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Bio-electrochemically extracted nitrogen from residual resources for microbial protein production

Ziyi Yang\textsuperscript{a,b}, Panagiotis Tsapekos\textsuperscript{b}, Yifeng Zhang\textsuperscript{c}, Yi Zhang\textsuperscript{d}, Irini Angelidaki\textsuperscript{b,#}, Wen Wang\textsuperscript{a,d,#,*}

\textsuperscript{a}Biomass Energy and Environmental Engineering Research Center, Beijing University of Chemical Technology, Beijing 100029, China;

\textsuperscript{b}Department of Chemical and Biochemical Environmental Engineering, Technical University of Denmark, DK-2800, Kgs Lyngby, Denmark;

\textsuperscript{c}Department of Environmental Engineering, Technical University of Denmark, DK-2800, Kgs Lyngby, Denmark;

\textsuperscript{d}College of Chemical Engineering, Beijing University of Chemical Technology, Beijing 100029, China.

\#Both authors contributed equally to this work.

To whom correspondence should be addressed:

* Wen Wang’s e-mail: wangwen@mail.buct.edu.cn, anne_wangwen@163.com;

Address: 507 Zonghe Building, Beijing University of Chemical Technology, 15 North 3rd Ring East Road, Beijing 100029, P. R. China
Abstract

Upcycling of nutrients from residual resources for producing microbial protein (MP) is an attractive method to valorize residues. In this study, we investigated bio-electrochemical methods to recover ammonia-N, for further production of MP. Reject water and digestate were used for ammonia-N recovery in microbial fuel cell (MFC) system. In one-stage process, ammonia-N recovery was 32 – 42% with 57 – 154 kJ/m³ waste stream of electricity generation. For further enhancing recovery efficiency, a two-stage process was developed, achieving efficiency of 53 – 61%. Subsequently, MP was grown with the extracted ammonia-N, and amino acid concentration was 421 and 272 mg/L under 25 °C and 35 °C, respectively. Similar essential amino acid content of MP (especially under 25 °C) with the one from fish demonstrated the attractiveness of upcycling residues to proteins. Based on simplified economic evaluation, the produced energy performed the potential to catch 1.63 – 6.54 €/m³ waste stream.

Key words: ammonia-N recovery; microbial fuel cell; microbial protein; methane oxidizing bacteria; temperature

1. Introduction

With the accelerating growth of population it is anticipated that food scarcity is going to become a crucial challenge requiring timely actions to be taken (Muthamilarasan & Prasad, 2021). Agricultural expansion results in increasing environmental pressure, and increment of usage amount of N-fertilizers was observed
with a relatively low utilization efficiency of fertilizers for production of plant-based 
and animal-based protein (Bouwman et al., 2013). In parallel, nearly 70% reactive 
nitrogen is lost to the environment, especially in the air and water (Alexander et al., 
2017). Therefore, recovering nitrogen from wastes and further producing products (such 
as protein) could be an effective method to prevent environmental decay and optimize 
nitrogen cycle process.

