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Separation and purification of L-phenylalanine from the fermentation broth by electrodialysis

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ABSTRACT

Electrodialysis (ED) is an electrochemical separation process during which ion exchange membranes use an electric potential as the driving force to separate and purify ionic species. In the experiment, the limiting current is firstly determined to avoid the polarization phenomenon. Then, the effects of the feed pH, feed concentration, and the glucose concentration on the performance of separation and purification are studied to determine the optimal operating conditions in terms of L-phenylalanine recovery and salt removal. The optimal operating conditions in this study are the limiting current of 0.75 A, feed pH of 5.91, feed concentration of 10 g/L, and the glucose concentration of 5 g/L, respectively. Under the optimal operating conditions, the L-phenylalanine recovery and salt removal can reach up to 84.3 and 98.5%, respectively.

Keywords: Electrodialysis; L-phenylalanine; Desalination; Simulated fermentation broth

1. Introduction

L-phenylalanine is one of the eight essential amino acids of the human body, which is also the main raw material of a new kind of sweetener aspartame. Because L-phenylalanine is an important component of the amino acid nourishment, the production of L-phenylalanine is enormous. In general, there are four methods for the production of L-phenylalanine, including the extraction from the natural protein, chemical synthesis, enzymatic reaction, and biological fermentation [1]. Compared with the four methods, the biological fermentation for L-phenylalanine production has obvious advantages [2]. The biological fermentation process in general is a biological and chemical reaction occurred at normal atmospheric pressure and temperature, and requirements of the process are relatively simple.

The fermentation broth is composed of the target amino acid, the residual sugar, and amounts of inorganic salts. Thus the component of the amino acid fermentation broth is quite complex. It is difficult to separate the L-phenylalanine from the amino acid fermentation broth for the acquisition of pure L-phenylalanine. At present, ion exchange is used to produce the pure L-phenylalanine in the fermentation industry [3]. However, when the ion exchange resin is regenerated, large amounts of waste acid and base are generated and difficult for treatment. What is worse, the large amounts of waste acid and alkali will cause

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serious environmental pollution. At the same time, the presence of inorganic salt from the L-phenylalanine broth needs to be removed by other methods, which will increase the load of the ion exchange.

Electrodialysis (ED) is an electrochemical separation process during which ion exchange membranes use an electric potential as the driving force to separate and purify ionic species. Due to its inherent advantages such as environmental friendliness, convenience of operation, and low energy consumption, ED has been widely applied in many fields. In recent years, ED technology has a wide application in the field of special separation. For example, ED can be used to remove electrolytes from organic solution, such as the demineralization of salted whey [4], natural sweet whey [5], cane sugar juice [6], glutamine fermentation broth [7], and neutral amino acids fermentation solutions [8]. Due to the amphoteric substance of the amino acid, ED has been applied in the production of L-phenylalanine. Grib et al. [9] have investigated the demineralization of L-phenylalanine fermentation broth by ED. However, the feed pH, the feed concentration, and the residual sugar in L-phenylalanine fermentation broth are not considered.

In this paper, ED is used to separate and purify the L-phenylalanine from the simulated fermentation broth. The limiting current is firstly determined to avoid the polarization phenomenon. Then, the effects of the feed pH, feed concentration, and the glucose concentration on the performance of separation and purification are optimized to optimize separation and purification performance in terms of L-phenylalanine recovery and salt removal. Finally, ED's huge potential is discussed to separate and purify the L-phenylalanine from the simulated fermentation broth.

2. Material and methods

2.1. Reagent and membranes

Sodium sulfate anhydrous, sodium hydroxide, sulfuric acid, and glucose were of analytical grade and used without further purification. The L-phenylalanine was of FCC IV food-grade provided by Shijiazhuang Shixing amino acid Co., Ltd, China. The L-phenylalanine fermentation broth was composed of L-phenylalanine and sodium sulfate. According to the real fermentation process, salt concentration is higher than the concentration of L-phenylalanine. Therefore, the concentration of sodium sulfate was two times the concentration of L-phenylalanine in the experiments. The concentrations of glucose may vary considerably in actual fermentation broth, so different amounts of glucose were prepared.

