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Green electricity-driven simultaneous ammonia recovery and in-situ upcycling for microbial protein production

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ABSTRACT

Currently, hydrogen-oxidizing bacteria (HOB) based power-to-protein is a promising approach to produce alternative microbial protein (MP), however, the nitrogen source used was either derived from commercial products or was firstly recovered from waste streams and then diluted for HOB growth. In the present study, simultaneous ammonium recovery from wastewater and in-situ utilization for the green MP (derived from *Cupriavidus necator* 335) production was successfully demonstrated using a microbial electrochemical recovery conversion cell (MERC). 0.41 ~ 0.82 g/L of dried biomass (protein content 49 ~ 63%) was yielded in 36 h with a power supply of 3, 4, and 5 V. *C. necator* 335 could grow in the MERC system receiving wastewater with a broad range of ammonium (0.05 ~ 8 g N/L) and the highest biomass production of 0.9 g/L (protein content 54%) was achieved at 2 g N/L. 2.69 g/L of dried biomass containing 57% protein was obtained in 120 h with an initial supply of 1 L CO₂ and 2 g N-NH₄⁺. Applied voltages and ammonium concentrations showed a minor impact on the amino acid profile. Furthermore, the MERC system was tested with real waste streams (e. g., municipal wastewater, and digestate) and 0.45 ~ 1.22 g biomass/L (protein content 52 ~ 62%) were harvested. The characteristics of the wastewater streams (e. g., ammonium concentration and conductivity) could significantly affect the system performances. The harvested MP from real wastewater showed a high quality of amino acid profile and implied a potential in substituting traditional plant/animal-based protein.

1. Introduction

It is predicted that the world's population will keep growing and by 2050 there will be 9 billion people on the planet [1]. As a consequence of the increasing world population, the demand for high-quality protein will be doubled by 2050 [2]. However, conventional protein production activities are now intensifying various environmental issues. For instance, a vast amount of fertilizers, pesticides, herbicides, and antibiotics have to be introduced to the processes to ensure high productivity and yield, resulting in negative impacts on water and soil environments and human health [3]. Meanwhile, enormous amounts of waste (e.g., straws, stems, manures, and urines) after the production must be well disposed of. Besides the solid wastes, the agriculture sector also contributed to about 13.5% of greenhouse gases (GHG) emissions [4], increasing the global concern about climate change. The competition of land and water by agriculture and grazing also threatens the biodiversity and the balance of the ecosystem [5]. Additionally, the

conventional ways of protein production are prone to be interfered by extreme weather (e.g., drought, waterlog, and flood). Therefore, a more sustainable way of protein production is urgently needed.

In this context, the power-to-protein (PtP) concept has been recently proposed [6–8]. In the PtP process, water is firstly split into hydrogen and oxygen with renewable electricity. Subsequently, the generated gases and carbon dioxide are assimilated as microbial biomass by a special group of bacteria (e.g., knallgas bacteria, or hydrogen-oxidizing bacteria (HOB)) [9,10]. The obtained biomass has been found with high crude protein content (approx. 70–75%) and therefore is referred to as microbial protein (MP) or single cell protein (SCP) [11]. The biological value of the synthesized MP is considered competent in replacing or supplementing conventional protein for feed and even food [12]. This MP production approach is regarded as sustainable because value-added MP is produced while the greenhouse gas (e.g., CO₂) is fixed using renewable electricity.

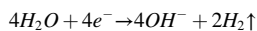
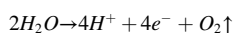
It has been found that the process of producing microbial protein

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using HOB through water electrolysis would become economically feasible if the production cost could be reduced by 13% [13]. According to a report from Avecom, a company in Belgium, which conducted a pilot-scale MP study using recovered nitrogen from wastewater, the total revenues almost equaled the total cost. Among the expenses, hydrogen generation through water electrolysis and ammonium recovery from wastewater through air stripping contributed the most (35% and 30% of the total costs, respectively) [7]. In these cases, the production process was generally separated into several steps and units, including water electrolysis unit (for supplying gas substrates), nitrogen recovery unit (for providing nitrogen), and HOB fermentation unit (for MP accumulation). To reduce the cost and simplify the production process, an in-situ water electrolyzer, in which simultaneous water electrolysis and HOB cultivation were achieved in one chamber, was recently reported [14]. However, during the MP synthesis in these studies, the nitrogen source, usually in the form of ammonium, was either directly supplied by dosing commercial compounds (e.g., ammonium chloride, and ammonium sulfate) or needed to be firstly recovered from waste streams by air stripping which is energy and chemicals supply intensive [7,12–16]. So far, MP production in a single reactor that can attain simultaneous water electrolysis, ammonium recovery, and microbial conversion has never been reported. If this ambitious hypothesis could be achieved, the MP production process could be simplified, more sustainable, and eco-friendly.

In the present study, an innovative microbial electrochemical recovery conversion cell (MERC) was for the first time developed and investigated for MP production. The MERC system consisted of two electrodes placed in two chambers separated by a cation exchange membrane (CEM) and driven by electricity. Ammonium from various waste streams was filled in anode chamber. With an external power supply, water splitting reactions (a) and (b) can happen in anode and cathode, respectively.



Furthermore, ammonium can be transferred into cathode chamber through the CEM due to both free diffusion and the potential difference between anode and cathode. Thus HOB can grow protein-rich biomass in the cathode chamber using the hydrogen and oxygen gas generated from water electrolysis, the ammonium recovered from waste streams, and the supplied carbon dioxide, trace elements, and vitamins. The MERC could therefore realize the ammonium recovery, water splitting, and MP production in a single system synchronously. The effects of applied voltage and ammonium concentration in the anode chamber on HOB growth were investigated using synthetic wastewater. Afterward, the applicability of MERC was further validated with different actual waste streams, including municipal sewage and digestate. The amino acid profile of the produced MP was also investigated. Finally, the energy transfer efficiency and prospectiveness were discussed.

2. Material and methods

2.1. MERC setup and operation

The MERC system was constituted of two identical polymethyl methacrylate frames separated by a CEM (CMI 7001, Membrane International, NJ, USA). Alloy mesh (4 × 4 cm) coated with IrO₂ was used as the anodic electrode, and titanium mesh (4 × 4 cm) was used as the cathodic electrode (Fig. 1). 170 mL of ammonium sulfate-rich synthetic wastewater was used as anolyte while the same volume of nitrogen-free medium was used as catholyte for HOB cultivation. The recipe of the medium was described previously [17] except removal of ammonium chloride and a supplement of sodium sulfate (2.35 g/L). The conductivity of the modified medium was around 7.5 mS/cm. Electricity was applied to the system by a direct-current power supply and a constant

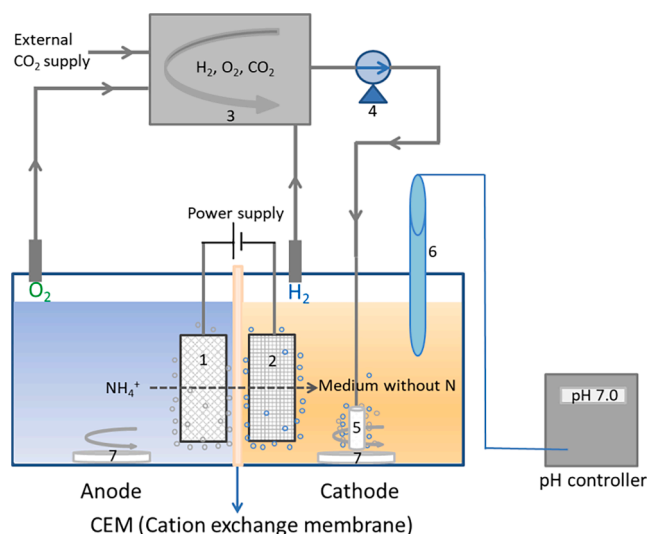


Fig. 1. The schematic graph of the MERC system, 1, anodic electrode; 2, cathodic electrode; 3, gas recycling bag; 4, pump; 5, aerator; 6, pH probe; 7, rotator.

output voltage (0, 2, 3, 4, or 5 V) was maintained during the whole operation period. The voltage across a resistor (10 Ω) was logged by a digital multimeter (Model 2700, Keithley Instruments, Inc., USA) every half an hour during operation. Oxygen and hydrogen gas generated from each chamber were mixed together in a gas recycling bag and flushed into cathode chamber at a speed of 5 mL/min through a peristaltic pump (Watson-Marlow Flexion A/S, UK) and an aerator (5 μm, Brewferm, Belgium). An automatic pH controller (ProSystem Aqua, Spain) was used to maintain the pH of the medium in cathode chamber at around 7.0. *Cupriavidus necator* 335 (DSM 531) purchased from the German Collection of Microorganisms and Cell Culture GmbH (DSMZ) was used as MP producer in this study. An adequate amount of the seed culture at log phase was inoculated to cathode chamber to assimilate the recovered ammonium for MP production.

