Therefore, stillage pretreatment is necessary before electrodialysis. Zhang used a flocculation–filtration system to treat FW fermentation broth and reduce SS and oil content [22]. Kim centrifuged the fermentation liquid to remove the suspended matter, followed by nanofiltration [10]. Many studies have used ultrafiltration (UF) for liquid purification, for example, coagulation–UF process for wastewater reclamation to remove viruses [23], UF membranes for raw apple juice purification [24], and UF for removing the residual lignin in ionic liquid solution, followed by electrodialysis to recover the ionic liquid 1-butyl-3-methylimidazolium bromide [25].

In the present study, the feasibility of using electrodialysis to separate LA from FW stillage was studied. The influence of different substances in the stillage on electrodialysis and recovery of lactic acid was studied by simulation experiments. Simultaneous recoveries of formic acid, acetic acid, and propionic acid in stillage were also compared, and the factors affecting the recovery rate of organic acids were investigated.

## 2. Materials and methods

### 2.1. Raw materials

In this experiment, the raw material for LA extraction is the fifth batch of stillage after distillation from FW ethanol fermentation. The specific stillage recycling fermentation procedure is shown in Fig. 1, and the fermentation procedure was described in our previous article [2]. Fig. 1 shows the process and the fifth batch of stillage used for pretreatment and electrodialysis (on the right in Fig. 1).

**Table 1**
The characteristics of the fifth batch stillage.

| Item | Unit | Value |
|---|---|---|
| TCOD | kg COD/m$^3$ | 161 ± 11 |
| SCOD | kg COD/m$^3$ | 146 ± 9 |
| Total Solids (TS) | kg TS/m$^3$ | 17 ± 4 |
| Volatile Solids (VS) | kg VS/m$^3$ | 14 ± 4 |
| Total Nitrogen (TN) | kg N/m$^3$ | 10.6 ± 0.3 |
| Organic acids | kg COD/m$^3$ | 112 ± 7 |
| pH | – | 4.1 ± 0.4 |

The properties of the stillage used in the experiment are summarized in Table 1. TS was approximately 17 kg TS/m$^3$, the organic acid content was approximately 112 kg COD/m$^3$, and the main component was LA, with a concentration of 92 ± 10 g/L.

### 2.2. Pretreatment of stillage

High-speed centrifugation was used to remove suspended solids with a rotation rate of 4000 rpm for 15 min. The supernatant was collected for UF. A hollow fiber UF device (Tianjin MOTIMO Membrane Technology Co., Ltd.) was used in the study. The device consisted of one module equipped with a tubular PVDF membrane with pore size and membrane surface area of 0.2 μm and 0.5 m$^2$, respectively. The stillage was injected into the UF membrane via a peristaltic pump with a flow rate of 100 rpm. When the system pressure reached 20–40 kPa and remained stable, the solution was collected and turbidity was measured. At the end of the experiment, the UF membrane was alternately washed with acid and alkali solution. Finally, 1% sodium bisulfite-filled membrane modules were used to prevent bacterial growth.

### 2.3. Electrodialysis by bipolar membrane equipment

A laboratory-scale BMED instrument was used (Shandong Tianwei Membrane Technology Co., Ltd.), with a membrane surface area of 10 cm × 20 cm and an effective area of 80%, it was assembled with 10 cell pairs. In the membrane stack, bipolar membranes (FUMATECH, Germany) and cation exchange membranes (Shandong Tianwei Membrane Technology Co., Ltd.) were arranged alternately. To adjust the conductivity at the beginning of the experiment, 10 g/L of LA was