In this experiment, the anion exchange membranes (AEM) and cation exchange membranes (CEM) were supplied by Qianqiu Environmental Protection and Water Treatment Corporation, China. The main characteristics are detailed in Table 1.

2.2. Experimental setup

The ED apparatus is shown in Fig. 1, which consists of three circulating systems. Each system comprises a tank, a flow-meter, a manometer, and a circulation pump. Each circulation pump could give a needed circulation flow during the operation process. The flow rate was controlled by flow-meter. The laboratory ED cell was composed of twelve pairs of anion and cation-exchange membranes. The membrane size was 200×120 mm and the effective membrane area was 110 cm^2.

The feed solution, concentrated solution, and electrode rinse were fed in the membrane stack of ED by pumps, and then they were recycled back to the chambers, respectively. The feed solutions were circulated through these compartments at a flow rate of 15 L h^{-1}. The direct current was supplied by a CV/CC regulated power supply (WYL1703x2, Hangzhou Siling Electrical Instrument Ltd.). During the whole ED process, 2-ml sample was taken every 20 min and determined.

2.3. Analysis method

The concentration of the L-phenylalanine was determined using an ultraviolet spectrophotometer (Shimadzu UV-2450) at 256 nm. Glucose can dehydrate to generate furfural derivative when it reacts with sulfuric acid, and furfural derivative reacts with phenolic compounds and generates red compound. The red compound could be determined using an ultraviolet spectrophotometer (UV-2450) at 484 nm [10]. The conductivity of the solution of salt was measured by conductivity meter (METTLER TOLEDO FE30).

2.4. Limiting current measurement

In the ED operation process, because the migration velocity of the counter-ions in the membrane matrixes was greater than that from the bulk solution, the concentration of ions near the membrane surface was greatly reduced in the desalination chamber. Thus, the concentration gradient was formed on the boundary layer. At the same time, the concentration of ions near the membrane surface on the desalination chamber

Table 1
Main characteristics of the ion exchange membranes

Membrane	Thickness (mm)	IEC (meq g^{-1})	Area resistance (Ω cm^2)	Selectivity (%)
AEM	0.6	1.8	15	>92
CEM	0.8	2.0	18	>94

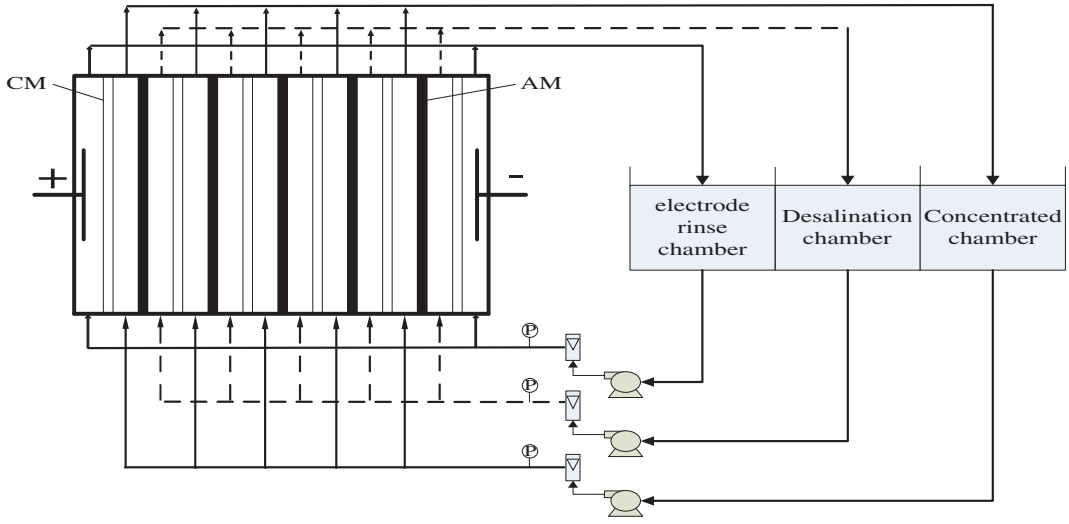

Fig. 1. Flow diagram of the experimental setup.

