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Recycling of acetate and ammonium from digestate for single cell protein production by a hybrid electrochemical-membrane fermentation process

Danfei Zeng, Yufeng Jiang, Carina Schneider, Yanyan Su, Claus Helix-Nielsen, Yifeng Zhang

Abstract
Digestate is a potential substrate for yeast to produce single cell protein (SCP), but its complex matrix constrains applications. This study developed a hybrid electrochemical-membrane fermentation process to recover nutrients from synthetic digestate and produce yeast SCP. Electrodialysis (ED) was first employed to recover acetate and ammonium. More nutrients were recovered at a higher voltage but with lower current efficiency. At 2 V, 42.2% and 60.1% of acetate and ammonium were reclaimed, and further concentrated by 14- and 10-fold via a forward osmosis (FO) process. Due to the complexity of real digestate, the recovery efficiency of ED in practical applications should be further investigated. Saccharomyces cerevisiae fed on raw and concentrated recovery achieved similar SCP productions of 0.76-0.86 g/L, with amino acid concentrations above the FAO recommendation by 1.5-4.2-fold. The total production cost was estimated to be €20.4/kg-SCP by single ED, while the hybrid ED-FO process cost 40 times more.

1. Introduction
By 2050, the world will need twice as much animal protein as today to support a population of around 9.7 billion (McLeod, 2011; United Nations, 2019). It is extremely difficult to sustain the expansion of food supply based on traditional agriculture which are highly dependent on natural resources. In this context, single cell protein (SCP), discovered a century ago, is now attracting attention as an alternative food protein source. SCP is the dried biomass of microorganisms and contains a high amount of protein. Compared with conventional livestock- and crop-derived protein sources, SCP has a shorter harvest period, less water or arable land demand, and lower climate dependence (Ritala et al., 2017). SCP can be produced by various microorganisms, including bacteria, yeast, filamentous fungi, and algae. Yeast stands out among the others because it has been widely accepted historically as a safe food supplement (Nasseri et al., 2011). Under the framework of circular economics, the substrate of yeast can be derived from waste streams. Currently, food and agriculture waste streams are primarily applied to produce yeast SCP, given the extraordinary ability of yeast to assimilate sugars and proteins. Especially, the yeast strain Saccharomyces cerevisiae is able to consume acetate and ammonia (NH₄-N) as the organic and nitrogen source (Molitor et al., 2019; Schüller, 2003). Acetate and NH₄-N are prevalent in the digestate from anaerobic digestion (AD) of various agricultural and industrial wastes. Thus, digestate could be alternative carbon and nitrogen feedstock for yeast to produce SCP, which is currently underestimated and lacks systematic investigation.

As SCP is the next-generation protein source for food or fodder, the growth substrate should be safe and reliable. Using the complex and fluctuant matrix of waste streams such as digestate directly as feedstock may cause health risks and quality uncertainty for the SCP produced. In order to address this challenge, pretreatment of digestate is necessary. Electrodialysis (ED) is an electrochemical separation process using electrical current to transport charged ions through a stack of ion exchange membranes and leaving the desired product in the concentrate stream. It is an effective approach to recover nutrients from waste streams and, at the same time to reject undesired substances, e.g., pathogens and micropollutants (Arola et al., 2019; Roman et al., 2020). In addition, ED saves more energy, produces less sludge, and requires less pretreatment than other nutrient recovering methods, such as chemical precipitation, membrane separation, absorption, etc.
ED has been applied in wastewater reclamation typically for one specific species, e.g., NH$_4$-N (Vecino et al., 2020; Ward et al., 2018), phosphorus (P) (Liu et al., 2017; Rotta et al., 2019), volatile fatty acids (Jones et al., 2021; Pan et al., 2018) or heavy metals (Gurreri et al., 2020; Wang et al., 2021). VFA and NH$_4$-N are dominant species in digestate (Cai et al., 2013; Park et al., 2010). However, few studies have been reported for the simultaneous recovery of VFA and NH$_4$-N using ED. Although the ED process can recover nutrients from digestate, the recovery is a mixture of multiple ions. Thus, downstream processes, e.g., gas stripping for ammonia, and adsorption for VFAs, are required to separate and manufacture the recovered resources into marketable products (Tharani and Ananthasubramanian, 2021; Ukwuani and Tao, 2016). Direct upcycling of the recovered resources mixture into high value products could reduce the considerable expense in downstream processes, which had not received attention it deserves. Furthermore, ED process has limited ability to up concentrate the recovery, which may hamper the upcycling processes that are dependent on the high concentration of feedstocks. In this context, forward osmosis (FO) could be a cost-effective method to concentrate the recovery (Schneider et al., 2019; Zarebska-Malgaard et al., 2022). It takes advantage of the natural osmotic pressure gradient across a semi-permeable membrane and enriches target chemicals by the exclusion mitigation of water molecules. Therefore, FO can be operated with low energy demand and typically with a lower risk of membrane fouling propensity. Moreover, all nutrients, including dissolved organic carbon, NH$_4$-N, and P, can be simultaneously retained in the concentrate by the FO process (Xue et al., 2015; Zhang et al., 2014).