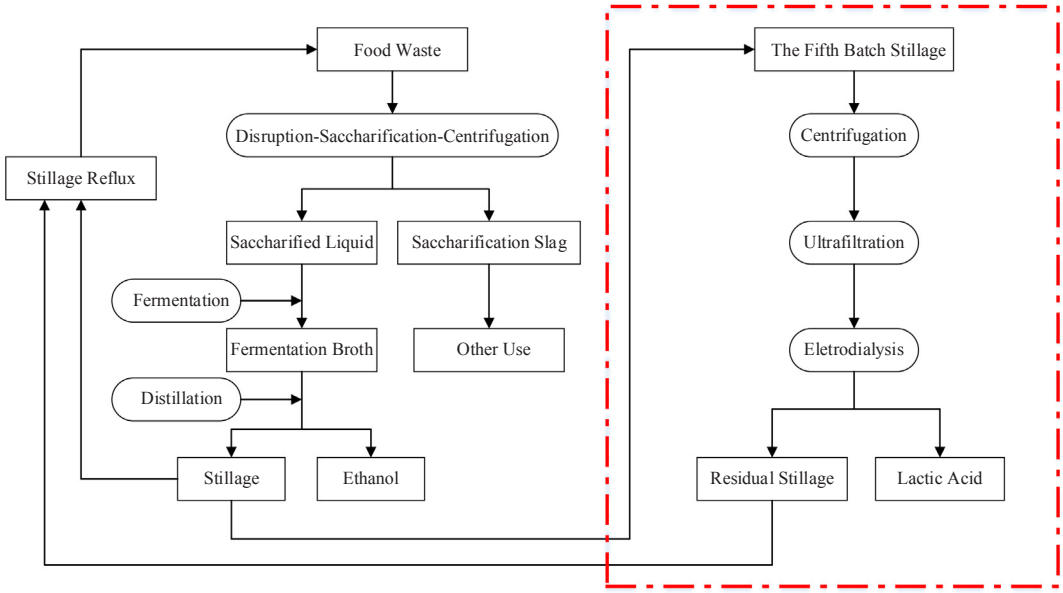


**Fig. 1.** Technology roadmap in this study.

added to the concentrate solution, and the electrode solution was 0.5 mol/L of $Na_2SO_4$. In the simulated experiment, artificial LA solution (80 g/L) was used as the dilute solution. For the real stillage experiment, the fifth batch of stillage was used as the dilute solution. Three peristaltic pumps were used to deliver the electrode and dilute and concentrate the solution to the ED module. The electrodialysis experiments were operated at a constant current of 1 A. All experiments were terminated when the contents of LA in the discharge and feed chambers became stable. At the end of each experiment, a cleaning procedure was performed as described in Section 2.2.

### 2.4. Analysis and calculation

Organic acids were analyzed with a high-performance liquid chromatograph (HPLC) system equipped with refractive index detector (RID-10A, SHIMADZU) and a SUGAR SH1011 (8.0 mmID * 300 mm) column (Shodex), using 0.005 M $H_2SO_4$ as the mobile phase. The liquid samples were prefiltered through a 0.45 μm membrane filter before injection into the HPLC system. The chloride ion was determined by ion chromatography (YC3000, Qingdao Ion Chromat Tograph Co., Ltd.) using 2.5 mmol/L of sodium carbonate and 1.7 mmol/L of bicarbonate as the mobile phase, with a flow rate of 0.6 mL/min. The pH and turbidity were measured with a pH meter and a turbidimeter (Turb 430 IR/T, Wissenschaftlich-Technische Werkstatten GmbH, Germany), respectively.

The recovery of organic acid was calculated using the formula

$$Recovery\ (\%) = \frac{Organic\ acid_{recovered\ by\ BMED}}{Organic\ acid_{recovered\ by\ BMED} + Organic\ acid_{discarded}}$$

where Organic $acid_{discarded}$ is the amount of organic acid discarded through the reactor effluent (g) and Organic $acid_{recovered\ by\ BMED}$ is the amount of carboxylates recovered from BMED (g).