decreases with on increasing of the current. At a ascertain current, a large number of water molecules are dissociated when the concentration of ions reaches zero, which leads to the result that the formed H^+ and OH^- ions participate in the current transfer. The phenomenon is the so-called polarization phenomenon, and then a limiting current is reached [11]. The occurrence of polarization phenomenon will reduce the current efficiency of ED, as well as the salt removal. Therefore, the determination of the limiting current is of great significance. In the experiment, different concentrations of sodium sulfate solution were prepared, and the limiting current (I_{lim}) was determined using the method of V-I curve [12].

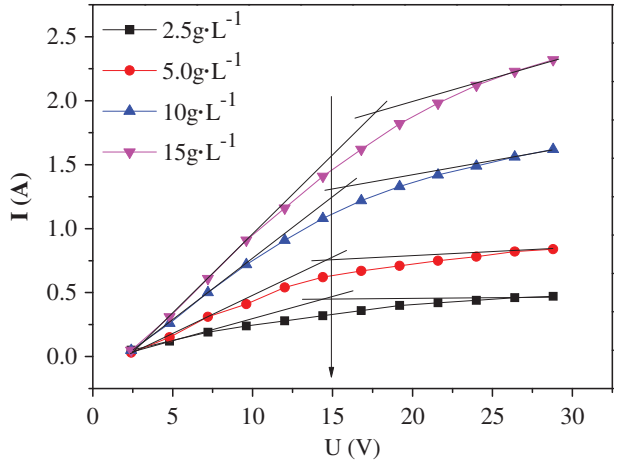

Fig. 2. Voltage–current curves under different concentrations of sodium sulfate solution.

3. Results and discussion

3.1. Voltage–current curves

The effects of the voltages on the current value are studied and the results are shown in Fig. 2. It can be seen from Fig. 2 that the current obviously increases with the electrolyte concentration increasing from 2.5 to 15 g/L^{-1}, which indicates that the electrolyte concentration has an important effect on the current. In terms of each curve, the current gradually increases with the voltages increasing from 2.4 to 28.8 V. Based on the analysis in Fig. 2, when the voltage is around 15 V, the current reaches up to the limiting current at the electrolyte concentration of 2.5 and 5.0 g/L,

respectively. In addition, the limiting current increases with the electrolyte concentration. According to above-mentioned analysis, the limiting current is about 0.75 A and the corresponding voltage is 15 V, while the electrolyte concentration is 5.0 g/L.

3.2. Effects of operating conditions on separation and purification performance

In order to achieve a better separation performance, the separation performance in terms of the desalination effect and the L-phenylalanine recovery was recorded in the experiments. Because the operating conditions of ED has an important effect on the desalination and the L-phenylalanine recovery, the influence of feed pH, L-phenylalanine concentration, and salt concentration were investigated in the ED process with the L-phenylalanine simulation fermentation broth. In the actual fermentation process, glucose is usually used as substrate, so the influences of glucose on the separation performance were also investigated at different glucose concentrations.