Nitrogen is mainly in the form of ammonia-N in wastes and wastewaters (Duan et 
al., 2020; Zhang & Angelidaki, 2015a). Free ammonia (NH₃) and ammonium (NH₄⁺), 
interchanging depending on pH and temperature, are the two primary forms of 
ammonia-N in aqueous solution (Chen et al., 2008). Several technologies have been 
proposed for ammonia-N recovery, such as gas stripping (Başakçilardan-Kabakci et al., 
2007), adsorption (ion exchange) (Lahav et al., 2013), micro and nanofiltration 
(Zarebska et al., 2015), etc. However, these methods are energy-consuming, using 
chemicals for pH control, inefficient and uneconomical (Domingo-Félez & Smets, 
2019). Recently, bio-electrochemical systems, mainly including microbial fuel cell 
(MFC) and microbial electrochemical cell (MEC), have been developed for ammonia 
recovery. These methods have advantages of relatively low energy consumption (even 
energy production) and no need of additional chemicals (Matassa et al., 2015; Zhang & 
Angelidaki, 2015a). MFC can operate without external energy input by using 
microorganisms as biocatalyst for oxidation of organic matter to gain electrons. 
Enrichment of exolectrogenic bacteria is a key step for establishment of a functional 
MFC. So far, exolectrogens are mainly belonging to Clostridia (Niessen et al., 2004),
Betaproteobacteria (Chaudhuri & Lovley, 2003), Deltaproteobacteria (Mei et al., 2018), and Gammaproteobacteria (Zheng et al., 2019). MFC, can convert chemical energy contained in wastewaters into electricity and thereby can be considered as a low-cost and green approach to recover ammonia from waste streams. Ammonia-N recovery efficiency is depending on the performance (mainly electricity production) and operational parameters (pH, temperature, etc.) in MFC (Ye et al., 2019). However, after ammonia-N recovery through MFC, 40 – 70% of nitrogen still remains in anodic chamber due to the limitations of current or concentration gradient of the ions (Khoshnevisan et al., 2020), resulting in a limited recovery efficiency. In this context, it is necessary to overcome the limitations and to optimize the recovering process based on MFC system, by creating an operational environment with high current or obvious concentration gradient.

Efficient assimilation of the extracted ammonia-N into protein is a valuable way to valorize the otherwise wasted nitrogen to a high-value product. It has been estimated that microbial protein (MP), has the potential to replace 10 – 15% plant-based protein (Pikaar et al., 2018). MOB could efficiently capture nitrogen into microbial biomass, i.e. MP. MP can present a similar quality to soybean (Matassa et al., 2016), performing an application potential on producing feed formulae, or even food (Valverde-Pérez et al., 2020). Also, MP exhibits the possibility to be considered as a kind of plant growth promoters (Kumar et al., 2019) and food additives (Anupama et al., 2000). Moreover, production of protein in the form of microbial biomass would have the advantage of being independent of the land crops cultivations requiring specific climatic conditions.
soils, irrigations etc. (Hulsen et al., 2018). Methane oxidizing bacteria (MOB) are proved to produce protein by using CH$_4$ and ammonia-N under 20 – 55 °C, and optimum growth occurs at 20 – 35 °C (Bowman et al., 1993). Temperature is a crucial parameter for MOB cultivation affecting enzyme activity (Arcus et al., 2020), molecular dynamics (Glowacki et al., 2012), and nutrients uptake (Grubecki & Wójcik, 2008). Previous studies have mainly focused on 20 – 25 °C (Acosta et al., 2020; Khoshnevisan et al., 2020). In the proper range of temperature, biological and chemical reaction rates of microorganisms can be improved by increasing temperature (Li et al., 2019). While, for MOB, the knowledge of microbial growth, MP production and MP quality at broader temperature spectrum is limited.

A concept recently introduced has proposed to upcycle organic wastes to microbial protein. The idea is to utilize both the organic matter by first converting it to biogas but also use the contained into the waste nitrogen, for production of MP. In this frame, developing an efficient ammonia recovery, for MP production, the main objectives of this study were: (i) to develop a bio-electrochemical ammonia-N recovery technology from different waste streams in MFC; (ii) to elucidate the response of mixed MOB cultures to different temperature to obtaining preferential condition for MP production; (iii) to assess the economic feasibility of using wastes as nitrogen source for MP production.

2. Materials and methods

2.1 Feedstock and inoculum
For ammonia-N recovery, two waste streams were used: a) reject water was the liquid fraction from centrifugal dewatering of effluent after anaerobic digestion, and it was obtained from Ejby Mølle wastewater treatment plant (Odense, Denmark) and b) digestate collected after anaerobic digestion experiment of biopulp which was the homogenized organic fraction of household waste collected in Copenhagen municipality. Characteristics of reject water and digestate were summarized in Table 1. A mixed MOB seed from Tsapekos et al. (Tsapekos et al., 2020), with bacterial relative abundance of 42% *Methylomonas* spp. and 33% *Methylophilus* spp., was used for MP production.