## 3. Results and discussion

### 3.1. Pretreatment of stillage

The IEMs in electrodialysis have excellent durability because of their high mechanical and chemical stability [26]; however, membrane fouling impairs the process performance and adversely affects IEM integrity [27]. Organic contaminants in the stillage should be removed as the presence of aromatic compounds can cause swelling of anion exchange and bipolar membranes. Moreover, stillage contains a large number of suspended substances, proteins, colloids, bacterial debris, and other impurities. The method of centrifugation combined with UF was carried out as described in Section2.2.

UF is a membrane separation technique driven by pressure. The solvent is smaller than the pore diameter of the membrane, which enables it to pass through. This technology can be used to remove harmful macromolecules that precipitate during electrodialysis [28]. UF is considered as a very promising process because of its compactness, easy automation, low cost, and high removal rate of turbidity and organic matters (such as humic substances); it also does not require chemical reagents [29,30]. As can be seen from Fig. 2, most of the suspended matter in the stillage were removed after centrifugation; however, there were still some fine suspended solids and soluble macromolecules, such as proteins, left in the stillage. These could be removed by UF. After UF treatment, the turbidity was significantly reduced (Table 2).

### 3.2. Effect of by-products in stillage on electrodialysis

In our previous studies, the stillage reflux in the first five batches was beneficial to ethanol production, and the production increased from the first batch (25 g/L) to the fifth batch (35 g/L) because the amino acids and peptides produced by autolysis of the yeast provided

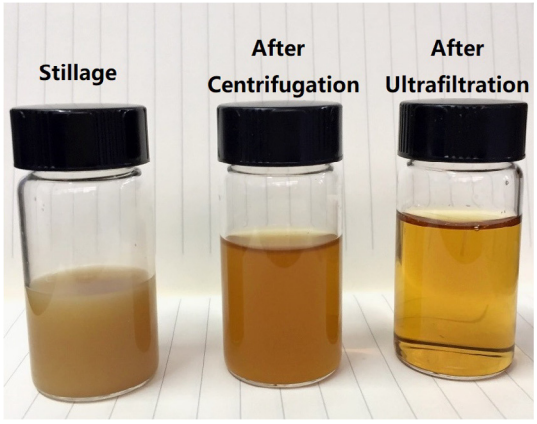


**Fig. 2.** Comparison of stillage with centrifugation and ultrafiltration treatment.

**Table 2**
Effect of centrifugation and ultrafiltration treatment on turbidity.

| | Stillage | After centrifugation | After ultrafiltration |
|---|---|---|---|
| Turbidity/NTU | 11,744 | 584 | 4.2 |

sufficient available nitrogen for fermentation [2]. However, with the further increase in reflux batch, harmful substances such as salt and high-boiling-point organic matter, which can inhibit ethanol fermentation, will accumulate.

Fig. 3 shows the organic acids and NaCl before and after distillation for fermentation of five batches. It shows that LA has become the main by-product in the reflux fermentation due to the presence of a large number of LA bacteria in FW. Along the reflux fermentation, LA accumulated in the stillage and gradually increased from the first batch of 15 g/L to the fifth batch of 96 g/L. In addition to LA, formic acid, acetic acid, and propionic acid can also accumulate with reflux. Butyric acid was also produced (approximately 20 g/L) in each batch of fermentation. As it can form azeotrope with water, it will evaporate with water vapor during distillation. The high concentration of NaCl is another characteristic of FW. NaCl increased from 0.14 mol/L in the first batch to 0.3 mol/L in the fifth batch. Glycerol, which maintains the redox balance of yeast cells, was the largest by-product of ethanol fermentation and had a concentration of 1.3 mol/L in the fifth batch. Among these by-products, the high content of LA and sodium chloride is an important reason for inhibiting ethanol fermentation, with LA exerting a greater impact [2].

