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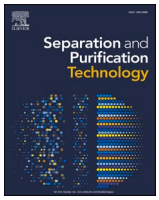

Recovery and upcycling of residual lactic acid and ammonium from biowaste into yeast single cell protein

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ABSTRACT

The yeast *Saccharomyces cerevisiae* is a potential candidate for converting waste resources into food-grade single cell protein (SCP). Food waste fermentation broth rich in lactic acid was tested as an alternative substrate source for *S. cerevisiae* in this study. An electrodialysis (ED) system was developed to recover lactate, acetate and ammonium ions (La^-, Ac^- and NH_4^+) from the broth. First, we evaluated the system resilience under various volatile fatty acids (VFAs) and pH levels. Initial acetate and lactate concentrations did not affect La^- and Ac^- recovery efficiencies but affected their ion fluxes and competitive ion transport. The migration of La^- was delayed by increasing the initial acetate concentration, while Ac^- migration was not affected by lactate concentrations. Increasing the broth pH reduced competitive H^+ ions and thus improved the NH_4^+ recovery efficiency. Further, broth recirculation and pH control were examined to identify potential challenges associated with continuous flow in real-life applications. Recirculation caused a greater loss of recovered Ac^- and NH_4^+ over the extended period. The pH control decreased VFAs recovery efficiencies because VFAs could not fully dissociate in the dilute, and more VFAs were lost in the concentrate. Finally, the recovery from lactate-rich fermentation broth was used as yeast substrate and contributed to a robust SCP yield of 0.62 g/g-C. Manufacturing one kilogram of SCP costs €6-10 in electricity and €3-6 in raw material. This study provides a better understanding of competitive ion transport during ED and uncovers potential biowaste resources for sustainable SCP production.

1. Introduction

Single cell protein (SCP) is edible dried biomass of algae, bacteria, yeast, or fungi containing a high protein level, regarded as an emerging source of protein for human or animal consumption. Since microorganisms have shorter harvest periods, use less water and land, and are not dependent on seasons, commercializing SCP will alleviate the global food shortage and reduce the environmental impact of conventional agriculture. The carbon and nitrogen sources in biowaste could also be used to produce SCP, which makes the process more circular and sustainable.

As one of the most promising candidates for producing SCP, yeast stands out for its larger size, low nucleic acid content, and ability to grow in acidic environments [1]. The unique characteristics of yeast make them easy to harvest, require minimal purification, and be adaptable to a wide range of waste streams. *Saccharomyces cerevisiae*, commonly known as brewer's or baker's yeast, has been utilized in food for centuries and has already been accepted for food use under the EU Novel Food Regulation [2]. The public acceptance will facilitate its future commercialization as an alternative dietary protein.

S. cerevisiae naturally inhabits a sugar-rich environment and has evolved mechanisms that ensure efficient sugar utilization. So food wastes, mainly fruit and agricultural waste, have been used as cheap substrates for *S. cerevisiae* in the current SCP applications [3,4]. *S. cerevisiae*, however, lacks the enzyme to degrade polysaccharides, so food wastes must be crushed into pulp, removed solids and fibers, and hydrolyzed with acid or heat into monosaccharides [5,6]. Food wastes were sometimes dried and ground into powder for better sugar dissolution and more accessible storage [6,7]. Both storage problems and pretreatment costs limit the industrial production of SCP from food waste. Besides sugar, our previous study revealed that *S. cerevisiae* could utilize lactate as the organic carbon source in the presence of acetate using ammonium as an inorganic nitrogen source [8]. The assimilation of *S. cerevisiae* requires more organic carbon than nitrogen in a ratio of

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10 g-chemical oxygen demand (COD) to 1 g-N [8]. Thus, waste streams with a high lactate content and some ammonia can be considered potential substrates for *S. cerevisiae*.

Ironically, in the same world where food shortages persist, food waste has become an increasingly recognized environmental issue. Many nations and cities are taking action to reduce and manage food waste [9]. During the fermentation of food waste, such as household biowaste and dairy industry waste, lactic acid is the main product of carbohydrate metabolism by lactic acid bacteria [10-12]. The process is called lactic acid fermentation. Lactic acid bacteria are autochthonous from vegetables or fruits or infused as bioaugmentation [13,14]. Although lactic acid fermentation can produce high yields (up to 1.11 g/g) and titers of lactic acid (up to 94 g/L) [14], downstream purification processes are required to produce commercially pure lactic acid. These processes lose approximately 50% of the produced lactic acid [15] and account for 20%-50% of the overall operation cost [16]. Additionally, other valuable components like acetic acid and ammonia, which co-exist in fermenters, are wasted because the downstream purification only targets lactic acid. Instead, the use of lactic acid-rich fermentation broth for SCP production could benefit the process in many ways by taking full advantage of the organic carbon and inorganic nitrogen present in the broth, circumventing expensive downstream processes, and creating value-added products. Further, human society and the food industry continuously generate a large amount of food waste, making lactate-rich fermentation broth readily available.

To use the lactic acid-rich fermentation broth for food-grade SCP production by *S. cerevisiae*, essential nutrients should first be recovered from the broth to eliminate potential hazards in food waste. Electrodialysis (ED) is a membrane separation process that can transport ionic species through ion exchange membranes under an electric field. ED can recover multiple chemicals from aqueous solutions, blocking harmful components like micropollutants and pathogens [17-19] and being energy efficient [20]. In lactic acid fermenters, it is common to experience composition fluctuations due to a variety of food wastes. For example, lactic acid concentration was reported in a wide range of 10–40 g/L and acetic acid concentration between 1.2 and 9 g/L [13,21-23]. However, there is a shortage of research on the impact of composition variations on ED recovery performance, which is necessarily fundamental to robust SCP productions. Furthermore, most ED applications targeted ammonia, nitrate, phosphate or a specific VFA from waste streams [24], whereas little research has been conducted on recovering ammonium and volatile fatty acids simultaneously.

ED was first developed in this work to recover lactate, acetate, and ammonium from the lactic acid fermentation broth. The resilience of the ED recovery system was evaluated by varying VFAs, ammonium, and pH levels in the fermentation broth. Additionally, recirculation and pH control effects on the overall recovery performance of ED systems were examined. Lastly, *S. cerevisiae* was fed with the ED recoveries from each scenario for SCP production, and its protein yield and amino acid profile were assessed. It is the first time yeast SCP has been produced using lactic acid-rich fermentation broth. The results demonstrate remarkable potential synergies between lactic acid fermentation of food wastes and yeast SCP production, which may reconfigure the biowaste value chain and bring more economic benefits.