3.2.1. Influence of feed pH on the performance of separation and purification

The pH of feed is an important parameter for the treatment of L-phenylalanine simulation fermentation broth in ED process due to the fact that the amino acid is an amphoteric substance and its ionization state varies with the feed pH. The effects of feed pH are shown in Fig. 3(a). It can be seen from Fig. 3(a) that the variation of L-phenylalanine recovery is different with the feed pH increasing from 2.0 to 10.0. In terms of each curve, the L-phenylalanine recovery gradually decreases on increasing the desalination time. This phenomenon can be explained by the electrolyzing characteristic of L-phenylalanine. When the feed pH is lower than the isoelectric point of amino acid, the L-phenylalanine is electropositive and the L-phenylalanine is transported from cation exchange membrane into the concentration chamber. However, when the feed pH is higher than the isoelectric point of amino acid, L-phenylalanine is electronegative and the L-phenylalanine is transported from anion membrane into the concentration chamber. Therefore, the L-phenylalanine recovery decreases when the feed pH is not consistent with the isoelectric point of amino acid. The desalination effect under different feed pH is shown in Fig. 3(b). It can be seen from Fig. 3(b) that the variation of the salt removal is different under different feed pH. That is to say, the difference of feed pH causes the different salt removal, which demonstrates that feed pH significantly influences the salt removal. Compared Fig. 3(a) and (b), it can be concluded that the recovery of L-phenylalanine and the salt rejection are much lower when the feed pH is 2.0, 8.0, and 10.0, respectively. However, when the feed pH is near electroneutrality at 4, 5.91, and 6, the migration ions were mainly the salt ions rather than L-phenylalanine. Thus, the L-phenylalanine recovery and salt removal are much higher during the desalination process.

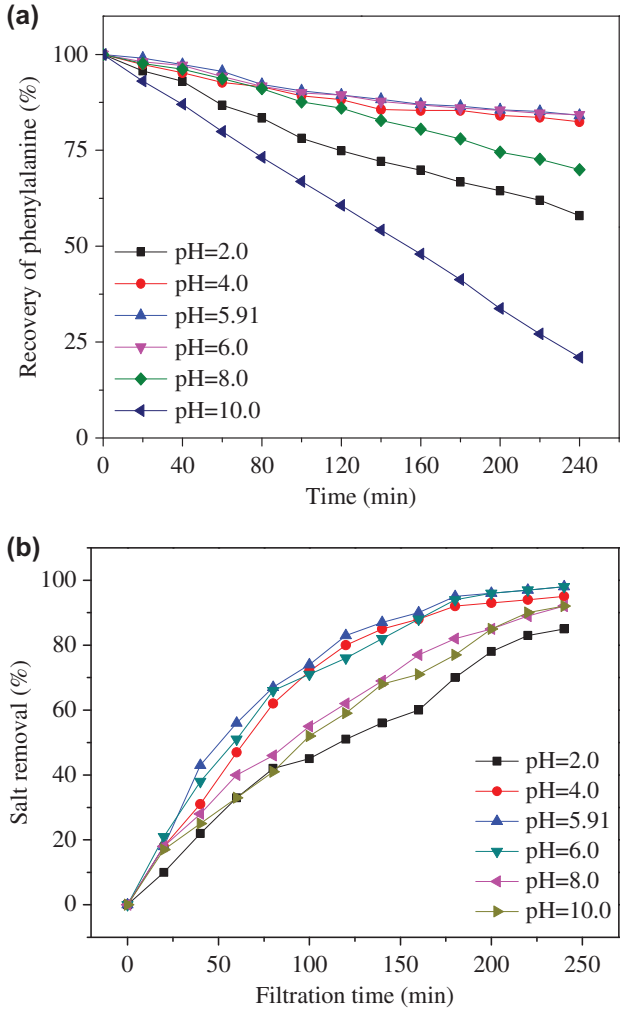

Fig. 3. Influence of feed pH on L-phenylalanine recovery (a) and salt removal rate (b).

Fig. 4 shows the effects of feed pH on the L-phenylalanine concentration ratio. It can be seen from Fig. 4 that the L-phenylalanine concentration ratios are all above 90%, which indicates that a better performance is achieved. Within 120 min, it can be seen that the L-phenylalanine concentration ratio gradually increases. The increase in L-phenylalanine

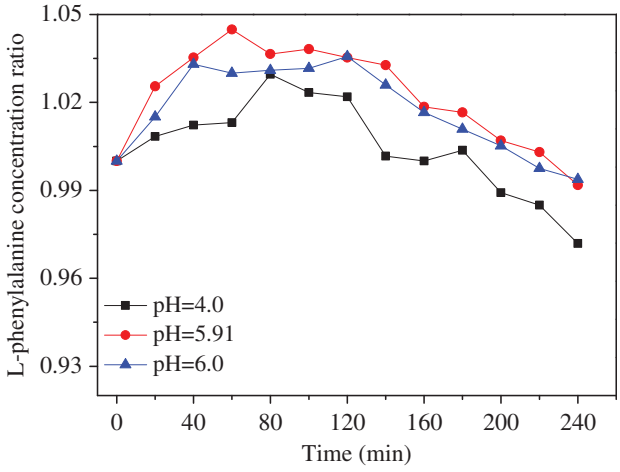

Fig. 4. L-phenylalanine concentration ratio of dilute chamber under different feed pH.