This study developed a novel process that integrated electrochemical recovery of ammonium and acetate from digestate, FO-based concentration, and fermentation processes for SCP production using yeast. The electrochemically recovered ammonium and acetate were collected to feed the brewer’s yeast S. cerevisiae directly or concentrated by a FO membrane module first before feeding the yeast. We investigated the voltage effect on the recovering performance of ED. An internal loop was set up as shown in Fig. 1. The reactors were made of polymethyl methacrylate. Silicone gasket and stainless screws were used to assemble the reactor. The effective volumes of the anode, middle, and cathode chamber were 200, 100, and 200 mL, respectively. The particular half volume of the middle chamber aims to better concentrate the migrated ions. The anode electrode was made of titanium alloy coated with IrO$_2$ with a dimension of 4 × 4 cm (MAGNETO special anodes, Evoqua Water Technologies, the Netherlands). The IrO$_2$ coating as a catalyst can induce oxygen evolution reaction and guarantee high stability in the acidic electrolyte, especially in the presence of halides such as Cl$^-$. The anode electrode, coated with 0.5 mg/cm$^2$ 20 wt% Pt/C (Johnson Matthey A/S, Denmark) (Xu et al., 2021). An external power supply (HQPS3003, Helmholt Elektronik A/S, Denmark) was connected with the anode and cathode electrode providing a specific voltage for ED. The anode and cathode chamber was filled with simplified synthetic digestate including 51.4 mM NH$_4$Cl (NH$_4$-N 0.72 g/L) and 86 mM sodium acetate (7 g/L, COD 5.5 g/L) (Jin et al., 2017). We chose the acetic acid concentration based on the experimental data obtained from an AD reactor inoculated with wastewater treatment plant sludge, where methanogenesis was inhibited to accumulate acetic acid (Hollinshead et al., 2014; Jin et al., 2017). The ammonia concentration was determined from a mesophilic biogas plant treating mixed manure and agriculture waste at an organic load of 2.8 kg VS·m$^{-3}·$d$^{-1}$ and a hydraulic retention time of 57 d (Franke-Whittle et al., 2014). The middle chamber contained 26 mM K$_2$HPO$_4$, and was adjusted to an initial pH at 5.6. The recovery process of electro dialysis lasted for 12 days, and 2 mL liquor was daily taken from both three chambers to measure pH, acetate,
and NH₄-N concentrations. In addition, 1, 2, 3 V were respectively applied in ED to determine the voltage effect on acetate and NH₄-N recovering performance. Open circuit (0 V) was the control group, which demonstrated native ion diffusions through ion exchange membranes. The real-time current of ED was registered across a 10 Ω resistor in the circuit by a battery testing equipment (CT-4008W, Neware, China). All the setups and experiments in this study were carried out in triplicate unless otherwise stated. The results reported for the reactors were the average value plus the deviations.

2.2. ED with an internal loop

The anode and cathode chamber were connected through an external circulation to recover both ammonium and acetate from the digestate completely. The configuration was named ‘internal loop’ (Fig. A.1). A peristaltic pump (Sci-Q 325, Watson-Marlow, UK) was operated at a 60 mL/min rate to extract liquid from the anode chamber to the cathode chamber. The overflow went back automatically from cathode to anode. The synthetic digestate was the same as Section 2.1. The ED reactor with an internal loop ran for 12 days, and 2 mL liquor from each chamber was taken daily for pH, acetate, and NH₄-N concentration measurement. The real-time current of ED was recorded in the same way as Section 2.1.

2.3. Configuration and operation of FO system

FO system included a FO module, draw solution (DS), and feed solution (FS) (Fig. A.2a). The configuration of the FO module was illustrated in Fig. A.2b. DS and FS channels were created using silicone pads with a rectangular hollow (5 × 5 cm) in the middle. The effective volume of DS and FS were 20 and 14 mL, respectively. A commercial flat-sheet FO membrane (5 × 5 cm) (Hydration Technology Innovations, USA) separated the DS and FS channel, and the active side was facing FS. The membrane was made of cellulose triacetate on nonwoven support (CTA-FO). The crossflow velocity was 2.5 cm/min. FS was placed on a digital scale (Kern SCD 4.0, Germany) which was connected to an online recording software (Kern, Germany) to monitor the weight change at 3 min intervals.

2.4. S. cerevisiae cultivation using ED recovery and SCP production

2.4.1. Growth medium and cultivation of S. cerevisiae

S. cerevisiae was cultivated in a shaking incubator (150 rpm) at 25°C. The yeast extract-peptone-dextrose (YPD) medium was applied, including 10 g/L yeast extract, 20 g/L peptone, and 20 g/L dextrose. The initial pH of the YPD medium was adjusted to 5.6. S. cerevisiae was pre-cultivated in the acetate-ammonia-based (AAB) medium for two generations before it was used for the SCP fermentation. AAB medium adopted acetate as the sole carbon source and NH₄-N as the sole nitrogen source. It contained 3.27 g/L sodium acetate, 1 g/L NH₄Cl, 0.75 g/L MgSO₄, 4.53 g/L K₂HPO₄, 10 mL/L trace element stock and 1 mL vitamin stock (Table A.1).

2.4.2. S. cerevisiae cultivation using ED recovery and SCP harvesting

SCP synthesis was conducted in two ways using acetate and NH₄-N recovered from ED reactors. One was directly using the recovery as a medium for S. cerevisiae fermentation. The recovered acetate and NH₄-N were collected from the middle chamber of ED reactor at the end of electrolysis. The recovery was distributed to conical flasks, 50 mL each, and supplemented with trace elements to the same concentration as the AAB medium. After autoclaving, MgSO₄ stock solution and vitamins were added through a 0.45 um membrane filter. Another way was to first concentrate the recovered nutrients by FO process and then use the concentrate as a feedstock for S. cerevisiae. Specifically, the concentrated feedstock was diluted by 10 times to 50 mL before use to avoid high osmotic pressure on S. cerevisiae. The following procedures for extra additives were the same as above.