2. Materials and methods

2.1. Setup and operation of ED recovery system

A three-chamber ED reactor was set up, including the outmost anode and cathode chamber (dilute chambers) and a central chamber (concentrate chamber) (Fig. A1 a). Two dilute chambers had an effective volume of 200 mL each, and the concentrate chamber had an effective volume of 100 mL. A cation exchange membrane separated the anode and middle chamber, allowing ammonium ion (NH_4^+) migration from dilute into concentrate. An anion exchange membrane between the cathode and middle chamber enabled the transport of lactate ion (La^-) and acetate ion (Ac^-) from dilute into concentrate. Each membrane had an effective area of 25 cm^2. The anode electrode was IrO_2-coated titanium alloy with dimensions of 4 × 4 cm and thickness of 1 mm (Magneto Special Anodes, Netherlands). The cathode electrode was titanium woven mesh with the same dimension as the anode and coated with 0.5 mg Pt/cm^2. Electrodes were connected to an external power supply (HQPS3003, Helmholt Elektronik A/S, Denmark) to provide a fixed voltage.

Two dilute chambers of the ED system were filled with synthetic lactic acid fermentation broth containing lactate, acetate, and ammonium. It included 3.8 g/L NH_4Cl (i.e., 1 g-N/L), 12.5–49.8 g/L sodium lactate (i.e., 10–40 g/L lactic acid), and 0.9–13.7 g/L sodium acetate (i.e., 1–10 g/L acetic acid). 4.5 g/L of K_2HPO_4 was added to the middle chamber to achieve a similar concentration to the acetate-ammonia-based (AAB) medium [25] while maintaining high conductivity. A magnetic stirrer continuously stirred the concentrate in the middle chamber throughout the ED process.

Multiple voltages of 0, 1.5, 2, 2.5, and 3 V were tested in the ED process. The synthetic lactic acid fermentation broths were identical with 1 g/L ammonium, 10 g/L lactic acid, and 2 g/L acetic acid. As a control group, the recovery at 0 V represented the natural diffusion of target ions through ion exchange membranes. We evaluated the recovery performance of La^-, Ac^-, and NH_4^+ after seven days of ED under each voltage. Every day, 3 mL of liquor was taken from each chamber for pH, VFAs and ammonium measurement. Unless otherwise noted, all batch experiments were conducted in duplicate, and fresh electrolyte was used in each batch. Based on the overall recovery performance, the best voltage for ED was selected for the following experiments.

2.2. Resilience of ED to fluctuations in fermentation broth composition

A variety of lactic acid fermentation broths were used to assess the system resilience of ED during recovery. Many factors influence the final composition of VFAs and pH of food waste fermentation broth, such as biowaste properties, lactic acid bacteria strains, and fermentation conditions. We simulated the composition fluctuation of real lactic acid fermentation broths by varying the lactate concentrations, acetate concentrations, and pH values. The following VFA concentrations, ammonium concentrations, and pH were designed based on actual household waste fermenters [13,21,22]. At first, lactic acid concentrations of 10, 20, 30, and 40 g/L were used, along with a fixed acetic acid concentration of 2 g/L. After that, different acetic acid concentrations of 1, 5, and 10 g/L were tested with a constant lactic acid concentration of 20 g/L. Furthermore, we tested fermentation broths with different pH values of 3, 4 and 5 with fixed lactic acid and acetic acid concentrations of 10 and 2 g/L, respectively. The ammonium concentration of fermentation broths remained the same at 1 g-N/L under all scenarios. We conducted duplicate experiments in two parallel ED reactors. Before each batch experiment, the chambers and ion exchange membranes were thoroughly rinsed with deionized water to remove any possible fouling from previous experiments. The same reactor Samples were collected in the same manner as Section 2.1.

2.3. Recirculation and pH control effect on ED performance

Recirculation and pH control of lactic acid fermentation broth in two dilute chambers were investigated to simulate a continuous flow in real-life applications, as shown in Fig. A1 b. Each dilute chamber was connected to an external serum bottle containing 500 mL of synthetic lactic acid fermentation broth. Consequently, dilute chambers were expanded from 200 mL to 700 mL. By circulating the dilute at a rate of 20 mL/min, we aimed to simulate a continuous flow in real ED applications and to reduce the internal resistance by a better mixture. Moreover, the pH in two dilute chambers was controlled at 4 ± 0.5 to simulate a acidic pH of real lactic acid fermentation broth supplied in a continuous mode [23].

A control group ran the ED reactor in a batch mode without recirculation bottles or pH control, being the same setup as those in previous sections. So three groups of ED systems were set up, i.e., recirculation (without pH control), recirculation with pH control, and no recirculation & no pH control. A baseline concentration of 1 g/L ammonium, 10 g/L lactic acid, and 2 g/L acetic acid was used in each group of experiments. In order to completely recover nutrients from expanded dilute chambers, the operation period of two recirculation groups was extended from 7 days to 20 days.. We conducted daily sampling in the same manner as previously described.

2.4. SCP production by S. cerevisiae using ED recoveries

S. cerevisiae was routinely grown on yeast extract-peptone-dextrose (YPD) medium in conical flasks [8]. Aseptic polypropylene film covered the flask, which allowed air exchange during incubation. Before the ED recoveries were used as substrates, *S. cerevisiae* grew on the AAB medium for two generations. Each recovery from previous ED experiments was adjusted to pH 5.6, separated into 50 mL flasks, and autoclaved for 15 min. Afterwards, $MgSO_4$, trace elements, and vitamins were supplemented through a 0.45 um membrane with the same concentration as the AAB medium [8]. Each bottle was inoculated with 2 mL of parent broth from the AAB medium during its exponential growth phase. *S. cerevisiae* was cultured at 25 °C in a shaking incubator for 6–9 days until the optical density (OD) was stable. A 1.5 mL broth sample was collected daily from flasks to measure OD, pH, ammonium, and VFA levels. In the case of pH values above 8, 1 M HCl was added to bring pH down to around 6. After incubation, 4 mL of broth was taken for the protein test and 30 mL for the biomass test.