concentration ratio can be attributed to the fact that water molecules are migrated into concentration chamber as the ion migration is transported from dilute chamber to concentration chamber in the form of hydrated ions. In the later period of ED process, the L-phenylalanine concentration ratio gradually decreases on increasing the desalination time. The decline in the L-phenylalanine concentration ratio can be attributed to the fact that the L-phenylalanine is migrated through membrane from dilute chamber into concentration chamber. However, the water migration is much higher than the ion migration [13]. Thus the L-phenylalanine concentration ratio decreases on increasing the desalination time. At the three feed pH, the L-phenylalanine recovery and salt removal are shown in Table 2. According to Table 2, salt removal is above 86% and the L-phenylalanine recovery is above 96% when the feed pH is 4.0, 5.91, and 6.0, respectively. The phenomenon can be attributed to the fact that the amino acid is electrically neutral in the tested range of feed pH. Especially when the feed pH is 5.91, the L-phenylalanine recovery can reached up to 98.53%. This is attributed to the fact that the feed pH of 5.91 is the isoelectric point of the L-phenylalanine.

Table 2
L-phenylalanine recovery and salt removal after 4 h of recycle

pH	4	5.91	6
L-phenylalanine recovery (%)	82.43	84.12	84.28
Salt removal (%)	96.14	98.53	98.23

According to the above-mentioned analysis, the feed pH has a significant effect on the desalination effect and the L-phenylalanine recovery. When the feed pH is 5.91, the performance of separation and purification of L-phenylalanine is much better. Thus, the feed pH of 5.91 is determined for the following experiments.

3.2.2. *Influence of feed concentration on separation and purification performance*

The influence of feed concentration on the separation performance was investigated with a feed pH of 5.91 and a voltage of 15 V. The results are shown in Fig. 5(a) and (b). It can be seen from Fig. 5(a) that the L-phenylalanine recovery decreases on increasing the

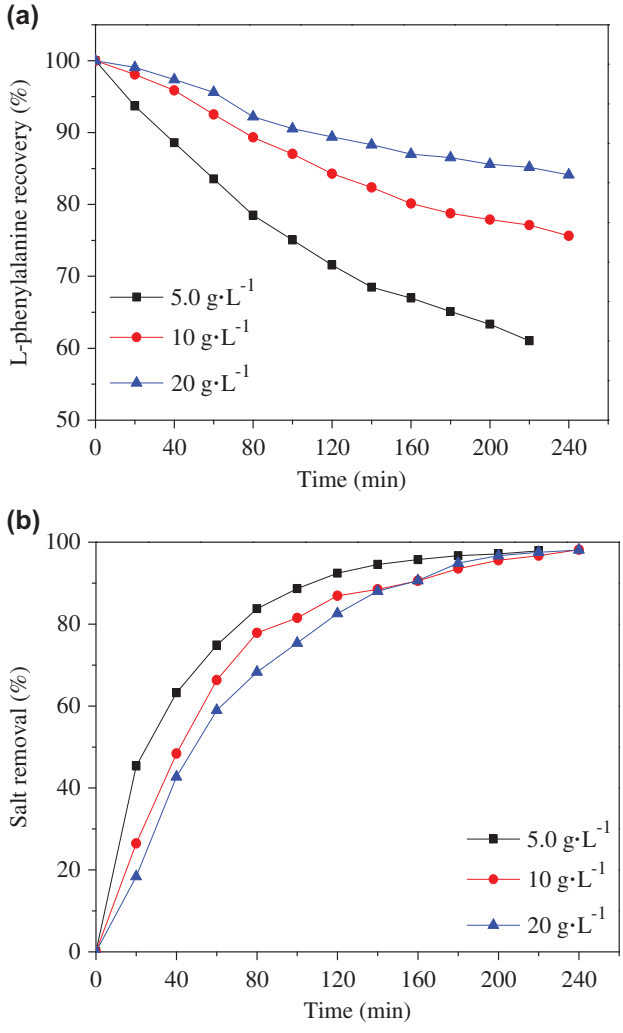

Fig. 5. Influence of feed concentration on L-phenylalanine recovery (a) and salt removal (b).