When the medium was ready, 2 mL parent broth from AAB medium was inoculated in the ED recovery and incubated at 25°C. The optical density (OD) of broth was measured every day at 600 nm absorbance by spectrophotometer (Varian-Cary 50, Varian, USA) and 2 mL broth was taken for VFA and NH₄-N test. Acetate concentration was tested by gas chromatography (GC) (TRACE 1300, Thermo Scientific, USA). NH₄-N concentration was determined using a commercial kit (Ammonium Test Kit 100683, Merek, Germany). At the end of incubation (Day 5), 2 mL broth was taken for protein test, and 35 mL was harvested as SCP. The 2 mL broth was pretreated with alkali (1M NaOH) and boiling (10 min), and then measured the protein concentration by the Lowry method at the absorbance of 550 nm (Lowry et al., 1951). The 35 mL broth was centrifuged at 8000 rpm for 10 min and the supernate was discarded. The same volume of deionized water was used to wash it twice to remove dissolvable inorganic salts from biomass. After lyophilization for 24 h, the biomass was dried as the SCP. The weight of dry biomass multiplied by broth volume was the biomass concentration. The SCP synthesis was conducted in two ways using acetate and NH₄-N as the sole nitrogen sources. In addition, 1, 2, 3 V were respectively evaluated how many percentages of acetate and NH₄-N loss were recovered. A modified Gompertz model was applied as a nonlinear fitting method to calculate the maximal growth rate of S. cerevisiae according to Eq. (1).

\[ OD = OD_{\text{max}} \times \exp \left\{ - \exp \left[ \frac{\mu_{\text{max}} \times e}{OD_{\text{max}}} \times (t - \tau) + 1 \right] \right\} \]  

(1)

where OD is the daily optimal density of S. cerevisiae at 600 nm (obs.), t is the incubation time (day), e is the natural base (2.71828). The fitting result of S. cerevisiae’s growth curve by Gompertz model will get the corresponding maximal OD (ODmax), maximal growth rate (\( \mu_{\text{max}} \)), and lag phase (\( \lambda \)).

2.5. Analytical and calculation method

2.5.1. Modelling maximal growth rate of S. cerevisiae

A modified Gompertz model was applied as a nonlinear fitting method to calculate the maximal growth rate of S. cerevisiae according to Eq. (1).
\[ NH_4 - N \text{ loss} = \left( C_{\text{Ac}} - C_{\text{Ac,1}} \right) \cdot V_{\text{Ac2}} - \left( C_{\text{N,Mid,1}} - C_{\text{N,Mid}} \right) \cdot V_{\text{Mid}} \times 100\% \] (3)

where \( C_{\text{Ac,t}} \) and \( C_{\text{N,t}} \) are the acetate and NH\(_4\)-N concentration in the cathode and anode chamber, respectively, on a specific day (t), \( C_{\text{Ac,Mid,t}} \) and \( C_{\text{N,Mid,t}} \) is the corresponding concentrations on the following day. \( C_{\text{Ac,Mid,1}} \) and \( C_{\text{N,Mid,1}} \) are the acetate and NH\(_4\)-N concentrations in the middle chamber on a specific day (t). \( C_{\text{Ac,Mid,1}} \) and \( C_{\text{N,Mid,1}} \) are the corresponding concentrations in the middle chamber on the following day.

The current efficiency of recovering acetate (\( \text{Ac}^- \)) or NH\(_4\)-N under different voltages was calculated by Eq. (4).

\[
\text{Current efficiency} \ (\%) = \frac{\int_0^t F \cdot I \cdot dt}{M} \times 100\% = \frac{\int_0^t (C_i - C_e) \cdot V \cdot dt}{M} \times 100\% \quad (4)
\]

where \( z \) is the ion valence of the ion, i.e. both 1 for \( \text{Ac}^- \) and NH\(_4^+\) ion, \( F \) is the Faraday constant, i.e. 96485 C/mol, \( n \) is the molar concentration of ion (mol/L), \( I \) is the real-time current of ED (A), \( t \) is the time interval for each recorded current (s), \( C_i \) is the final ion concentration in the middle chamber at different voltages, \( C_e \) is the final ion concentration in the middle chamber with an open circuit (0 V). The integration value in the equation was calculated by the ‘trapz ()’ function in Matlab.

2.5.3. Evaluation of FO performance

The water flux (\( J_w \)) was determined by the online weight change of FS divided by the time interval and the effective area of the FO membrane. The reverse salt flux (\( J_s \)) of Na\(^+\) and Cl\(^-\) were determined by the concentration increase in FS divided by membrane area and running period, see Eq. (5). Ion chromatography ( Dionex ICS-5000, Thermo Scientific, USA) and inductive coupled plasma-optical emission spectrometer (ICP-OES, Perkin Elmer Avio 200, USA) were used to examine the Cl\(^-\) and Na\(^+\) concentration, respectively.

\[
J_s = \frac{C_e \cdot V_e - C_i \cdot V_i}{A \cdot t} \quad (5)
\]

where \( C_i \) and \( C_e \) are the final and initial concentration of Na\(^+\) or Cl\(^-\) in ED recovery as FS (g/L), \( V_e \) and \( V_i \) are the final and initial volume of FS (L), \( A \) is the effective FO membrane area (m\(^2\)), \( t \) is the running period of FO process (h).