2.5. Analytical and calculation methods

2.5.1. Evaluation of ED recovery performance

The ED performance was evaluated in terms of the recovery efficiency, maximum ion flux, and current efficiency in recovering La^-, Ac^-, and NH_4^+ ions. They were calculated according to Eq. (1)–(3), respectively.

$$RE_i = \frac{C_{i,m} \bullet V_m}{C_{i,0} \bullet V} \times 100\% \quad (1)$$

where RE_i denotes the recovery efficiency of a specific ion (%), i.e., La^-, Ac^-, or NH_4^+; $C_{i,m}$ is the final concentration of the ion in the concentrate chamber of ED (mM); $C_{i,0}$ is the initial ion concentration in the dilute chamber (mM); V_m is the effective volume of the concentrate chamber, 100 mL; V is the effective volume of the dilute chamber, 200 mL.

$$J_i = \frac{C_{i,t} \bullet V_m}{A \bullet t} \quad (2)$$

where J_i is the ion flux of La^-, Ac^-, or NH_4^+ ($mmol \cdot m^{-2} \cdot h^{-1}$); $C_{i,t}$ represents the ion concentration in the concentrate chamber at time t (mM), A is the surface area of ion exchange membrane (m^2), t is the course of time (h). A maximum flux was adopted to compare the ion flux in different scenarios, given that the target ions showed different recovery rates at the beginning and end of ED. During seven days of ED, the maximum flux was calculated based on the period with the most rapid recovery of target ions, i.e., the first five days for La^- and Ac^- (t = 5) and the first three days for NH_4^+ (t = 3). When ED had an external recirculation of dilute, an average ion flux over 20 days (t = 20) was calculated in addition to the maximum ion flux in order to assess the long-term recovery performance.

$$CE_i = \frac{z \bullet F \cdot C_{i,m} \cdot V_m}{\bar{I} \cdot t \cdot M} \times 100\% \quad (3)$$

where CE_i is the current efficiency of La^-, Ac^-, or NH_4^+ (%); z is the ion valence of the ion; F is the Faraday constant, 96,485 C/mol; $\bar{I}$ is the average electrical current during ED (A); t is the course of operation (s); M is the molar mass of the ion (g/mol). The total current efficiency of the ED process is the sum of the current efficiencies of La^-, Ac^-, and NH_4^+.

The separation factor is a parameter expressing membrane selectivity between two ions [26]. In this study, La/Ac separation factor (α_A^L) was introduced to assess the different migration abilities between La^- and Ac^- (Eq. (4)). The equation allows an accurate comparison of migration rates because it incorporates the initial concentrations of both ions. α_A^L is a positive number. The α_A^L value between 0 and 1 indicates that La^- migrates slower than Ac^-. When La^- moves at the same speed as Ac^-, the α_A^L value equals 1. When the α_A^L value is greater than 1, it means La^- migrates faster than Ac^-.

$$\alpha_A^L = \frac{1 - C_{L,t}/C_{L,0}}{1 - C_{A,t}/C_{A,0}} \quad (4)$$

where α_A^L is the La/Ac separation factor when La^- and Ac^- simultaneously cross the anion exchange membrane; $C_{L,t}$ and $C_{A,t}$ represent the concentration of lactic acid and acetic acid in the cathode chamber at time t, respectively; $C_{L,0}$ and $C_{A,0}$ represent the initial concentration of lactic acid and acetic acid in the cathode chamber, respectively.

2.5.2. Analytical methods

VFA concentrations, ammonium concentrations, and pH were tested for the liquor samples taken from ED chambers and yeast broths. Ammonium concentrations were determined by an automated wet chemistry analyzer (San series, Skalar, Netherlands). Acetic acid concentrations were tested by gas chromatography (GC) (TRACE 1300, Thermo Scientific, USA). Lactic acid concentrations were examined by a high-performance liquid chromatograph (HPLC) equipped with a RID-10A refractive index detector (Nexera, Shimadzu, Japan). The dissociation and species distribution of acetate and lactate at different pHs were calculated using CurTiPot freeware V4.3.1.

2.5.3. Determination of biomass and SCP

The OD of *S. cerevisiae* was measured at 600 nm. Biomass concentration was determined by the gravimetric method. Briefly, 30 mL broth was centrifuged at 8000 rpm for 10 min. The pellet was resuspended twice with 30 mL of deionized water to remove soluble salts. The remaining pellet was lyophilized in a freeze dryer (CoolSafe, ScanVac, Denmark) for 24 h. The weight of the dry biomass (g) divided by the broth volume (0.03 L) was the biomass concentration (g/L). 10 mg of the dehydrated biomass was used to detect the amino acid profile. It was first dissolved in 500 μL 6 N HCl and hydrolyzed at 130 °C in a microwave digestion system (3000 SOLV, Anton-Paar®, Austria) for 30 min. After hydrolysis, the hydrolysate could be analyzed by HPLC-MS-MS (1290 Infinity II-6470 QQQ, Agilent Technologies, USA) for amino acid content. SCP production of *S. cerevisiae* was evaluated by determining the protein concentration in broth. In short, 2 mL broth was centrifuged for 3 min at 10000 rpm and washed twice with deionized water. The pellet was resuspended in 2 mL of 1 M NaOH and boiled for 10 min in a glass tube. The thermal and alkaline treatments could better dissolve the cellular protein in the liquor. After that, the liquor was ready for the Lowry protein assay [26]. Protein yield was calculated by multiplying the protein concentration in broth (g/L) by the total organic carbon consumption during incubation, including lactic acid and acetic acid (g-C/L). SCP manufacturing cost was estimated by adding up the electricity cost of ED and the chemical addition cost for yeast growth and then divided by the net protein weight.