desalination time. The decline in the L-phenylalanine recovery is due to the fact that the L-phenylalanine is migrated through membrane from dilute chamber into concentration chamber. In terms of each curve, the L-phenylalanine recovery increases with the feed concentration increasing from 5.0 to 20.0 g/L. The phenomenon can be attributed to the fact that a high feed concentration leads to a high ion concentration. Thus, the mass ions are migrated from the dilute chamber to the concentration chamber in ED process and the volume of migration is correspondingly decreased. As a result, higher feed concentration can achieve a higher L-phenylalanine recovery.

Fig. 5(b) shows the effects of feed concentration on the salt removal. It can be seen from Fig. 5(b) that the salt removal decreases on increasing the desalination time. At the end of the experiment, the salt removal is 97.86, 98.08, and 98.13%, respectively when the feed concentration was 5.0, 10.0, and 15.0 g/L, respectively. The results demonstrate that the ED process has achieved a better desalination effect with no correspondence with the feed pH. As seen in Fig. 5(b), it also can be concluded that a high feed concentration leads to a lower salt removal velocity. The trade-off reasons cause the phenomenon. Although higher feed concentration can increase current density of ED, higher feed concentration has a large base of ions, which leads to decrease the salt removal. And higher feed concentration can lead to higher energy consumption. As a result, lower feed concentration is a good choice for salt removal.

According to Fig. 5(a) and (b), the higher feed concentration can achieve a higher L-phenylalanine recovery, but the salt removal is lower. Nevertheless, the lower feed concentration leads to much lower L-phenylalanine recovery. Therefore, taking into consideration all factors, the feed concentration 10 g/L is determined for following experiments.

3.2.3. *Influence of glucose concentration on separation and purification performance*

The influence of glucose concentration on the separation performance was investigated with a feed pH of 5.91, a feed concentration of 10 g/L, and a voltage of 15 V. The results are shown in Fig. 6(a) and (b). It can be seen from Fig. 6(a) that L-phenylalanine recovery decreases with the glucose concentration increasing from 0 to 50 g/L. In addition, the L-phenylalanine recovery also decreases on increasing the desalination time. Two reasons cause the decline in the L-phenylalanine recovery. On the one hand, the addition of glucose makes the fermentation broth complex, which