The solute concentration factor (SCF) was the final conductivity of DS versus its initial conductivity to evaluate the dilution factor of DS. The water concentration factor (WCF) was the final volume of FS versus its final volume to assess the concentration factor of FS. The concentration ratios of targets were calculated by the final concentration of NH\(_4\)-N or Ac\(^-\) in FS divided by the corresponding initial concentration in FS. The rejection ratio of the target ion was to determine how much Ac\(^-\) or NH\(_4\)-N was safely retained in FS rather than permeating into DS and was calculated by Eq. (6).

\[
\text{Rejection ratio} (\%) = \frac{C_{\text{Ac,FS}} \cdot V_{\text{FS}} - C_{\text{Ac,DS}} \cdot V_{\text{DS}}}{C_{\text{Ac,FS}} \cdot V_{\text{FS}}} \times 100\% \quad (6)
\]

where \( C_{\text{FS}}\) and \( V_{\text{FS}}\) are the initial concentration (mg/L) of Ac\(^-\) or NH\(_4\)-N in FS and the FS volume (L), \( C_{\text{DS}}\) and \( V_{\text{DS}}\) are the final concentration (mg/L) of Ac\(^-\) or NH\(_4\)-N in DS and the DS volume (L).

2.5.4. Energy consumption and cost calculations

The majority of energy consumption for SCP production was derived from the pumps used in DS and FS circulation during the FO concentration process and the voltage input for the ED cell. The energy consumption of FO pump (\( E_{\text{FO}}\), kWh) was calculated by multiplying electrical power (P, W) with the total operation period (T, h). Given the constant voltage of the ED cell, the energy consumption of ED (\( E_{\text{ED}}\), kWh) was calculated by the constant voltage (U, V) times the numerical integration of electrical current (I, A) over time (t, h). The integration value was calculated using the ‘trapz ()’ function in Matlab. The specific energy consumption in manufacturing SCP (SEC, kWh/kg) was calculated by the total energy consumption divided by the corresponding SCP production (M, kg), according to Eq. (7).

\[
\text{SEC} = \frac{E_{\text{FO}} + E_{\text{ED}}}{M} = \frac{U \cdot \int_0^t I \cdot dt + P \cdot T}{M} \quad (7)
\]

The energy cost was then calculated based on the latest electricity price in Denmark for non-households, which was 1.1559 DKK/kWh (approx. 0.1554 Euro/kWh) in 2021 (Statistics Denmark, 2021).

3. Results and discussion

3.1. Acetate and NH\(_4\)-N recovery from digestate

The three-chamber ED was supplied with 1, 2, and 3 V of voltage, respectively. Fig. 2 illustrated the acetate concentration, pH, and current density profile in the middle chamber within 12 days. The acetate recovery increased from 22.3% to 64.4%, with increasing voltages from 1 to 3 V (Table 1). The maximal acetate concentration of 6.8 g/L was achieved under 3 V, followed by 4.5 g/L acetate under 2 V. Additionally, the maximal acetate recovery rate was elevated by 2.2 times at 2 V, and by 4.5 times at 3 V compared with the scenario at 1 V. Despite the decent acetate recovery at a high voltage, the current efficiency for acetate recovery was undesirably declined, i.e., 96%, 50%, and 7% at 3, 2, and 1 V, respectively. As for NH\(_4\)-N, a maximum of 60.1% NH\(_4\)-N was recovered at 2 V, achieving a final concentration of 0.76 g/L at the end of the electrodialysis process. Furthermore, the system with 2 V exhibited the highest current efficiency at 25% for ammonium recovery.

As shown in Fig. 2d, the current density of ED at 3 V was almost an order of magnitude greater than that at 2 V. When a high voltage was used, the current was substantially wasted for untargeted electrochemical redox reactions. The IrO\(_2\) catalyst on anode contributed to the oxygen and chlorine evolution (Le Luu et al., 2015), and the Pt/C catalyst on cathode contributed to hydrogen evolution. Furthermore, the current decreased with time at all voltages. It was due to the increment of internal resistance during operation particularly the transmembrane transport resistance, electrode resistance, and pH gradient resistance (Sleutels et al., 2009; Van Eerten-Jansen et al., 2012). At 1 V, the pH in the middle chamber was stabilized at 5.5–6.5, while at 2 and 3 V, the pH radically changed, continuously increasing to 7.6 at 2 V, and decreasing to 4.1 at 3 V (Fig. 2c). This observation can be explained by the extreme acidic and alkaline conditions in the anodic and cathodic chamber (Fig. A.3) and the unbalanced permeation of proton (H\(^+\)) and hydroxyl ions (OH\(^-\)) across membranes into the middle chamber.

In general, the ED reactor with 2 V obtained better performance of acetate and nitrogen recycling in terms of recovery efficiency and current efficiency.

3.2. Transform ED design for optimization of recovery

To further increase the recovery of the acetate and ammonium from digestate, the anode and cathode chamber of ED were connected through an internal loop. Table 1 and Fig. 3 show the recovery performance of ED at 2 V with the new configuration. The maximal acetate concentration reached 6.4 g/L on Day 8, compared with 4.5 g/L using independent anode and cathode configuration. A relatively higher NH\(_4\)-N concentration of 0.9 g/L was also observed in the middle chamber at the end of electrodialysis. It was attributed to the new configuration that expanded the volume of anolyte and catholyte and continuously refilled acetate and NH\(_4\)-N across membranes. The current density of ED with an internal loop was 0.2-0.4 mA/cm\(^2\) during 12 days (Fig. 3d), compared with 0.05-0.2 mA/cm\(^2\) without having the internal loop (Fig. 2d).
implied a lower internal resistance of ED with the internal loop because the circulation made electrolytes more homogeneous and less energy loss in ion transport. Nevertheless, the current efficiency in recovering acetate and NH$_4$-N decreased by 3- and 1.6-fold. The side reactions under a neutral pH could be one of the reasons for the decline. For example, with the internal loop, an alkaline condition in the anode (Fig. 3c) was thermodynamically favorable for Cl$^-$ oxidation and generating undesirable hypochlorite ions (Mohan et al., 2007). Meanwhile, lower pH (6.4-8.2) in the internal loop induced more active H$_2$ evolution in the cathode (Durst et al., 2014) than in the scenario having high cathodic pH (6.5-10.8) without the loop. Acetate loss was observed as early as Day 2, whereas NH$_4$-N loss occurred gradually from Day 6 (Fig. 3a, 3b). 83% of acetate was lost in the internal loop at 2 V compared with 28% lost in the scenario without the loop (Table 1, Fig. A.6). Concerning NH$_4$-N, the loss also increased from 12% to 36% when the ED setup switched on the internal loop (Table 1).