3. Results and discussion

3.1. VFA and ammonium recovery by the ED system

ED reactors were operated at 1.5, 2, 2.5 and 3 V, respectively. The recovery efficiency, ion flux and current efficiency of La^-, Ac^-, and NH_4^+

at different voltages were compared in Fig. 1. A higher voltage was generally associated with higher La^-, Ac^-, and NH_4^+ recovery efficiencies, except at 3 V for NH_4^+ (Fig. 1a). La^-, Ac^-, and NH_4^+ had a maximum recovery efficiency of 49%, 53%, and 51%, respectively. According to Fig. 1b, maximum ion flux followed the same trend as recovery efficiency. At 3 V, NH_4^+ showed the highest flux of 48 $mmol \cdot m^{-2} \cdot h^{-1}$, followed by La^- flux at 30 $mmol \cdot m^{-2} \cdot h^{-1}$, and Ac^- flux at 19 $mmol \cdot m^{-2} \cdot h^{-1}$. The flux ratio between NH_4^+, Ac^- and La^- was 3:2:1. When we calculated the product value of the initial ion concentration (C_i) in dilute chambers and the corresponding diffusion coefficient of three ions (D_i) (Table A1), it showed the same ratio of 3:2:1 as ion flux. The result revealed that electromigration was the dominant mechanism driving the ion flux in the early period of ED compared to diffusion and convection when the Nernst-Plank equation (Eq. A1) can be simplified to Eq. A2. Therefore, given the same voltage ($\nabla\varphi$), ion flux (J_i) was proportional to $D_i{*}C_i$.

Fig. 1c illustrates the current efficiency in recovering La^-, Ac^-, and NH_4^+. A seven-day ED process was separated into three stages to better compare the variation over time. The figure did not show the current efficiency at 1.5 V, given the shallow electrical current. 2 V exhibited the highest and most stable total current efficiency at 66–76%. In contrast, at 2.5 and 3 V, the total current efficiency was less than 30%. Follow-up batch experiments were all conducted at 2.5 V, considering both the current efficiency and the total amount of recovery. The efficiencies were far from 100% because Na^+ and H^+ in the anode chamber and Cl^- and OH^- in the cathode chamber were co-transported with target ions by the current into the middle chamber. A higher current can further reduce membrane selectivity [27]. More untargeted ions such as Na^+, H^+, Cl^-, and OH^- rushed into the membrane and reduced the current efficiency of NH_4^+, Ac^-, and La^-. Another reason was that higher electrical current triggered side electrochemical reactions (such as water hydrolysis and chlorine formation) rather than simply transporting ions. Current efficiency is also a good indicator of the priority of ion migration based on its evolution over time. NH_4^+ was predominantly recovered on Day 1–3, followed by La^- being mainly recovered on Day 3–5, and the recovery of Ac^- lasted the longest period from Day 3 to Day 7 (Fig. 1c). The difference in the diffusion ability of competitive ions was responsible for this phenomenon. The $D_i{*}C_i$ value determines the ion flux at a fixed voltage (Eq. A2). The initial $D_i{*}C_i$ value of Cl^-, La^- and Ac^- were 145, 114 and 36 $M \cdot 10^{-2} \cdot cm^2 \cdot s^{-1}$, respectively. Consequently, Cl^- could pass the anion exchange membrane as soon as ED started, while La^- and Ac^- caught up later. The recovery of Ac^- lasted longer than La^- because the current decreased with time (Fig. A3), and Ac^- with a smaller molar mass had priority to migrate under low currents [27]. In contrast, the migration of NH_4^+ through the cation exchange membrane started rapidly during ED due to a high $D_i{*}C_i$ value of 138 $M \cdot 10^{-2} \cdot cm^2 \cdot s^{-1}$. After three days, however, the current efficiency of NH_4^+ plummeted (Fig. 1c). This was due to the continuous formation of H^+ in the anode, which had a 5-fold higher diffusion coefficient than NH_4^+ (Table A1). The $D_i{*}C_i$ value revealed that, at 2.5 V and 3 V, H^+ was more competitive than NH_4^+ to cross the membrane after 3–5 days (Fig. A4), so H^+ occupied the ion channel of the membrane and blocked the further migration of NH_4^+.

Additionally, Fig. A2 plotted La^-, Ac^-, and NH_4^+ concentrations in the concentrate as a function of time. A consistent saturation of La^- and Ac^- concentrations was observed regardless of applied voltage, recovering quickly on the first three or five days and slowing down on subsequent days. The stagnant period was previously reported in an ED for chromium removal, where the plateaus intensified at higher initial chromium concentrations [28]. One possible explanation for the behavior was the depletion of charged ions over time, but there was no apparent drop in the conductivity of dilute. It is more likely due to concentration polarization. Driven by the electrical current, the ions continuously migrated, bounded to the membrane surface, and accumulated with