2.2. Bacterium growth and microbial protein quantification

To track the bacterial growth, optical density at the wavelength 660 nm (OD₆₆₀) of the catholyte was measured by a spectrophotometer every 6 or 12 h and the values were used for determining the specific growth rate [17]. After cultivation in the MERC, the liquid in cathode chamber was collected and centrifuged at 8000 rpm for 10 min. The pellets were washed with deionized water three times and then lyophilized. The weight of the dried biomass, in other words, the produced MP, was termed as cell dry weight (CDW). The CDW production (P_{CDW}, g/L) was defined as the weight of CDW harvested per liter of the medium in the cathode chamber. The amino acid profile of the MP was examined by an HPLC-MS/MS according to the method described before [17]. Of note, neither tryptophan nor cysteine was able to be detected by this method. The ratio of the total amount of all the amino acids detected and the weight of the CDW analyzed was termed the protein content (%) of the MP.

2.3. Experiments procedure

Initially, the MERC was operated at different voltages (0, 2, 3, 4, and 5 V). The synthetic wastewater used as anolyte contained 1 g N/L of nitrogen in the form of ammonium sulfate and the conductivity was 7.9 mS/cm. 250 mL of CO₂ gas was injected into the gas recycling bag before starting. Operation of the MERC was stopped when bacterial growth ceased. Subsequently, synthetic wastewater with different ammonium concentrations was filled in the anode chamber of the MERC.

Additionally, 250 mL of CO₂ was supplied in the recycling bag. The system was operated for a longer period by re-injecting CO₂ in the recycling bag and supplying more ammonium in anode chamber. Finally, wastewater streams, including influent from a municipal wastewater treatment plant (WWTP, Lyngby, Denmark) and effluent from a lab-scale continuously stirred anaerobic reactor (CSTR) treating municipal solid waste [18], were used to verify the feasibility of MERC. After running the system with municipal sewage (WW-1 group), the gas generated from the anode chamber was not connected with the gas recycling bag in the other three groups (WW-2, AD, and AD/WW) to get rid of the toxicity of chlorine. Accordingly, 500 mL of oxygen was directly supplied in the recycling bag initially in the three groups. In the group AD/WW, wastewater influent and effluent from CSTR were mixed as a specific ratio to reach a 7.9 mS/cm conductivity.

2.4. Analysis and calculations

Gas components including H₂, O₂, and CO₂ were measured by gas chromatography (Trace 1310, Thermo Scientific, USA) equipped with different columns. The detailed measurement methods for H₂ and CO₂ were described previously [19] and O₂ was detected with hydrogen simultaneously using the same method. Ammonium in liquid phase in each chamber was measured using a commercial kit (Ammonium Test Kit 100683, Merck, Germany). Fixed nitrogen in protein was calculated according to Eq. (1). The conversion factor 5.6 was derived from the previous literature [20].

$$N_{fixed}(\text{mg/L}) = [P_{CDW} \times \text{Protein content}] / 5.6 \times 1000 \quad (1)$$

The differences between each amino acid content in the protein harvested from different culture conditions were tested through a one-way analysis of variance (ANOVA). The tests were realized via using the function of “aov” and “TukeyHSD” in RStudio (version 1.3.1056).

The total input electricity (W_T) was calculated through Eq. (2), while the electricity consumed by the external resistor (W_R) was calculated by Eq. (3),

$$W_T(\text{kWh}) = \frac{\sum_{t=0}^m UI \Delta t}{1000} \quad (2)$$

$$W_R(\text{kWh}) = \frac{\sum_{t=0}^m I^2 R \Delta t}{1000} \quad (3)$$

in where, U (V) was the voltage supplied by the external power supply, I (A) was the current across the resistor calculated according to Ohm's law, R was the resistance of the resistor (10 Ω), and $\Delta t = 0.5$ h. The energy converted into H₂ (W_{H_2}) was calculated through Eq. (4),

$$W_{H_2}(\text{kWh}) = [(V_{H_2} \times 10^{-3}) / V_m] \times M_{H_2} \times 0.001 \times 8 \times 4 \quad (4)$$

in where V_{H_2} (mL) was the volume of hydrogen gas generated, V_m (24.5 L/mol) was the molar volume of gas at 25°C, 1 atm, M_{H_2} (2 g/mol) was the molar mass of hydrogen gas, 8 was the conversion factor one kilogram of hydrogen to H₂-COD (kg), and 4 was the conversion factor of one kilogram of chemical energy in the form of COD to electricity (kWh) [13].

Therefore, the energy efficiency of water electrolysis was derived through Eq. (5). The energy converted into biomass was calculated through Eq. (6),

$$\eta_E(\%) = \frac{W_{H_2}}{W_T - W_R} \times 100\% \quad (5)$$

$$W_B(\text{kWh}) = \frac{P_{CDW} \times V}{F_{H_2-Biomass}} \times 0.001 \times 8 \times 4 \quad (6)$$

in where CDW (g/L) was the biomass production, V (L) was the volume of the medium in cathode chamber, $F_{H_2-Biomass}$ was the conversion factor of one gram of hydrogen to the biomass of HOB, 8 and 4 were the conversion factor described in Eq. (4). The $F_{H_2-Biomass}$ was the conversion factor of one gram hydrogen to biomass and referred from

the stoichiometric equations derived from the dynamic autotrophic growth of *C. necator* [21]. Therefore, the hydrogen utilization rate (U_{H_2}) was calculated through Eq. (7). The energy efficiency of MP production (η_B) was obtained from Eq. (8), and the energy efficiency of the whole system (η_S) was calculated via Eq. (9).

$$U_{H_2} = \frac{\frac{P_{CDW} \times V}{F_{H_2-Biomass}}}{\frac{P_{CDW} \times V}{F_{H_2-Biomass}} + V_{H_2} \times M_{H_2}} \times 100\% \quad (7)$$

$$\eta_B(\%) = \frac{W_B}{W_T - W_R} \times 100\% \quad (8)$$

$$\eta_S(\%) = \frac{W_B + W_{H_2}}{W_T - W_R} \times 100\% \quad (9)$$

3. Results and discussion

3.1. The effect of applied voltage on the MERC performance

The performance of the MERC under different applied voltages (0, 2, 3, 4, and 5 V) was investigated (Fig. 2). *C. necator* did not grow when the applied voltage was 0 or 2 V (Fig. 2A). With higher applied voltages (3, 4, and 5 V) the growth stopped after 36 h and fell in a stationary phase afterward. The harvested CDW was 0.41, 0.82, and 0.81 g/L at 3, 4, and 5 V, respectively. The maximum specific growth rate (μ_{max}) of 0.139 h⁻¹ was achieved at 4 and 5 V, while it was 0.122 h⁻¹ at 3 V. In the anode chamber, no bacterial growth was observed at any conditions because of the lack of nutrients and the dramatically decreased pH. The gases (H₂ and O₂) production rate, i.e., the water electrolysis rate was significantly affected by voltages supplied (Fig. 2B and Fig. S1) and eventually affected the bacterial growth. For instance, the bacterium could not grow under 0 and 2 V because of the absence of H₂ (Fig. S1B). Hydrogen evolution was promoted as the lifted applied voltage (3, 4, and 5 V) (Fig. 2B and Fig. S1B) and as a consequence, the bacterium grew better at 4 and 5 V than that at 3 V (Fig. 2A). The growths at 4 and 5 V were close which might be because at both conditions the H₂ generated was sufficient for growth and became excess in the recycling bag. As the product of the other concomitant half-reaction of water splitting in the anode chamber, the O₂ amount in the bag changed as a similar trend with H₂ (Fig. S1C).

Nitrogen essential for bacterial growth was present in NH₄⁺ (1 g N/L) in the anode chamber at the beginning of the batch run. The positively charged ammonium ions could pass through the CEM slowly by free diffusion without external power supply (0 V) and reached a balance between the two chambers after 60 h (469 and 548 mg/L in anode and cathode chamber, respectively) (Fig. 2C). Besides free diffusion, the ammonium migration could be driven by electroosmosis. The higher voltage (or the higher current) was applied, the more ammonium ions migrated to the cathode chamber, and the migration speed was faster (Fig. S2). Only 3.56% of ammonium (37 mg N/L) was left in the anode chamber under 5 V, while 38.3% of ammonium (392 mg N/L) remained in the anode chamber under 2 V. Ammonium was fixed into biomass mainly in the form of amino acids during the growth of the HOB in the cathode chamber. The protein accounted for over half of the CDW (48.9 \pm 6.9%, 56.8 \pm 7.5%, and 63.3 \pm 9.6% at 3, 4, and 5 V, respectively). Parts of the ammonium might be assimilated as nucleic acids, and be lost in either the gas phase because of stripping, or precipitated with metal ions (e. g., struvite), or due to the direct/indirect oxidation in the anode chamber [22]. It was reported that the content of nucleic acids in dried biomass could reach 5 ~ 16% [23,24]. Fig. 2D shows the current at different applied voltages. As expected the highest current of approx. 120 mA was achieved at 5 V, while the current was neglectable at 2 V. Furthermore, the change of current was in line with the gas generation rate.