This study aimed to use BMED to extract lactate ions from the

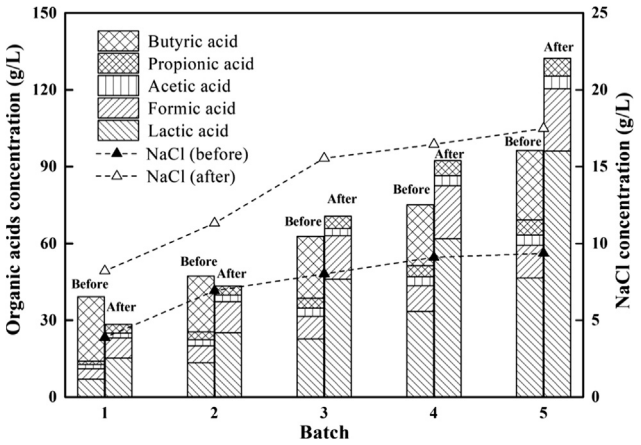


**Fig. 3.** Content of organic acids and NaCl in stillage before and after distillation.

**Table 3**
The composition of the solutions in each experiment group.

| | Control group | NaCl | Glycerol | Formic acid | Acetic acid | Propionic acid |
|---|---|---|---|---|---|---|
| Solution A | 80 g/L LA | 0.1 mol/L | 1.3 mol/L | 30 g/L | 30 g/L | 30 g/L |
| Solution B | 10 g/L LA | 10 g/L LA | 10 g/L LA | 10 g/L LA | 10 g/L LA | 10 g/L LA |

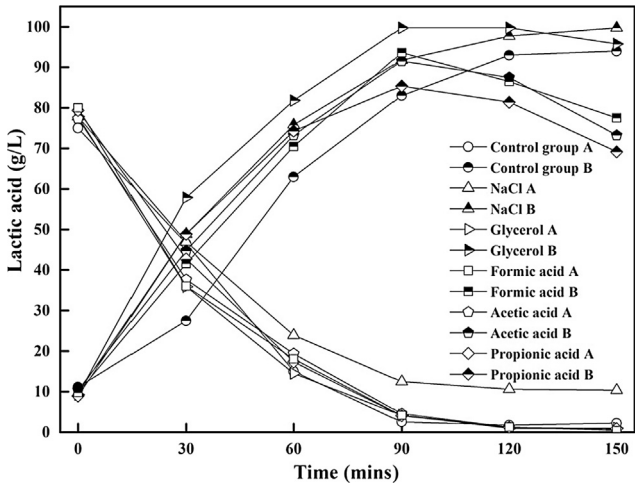


**Fig. 4.** Change of lactic acid content in electrodialysis process.

stillage. CED techniques can only separate anions and cations with different charges and are not able to distinguish anions of the same electrical property. However, the stillage contains many anions, such as chlorine, formate, acetate, and propionate ions. To investigate the effects of several major substances in the stillage on the extraction of LA, a single-factor simulation experiment was conducted using artificial simulated stillage. To investigate the effects of several major substances in the stillage on the extraction of LA, a single-factor simulation experiment was conducted using artificial simulated stillage. The LA solution A was used as simulated stillage. It was added with different ingredients in different groups according to the composition of the real stillage (Table 3). On the other hand, the LA solution B was used as concentrate solution.

Fig. 4 shows the LA content during electrodialysis in the simulated solution with different types of ingredients added (in Table 3). Solution A passed through the feed chamber (dilute chamber), and solution B passed through the discharge chamber (concentration chamber). As can be seen from the figure, the trend for LA concentration in the dilute chamber kept stable when different substances were added. The concentration rate at 0–60 min decreased rapidly and gradually decreased at 60–90 min. The reaction was almost completed at 90 min. The minimum LA concentration in the dilute chamber of the sodium chloride group remained above 10 g/L (10.38 g/L). In other groups, it was below 1 g/L. In the glycerol, formic acid, acetic acid, and propionic acid groups, the LA concentration decreased after the peak value.