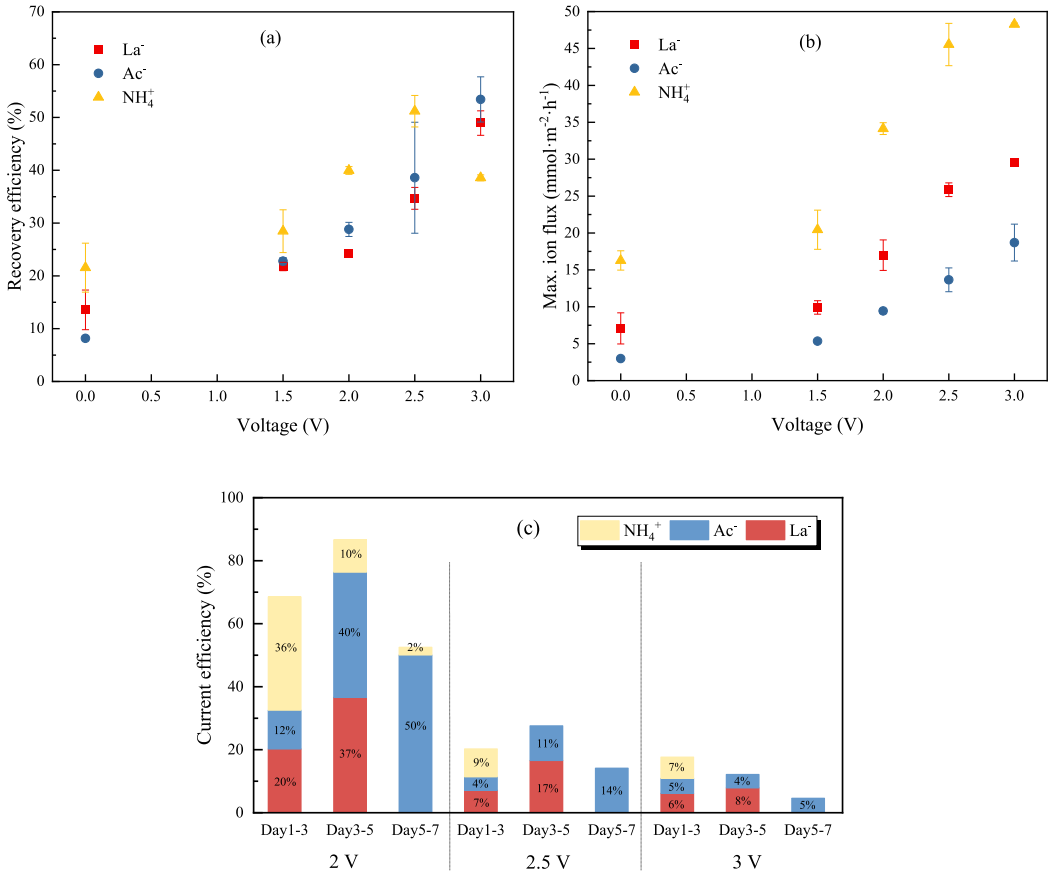

Fig. 1. Voltage effect on the (a) recovery efficiency, (b) ion flux of La^-, Ac^- and NH_4^+, and (c) current efficiency of ED.

time. Only 16.5% of targeted ions managed to enter the concentrate, while the membrane retained 83.5% of target ions, according to a previous study on the ED process [28]. Concerning NH_4^+, the recovery rate declined on the second or third day, earlier than La^- and Ac^- (Fig. A2 e), because anolyte acidification formed more H^+, which was more competitive than NH_4^+ to pass the membrane.

Based on the findings, an applied voltage of 2.5 V was selected for subsequent experiments to maximize the recovery efficiency while maintaining acceptable current efficiencies. At this voltage, electrodialysis required five days to achieve the highest possible recovery of La^-, Ac^- and NH_4^+ ions.

3.2. Impact of lactate concentration on the ED recovery performance

As seen from Fig. 2a, different lactate concentrations in the range of 10–40 g/L had no impact on the recovery efficiency of the three target ions. The recovery efficiency of La^-, Ac^-, and NH_4^+ were 31–39%, 27–46%, and 41–51%, respectively.

As La^- and Ac^- showed different recovery rates at the beginning and end of the ED process, the maximum ion flux during the first five days was applied instead of a seven-day average ion flux to compare the recovery potential better. The α_A^L value was also calculated based on two phases, Day 1–5 and Day 5–7. Fig. 2b shows that higher initial lactic acid concentrations from 10 to 30 g/L increased the La^- flux but decreased the NH_4^+ flux. There was no further flux change with 40 g/L lactate. At 10 g/L of initial lactate, La^- and NH_4^+ flux were 26 and 46 mmol/(m^2·h), respectively. At 40 g/L of lactate, La^- flux increased to 61 mmol/(m^2·h) while NH_4^+ flux decreased to 34 mmol/(m^2·h). The increase of La^- flux was because ion flux across the membrane is proportionate to the initial ion concentration. The NH_4^+ flux was adversely affected because more competitive Na^+ was introduced at higher initial lactate concentrations (added as sodium lactate). The α_A^L value was always below 1, indicating Ac^- migrated faster than La^- (Fig. 2b). Moreover, the α_A^L value was stable at different lactate concentrations, suggesting that a large amount of La^- would not pose a competitive threat to the migration of Ac^-.

3.3. The impact of acetate concentration on the ED recovery performance

With higher acetate concentrations in the lactic acid fermentation broth, the recovery efficiency of three target ions was not affected in the ED system (Fig. 3a). The recovery efficiency of La^-, Ac^-, and NH_4^+ was 26–36%, 35–50%, and 41–47%, respectively. However, it significantly impacted the ion flux and α_A^L (Fig. 3b). The ion flux of Ac^- increased by a factor of 7, while the La^- flux decreased to 74% when the acetate concentration increased from 1 to 10 g/L. The trend of Ac^- flux corresponded to the positive correlation between ion flux and initial ion concentration (Eq.A2). The result also revealed that Ac^- transport was preferential over La^- transport, and Ac^- could occupy ion channels in the membrane by increasing acetate concentrations. Several factors can affect the transport rate of different ions, including their charge, size (Stokes radius), molar mass, the ionic strength of electrolyte, and the mass ratio of co-existing ions [29-31]. Since Ac^- and La^- were in the same electrolyte and had similar Stokes radius (Table A1), ion conductivity [32], and charge, the superiority of Ac^- could be due to its smaller molar mass. As indicated by the α_A^L value, there was a considerable change in the selectivity between La^- and Ac^- across the anion exchange membrane (Fig. 3b). The α_A^L value during Day 1–5 decreased significantly as acetate concentration increased from 1 to 10 g/L, revealing that La^- migration slowed down while Ac^- accelerated. Interestingly, the α_A^L value was above one when the initial acetate concentration was 1 g/L. It implies that La^- could take precedence to be transported through membrane at a shallow concentration of acetate. During Day 5–7, there was an opposite trend of α_A^L with increasing acetate concentrations, suggesting an accelerated La^- migration. Since Ac^- could easily migrate through the membrane on Day 1–5, the migration of La^- was inferior at that time. As long as Ac^- was depleted, La^- migration would took over. Apart from that, the α_A^L value on Day 5–7 was smaller than the value on Day 1–5 when the initial acetate concentration was 1–5 g/L. The gap between two values further demonstrated the preferential of Ac^- migration over La^- during the early phase of ED.

3.4. pH effect on the ED recovery performance

We investigated the recovery performance of ED treating lactic acid-rich fermentation broth at different pH levels. The broth pH had the largest impact on NH_4^+ recovery, increasing its recovery efficiency from 35% to 62% when pH increased from 3 to 5 (Fig. 4a). A reduced ion competition between NH_4^+ and H^+ was responsible for the increase in efficiency. The anolyte at a higher pH contained less H^+, which has a diffusion coefficient (9.3×10^{-5}·cm^2·s^{-1}) five times greater than NH_4^+ (2.0×10^{-5}·cm^2·s^{-1}) (Table A1). Fig. 4b illustrates the product of the ion diffusion coefficient and ion concentration of H^+ and NH_4^+ in the anode chamber (D_i*C_i). The D_i*C_i value assesses the migration ability of H^+ and NH_4^+ across the membrane. At pH 3, H^+ had 5–19 times greater D_i*C_i values than NH_4^+ since the second day of ED. It indicated that NH_4^+ was fundamentally less competitive than H^+ in migration, contributing to the lowest recovery efficiency. As pH increased, the D_i*C_i value of H^+ decreased dramatically. NH_4^+ had higher D_i*C_i values than H^+ during the first four days at pH 4, and the superiority was more pronounced at pH 5. Therefore, with fewer competitive H^+ ions, more NH_4^+ could be recovered.