Table 1. Characteristics of reject water and digestate

### 2.2 Experimental design and procedure

#### 2.2.1 Experimental setup for ammonia-N recovery

MFC system was composed of two chambers (10 cm × 10 cm × 3 cm), separated by a cation exchange membrane (CEM; CMI 7000, Membrane international, NJ). The contact area of CEM with liquid was about 64 cm² (8 cm × 8 cm). A 1 kΩ resistance was used and the systems was operated in MFC mode. Effective volume of each chamber was 250 mL. The anode electrode was carbon fiber brush (4 cm diameter, 4 cm length). The carbon fiber brush was pretreated at 450 °C for 30 min before using. After pretreatment, the brush was further pre-acclimated in digestate, operating as two-chamber MFC. 50 mL digestate and 200 mL medium was added in the anode chamber,
with 50 mM K$_3$[Fe(CN)$_6$] added into cathode chamber. Medium used in anode chamber was prepared with adding 0.31 g NH$_4$Cl, 0.13 g KCl, 4.33 g Na$_2$HPO$_4$, 2.69 g NaH$_2$PO$_4$$\cdot$2H$_2$O, 0.1 g CaCl$_2$$\cdot$2H$_2$O, 0.1 g MgCl$_2$$\cdot$6H$_2$O, 12.5 mL trace element solution, 12.5 mL vitamin solution, and then added distilled water, constating volume to 1 L. After operating for 3 runs (about 16 days), the voltage could be kept over 0.6 V for about 5 days. Stainless steel woven mesh (4 cm × 4 cm) coated with 0.5 mg/cm$^2$ Pt, was used as cathode electrode. The tested wastes (reject water and digestate) were added in the anodic chamber, while in the cathodic chamber a mixture 40 mmol KH$_2$PO$_4$ and 40 mmol Na$_2$HPO$_4$ were added. The selection of buffer solution (KH$_2$PO$_4$ and Na$_2$HPO$_4$) was according to the wished pH in the subsequent MP fermentation experiment. The experiment for ammonia-N recovery from each waste was carried out in three runs, to detect the stability and reproducibility of system. Liquid in both anodic and cathodic chamber was refilled in each new run to maintain the same initial state. In order to increase the ammonia recovery from the wastes, a two-stage recovery experiment was run. In this test, when the average recovery rate of ammonia-N from the anodic to the cathodic chamber was lower than 5 mg/L/h and 10 mg/L/h in reject water and digestate, respectively, the liquid in cathodic chamber was refilled and the experiment continued for extracting an additional amount of ammonia. In order to detect the contribution of two dominant ways of ammonia-N transportation through CEM (NH$_4^+$ migration and NH$_3$ diffusion), open circuit (= without current flow) MFC reactors were set as the control experiments. The mixture liquid of 40 mmol KH$_2$PO$_4$ and 40 mmol Na$_2$HPO$_4$ was added into the cathodic
chamber, along with feeding reject water and digestate into the anodic chamber, respectively.

2.2.2 Experimental setup for microbial protein production

Two continuously stirred tank reactor (CSTR) fermenters were operated at 25 °C and 35 °C for MP production. The total and working volume of the fermenters were 2.5 L and 2 L, respectively. Hydraulic retention time (HRT) was set as 4 days. In order to start up the fermenters, about 1 L MOB seed and 1 L diluted ammonia-N mineral salt (dAMS) medium was added. 9 L synthetic gas mixture (CH₄/O₂ = 2/3 (v/v)) collected in a gasbag was supplied into each fermenter for one HRT (4 days) at the recirculation rate of 50 mL/min. The experiments for MP production were conducted in three periods. In period I, NH₄Cl was used as ammonia-N resource; while ammonia-N collected from reject water and digestate (effluent of cathodic chamber in Section 2.2.1) was used in period II and period III, respectively. In order to keep a similar operational environment during the whole experiment, ammonia-N concentration in the fermenter was controlled at 28 mg N/L. In period II and period III, after testing the ammonia-N concentration of effluent (C_{ammonia-N}, mg/L) from cathode in MFC, the added volume of effluent (V_{added}, mL) into each litter medium was calculated based on the Equation (1).