In previous reports about HOB cultivation in a single chamber water electrolyzer, the bacteria could keep a linear growth with enough CO₂

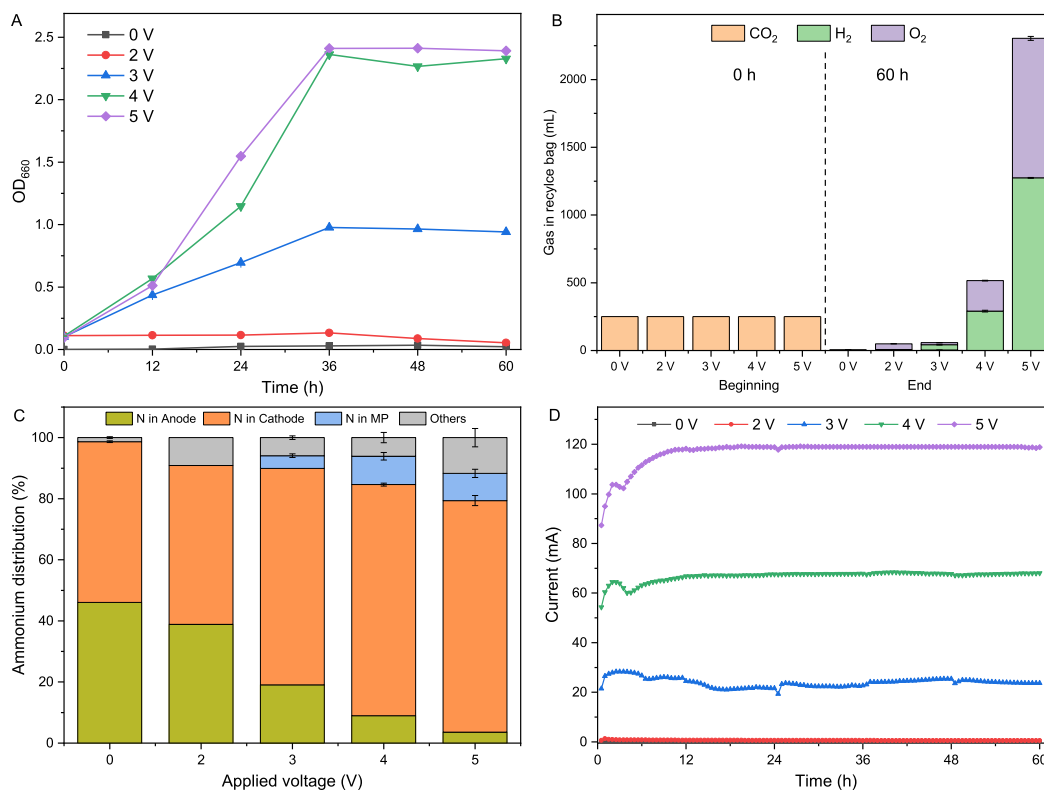


Fig. 2. The performances of the MERC when supplied with different voltages (initial ammonium in anolyte: 1 g N-NH₄⁺/L). A) OD_{660} change of the medium in the cathode chamber; B) gas components in gas recycling bag at 0 and 60 h; C) ammonium distribution in the system at 60 h; D) current change during cultivation.

and NH₄⁺, and the growth rate was determined by the H₂ generation rate [14,15]. In the present study, the bacterial growth did not follow a linear mode mainly because carbon source (CO₂) was not provided continuously and was becoming the limiting factor for growth (250 mL). The μ_{\max} (0.139 h⁻¹) at 4 and 5 V was close to the reported value by the single chamber water electrolyzer (0.14 h⁻¹) [14]. The specific growth rates were higher than the chemolithotrophic (CO₂, H₂, and O₂) specific growth rates of *C. necator* reported previously, which were 0.094 and 0.12 h⁻¹ under 1 and 4 atm, respectively [25] and were slightly lower than the μ_{\max} (0.154 h⁻¹) when cultivated in a shake flask in our previous investigation [17]. The results of gas generation implied that water electrolysis in the MERC required an external voltage of 3 V or higher. Compared with the theoretical potential of 1.23 V for water electrolysis, the results implied the overvoltages of about 1.77 V were presented in the system. The voltage losses might relate to characteristics of the electrode materials, conductivity of the solution, and mass transfer limitations [15]. The protein contents in yielded MP (48 ~ 63%) were a bit lower than those reported in previous studies (60 ~ 70%) [12,26,27], which might be due to different analytical methods or differences in the experimental setups. In these studies, the amounts of crude protein were determined by multiplying the fixed nitrogen amounts by the nitrogen conversion factor of 6.25, which was proved would overestimate the protein content [20,28]. However, the protein contents in the current research were obtained by summing up all the amino acids amounts detected by HPLC-MS/MS. In respect to amino acids analysis, some amino acids (e.g., tryptophan and cysteine) could not be detected using our current method thus underestimated protein contents were resulted in. Besides, metal ions (e.g., Fe²⁺, Ca²⁺, and Mg²⁺) that existed in the medium in cathode chamber might form precipitation [29] that were hardly washed out from pellets and therefore lowered the protein contents.

In summary, the applied voltage had a significant influence on gas substrates production and ammonium migration, and therefore affected the MP production in cathode chamber. With an appropriate external

voltage, the MERC achieved simultaneous water electrolysis, ammonium migration, and nitrogen upcycling into *C. necator* biomass. In the subsequent experiments, 5 V was supplied to the system to ensure plenty of gas substrates.

3.2. The effect of initial ammonium concentration on the MERC performance

The ammonium concentrations in wastewater streams are quite distinct [30]. It may vary from dozens of milligrams per liter in municipal wastewater [31] to thousands of milligrams per liter in digestate [32]. High ammonium concentration could be toxic to HOB because of the potential inhibition of the activity of hydrogenase which is crucial during carbon dioxide assimilation [33,34]. Herein, synthetic wastewaters with different initial ammonium concentrations (0.05, 0.5, 1, 2, 4, and 8 g N/L) were fed into the anode chamber to investigate the effect on the MERC performance.

Clearly, *C. necator* survived under all the conditions (Fig. 3A). In the 0.05 ~ 2 g N/L range, the bacterium grew better with a higher ammonium concentration. Comparatively, it harmed microbial growth when ammonium concentration was above 4 g N/L. The yielded CDW was 0.901 ± 0.003 , 0.623 ± 0.002 , and 0.796 ± 0.002 g/L with 2, 0.05, and 8 g N/L, respectively. Similarly, the highest growth rate of 0.163 h⁻¹ was observed at 2 g N/L. The initial ammonium concentration in anolyte had an effect on water electrolysis and gas evolution (Fig. 3B and Fig. S3) which was mainly caused by the change of anolyte conductivity. At low ammonium concentration (0.05 g N/L), the generated H₂ gas was relatively low and was insufficient to meet the growth requirements (Fig. S3C). The low H₂ production might be due to the low conductivity of anolyte (0.5 mS/cm), which significantly raised the internal resistance of the system. The gas components in the recycling bag were similar when the ammonium concentration was at 0.5 to 4 g N/L. At the highest ammonium concentration (8 g N/L), H₂ and O₂ production were significantly higher than under other concentrations. The water