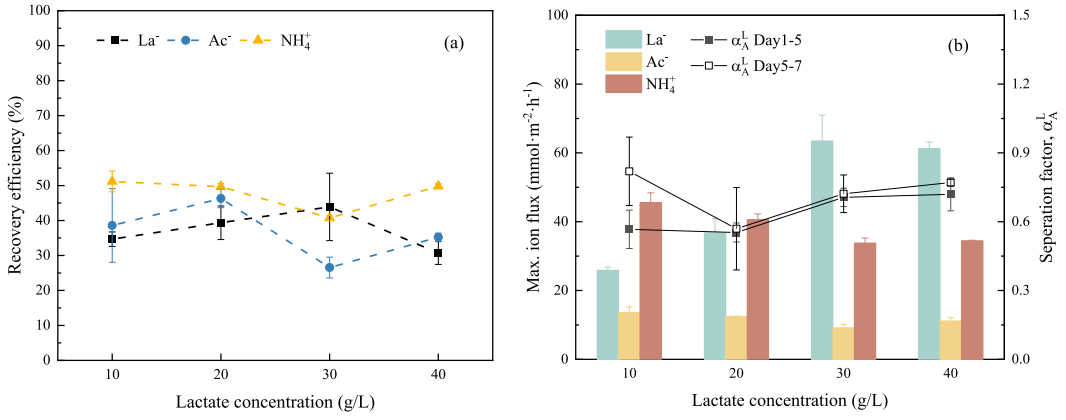

Fig. 2. Lactate concentration effect on the (a) recovery efficiency, and (b) maximum ion flux of La^-, Ac^- and NH_4^+, and La/Ac separation factor (α_A^L).

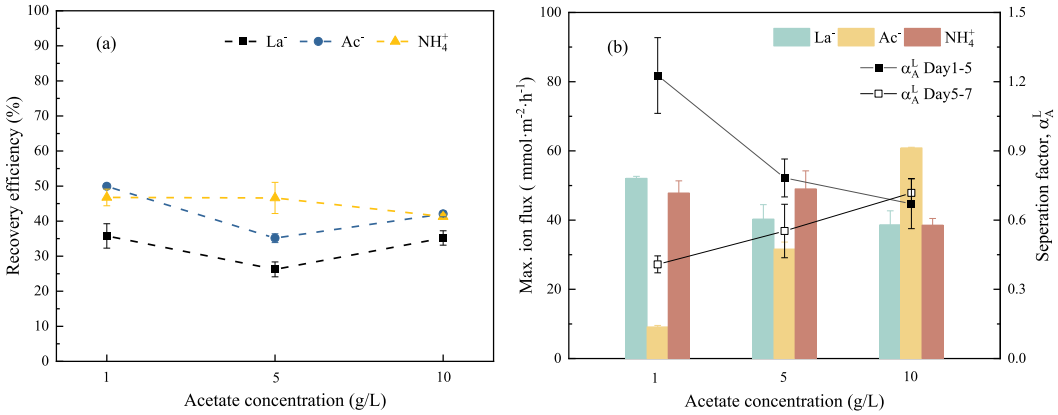

Fig. 3. Acetate concentration effect on the (a) recovery efficiency, and (b) ion flux of La^-, Ac^- and NH_4^+.

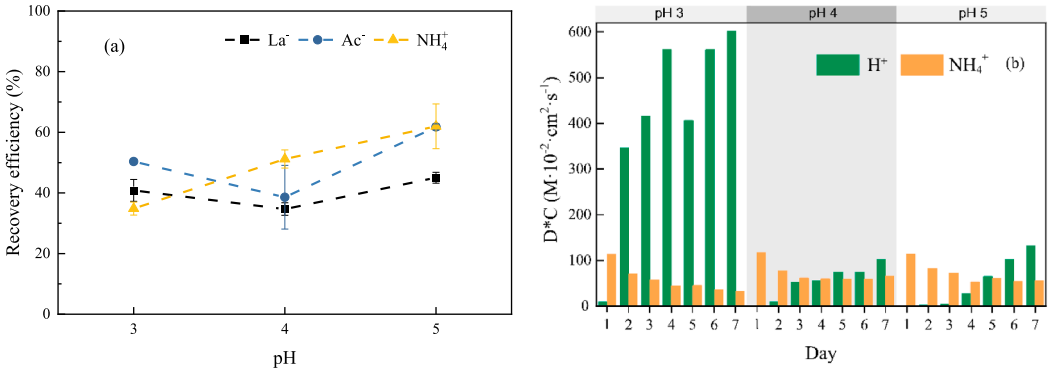

Fig. 4. Ph effect on the (a) recovery efficiency of la^-, Ac^- and NH_4^+, and (b) the product of ion diffusion coefficient and ion concentration (D_i*C_i) of H^+ and NH_4^+.

3.5. ED recovery performance with broth recirculation and pH control

Fig. 5 shows the recovery results of ED systems with recirculation and pH control in dilute chambers. In the control group without recirculation and pH control, the maximum recovery efficiency was 38% for La^-, 34% for Ac^-, and 51% for NH_4^+ during seven days of ED process (Fig. 5d). For maximum recovery, ED ran 20 days in the new setup with recirculation. Even though more La^- and Ac^- were recovered in the middle chamber at the end of ED (Fig. 5a, 5b), the overall recovery efficiency was extremely low at 22–28% for La^- and Ac^- (Fig. 5d). They both decreased to 9% when the dilute pH was controlled at 4. The low efficiency was because only 18% acetate dissociated into ionic Ac^- and 64% lactate into La^- at pH 4. The undissociated VFAs were uncharged and could not be electro-migrated. Regarding NH_4^+, pH control did not affect NH_4^+ recovery. The NH_4^+ concentrations reached 129–132 mM without or with pH control. In both cases, NH_4^+ in the concentrate was gradually lost after one week (Fig. 5c), resulting in a low actual recovery efficiency of 8–13% (Fig. 5d). Similar loss of Ac^- was observed in the ED system with recirculation and pH control (Fig. 5b). So the actual recovery efficiency of Ac^- was only 4% compared to its maximum recovery efficiency of 9% (Fig. 5d). With pH control in dilute chambers, a stable pH of concentrate was thereby achieved around pH 4. As a result, external heterotrophs spoiled recoveries under unsterilized conditions over a long time. By contrast, without pH control in dilute chambers, the pH of the concentrate was around 2 after one week, effectively preventing microbial contamination.