\[ V_{\text{added}} (\text{mL}) = \frac{28 \text{ mg}}{C_{\text{ammonia-N}} (\text{mg/L})} \times 1000 \]  (1)

The pH was adjusted to 6.8 by addition of a mixture of KH₂PO₄ and Na₂HPO₄ every day during the whole operational period.

dAMS medium
Dilute 20 mL of Stock A to 800 mL, and then add 1 mL FeNaEDTA Stock and 1 mL trace solution. Dissolve and bring to 1 L. After sterilization, adjust pH to 6.8 by KH₂PO₄ stock and Na₂HPO₄ stock at room temperature. Stock A: 10 g MgSO₄·7H₂O, 5 g NH₄Cl, 1.5 g CaCl₂·2H₂O, and then 1 L distilled water was added. FeNaEDTA Stock: 0.5 g FeNaEDTA, and then 100 mL distilled water was added.

**KH₂PO₄ stock**

27.2 g KH₂PO₄, and then 1 L distilled water was added.

**Na₂HPO₄ stock**

71.7 g Na₂HPO₄·12H₂O, and then 1 L distilled water was added.

### 2.3 Analytical methods

Voltage was monitored by a multimeter (MESTEK, China). pH was measured by pH meter (METTLER, Switzerland). Soluble chemical oxygen demand (SCOD), total chemical oxygen demand (TCOD), total Kjeldahl nitrogen (TKN) and ammonia-N were tested according to the guidelines of standard methods (APHA, 2005). TKN was quantified by Kjeldahl Apparatus (Foss™ Kjeltec™ 8100, FOSS, Denmark). Optical density at 600 nm (OD₆₀₀) was used to evaluate the biomass growth by using spectrophotometer (Helios™ Epsilon visible spectrophotometer, Thermo Scientific, USA), and culture medium without microorganisms was used as baseline for spectrophotometer calibration. After centrifuged at 9280 g (10,000 rpm) for 20 min, the sediment of the samples without supernatant were freeze-dried to quantify cell dry weight (CDW) and then determine the amino acid profile. Amino acid profile was
measured by high pressure liquid chromatography (Dionex UltiMate 3000 UHPLC, Thermo Scientific, USA) according to Tsapekos et al. (Tsapekos et al., 2019). Gas compositions (including CH₄, CO₂, and O₂) were monitored via gas chromatograph (GC-TRACE 1310, Thermo Scientific, USA) based on previous study (Khoshnevisan et al., 2018).

2.4 Data analysis

Gas volume was calibrated to standard temperature and pressure (STP; 273 K, 1 atm). Fluctuation of data less than 10% was considered as steady state condition, in experiment for microbial protein production.

Electricity production was integrated based on Equation (2) by OriginPro 2016. Where “W” stands for electric energy (J), “U” represents the voltage of MFC (V), “t” means operational time (h), and “R” stands for the external resistance, which was 1000 Ω in this study.

\[
W = \int_{0}^{t} \frac{U^2}{R} dt \times 3.6 \times 10^3 \hspace{1cm} (2)
\]

The average recovery rate of ammonia-N in MFC system was calculated according to Equation (3). Where “r” represents the average recovery rate of ammonia-N, “ΔC_{ammonia-N}” means the increment of ammonia-N concentration, and “Δt” stands for operational time.

\[
r \text{ (mg/(L•h))} = \frac{ΔC_{ammonia-N}}{Δt} \hspace{1cm} (3)
\]

The CDW yield on carbon and nitrogen was calculated by Equation (4) and (5). Where “C consumption” was evaluated based on methane consumption, and “N consumption” was evaluated based on ammonia-N consumption.

\[
\frac{ΔC_{carbon}}{Δt} = \frac{ΔC_{ammonia-N}}{Δt} \hspace{1cm} (4)
\]

\[
\frac{ΔC_{nitrogen}}{Δt} = \frac{ΔC_{ammonia-N}}{Δt} \hspace{1cm} (5)
\]
consumption” was converted according to the decrement of ammonia-N concentration.