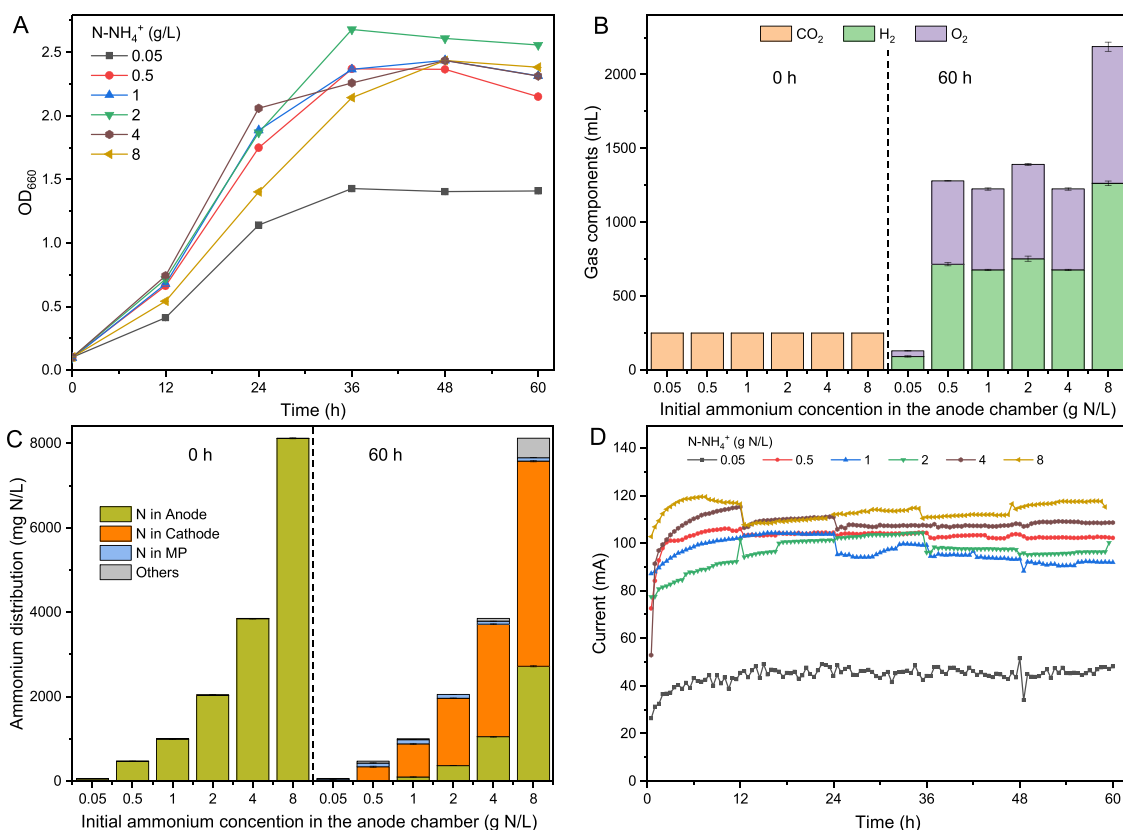


Fig. 3. The performances of the MERC when fed with synthetic wastewater of different initial ammonium concentrations in the anode chamber. A) OD_{660} change of the medium in cathode chamber; B) gas components in gas recycling bag at 0 and 60 h; C) ammonium distribution in the system at 0 and 60 h; D) current change during cultivation.

electrolysis efficiency might be enhanced by the extremely high conductivity of anolyte (49.3 mS/cm).

Ammonium migration was also affected by the initial NH_4^+ concentration in anolyte (Fig. 3C). Ammonium was completely removed when low ammonium concentration was added to the anode (0.05 g N/L). 83.55% of the ammonium was eventually assimilated into MP despite potential losses. With higher ammonium concentrations, ammonium exceeded the nutritional requirement of bacterium, and CO_2 became the growth limiting factor, and therefore ammonium accumulated in the cathode chamber. In the anode chamber, the ammonium removal efficiency decreased with the increase of initial ammonium concentration. Specifically, more than 90% of ammonium could be transported to the cathode when the initial ammonium concentration was lower than 1 g N/L. Comparatively, it was only 66.51% when the initial ammonium concentration was 8 g N/L. 14.7 ~ 17.6 mg N of ammonium was fixed as MP when the ammonium was above 0.5 g N/L.

Fig. 3D showed the current during operation of the MERC. It was in the 80 ~ 120 mA range when the ammonium concentration was higher than 0.5 g N/L. The lowest current (around 40 mA) at 0.05 g N/L was evidence of the low efficiency of water electrolysis. Notably, the current showed a slow decay after a period of operation indicated a corruption of the electrodes during electrolysis. Therefore, novel electrodes with a longer lifespan would need to be developed.

It should be mentioned that to obtain a good growth of microorganisms, the concentration of nitrogen source should be controlled in a proper range [33,35,36]. In most previous studies, HOB were cultivated at around 0.26 g N/L, which was considered the optimal concentration for the HOB's growth [12,27,37]. Besides, it was reported that ammonium concentrations higher than 4 g N/L could completely inhibit the growth of *C. necator* [17]. However, in the present MERC, *C. necator* grew at all the tested ammonium concentrations (0.05 ~ 8 g N/L). This

may be because the ammonium migration in the system was relatively slow and the bacterium had already stopped growth (36 h) due to limited CO_2 supply (250 mL) before the ammonium in the cathode chamber reached a harmful level (60 h). It would be worth investigating the threshold ammonium concentration leading to ammonia toxicity in the proposed MERC. Regarding ammonium migration, though higher ammonium concentration in the anode chamber meant a higher concentration gradient which should promote the ion diffusion, it would also lead to a high solution viscosity which may lower mass transfer efficiency [38].

Overall, the HOB *C. necator* was successfully cultivated in the MERC at a broad range of ammonium (0.05 ~ 8 g N/L). The results imply that the MERC has a promising potential in ammonium recovery and capturing in the form of MP, given the applicability to the broad range of ammonium concentrations (from municipal wastewater to digestate).

3.3. Extended batch run with repeated CO_2 supply

The batch run of the MERC was extended by supplying more CO_2 (Fig. 4). In the control group (a), 250 mL of CO_2 was added into the recycling bag in the beginning. In the group (b), 500 mL of CO_2 was initially supplied, subsequently, 200 mL and 300 mL CO_2 were added respectively to the recycling bag at 42 and 66 h (red arrow in Fig. 4A) when the CO_2 was <10% in the recycling bag. Finally, in the group (c), 1000 mL of CO_2 was initially supplied, and 2 g N/L of ammonium was spiked into the anode chamber.

Compared with the growth from the group (a), the growth of *C. necator* was extended from 36 to 72 and 114 h with more CO_2 or ammonium supply in the group (b) and (c), respectively, and the growth rates were close (Fig. 4A). After cultivation, 2.0 ± 0.01 and 2.69 ± 0.01 g/L of CDW was obtained from the group (b) and (c), which was 2.42

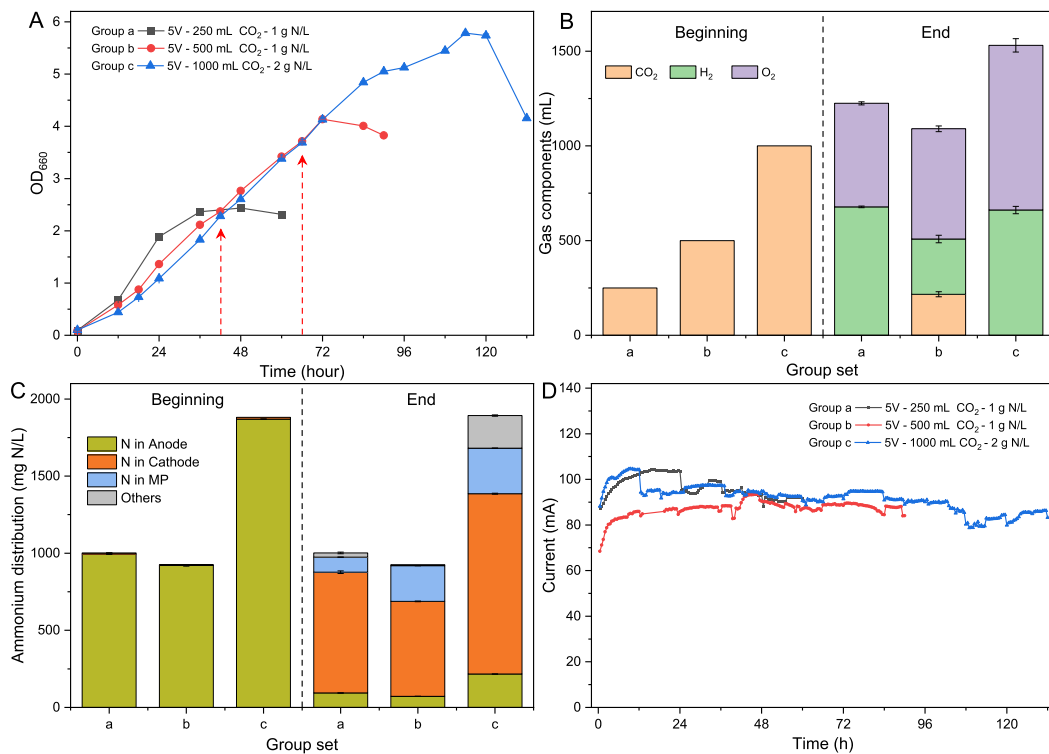


Fig. 4. The MERC was operated at three different conditions, A) Change of OD_{660} in the cathode chamber, the red arrow indicated the repeated CO_2 supply in the group (b), B) gas components in gas recycling bag at the beginning and end of the test; C) ammonium distribution in the system at the beginning and end of the test; D) current change during operation. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