The migration of water is an important factor affecting the concentration of LA in the discharge chamber [31]. The volume of solution in the discharge chamber of each group increased at the end of electrodialysis. For the groups where in the final concentration of LA fell below 1 g/L, the volume increase occurred in the glycerin, formic acid, acetic acid, and propionic acid groups. Although the remaining LA content in the feed chamber was basically the same, the LA concentration in the discharge chamber varied due to the increase in the volume.

Most of the ions in the solution were hydrated ions. As the anions entered the concentration chamber, the volume of the concentration chamber solution increased. A volume change phenomenon caused by lactate ion migration was found by Habova et al. using a two-compartment BMED [32]. For LA solutions containing formic acid, acetic acid, and propionic acid, other acid ions entered the concentration chamber (aside from the lactate ions), which increased the volume of the concentration chamber. In Arslan's study, water migration was also observed to be proportional with carboxylate ion passage [33]. On the other hand, because of the concentration difference between the dilute chamber and the concentration chamber, some water molecules will permeate into the concentration chamber, that is why the LA concentration began to decline in the later period of electrodialysis.

For the glycerin group, the presence of glycerol maintained the concentration of the solution in the dilute chamber in the later period of electrodialysis. Thus, the migration effect of water decreased compared with other groups, and the concentration of LA in the concentration chamber could be maintained at a higher level.

The transport of strongly hydrated anions, such as sulfate and fluoride ions, through an anion exchange membrane is reduced in comparison to that of chloride ions [34]; that is to say, the lower the degree of ion hydration, the easier it is to pass through the anion exchange membrane. The concentration of sodium chloride in the stillage is high, and it is completely ionized as a strong electrolyte. Compared with lactic ions, the hydration degree of chloride ions is very low, so they have a strong competitive relationship with lactate ions. Chloride ions are removed preferentially to lactate ions via the anion exchange membranes. After most of the chloride ions are transferred, the electrical energy is utilized predominately to remove lactate ions. A similar phenomenon has been described in George Q's study [15].

The electrodialysis experiment of simulated stillage showed that the migration of water was an important factor affecting the concentration of LA in the concentration chamber. Water transport capacity depends on many parameters, such as membrane specific resistance, current efficiency, type of ion, and concentration gradient between compartments. In our study, maintaining the solution concentration of the dilute chamber and reducing the transport of other anions may be an effective way to reduce the volume increase in the concentration chamber. In addition, the presence of chloride ions in the stillage can seriously affect the extraction of lactate ions. However, as chloride ions are the least hydrated anions, their transport via AEM is inevitable. To avoid the reduction of LA concentration in the concentration chamber in later period of electrodialysis, LA should be measured in time to control the electrodialysis time.

### *3.3. Comparison of the effect of electrodialysis between real stillage and simulated stillage*

Fig. 5 shows the LA change in electrodialysis using pretreated real stillage. Compared with the simulation experiment, the time of the real stillage was obviously prolonged from 90 min to 240 min. When it reached 210 min, LA separation began to slow down and tended to be stable. When the concentration of LA in the concentration chamber accumulates to a high level, concentration gradient of lactate ions between the concentration chambers and the neighbor chambers will be enlarged. This will increase the back-diffusion of lactate ions, which inevitably decreases the performance of EDBM process [19]. However, compared with the simulated stillage, the range of LA reduction became smaller. At the end of the experiment, the LA concentration in the stillage was 20.51 g/L, and that in the concentration chamber increased from 10 g/L to 71 g/L.