**Table 1**
Voltage and ED configuration effect on acetate and ammonia recovery.

<table>
<thead>
<tr>
<th>Voltage</th>
<th>HAc recovery (%)</th>
<th>Max. HAc recovery rate (g/(m$^2$ h))</th>
<th>Max. HAc (g/L)</th>
<th>Acetate loss in cathode</th>
<th>Current efficiency for HAC</th>
<th>NH$_4$-N recovery (%)</th>
<th>Max. NH$_4$-N (g/L)</th>
<th>NH$_4$-N loss in anode</th>
<th>Current efficiency for NH$_4$-N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without internal loop</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0V</td>
<td>10.5±1.5%</td>
<td>0.21±0.01</td>
<td>0.9±0.1</td>
<td>50%</td>
<td>-</td>
<td>21.4±3.3%</td>
<td>0.38±0.06</td>
<td>8%</td>
<td>-</td>
</tr>
<tr>
<td>1V</td>
<td>22.3±1.1%</td>
<td>0.52±0.01</td>
<td>1.8±0.09</td>
<td>32%</td>
<td>96%</td>
<td>22.3±1.1%</td>
<td>0.36±0.03</td>
<td>26%</td>
<td>10%</td>
</tr>
<tr>
<td>2V</td>
<td>42.2±1.9%</td>
<td>1.15±0.05</td>
<td>4.5±0.2</td>
<td>28%</td>
<td>50%</td>
<td>60.1±1.4%</td>
<td>0.76±0.09</td>
<td>12%</td>
<td>20%</td>
</tr>
<tr>
<td>3V</td>
<td>64.4±3.7%</td>
<td>2.34±0.07</td>
<td>6.8±0.4</td>
<td>24%</td>
<td>7%</td>
<td>54.2±0.7%</td>
<td>0.81±0.00</td>
<td>40%</td>
<td>2%</td>
</tr>
<tr>
<td>With internal loop</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2V</td>
<td>22.7±0.2%</td>
<td>1.49±0.20</td>
<td>6.4±0.3</td>
<td>83%</td>
<td>19%</td>
<td>36.6±3.7%</td>
<td>0.9±0.09</td>
<td>36%</td>
<td>16%</td>
</tr>
</tbody>
</table>
Consequently, the overall recovery of acetate and NH$_4$-N were 22.7% and 36.6%, respectively, corresponding to only half of the scenario with independent anode and cathode chambers (Table 1). The main reason for acetate loss was that the digestate was spoiled and degraded by airborne bacteria because of the unhygienic experimental condition. Increasing turbidity of anolyte and catholyte was observed during the electrodialysis. In ED with an internal loop, the mild pH of anolyte and catholyte (pH 7.9 on Day 12) even facilitated microbial spoilage, compared with the acidic anolyte (pH 3.4 on Day 12) and alkaline catholyte (pH 10.8 on Day 12) in the ED with independent chambers (Figs. 3c, A.3). Similarly, dramatic NH$_4$-N loss (36%) occurred compared to the 12% loss in ED with independent chambers. Ammonia can be electrochemically oxidized in NH$_3$ instead of NH$_4^+$, so the oxidation process is sensitive to pH given the availability of NH$_3$ reactant (Zöllig et al., 2015). Acidic anolyte could diminish NH$_3$ species available for oxidation. Therefore, ED with independent chambers had less NH$_4$-N loss than the reactor with an internal loop.

### 3.3. FO as a downstream process to concentrate the recovered nutrients

After electrodialysis, an FO system was applied to concentrate the recovered nutrients for *S. cerevisiae*. The performance is shown in Table 2. Acetate and NH$_4$-N as the target ions were concentrated by 14 and 10 times, respectively. The solute concentration factor (SCF) was 0.92, indicating a slight dilution of DS and a chance for repetitive use in the FO process. As indicated by the water concentration factor (WCF), the volume reduction factor (up-concentration) of FS was ~20. Fig. A.4 illustrates the water flux variation during the FO process. Driven by the transmembrane osmotic pressure difference, water molecules migrated from FS to DS. The water flux declined by 28%, from the initial maximal