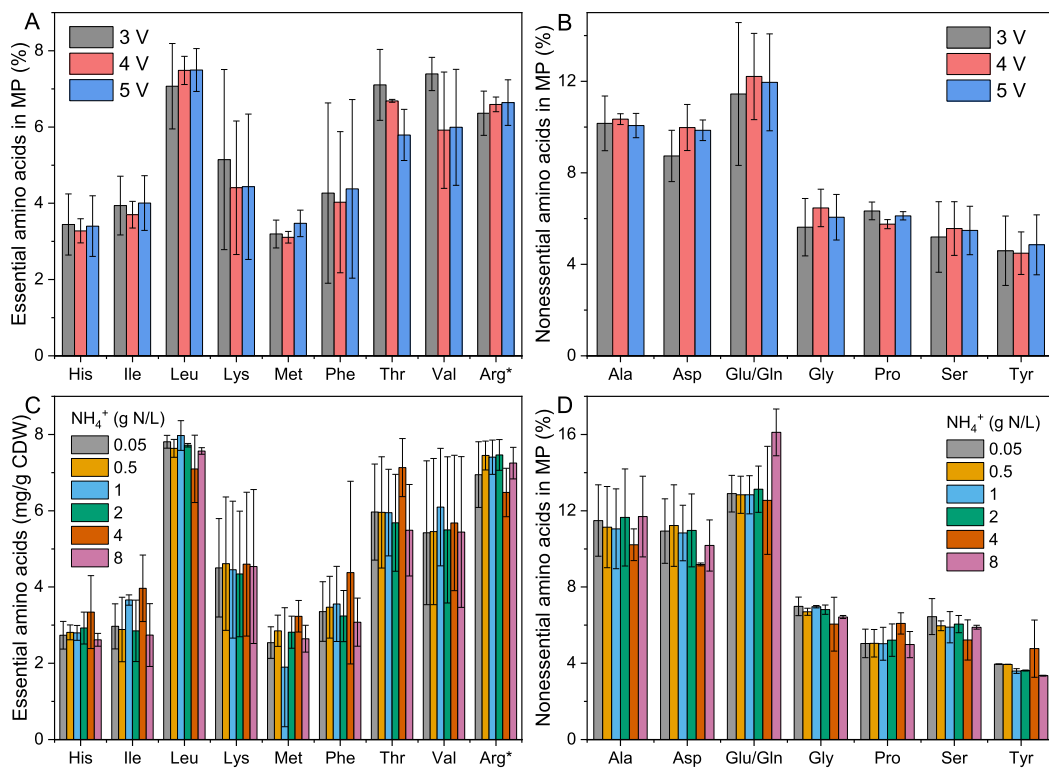


Fig. 5. The amino acid composition of the yielded MP under different operation conditions (His: histidine, Ile: isoleucine, Leu: leucine, Lys: lysine, Met: methionine, Phe: phenylalanine, Thr: threonine, Val: valine, Arg: arginine, Ala: alanine, Asp: aspartic acid and asparagine, Glu/Gln: glutamic acid and glutamine, Gly: glycine, Pro: proline, Ser: serine, Tyr: tyrosine).

and 3.24 times of that yielded from the group (a). No gaseous CO₂ was left in recycling bag in the group (a) and (c), however, 261 mL of CO₂ remained in the bag at 90 h in the group (b) (Fig. 4B). A similar amount of H₂ gas was detected in the bag in the group (a) and (c) while the lowest H₂ was in group (b) (Fig. 4B). With the extended operation, more ammonium was recovered and upcycled (Fig. 4C). Nitrogen contents of 97.6 ± 0, 231.3 ± 0.03, 294.9 ± 0.03 mg N were assimilated into MP in the three groups. The protein contents in biomass obtained from three groups were similar (65.86 ± 0.15%, 64.49 ± 1.81%, and 61.44 ± 2.49%, respectively). The currents in the group (b) and (c) were similar, and both were a bit higher than that in the group (a) (Fig. 4D). However, all the currents were lower than 120 mA observed before.

With repeated CO₂ and ammonium supply, *C. necator* could keep growing for more than 110 h. In a reported single chamber water electrolyzer, lower than 1.6 g/L of biomass was accumulated in 140 h [14]. Comparatively, in the present study, the biomass harvested with less reaction time (120 h) was 1.68 times higher. The results show that the MERC probably has a better performance on biomass accumulation than a single chamber water electrolyzer. Besides, ammonium can be recovered from wastewater. The results also reveal the possibility of operating the MERC system for an extended period.

3.4. The amino acid profile under different operating conditions

The amino acid profiles of the produced MP under different operating conditions were analyzed to assess the corresponding protein quality (Fig. 5). In general, the MP yielded at different voltages showed a similar amino acid profile (p greater than 0.05). The effect of applied voltage on the amino acid profile of MP has never been investigated in the past. It has been reported that the current density (2.14 vs 4.29 mA/cm²) could significantly shift the community structure of HOB in a mixed culture fed with nitrate [39], but the impact of potential on protein synthesis was not examined. It was observed in this study that the amino acid profile of the MP derived under different voltages (3 ~ 4

V, in the range of 1.25 ~ 7.5 mA/cm²) was not changed significantly. The results imply that the protein produced by *C. necator* 335 in such an electricity-driven system may keep a steady quality even at a fluctuating power supply.

When the MERC operated with the synthetic wastewater containing different ammonium concentrations, the amino acid profile also showed little difference (p greater than 0.05). The results were consistent with the observation in our previous work [17]. The results indicate that the protein quality would not be significantly affected even when the proposed system receives waste streams that contain ammonium in a wide range.

3.5. Operation with real waste streams

The system was then tested with actual wastewater streams (Fig. 6). When the system was tested with the influent from a WWTP (group WW-1), the bacterium grew at a very low rate and stopped growing after 72 h. Chloride ions are widely found in the influent of WWTP [39], which could be oxidized into chlorine gas, and then inhibited the growth of *C. necator*. To verify this hypothesis, the gas generated from anode chamber was disconnected from the gas recycling bag, and 500 mL of O₂ was directly supplied in the bag (group WW-2). Consequently, the bacterium growth rate was close to that in group WW-1 in the first 42 h but was higher afterward until 60 h. It seemed that chlorine only showed a slightly negative effect on microbial growth. This may be because that the chloride in municipal wastewater is relatively low [40,41]. The OD₆₆₀ stopped increasing mainly because of the exhaustion of ammonium (Fig. 6 C) since the influent only contained 46.82 ± 0.11 mg N-NH₄⁺/L of ammonium. 96.1 ± 0.24 % and 95.2 ± 0.24% of ammonium were removed from wastewaters (group WW-1 and WW-2, respectively) in anode chamber. At the end of batch run, 94.2% and 93.5% of the recovered ammonium was eventually fixed into 0.48 ± 0.001 and 0.45 ± 0.003 g/L of CDW (51.9 ± 4.1% and 54.9 ± 3.1% of protein), respectively.