Although the stillage was pretreated by centrifugation and UF,

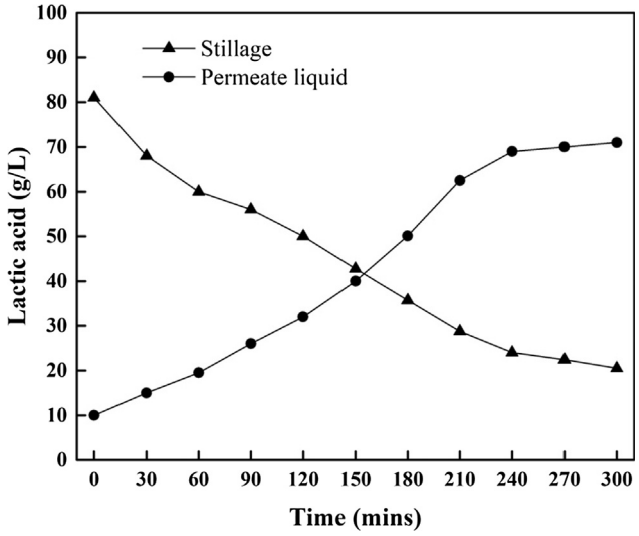


**Fig. 5.** Change of lactic acid concentration of real stillage in electrodialysis.

multivalent cations and some peptides and amino acids still remained, which are found can affect the electromembrane process by fouling [35]. The fouling of the ion exchange membranes (IEM) impaired the process performance and adversely affected the IEMs' integrity, which led to the decrease of electrodialysis efficiency and prolongation of electrodialysis time [36]. Meanwhile, bacteria in stillage may adhere on the membrane surface, grow and create a colony, finally formed the biofilm, and led to biofouling [26].

The effect of electrodialysis in each group was compared from the recovery rate of LA (Fig. 6). In the simulation experiment, the recovery rates of LA in the control group and glycerol, formic acid, acetic acid, and propionic acid groups were all above 98%. The recovery rate of LA in the NaCl group was relatively low (92.4%). The recovery rate of LA from the real stillage was significantly lower than that of the simulated stillage, which was approximately 70%.

In addition to the above-mentioned membrane fouling and stillage viscosity, another major reason for lower LA recovery rate in real stillage is that the composition of the simulated stillage is simple, and LA content accounted for an absolute advantage; however, the real stillage contains various organic acids, inorganic salts, and other impurities which cannot be removed at the pretreatment stage. The anion exchange membrane can allow lactate ions to pass through, so the lactate ions in the stillage must compete against other anions, such as chloride

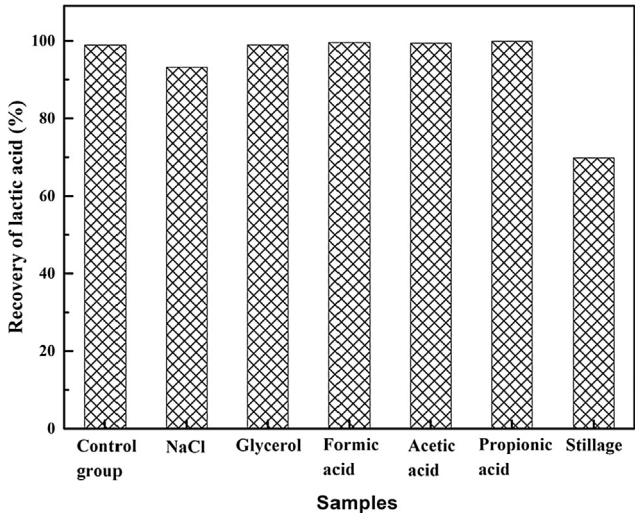


**Fig. 6.** Comparison of lactic acid recovery between simulated and real stillage.

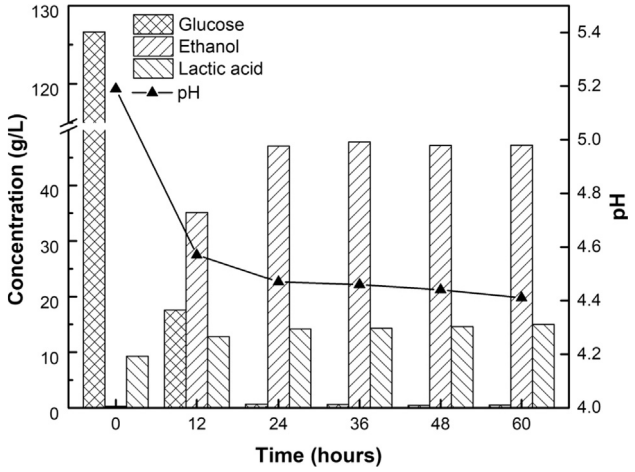


**Fig. 7.** Change of pH and the concentration of glucose, ethanol and lactic acid.

ions and organic acid radical ions, which hinder the extraction process of LA.