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Concentration performance of forward osmosis system.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Solute concentration factor (SCF)</strong></td>
<td>5 M NaCl as solute</td>
</tr>
<tr>
<td><strong>Water concentration factor (WCF)</strong></td>
<td>20.17 ± 2.33</td>
</tr>
<tr>
<td><strong>Water flux ($J_w$)</strong></td>
<td>7.55-4 L/(m$^2$·h)</td>
</tr>
<tr>
<td><strong>Concentration ratio</strong></td>
<td>Ac$^-$</td>
</tr>
<tr>
<td></td>
<td>NH$_4$-N</td>
</tr>
<tr>
<td><strong>Rejection of target</strong></td>
<td>Ac$^-$</td>
</tr>
<tr>
<td></td>
<td>NH$_4$-N</td>
</tr>
<tr>
<td><strong>Reverse salt flux ($J_s$)</strong></td>
<td>Na$^+$</td>
</tr>
<tr>
<td></td>
<td>Cl$^-$</td>
</tr>
</tbody>
</table>
7.55 to the end of 4 L/(m²·h) even in a short-term run of 260 min. The trend of fast decline was also observed in other studies (Ezugbe et al., 2021; Tang et al., 2010). In light of the short period and the absence of visible changes in the membrane surface, flux decline cannot be ascribed to membrane fouling. Dilution of DS over time could in principle explain the decrease, but DS conductivity was only reduced by ~8% (from 243 to 225 mS/cm) during the concentration process indicating a fairly stable osmotic driving force. Thus we ascribe primary reason for the decline to concentration polarization of the FO membrane, including internal concentration polarization (ICP) and external concentration polarization (ECP). The CTA-NW membrane used in this study has an asymmetric structure and is made of cellulose acetate which constitute the active (selective) layer supported by nonwoven fibrous support mesh. The total thickness of the membrane is ~500 µm. ICP occurs in the porous support layer and is affected by membrane orientation (Gray et al., 2006; Li et al., 2015). In this study, the dense active layer of the FO membrane faced the FS as this orientation has higher resistance to membrane fouling compared to more open support mesh (Tang et al., 2010). Consequently, ICP will occur in the porous material and generally has more severe impact on reducing water flux than ECP at high DS concentrations (Tan and Ng, 2013; Tang et al., 2010; Yu et al., 2017) (Fig. A.5). The end result is that the effective osmotic driving force across the active layer was reduced thereby diminishing the water flux.

Ideally, the FO membrane only allows water molecules to pass and can maintain all targeted acetate and NH₄-N ions in the FS. However, in reality, a small portion of targeted ions in the FS will permeate through the membrane. In this study, the rejection ratios of acetate and NH₄-N were 77% and 64%, respectively (Table 2). Conversely, Na⁺ and Cl⁻ in the DS also permeated into the FS with a flux of 3.53 and 12.36 g/(m²·h). The diffusion of Cl⁻ was significantly higher than that of Na⁺ across the FO membrane. Devia and coworkers also observed that three times more Cl⁻ than Na⁺ was diffused into FS using MgCl₂ as the DS (Devia et al., 2015). The uneven diffusion of anion and cation across FO membrane often occurs and is related to membrane materials. For instance, the reverse flux of chloride was nearly double that of sodium through a CTA membrane, which was different from the results with a polyamide thin-film composite (TFC) membrane (Cody et al., 2013). The rejection ratio of target ions and reverse salt flux are affected by many factors, including membrane material, flow velocity, DS types, and DS solute concentration. The HTI CTA-NW membrane used in this study has a lower reverse salt flux than the CTA membrane with embedded polyester screen (HTI CTA-ES) and the TFC membrane with an embedded polyester screen (HTI TFC-ES) (Zheng et al., 2015).

Na⁺ is an essential element for the growth of S. cerevisiae. Thus, the reverse Na⁺ flux in the FO process was a bonus for the enriched feedstock, which will reduce the cost of adding extra Na⁺ in S. cerevisiae’s substrate. However, the reverse Cl⁻ flux will possibly inhibit the growth of S. cerevisiae at high concentrations. Based on our previous study, 500 mM Cl⁻ resulted in a more extended lag phase of S. cerevisiae (Zeng et al., 2022). Chloride salts showed antimicrobial effects on S. cerevisiae; for instance, 33.5-140 g/L of CaCl₂ dramatically decreased its maximal specific growth rate and increased the lag phase (Bautista-gallego et al., 2008). In future studies, it is possible to alleviate the reverse ion diffusion by using non-ionic solutes with lower diffusion coefficient and larger Stokes radius, such as glucose (Ryu et al., 2020; Xie et al., 2012). In that case, even a small amount of glucose that permeates into FS could be utilized as a carbon source by S. cerevisiae.

Overall, the FO system could effectively concentrate the acetate and NH₄-N recovered from the ED process, albeit with 23% and 36% losses. The weakness of the FO system tested with the HTI CTA-NW membrane included a fast decline in water flux and adverse Cl⁻ diffusion into the concentrated product. Future improvements of the FO step thus entails identifying FO membranes with lower ICP and reverse salt flux.

3.4. SCP production from the recovered and concentrated nutrients

S. cerevisiae was directly fed with the ED recovery or the FO-concentrated feedstock for SCP synthesis. The OD, pH, and acetate consumption are shown in Fig. 4. After ED recovery, the initial acetate concentration was above 4 g/L, higher than that in the AAB medium. So a higher maximal OD at 2.2 was observed using ED recovery (Fig. 4a). OD reached a plateau from Day 3 to Day 5, because the pH increased to above 8 before acetate was completely consumed (Fig. 4b), which inhibited further growth of S. cerevisiae (Peña et al., 2015). The pH increase was concomitant with acetate consumption because acetate was transported into the cytoplasm via proton symporter, which squandered protons in the broth (Casal et al., 1996). In comparison with the AAB medium, OD was stably around 1.2 at the end of incubation as long as acetate was depleted. When FO concentrate was applied, a maximal OD of 2.0 was obtained on Day 3 and it slightly declined in the following two days (Fig. 4a). Compared to the exhaustion of acetate in ED recovery and AAB medium, 18% of acetate eventually remained in the FO concentrate medium (Fig. 4c). It revealed that acetate was not the limiting factor to S. cerevisiae growth in the scenario. The growth was hindered by alkaline conditions (Fig. 4b, 4c). In all the scenarios, NH₄-N was not completely consumed. The total nitrogen consumption by S. cerevisiae was 331-332 mg/L using ED recovery and FO concentrate, and 96 mg/L using AAB medium. The maximal growth rate of S. cerevisiae was 2.3 and 2.1 obs/day using ED recovery and FO concentrate, respectively, slightly lower than the control of 3.2 obs/day using AAB medium. The protein concentrations were 0.76-0.86 g/L, accounting for 47-48% of the biomass.