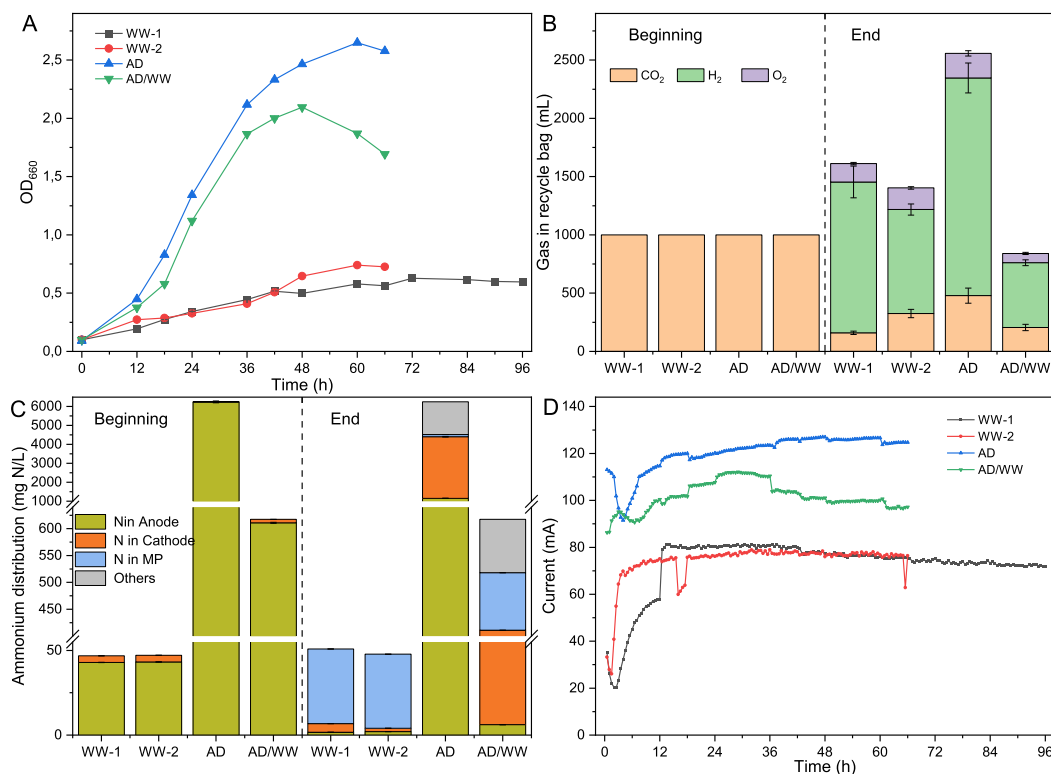


Fig. 6. The performance of the MERC treating real waste streams. A) the OD₆₆₀ change of medium in cathode chamber; B) gas components in gas recycling bag at the beginning and end of the test; C) ammonium distribution in the system at the beginning and end of the test; D) current change during operation.

Subsequently, ammonium-rich digestate (approx. 6238.5 ± 47.7 mg N/L) obtained from a CSTR reactor was fed into the MERC system (group AD). The bacterium grew well in the cathode chamber, and 1.22 ± 0.003 g/L CDW was harvested in 60 h with a protein content of $51.8 \pm 3.1\%$. In the group of AD/WW (a mixture of municipal influent and digestate), 0.96 ± 0.006 g/L of CDW was yielded with a protein content of $62.3 \pm 4.4\%$.

The conductivity of influent from WWTP was only 1.6 mS/cm, resulting in a low current of 80 mA in the MERC. Once the MERC was tested with the wastewater (AD/WW) with a relatively higher conductivity (7.9 mS/cm), the current reached 100 mA. A higher current (around 120 mA) was observed while treating the digestate which had an extremely high conductivity (64.5 mS/cm). The high current would contribute to the high efficiency of water electrolysis. Therefore, the growth of HOB was faster with a higher current due to the enhanced production of H_2 and O_2 .

The amino acid profiles of the MP produced during the tests were also examined (Fig. S4, Fig. 7). Though real waste streams contained different ammonium concentrations, they showed little effect on amino acid profile except for Glu/Gln (glutamic acid and glutamine) and Tyr (tyrosine) (Fig. S4). The protein produced from digestate (group AD) had a significantly high amount of Glu/Gln compared with other groups ($p < 0.05$). Nevertheless, it had a lower content of Tyr ($p < 0.05$ compared with group AD/WW, $p < 0.01$ compared with group WW-1 and WW-2).

The amino acid profiles were further compared with the recommended recipe from World Health Organization (WHO) [42]. In general, the protein produced by *C. necator* 335 in the proposed MERC using various real waste streams is competent to be a feed or food alternative. Several amino acids were clustered as sulfur amino acids (SAA, including Met and Cys), branched-chain amino acids (BCAA, including Leu, Ile, and Val), total aromatic amino acids (TAAA, including Phe and Tyr), and dispensable amino acids (DAA, including Arg, Ala, Asp, Glu/Gln, Gly, Pro, and Ser). The protein produced from real waste streams contained higher amounts of His, Thr, BCAA, and TAAA, competent amounts of Lys, SAA, and DAA (Fig. 7).

3.6. The energy efficiency of the system

Firstly, a control test was performed to evaluate the efficiency of

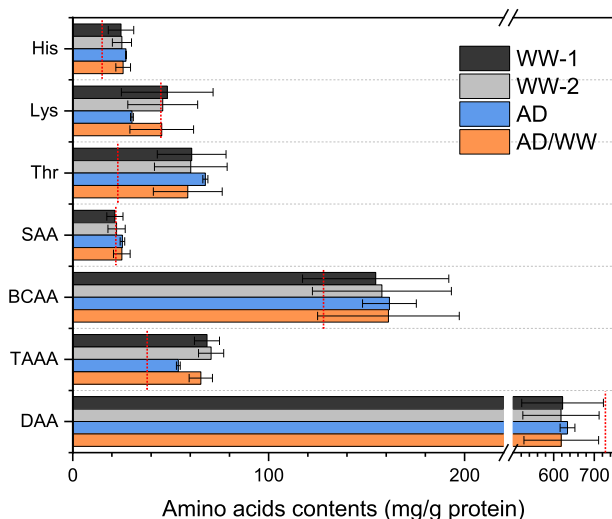


Fig. 7. Distribution of amino acids in the MP produced from ammonium contained real waste streams and the nutritional requirements of adults recommended by WHO were indicated by the red dot lines in figure. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

water electrolysis in the MERC. Synthetic wastewater containing 1 g $N-NH_4^+/L$ was filled into anode chamber and nitrogen-free HOB medium was used as catholyte. The MERC system was operated for 84 h with 5 V applied voltage. The hydrogen evolution rate was about 26 mL/h ($R^2 = 0.996$) (Fig. S5), and the energy efficiency was 20.1%. In the conventional single chamber electrolyzer, the hydrogen evolution rate was 5.3×10^{-7} kg/h, i. e., 6.5 mL/h, but the energy efficiency was 78% [14]. Industrial water electrolysis could reach an energy efficiency of 70% [13]. It should be noted that the MERC was developed for proof of concept in this study. The energy efficiency of water splitting in the MERC needs to be further improved during scaling-up in the future.

The hydrogen utilization rate and the energy efficiencies of the system while treating various real waste streams were calculated and summarized in Table 1. Generally, the hydrogen utilization rates were lower in the tests of WW-2 (45 ~ 58%) and AD (49 ~ 62%) compared to the results of group AD/WW (79 ~ 87%). In the WW-2 group, the low ammonium concentration suppressed the growth of *C. necator* 335, thereby leading to a waste of hydrogen. In the group AD, the exceptionally high conductivity of the digestate enhanced the efficiency of water electrolysis by lowering the internal resistance. In this context, the gas generation rate was higher than the growth rate of HOB, and the gas mass transfer could also limit the hydrogen utilization rate. Higher hydrogen utilization rates were achieved in single chamber electrolyzer (close 100%) [14], indicating that the MERC needs further optimization with an adequate external power supply and higher gas mass transfer.

Regarding the energy efficiency of biomass conversion (i. e., MP production), the system performed better in the group AD and AD/WW (74 ~ 142%) because of the better growth of HOB. Similarly, the energy efficiency of the whole system was higher in the group AD and AD/WW (86 ~ 147%) mainly because of the higher biomass production. 121% of energy efficiency was achieved when the current density was 2.14 mA/cm² in a single chamber electrolyzer for the growth of a mixed HOB culture. The energy efficiency quickly decreased to 14% when the current density was shifted to 4.29 mA/cm² [39]. In the present study, *C. necator* 335 showed higher tolerance to high current density (5 ~ 7.5 mA/cm²) than the mixed culture fed with low nitrate concentration (around 80 mg NO_3^-/L).

4. Perspective and limitations

The protein-rich MP was produced by a HOB strain in the designed MERC using ammonium from various waste streams. The process can be driven by renewable electricity and have a low environmental footprint. Without considering the environmental benefits, the electricity cost on MP production in the proposed MERC was in the range of 97 ~ 168 kWh/kg CDW. Given that the renewable electricity price is 0.05 €/kWh (e. g., the off-shore and on-shore wind electricity prices were 0.05 ~ 0.07 €/kWh and 0.03 €/kWh in 2016) [43] and protein content is 60%, the CDW and pure protein price produced from the MERC will be 4.85 ~ 8.4 €/kg CDW and 8.08 ~ 14 €/kg protein, respectively. The price is higher than the cost reported from a single chamber electrolyzer (3.4 €/kg CDW and 5.4 €/kg protein) [44], but the MERC could upcycle ammonium from wastewaters which would provide additional environmental and economic benefits.