\[ Y_{CDW/C} = \frac{CDW (g/L) \times \text{Working volume (L)}}{C \text{ consumption (g)}} \]  
\[ Y_{CDW/N} = \frac{CDW (g/L) \times \text{Working volume (L)}}{N \text{ consumption (g)}} \]  

3. Results and discussion

3.1 Ammonia-N recovery and electricity production from different waste streams

Reject water and digestate were used as sources for ammonia-N recovery by MFC. Reactor performance, including voltage, ammonia-N concentration and SCOD, is shown in Figure 1 and Figure 2. The evaluation indexes and ammonia-N recovery efficiency, are summarized in Table 2.

When reject water was used as waste stream, from batch 1 to batch 3, voltage was increased gradually, with a 2-fold increment on electricity energy production (from 14.4 J to 38.5 J; Table 2). This phenomenon was attributed to the adaptation of microorganisms in anodic chamber to the reject water. According to ammonia-N balance, the loss of ammonia-N during the operational period was about 5%. 32 – 36% of ammonia-N from reject water was recovered in cathodic chamber, with the recovery rate of 4.6 – 5.2 mg/L/h. \( \text{NH}_4^+ \) migration and \( \text{NH}_3 \) diffusion, which was mainly depended on current intensity and environmental factors (such as concentration gradient, pH, temperature), respectively, were considered as the two dominant ways of ammonia-N transportation through CEM (Zhang & Angelidaki, 2015b). As shown in Figure 3, from batch 1 to batch 3, the increase on voltage could enhance the transfer rate of \( \text{NH}_4^+ \) at the beginning of experiment (0 – 30 h). In addition, in all batches, pH in
anodic and cathodic chamber was in the range of 8.0 – 8.4 and 9.0 – 9.4, respectively.

Thus, the same tendencies were observed for NH$_3$ diffusion. Stable recovery efficiency and recovery rate of ammonia-N could be obtained in MFC system in the three batches. These results indicated that, environment and not voltage, was the decisive factor of the final ammonia-N concentration in cathodic chamber. As shown in Figure 1, after 30 h, no significant changes of ammonia-N concentration in two chambers appeared, with a downward tendency on voltage simultaneously. In this study, the driving force for ammonia-N transfer was concentration gradient. After 30 h, the gradient between anodic and cathodic chamber was low, resulting in a weak transport of ammonia-N. The transport of electrons, which was mainly depended on the imbalanced charge between two chambers (Haddadi et al., 2014), was weaken due to the approximate balance of ammonia-N, leading to a decrement on voltage.

SCOD in anodic chamber dropped dramatically in 10 hours, and then nearly kept steady. However, electricity production was maintained after the 10 hours. In MFC system, electricity was generated by the degradation of soluble organics by exoelectrogenic microorganisms and released electrons were transferred to anode electrode (Feng et al., 2010). According to organic matter solubilization rate (Table 2), over 12% organic insoluble matter was converted into SCOD, leading to stable SCOD concentration.
Reject water and digestate are two typical kinds of real nitrogen-rich wastes. As shown in the Table 1, the ammonia-N concentration and TKN concentration in digestate were about 2-fold of those in reject water. In order to detect the stability and efficiency of MFC fed with different ammonia-N concentration, digestate (about 1800 mg N/L) was tested as another substrate. As shown in Figure 2, in three batches, electric energy production was in the range of 15.5 - 27.7 J. According to Table 2, recovery efficiency of ammonia-N from digestate was in the range of 40 – 42% in three batches, which was higher than that in MFC fed with reject water. Recovery rate of ammonia-N was about 12 mg/L/h, which was about 2.5 times higher than that from reject water (about 5 mg/L/h). The results revealed that higher initial ammonia-N concentration could boost the ammonia-N transfer in MFC, resulting in a higher recovery efficiency as well as a higher recovery rate. The SCOD concentration in the anodic chamber decreased approx. 600 mg/L, and 16 – 24% of the insoluble organic content was solubilized, leading to a stable SCOD concentration of approx. 1600 mg/L.