The amino acid profile of biomass is shown in Fig. 5. S. cerevisiae fed with ED recovery and FO concentrate both had a similar and well-balanced distribution of amino acids. Most essential amino acids have met the FAO recommendation as a high-quality protein for adults. Using ED recovery, for example, leucine, lysine, threonine, and valine were especially above the recommendation by 1.9, 3.7, 3.3, and 1.5-fold. Likewise, 1.6-4.2 folds of these amino acids were detected beyond the recommendation using FO concentrate as S. cerevisiae’s substrate. In all cases, histidine was exceptionally lower than FAO recommendation by 18-22%. Overall, S. cerevisiae can provide a superb SCP product using the organic and nitrogen that was directly recovered from digestate by ED, or concentrated by the FO process. Acidic pH control is recommended in future studies to enable robust SCP production.

3.5. Energy consumption and economic cost of SCP production

Table 3 revealed the energy consumption, raw material expenditure, and cost estimation of the ED and FO system. In the electrodialysis process, 42.2% of acetate and 60.1% of NH₄-N were recovered by ED from 250 mL of synthetic digestate. S. cerevisiae which grew on the ED recovery, produced 35 mg SCP product. Accordingly, 7143 L digestate was needed to produce one kilogram SCP. In contrast, using the recovery of hybrid ED-FO system, S. cerevisiae produced 66 mg SCP product. The hybrid ED-FO process reduced the volume requirement of digestate by two-fold. With the same recovery efficiency of ED, 3788 L digestate was sufficient for S. cerevisiae to produce one kilogram SCP via the hybrid system.

However, given the energy consumption, the electricity consumption of the hybrid ED-FO system dramatically increased compared with the single ED system. ED consumed 0.00065 kWh during 12-day electrodialysis, while the pump for DS and FS circulation consumed 0.21667 kWh within the 260 min FO concentration process. The low current density (0.02-0.21 mA/cm²) and voltage input (2 V) led to relatively low energy consumption in ED. Thus, the specific energy consumption of the hybrid ED-FO system was 3297.9 kWh/kg SCP, increased by 177 times over the single ED system (18.6 kWh/kg SCP). According to the current electricity price in Denmark in 2021, it cost €2.9 to produce 1 kg SCP by single ED, and cost €512.4 by coupled ED-FO system.
Besides the energy cost, raw material accounts for the most considerable portion of the operation cost in manufacturing commercial SCP products (Voutilainen et al., 2021). The carbon and nitrogen sources were all derived from the digestate in this study. The additional cost of raw material only included MgSO$_4$, H$_2$HPO$_4$, vitamins, and trace elements that were added before inoculation. The expense of chemical supplements was €0.0032/L, corresponding to €9.7/kg SCP using the single ED system and €5.3/kg SCP using the hybrid ED-FO system. An annual operation cost of SCP products consists of variable and fixed costs. Variable cost includes raw material and energy consumption, and fixed cost includes labor cost and maintenance. The average variable cost accounts for 62% of the total expenditure based on empirical costs of four commercial SCP products, i.e. Pekilo (Paecilomyces variotii), Torula (Candida utilis), Fusarium (Fusarium venenatum), and an acellular recombinant protein (Voutilainen et al., 2021). Therefore, in this study, the total operation cost of manufacturing SCP products of S. cerevisiae was estimated to be €20.4 by single ED, and €835.0 by coupled ED-FO system. Using real digestate will result in a slightly higher cost since the complex compositions in real wastewater increase the internal electrical resistance of ED and possibly cause membrane fouling (Mohammadi et al., 2021).

A limited number of studies have evaluated the economics of manufacturing yeast SCP products. To our best knowledge, the cost ranged from €0.74 to €7.3/kg SCP (Table A.2). In these studies, thermotolerant yeast (Kluyveromyces marxianus), torula yeast (Candida utilis), or yeast mixed with molds or lactobacilli produced SCP from waste streams such as wheat straw hydrolysate, dairy manure anaerobic digestate, and various food waste. However, different expenditure factors were considered in these studies, which made them incomparable. The prices of various food proteins in daily life were compared in a previous study, including egg white, casein powder, tofu, soy chunks, etc., ranging from €9-56/kg protein (Voutilainen et al., 2021). Quorn is a well-known meat-free food brand making SCP products with meat texture by Fusarium Venenatum. A Quorn product contains 13.8% protein at a corresponding price of €48/kg protein (Voutilainen et al., 2021). Therefore, the cost of nutrient recycling from digestate by ED and subsequent SCP synthesis by S. cerevisiae is competitive with the existing commercial SCP product and has the potential for scaling up.

To sum up, a single ED was more economical than a hybrid ED-FO system to recover nutrients for SCP production. It cost only €20.4 to produce one kilogram SCP by S. cerevisiae fed with recovery at 2 V. Meanwhile, 42.2% acetate and 60.1% NH$_4$-N were recovered from 7143 L digestate. The single ED system followed by yeast fermentation is advantageous when the recovery is instantly used as the substrate for S. cerevisiae from an economic perspective. However, when remote transport or long-term storage is required, the hybrid ED-FO system is
Fig. 5. Essential and nonessential amino acids in SCP product of S. cerevisiae.