Therefore, if lactic acid fermentation broth is continuously supplied to ED systems in real-world applications, a strong acidic pH may hinder VFA recovery. Instead, by using the batch mode to fill the fermentation broth, natural alkalization in the cathode chamber can promote VFA recovery. Moreover, a short operation cycle of ED is necessary to avoid losing recovered components.

3.6. SCP yield and economic cost

All the recovered resources from ED systems were collected with variable La^-, Ac^-, and NH_4^+ concentrations. They were directly used as substrates for *S. cerevisiae* after regulating pH to 5.6 and adding essential trace elements and vitamins. After a few days of incubation at 25 °C, *S. cerevisiae* reached a maximum OD of 3.6 (Fig. 6a). Proteins accounted for 49% of the biomass, with an average yield of 0.62 ± 0.25 g/g-C. Essential amino acids were 1.7–3.3 times higher than the Food and Agriculture Organization of the United Nations (FAO) recommendations regarding high-quality protein for adults. The SCP products of *S. cerevisiae* were most affluent in glutamine/glutamic acid as nonessential amino acid and leucine as essential amino acid (Fig. 6b).

In the lab-scale manufacture of SCP, electricity usage in the ED system and chemical supplements in yeast broth were two main components that contributed to the cost. The two cost aspects are illustrated in Fig. A5. Costs were significantly affected by the voltage applied to the ED system. Higher voltages from 1.5 V to 3 V increased electricity costs (from €1 to €17/kg SCP) but reduced raw material costs (from €8 to €3/kg SCP) because more VFAs and NH_4^+ were recovered, contributing to more SCP production with the same amount of chemical supplement. Under various VFA concentrations, pH levels, and a new ED configuration with recirculation, the specific electricity cost at 2.5 V was stable at €6-10/kg SCP, and the raw material cost was steady at €3-6/kg SCP. To obtain a final protein product, downstream processes such as protein separation and purification are necessary, which would increase the overall production cost. It is also important to note that the ED system and yeast SCP production on a commercial scale may lead to quite different expenditures. Therefore, a comprehensive economic

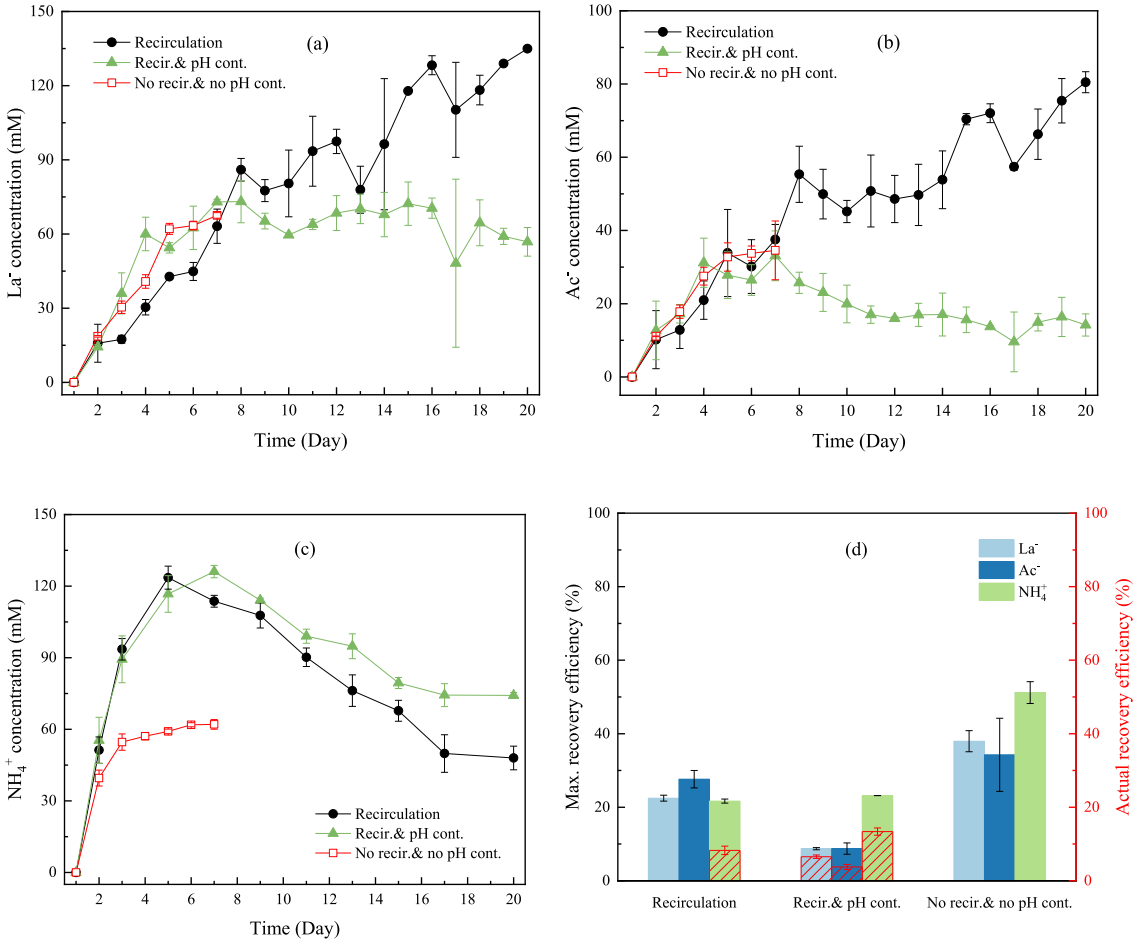

Fig. 5. Recirculation and pH control effect on (a) La^- recovery, (b) Ac^- recovery, (c) NH_4^+ recovery within 20 days, and (d) recovery efficiencies. The maximum recovery efficiency was based on the maximum ion concentration in ED process; the actual recovery efficiency was based on the final ion concentration at the end of ED process.