In general, the energy costs need to be further reduced to compete with other protein sources. Firstly, the efficiency of water electrolysis could be significantly promoted by employing novel electrodes. For instance, as high as 85% of energy efficiency was reported using ionic activated electrodes [46]. Assuming that the water-splitting efficiency in the MERC could be increased from 21% to 60%, the costs on protein may be reduced to 2.7 €/kg protein. Secondly, the cost of the process can be reduced by optimizing gas mass transfer and gas components ratio controlled. Thirdly, the synchronous synthesis of prebiotic (polyhydroxybutyrate) by *C. necator* could further increase the commercial price of the HOB meal [13]. Besides, HOB strain/culture with a higher growth rate may be isolated or enriched to increase protein production

Table 1

The energy efficiencies of the present MERC system while treating various real waste streams.

Group	W_T (Wh) ^d	W_R (Wh)	W_{H_2} (Wh)	W_B (Wh)	U_{H_2} (%)	η_B (%)	η_S (%)
WW-2 ^a	22.049	3.311	1.922	7.367 ^e	44.60	39.31	49.57
				10.637 ^f	53.76	56.77	67.02
				12.788 ^g	58.29	68.24	78.50
AD ^b	35.677	8.596	4.443	20.118 ^e	48.74	74.29	90.69
				29.049 ^f	57.86	107.27	123.67
				34.921 ^g	62.27	128.95	145.36
AD/WW ^c	24.46	5.060	0.865	15.882 ^e	79.40	81.86	86.32
				22.932 ^f	84.77	118.21	122.67
				27.568 ^g	87.00	142.10	146.56

^a Group WW-2, influent from a municipal WWTP was used as anolyte.^b Group AD, effluent from a lab-scale CSTR was used as anolyte.^c Group AD/WW, a mixture of the influent from WWTP and the effluent from CSTR was used as anolyte.^d W_T , total input energy by power supply. W_R , energy cost by external resistor. W_{H_2} , the energy which was converted into H_2 . W_B , the energy which was converted into biomass. U_{H_2} , hydrogen utilization rate. η_B , energy efficiency of MP production. η_S , energy efficiency of the whole system.^e The result was calculated using the $F_{H_2-Biomass}$ of 1.57 derived from fast autotrophic growth[21].^f The result was calculated using the $F_{H_2-Biomass}$ of 1.09 derived from slow autotrophic growth[21].^g The result was calculated using the $F_{H_2-Biomass}$ of 0.91 derived from stationary autotrophic growth[21].

and productivity.

Besides energy efficiency, some other parameters could also be optimized in future studies. For instance, the energy in the waste streams was lost from the anode chamber through removal other than recovered. Therefore, the configuration of the MERC system needs to be optimized (e. g., coupling with a microbial fuel cell to recover the energy from waste streams). The hydrogen utilization rate needs to be enhanced to reduce the risk of explosion. The pH of anolyte and catholyte is changed drastically during cultivation. Thus, alkalis are required to neutralize the wastewater before discharging, and acids must be amended to the medium in cathode chamber to maintain an optimal pH for bacterial growth. The expensive costs of cation exchange membrane and electrodes are still hindering the application of the MERC system, which needs to be further reduced.

5. Conclusion

An electricity-driven microbial electrochemical recovery conversion cell was demonstrated in this study. With a power supply of higher than 3 V, protein-rich biomass was produced in the form of the hydrogen oxidizing bacterial (HOB) strain *C. necator* 335, in the cathode chamber. Ammonium recovered from the anode chamber was used as nitrogen source to *C. necator*. Ammonium solution was added in the anode chamber at a concentration range of 0.05 ~ 8 g N-NH₄⁺/L. Neither supplied voltage (3 ~ 5 V) nor ammonium concentration (0.05 ~ 8 g N-NH₄⁺/L) showed a significant impact on the amino acid profile of the produced MP. The system can be applied to real ammonium-rich waste streams, and the performance is mainly affected by the characteristics of the waste streams. High energy efficiency can be achieved when treating ammonium-rich wastewater. The produced protein has a good amino acid profile, containing essential amino acids and can meet the nutritional requirements of adults according to WHO recommendation [42].

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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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.cej.2021.132890>.

References

- [1] World population growth is projected to flatten in coming decades, Pew Res. Cent. (2019). https://www.pewresearch.org/fact-tank/2019/06/17/worlds-population-is-projected-to-nearly-stop-growing-by-the-end-of-the-century/ft_19-06-17_worldpopulation_world-population-growth-projected-flatten/ (accessed October 27, 2020).
- [2] M. Henchion, M. Hayes, A. Mullen, M. Fenelon, B. Tiwari, Future protein supply and demand: strategies and factors influencing a sustainable equilibrium, *Foods*. 6 (2017) 53, <https://doi.org/10.3390/foods6070053>.
- [3] J. Pretty, Intensification for redesigned and sustainable agricultural systems, *Science*. 362 (6417) (2018) eaav0294, <https://doi.org/10.1126/science.aav0294>.
- [4] S. Mohammed, K. Alsafadi, I. Takács, E. Harsányi, Contemporary changes of greenhouse gases emission from the agricultural sector in the EU-27, *Geol. Ecol. Landscapes*. 4 (4) (2020) 282–287, <https://doi.org/10.1080/24749508.2019.1694129>.
- [5] N. Dudley, S. Alexander, Agriculture and biodiversity: a review, *Biodiversity*. 18 (2–3) (2017) 45–49, <https://doi.org/10.1080/1488386.2017.1351892>.
- [6] A. Mishra, J.N. Ntuhuga, B. Molitor, L.T. Angenent, Power-to-Protein: Carbon fixation with renewable electric power to feed the world, *Joule*. 4 (6) (2020) 1142–1147, <https://doi.org/10.1016/j.joule.2020.04.008>.
- [7] F. Oosterholt, S. Matassa, L. Palmen, K. Roest, W. Verstraete, Pilot scale production of single cell proteins using the power-to-protein concept, in: 2nd Int. Resour. Recover. Conf., 2017: pp. 1–16.
- [8] B. Molitor, A. Mishra, L.T. Angenent, Power-to-protein: Converting renewable electric power and carbon dioxide into single cell protein with a two-stage bioprocess, *Energy Environ. Sci.* 12 (12) (2019) 3515–3521, <https://doi.org/10.1039/C9EE02381J>.
- [9] J. Yu, A. Dow, S. Pingali, The energy efficiency of carbon dioxide fixation by a hydrogen-oxidizing bacterium, *Int. J. Hydrogen Energy*. 38 (21) (2013) 8683–8690, <https://doi.org/10.1016/j.ijhydene.2013.04.153>.
- [10] B. Bowien, H.G. Schlegel, Physiology and biochemistry of aerobic hydrogen-oxidizing bacteria, *Annu. Rev. Microbiol.* 35 (1) (1981) 405–452, <https://doi.org/10.1146/annurev.mi.35.100181.002201>.
- [11] I. Pikaar, J. de Vrieze, K. Rabaey, M. Herrero, P. Smith, W. Verstraete, Carbon emission avoidance and capture by producing in-reactor microbial biomass based food, feed and slow release fertilizer: Potentials and limitations, *Sci. Total Environ.* 644 (2018) 1525–1530, <https://doi.org/10.1016/j.scitotenv.2018.07.089>.
- [12] S. Matassa, W. Verstraete, I. Pikaar, N. Boon, Autotrophic nitrogen assimilation and carbon capture for microbial protein production by a novel enrichment of hydrogen-oxidizing bacteria, *Water Res.* 101 (2016) 137–146, <https://doi.org/10.1016/j.watres.2016.05.077>.
- [13] R.D. Msc, J. de Wilt, Power to gas to protein, Protein at farm scale from feed compatible components (2015), <https://doi.org/10.1007/s12010-018-2824-1>.
- [14] G. Givirovskiy, V. Ruuskanen, L.S. Ojala, P. Kokkonen, J. Ahola, In situ water electrolyzer stack for an electrobioreactor, *Energies*. 12 (2019) 1904, <https://doi.org/10.3390/en12101904>.
- [15] G. Givirovskiy, V. Ruuskanen, L.S. Ojala, M. Lienemann, P. Kokkonen, J. Ahola, Electrode material studies and cell voltage characteristics of the in situ water