Table 3
Energy consumption and financial cost of SCP production.

<table>
<thead>
<tr>
<th>ED configuration</th>
<th>Single ED</th>
<th>Coupled ED-FO</th>
</tr>
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<tbody>
<tr>
<td>Recovery efficiency and SCP production</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Synthetic AD effluent volume (mL)</td>
<td>250</td>
<td></td>
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<tr>
<td>ED recover efficiency (% acetate)</td>
<td>42.2±1.9%</td>
<td></td>
</tr>
<tr>
<td>ED recover efficiency (% NH₄-N)</td>
<td>60.1±1.4%</td>
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<tr>
<td>Harvested biomass volume (mL)</td>
<td>100</td>
<td>150</td>
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<tr>
<td>SCP production (mg)</td>
<td>35</td>
<td>66</td>
</tr>
<tr>
<td>Recovered AD effluent (L/kg SCP)</td>
<td>7143</td>
<td>3788</td>
</tr>
<tr>
<td>Energy consumption</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy consumption at 2V (kWh)</td>
<td>0.00065</td>
<td>0.00065</td>
</tr>
<tr>
<td>Total energy consumption (kWh)</td>
<td>0.00065</td>
<td>0.21731</td>
</tr>
<tr>
<td>Specific energy consumption (kWh/kg SCP)</td>
<td>18.6</td>
<td>3297.9</td>
</tr>
<tr>
<td>Non-household electricity price in Denmark in 2021 (€/kWh)</td>
<td>1.1559</td>
<td>835.0</td>
</tr>
<tr>
<td>Energy cost (€/kg SCP)</td>
<td>2.9</td>
<td>512.4</td>
</tr>
<tr>
<td>Raw material</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inorganics supplement (MgSO₄, trace element, vitamin) (€/L)</td>
<td>0.00022</td>
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</tr>
<tr>
<td>K₂HPO₄ in the middle chamber of ED (€/L)</td>
<td>0.0032</td>
<td></td>
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<tr>
<td>Inorganics supplement cost (€)</td>
<td>0.00002</td>
<td>0.00003</td>
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<tr>
<td>K₂HPO₄ cost (€)</td>
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<tr>
<td>Total chemical cost (€/kg SCP)</td>
<td>9.7</td>
<td>5.3</td>
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<tr>
<td>Estimated total cost</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cost of raw material and energy consumption (€/kg SCP)</td>
<td>12.6</td>
<td>517.7</td>
</tr>
<tr>
<td>Reference ratio of raw material and energy to total operation cost (Voutilainen et al., 2021)</td>
<td>62%</td>
<td></td>
</tr>
<tr>
<td>Estimated operation cost in manufacturing SCP (€/kg SCP)</td>
<td>20.4</td>
<td>835.0</td>
</tr>
</tbody>
</table>

* The currency exchange rate of 7.44 dkk/€ was used to convert Danish Krone (dkk) to Euro (€).

3.6. Future perspective

Even though the current study demonstrated the techno-economic feasibility of lab-scale SCP production from anaerobic digestate by S. cerevisiae, more work is necessary to achieve robust and economic SCP production in large-scale applications. For the yeast fermenter, a commercial fermenter on a larger scale often incorporates a sterilize-in-place system and more precise control of pH, temperature, aeration, and agitation, which would be necessary and beneficial for robust SCP production. Furthermore, purification and sanitation procedure is crucial to improve the market value of SCP before serving it as a food commodity. In the ED process, anolyte and catholyte can be circulated at different rates considering the low recovery efficiency and S. cerevisiae’s imbalanced need for acetate and ammonia. It is also possible to eliminate undesirable redox reactions of ED by galvanostatic operation or by selecting proper electrode materials. In addition, a sterile middle chamber can be directly connected to yeast fermenters. It will guarantee sufficient carbon and nitrogen supply for S. cerevisiae and reduce the expenditure on collection, concentration (e.g., FO) and transportation of the recovery. Last but not least, a holistic economic assessment encompassing the whole supply chain of SCP is necessary to evaluate the actual market potential of yeast SCP products in large-scale applications.

4. Conclusions

This work demonstrated the feasibility of SCP generation from digestate through a hybrid process using brewer’s yeast S. cerevisiae for the first time. Acetate and NH₄-N were simultaneously extracted from the digestate by 42.2% and 60.1%, respectively, at 2 V in a three-chamber ED cell. A FO system was applied to concentrate the recovery further, achieving 14- and 10-fold concentrated acetate and NH₄-N. However, it contributed to the most significant nutrient recovery expenditure. Without the FO system, the operation cost of manufacturing SCP was estimated at €20.4/kg SCP, which exhibited great market potential. The hybrid ED-FO system cost more (€835.0/kg SCP) but produced a higher yield of SCP (1 kg from 3788 liters of digestate). Furthermore, most amino acid compositions of the yeast’s SCP using the recovered nutrients from digestate have met the FAO recommendation as a high-quality protein, especially in terms of leucine, lysine, threonine, and valine. The hybrid electrochemical-membrane system opened up a new avenue in the economical SCP production by S. cerevisiae through a successive recovery and concentration of the nutrients from digestate.

CRediT authorship contribution statement

Danfei Zeng: Data curation, Methodology, Visualization, Validation, Writing – original draft. Yifeng Jiang: Validation. Carina Schneider: Software, Writing – review & editing. Yanyan Su: Writing – review & editing. Claus Helix-Nielsen: Writing – review & editing. Yifeng Zhang: 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.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.resconrec.2022.106705.

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