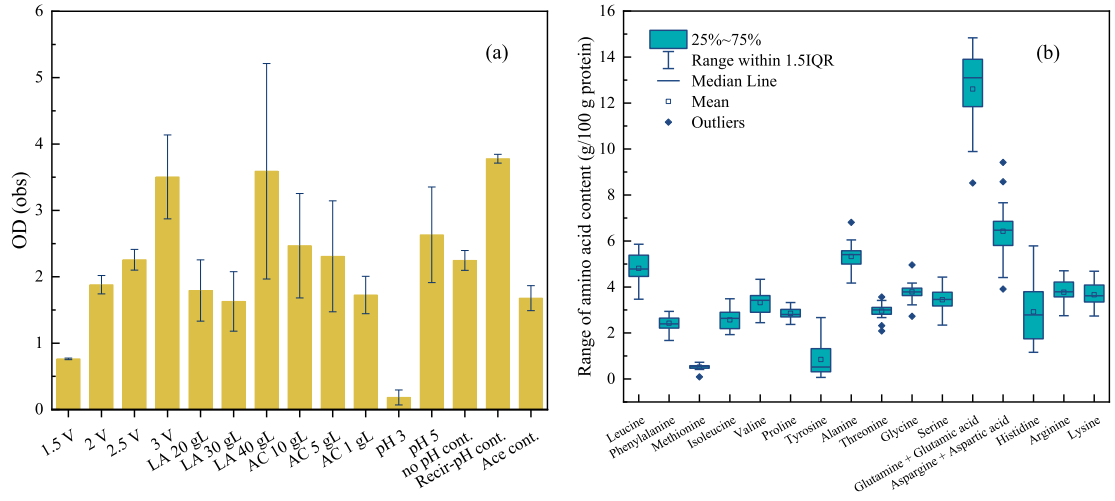

Fig. 6. Maximum optimal density of *S. cerevisiae* using ED recoveries (a), and amino acid profile of SCP products (b).

evaluation of the yeast production process is required in future studies. This evaluation should consider additional costs such as equipment, packaging, transportation, labor, and other related expenses to determine the feasibility of the process in a commercial setting.

3.7. Future perspective

While it is feasible to hybrid ED-based nutrient recovery from artificial biowaste fermentation broth and yeast-based SCP production, the process is still limited by the low recovery efficiency of target ions. It is

possible to improve the recovery in future studies from two aspects. First, the configuration of ED system can be upgraded by introducing more pairs of cells and making thinner chambers [20]. In the multi-cell ED stack, the electrolyte in the chamber with electrodes should exclude Cl^- to avoid chlorine formation. Second, the electrode materials can be optimized to reduce side redox reactions so that the current can be efficiently used for ion migrations. In addition, it would be worthwhile to investigate the co-transport of untargeted inorganic ions during ED, which are present in the real lactic acid-rich biowaste fermentation broth. For example, synchronous recovery of Na^+, K^+, and HPO_4^{2-} into the concentrate could be advantageous for subsequent SCP production since both are essential elements for yeast. It can further reduce the cost of raw material usage in yeast SCP production. After that, real biowaste fermentation broth can be used to determine the efficiency of ED process and the cost-effectiveness of manufacturing SCP.

When it comes to the market potential of yeast SCP products, one of the primary challenges for marketing is meeting regulatory requirements for food safety. In European countries, for instance, new food products must pass the Novel Food Regulation by the EU. While *S. cerevisiae* has already been approved for food use [2], using waste streams as its substrate requires a thorough evaluation of its safety. In this study, electrodialysis was used to recover nutrients from food waste fermentation broth, which to some extent ensured the safety of the substrate by avoiding pathogens and micropollutants [17-19]. However, before using the recovery from real food waste fermentation broth as a yeast substrate, its safety must be thoroughly examined by detecting and excluding harmful chemicals and pathogens that may pose risks to yeast growth and/or human consumption. Additionally, customizing the downstream process for yeast protein purification is crucial to bringing the new food protein to market.

4. Conclusion

In this study, ED and SCP biosynthesis were integrated to recover La^-, Ac^-, and NH_4^+ from biowaste and use these directly as fundamental nutrients of *S. cerevisiae* to produce SCP. We elucidated the effect of composition fluctuation in biowaste fermentation broth on the recovery performance and deciphered the ion competitions during ED. The initial acetate (1–10 g/L) and lactate concentrations (10–40 g/L) were found to have no impact on the recovery efficiency of the three target ions but to have an effect on ion flux and ion competition between La^- and Ac^-. A higher initial lactate concentration increased La^- flux and decreased NH_4^+ flux, while a higher initial acetate concentration increased the Ac^- flux and decreased La^- flux. As acetate concentration increased, Ac^- migration accelerated while La^- migration slowed down. Ac^- usually passed the membrane faster than La^-, but La^- would take precedence with deficient acetate. A pH increase in lactic acid fermentation broth improved NH_4^+ recovery efficiency by less ion competition from H^+. The dilute recirculation of ED resulted in a loss of Ac^- and NH_4^+ over an extended period. The pH control of dilute decreased VFA recovery efficiency as more uncharged VFAs could not pass membranes, and more recovered VFAs were lost. Using ED recoveries, the average SCP yield was 0.62 ± 0.25 g/g-C with all essential amino acids above the FAO guideline. SCP was produced using the nutrients from fermentation broth at the cost of €6-10/kg SCP in electricity and €3-6/kg SCP in raw material. This study will improve the understanding of ion competitions during multiple species recovery by ED and guide the future development of yeast SCP using biowaste nutrients.

CRediT authorship contribution statement

Danfei Zeng: Investigation, Data curation, Methodology, Visualization, Validation, Writing – original draft. **Song Wang:** Resources, Validation. **Yufeng Jiang:** Resources, Validation. **Yanyan Su:** Supervision, Writing – review & editing. **Yifeng Zhang:** Conceptualization, Supervision, Project administration, Funding acquisition, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.seppur.2023.123632.

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