### 3.4. Stillage after electrodialysis was used for ethanol fermentation

During electrodialysis extraction, lactate ions in the stillage migrated into the concentrated solution, and LA content decreased significantly from 81.34 g/L to 20.51 g/L in the stillage. The inhibition of LA on yeast ethanol production has been reported many times. However, Graves found that by using corn pulp as raw material to simulate industrial ethanol fermentation, yeast can tolerate up to 40 g/L of LA, and under pH $<$ 5, the LA concentration can be reduced to 30 g/L [37]. In the present study, the concentration of LA has been reduced to approximately 20 g/L after electrodialysis. After mixing with FW, the LA concentration in fermentation broth decreased to approximately 9 g/L. Theoretically, ethanol fermentation will not be inhibited. To verify the hypothesis, the treated stillage was reused for ethanol fermentation.

As seen from Fig. 7, the glucose content in the fermentation broth was 126.63 g/L and then decreased rapidly within 12 h. At 24 h, the glucose content was reduced to 0.65 g/L and was almost completely consumed. Before fermentation, there was no ethanol in the fermentation broth. After 12 h, the ethanol concentration increased rapidly to approximately 35 g/L and then decreased. The concentration reached 47 g/L at 24 h. Between 24 h and 60 h, the ethanol concentration was maintained at approximately 47 g/L, which implied that ethanol fermentation has been completed at 24 h. This result is similar with the fermentation time in the first two batches of the previous experiment.

For LA, 20 g/L of LA remained in the stillage after electrodialysis. In addition, because FW contains LA bacteria, it will produce a certain amount of LA in the process of preservation [38]. Therefore, approximately 9 g/L of LA was found in the initial fermentation broth. After 12 h of fermentation, the content increased to approximately 13 g/L and slightly increased by approximately 2 g/L in 12–60 h. The results showed that LA was still produced in small amounts after ethanol fermentation stopped.

In the initial fermentation broth, the pH was 5.2. After fermentation for 12 h, it decreased to 4.6 and then decreased to 4.5 after 24 h. During electrodialysis, the $OH^-$ produced by bipolar membrane electrolysis enters the feed chamber (stillage) under the action of electric field to supplement the lost lactate and other anions, increasing the pH of the stillage. The pH of the stillage was approximately 2.5 before electrodialysis and reached 6.5 after electrodialysis. Wu Y et al. used fruit and vegetable waste for ethanol-type acidogenic fermentation and found the maximum ethanol concentration at pH 5.5 [39]. Therefore, in the present study, the pH of the stillage was increased by electrodialysis,

which could promote the fermentation of ethanol. In the later stages of fermentation (24–60 h), the pH value decreased slightly, which may be due to the production of LA and formic acid.

In summary, after electrodialysis, the stillage could be reused for fermentation, and the fermentation time was shortened to 24 h. Meanwhile, the production of ethanol was also higher than before, indicating that electrodialysis not only eliminates the inhibitory effect of LA in the stillage but also promotes the fermentation of ethanol because of the increase in alkalinity of the stillage.

## 4. Conclusion

BMED is an effective way to extract LA from the stillage. The combination of centrifugation and UF can be used to remove impurities such as suspended solids in the stillage. The recovery rate of LA from stillage can reach 71.2%, lower than that of the simulated stillage, due to the presence of anion impurity in the stillage, which forms a competitive relationship with lactate ions. After electrodialysis, the stillage can be reused in ethanol fermentation, the fermentation time was shortened to 24 h, and the ethanol yield increased.

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