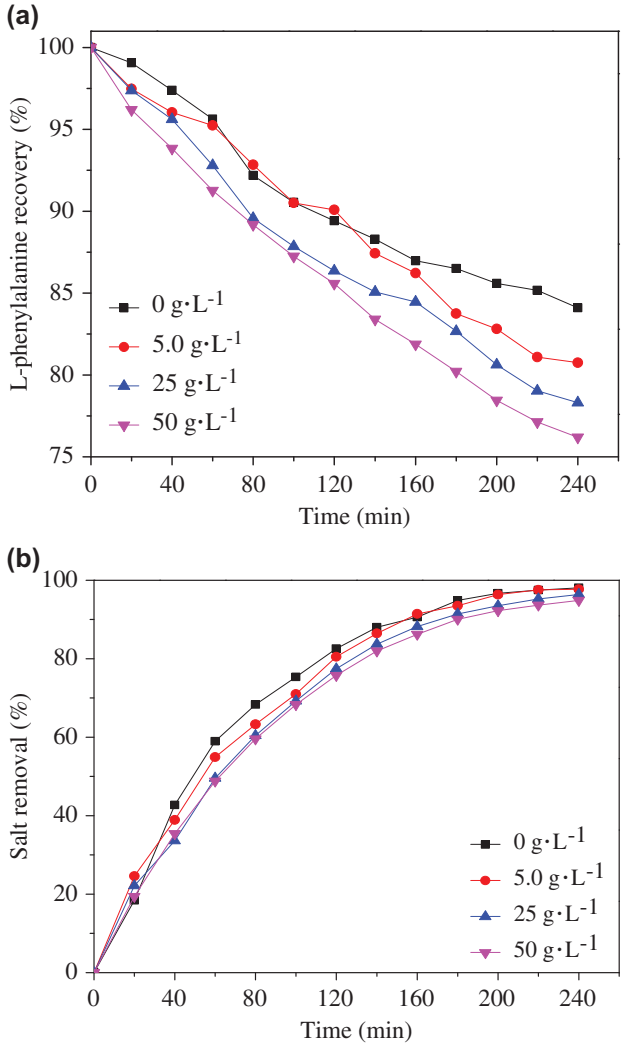

Fig. 6. Influence of glucose concentration on L-phenylalanine recovery (a) and salt removal (b).

will reduce the current efficiency. On the other hand, amount of glucose in the fermentation broth might be precipitated on the membrane surface, which causes membrane fouling. The effects of glucose concentration on the salt removal are shown in Fig. 6(b). It can be seen from Fig. 6(b) that there is no significant effect on salt removal regardless of the glucose concentration. The phenomenon can be attributed to the fact that the simulated fermentation broth has relatively high concentration. The addition of the glucose only further enlarges the feed concentration. In other words, the concentration driving force increases, which causes that L-phenylalanine is more prone to migrating from the dilute chamber to concentration chamber. The results are shown in the Fig. 6(a).

The variation of glucose concentration during ED process is shown in Fig. 7. It can be seen from Fig. 7 that

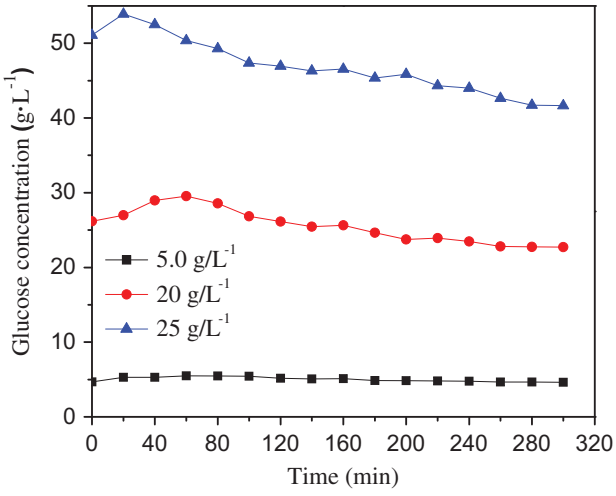

Fig. 7. Variation of glucose concentration in the desalination chamber.

the glucose concentration increases at an early of stage, which is caused by the leakage of water. And then, the glucose concentration gradually declines on increasing the desalination time. This is because high glucose results in the concentration driving force. As the desalination goes on, part of glucose can penetrate through the ion exchange membrane into the concentration chamber under the differential concentration driving force [14].

According to above-mentioned analysis, the glucose concentration doesn't have significant effect on the salt removal, but it obviously influences the L-phenylalanine recovery. The higher the concentration of glucose is, the lower the recovery of L-phenylalanine is. The optimal glucose concentration can be a compromise between L-phenylalanine recovery and salt removal. Therefore, the optimal glucose concentration is determined to be 5 g/L.

4. Conclusion

In this paper, the ED has achieved a better performance for separation and purification of the L-phenylalanine from the simulation fermentation broth. The optimal operating conditions in this study are the limiting current of 0.75 A, feed pH of 5.91, feed concentration of 10 g/L, and the glucose concentration of 5 g/L, respectively. Under the optimal operating conditions, the ED system has achieved a better L-phenylalanine recovery and salt removal, which are 84.3 and 98.5%, respectively.

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