- electrolysis performed in a pH-neutral electrolyte in bioelectrochemical systems, *Heliyon*. 5 (2019), e01690, <https://doi.org/10.1016/j.heliyon.2019.e01690>.
- [16] M.E.R. Christiaens, S. Gildemyn, S. Matassa, T. Ysebaert, J.o. De Vrieze, K. Rabaey, Electrochemical ammonia recovery from source-separated urine for microbial protein production, *Env. Sci Technol.* 51 (22) (2017) 13143–13150, <https://doi.org/10.1021/acs.est.7b02819>.
- [17] X. Yang, M. Xu, R. Zou, I. Angelidaki, Y. Zhang, Microbial protein production from CO₂, H₂, and recycled nitrogen: Focusing on ammonia toxicity and nitrogen sources, *J. Clean. Prod.* 291 (2021) 125921, <https://doi.org/10.1016/j.jclepro.2021.125921>.
- [18] M. Yan, L. Treu, S. Campanaro, H. Tian, X. Zhu, B. Khoshnevisan, P. Tsapekos, I. Angelidaki, I.A. Fotidis, Effect of ammonia on anaerobic digestion of municipal solid waste: inhibitory performance, bioaugmentation and microbiome functional reconstruction, *Chem. Eng. J.* 401 (2020) 126159, <https://doi.org/10.1016/j.cej.2020.126159>.
- [19] C. Li, X. Zhu, I. Angelidaki, Carbon monoxide conversion and syngas biomethanation mediated by different microbial consortia, *Bioresour. Technol.* 314 (2020) 123739, <https://doi.org/10.1016/j.biortech.2020.123739>.
- [20] F. Mariotti, D. Tomé, P.P. Mirand, Converting nitrogen into protein - beyond 6.25 and Jones' factors, *Crit. Rev. Food Sci. Nutr.* 48 (2) (2008) 177–184, <https://doi.org/10.1080/10408390701279749>.
- [21] J. Yu, Y. Lu, Carbon dioxide fixation by a hydrogen-oxidizing bacterium: biomass yield, reversal respiratory quotient, stoichiometric equations and bioenergetics, *Biochem. Eng. J.* 152 (2019) 107369, <https://doi.org/10.1016/j.bej.2019.107369>.
- [22] K. Siddharth, Y. Chan, L. Wang, M. Shao, Ammonia electro-oxidation reaction: recent development in mechanistic understanding and electrocatalyst design, *Curr. Opin. Electrochem.* 9 (2018) 151–157, <https://doi.org/10.1016/j.COELC.2018.03.011>.
- [23] M. Overland, A.-H. Tauson, K. Shearer, A. Skrede, Evaluation of methane-utilising bacteria products as feed ingredients for monogastric animals, *Arch Anim Nutr.* 64 (3) (2010) 171–189, <https://doi.org/10.1080/17450391003691534>.
- [24] T.G. Volova, V.A. Barashkov, Characteristics of proteins synthesized by hydrogen-oxidizing microorganisms, *Appl. Biochem. Microbiol.* 46 (6) (2010) 574–579, <https://doi.org/10.1134/S0003683810060037>.
- [25] J. Yu, P. Munasinghe, Gas fermentation enhancement for chemolithotrophic growth of *Cupriavidus necator* on carbon dioxide, *Fermentation*. 4 (2018) 63, <https://doi.org/10.3390/fermentation4030063>.
- [26] J. Dou, Y. Huang, H. Ren, Z. Li, Q. Cao, X. Liu, D. Li, Autotrophic, heterotrophic, and mixotrophic nitrogen assimilation for single-cell protein production by two hydrogen-oxidizing bacterial strains, *Appl. Biochem. Biotechnol.* 187 (1) (2019) 338–351, <https://doi.org/10.1007/s12010-018-2824-1>.
- [27] X. Hu, F.-M. Kerckhof, J. Ghesquière, K. Bernaerts, P. Boeckx, P. Clauwaert, N. Boon, Microbial protein out of thin air: fixation of nitrogen gas by an autotrophic hydrogen-oxidizing bacterial enrichment, *Env. Sci Technol.* 54 (6) (2020) 3609–3617, <https://doi.org/10.1021/acs.est.9b06755>.
- [28] N. Sriperum, G.M. Pesti, P.B. Tillman, Evaluation of the fixed nitrogen-to-protein (N:P) conversion factor (6.25) versus ingredient specific N:P conversion factors in feedstuffs, *J. Sci. Food Agric.* 91 (7) (2011) 1182–1186, <https://doi.org/10.1002/jsfa.4292>.
- [29] V. Ruuskanen, G. Givirovskiy, J. Elfving, P. Kokkonen, A. Karvinen, L. Järvinen, J. Sillman, M. Vainikka, J. Ahola, Neo-carbon food concept: a pilot-scale hybrid biological–inorganic system with direct air capture of carbon dioxide, *J. Clean. Prod.* 278 (2021) 123423, <https://doi.org/10.1016/j.jclepro.2020.123423>.
- [30] Y. Ye, H.H. Ngo, W. Guo, Y. Liu, S.W. Chang, D.D. Nguyen, H. Liang, J. Wang, A critical review on ammonium recovery from wastewater for sustainable wastewater management, *Bioresour. Technol.* 268 (2018) 749–758, <https://doi.org/10.1016/j.biortech.2018.07.111>.
- [31] T. Luukkonen, K. Věžníková, E.-T. Tolonen, H. Runtti, J. Yliniemi, T. Hu, K. Kempainen, U. Lassi, Removal of ammonium from municipal wastewater with powdered and granulated metakaolin geopolymer, *Environ. Technol.* 39 (4) (2018) 414–423, <https://doi.org/10.1080/09593330.2017.1301572>.
- [32] A. Schievano, G. D'Imporzano, S. Salati, F. Adani, On-field study of anaerobic digestion full-scale plants (Part I): an on-field methodology to determine mass, carbon and nutrients balance, *Bioresour. Technol.* 102 (17) (2011) 7737–7744, <https://doi.org/10.1016/j.biortech.2011.06.006>.
- [33] D.E. Atkinson, The biochemistry of hydrogenomonas III.: The effect of inorganic nitrogen compounds on hydrogen uptake, *J. Bacteriol.* 70 (1955) 78–81.
- [34] M. Aragno, H.G. Schlegel, in: *The Prokaryotes*, Springer Berlin Heidelberg, Berlin, Heidelberg, 1981, pp. 865–893, https://doi.org/10.1007/978-3-662-13187-9_70.
- [35] V.M. Vadivelu, J. Keller, Z. Yuan, Effect of free ammonia on the respiration and growth processes of an enriched *Nitrobacter* culture, *Water Res.* 41 (4) (2007) 826–834, <https://doi.org/10.1016/j.watres.2006.11.030>.
- [36] Györgyi Nyerges, S.-K. Han, L.Y. Stein, Effects of ammonium and nitrite on growth and competitive fitness of cultivated methanotrophic bacteria, *Appl. Environ. Microbiol.* 76 (16) (2010) 5648–5651, <https://doi.org/10.1128/AEM.00747-10>.
- [37] J. Yu, Fixation of carbon dioxide by a hydrogen-oxidizing bacterium for value-added products, *World J. Microbiol. Biotechnol.* 34 (2018) 89, <https://doi.org/10.1007/s11274-018-2473-0>.
- [38] Z. ZHU, Z. HAO, Z. SHEN, J. CHEN, Modified modeling of the effect of pH and viscosity on the mass transfer in hydrophobic hollow fiber membrane contactors, *J. Memb. Sci.* 250 (1-2) (2005) 269–276, <https://doi.org/10.1016/j.memsci.2004.10.031>.
- [39] W. Zhang, F. Zhang, Y. Niu, Y.-X. Li, Y. Jiang, Y.-N. Bai, K. Dai, R.J. Zeng, Power to hydrogen-oxidizing bacteria: effect of current density on bacterial activity and community spectra, *J. Clean. Prod.* 263 (2020) 121596, <https://doi.org/10.1016/j.jclepro.2020.121596>.
- [40] S. Hosseini-pour Dizgah, K. Taghavi, J. Jaafari, E. Roohbakhsh, S.D. Ashrafi, Data on pollutants content in the influent and effluent from wastewater treatment plant of Rasht in Guilan Province, Iran, *Data Br.* 16 (2018) 271–275, <https://doi.org/10.1016/j.dib.2017.11.042>.
- [41] C.C. Hong, S.-K. Chan, H. Shim, Effect of chloride on biological nutrient removal from wastewater, *J. Appl. Sci. Environ. Sanit.* 2 (2007) 85–92.
- [42] Joint FAO/WHO/UNU, Protein and amino acid requirements in human nutrition: report of a joint FAO/WHO/UNU expert consultation, 2007. <https://apps.who.int/iris/handle/10665/43411> (accessed May 14, 2021).
- [43] T. Kåberger, Progress of renewable electricity replacing fossil fuels, *Glob. Energy Interconnect.* 1 (2018) 48–52, <https://doi.org/10.14171/j.2096-5117.gei.2018.01.006>.
- [44] M. Wuokko, Chemolithoautotrophic growth of Knallgas bacteria in an electrobioreactor using in situ water electrolysis, Aalto University, 2017. <https://aaltodoc.aalto.fi/443/handle/123456789/26114> (accessed May 8, 2021).