Compared with reject water case, although the digestate had a higher potential for electricity production than reject water due to higher SCOD concentration, no noticeable difference in electricity production was observed, even lower electricity production in digestate case was detected. Considering the higher recovery efficiency of ammonia-N in digestate case (about 41%) than that in reject water case (about 33%), a
relatively weak influence of electrochemical process was proved. The lower voltage and electricity voltage in digestate case was attributed to the relatively high SCOD content (> 2200 mg/L) and high pH (> 8.8) in anodic chamber, which might inhibit exoelectrogenic microorganisms. According to previous studies, exoelectrogenic microorganisms in microbial electrochemical system are usually favored at SCOD concentration and pH in the range of 200 – 1500 mg/L and 6.5 – 8.5, respectively (Zhang & Angelidaki, 2015a; Zhang & Angelidaki, 2015b).

In general, the current density in reject water case (Figure 1) and digestate case (Figure 2) was in the range of 60 – 70 mA/m², which was lower than that in previous studies, presenting the current density of 88 – 200 mA/m² in two-chamber MFCs (Min & Logan, 2004; Zhao et al., 2018; Schaetzle et al., 2008). The reasons might be due to 1) the high external resistance could perform the inhibition on the current density (Katuri et al., 2011); and 2) substrate fed to MFC is a crucial parameter to significantly affect microbial activity and power generation, thus, the complex components in the real waste streams could be considered as an influencing factor (Gul et al., 2021).
Focused on the contribution of NH$_4^+$ migration and NH$_3$ diffusion in MFC recovery system, the ammonia-N concentration from open circuit experiment and the results from MFCs were compared, obtaining the similar tendency in two cases. In the open circuit experiments, the final ammonia-N concentration in cathodic chamber was about 290 and 630 mg/L in reject water case and digestate case, respectively. Thus, the contribution of diffusion in MFC recovery system was about 81% and 86%, respectively. Based on above discussion, electrochemical process has no significant effect on the transport of ammonia-N from anodic chamber to cathodic chamber with low current density (< 70 mA/m$^2$), but that it is mainly diffusion.

3.2 Two stage ammonia-N recovery system

In the one-stage tests the ammonia-N recovery efficiency was lower than 42%. In order to improve the recovery efficiency, a two-stage process was examined as alternative method. The cut-off point was chosen when the fluctuation of ammonia-N concentration was lower than 10% of the total ammonia-N concentration after operating for 24 h, combining with the ammonia-N concentration of reject water (about 1000 mg/L) and digestate (about 1800 mg/L), the recovery rate of ammonia-N in cathodic chamber needed to be lower than about 4 mg/L/h and 8 mg/L/h, respectively. Ammonia-N recovery rate in MFC from reject water and digestate was shown as Figure 3. 48 h and 40 h were chosen as the cut-off point of two stages in MFC fed with reject water and digestate, respectively. At the cut-off point, the liquid in cathodic chamber (a mixture 40 mmol KH$_2$PO$_4$ and 40 mmol Na$_2$HPO$_4$) was refilled and the experiment
continued for extracting an additional amount of ammonia.

In general, compared with one-stage recovery, similar pH alternations were recorded. Also, no obvious differences were observed in voltage output (Figure 4) as well as electricity production in two-stage operation (Table 2). Ammonia-N balance was higher than 93%, indicating the feasibility and stability of two-stage ammonia-N recovery. Two-stage operation achieved high ammonia-N recovery efficiencies in cathodic chamber (Figure 4). MFC fed with reject water and digestate, total ammonia-N efficiencies in cathodic chamber were 60.5% and 53.6%, respectively, corresponding to about 70% and 25% higher than that in one-stage operation. Likewise, as shown in Table 2, the ammonia-N recovery rates in two-stage recovery process (8.5 mg/L/h in reject water case and 15.9 mg/L/h in digestate case) were higher compared to one-stage process. At the beginning of Stage II, a rapid enhancement on voltage was observed, and then decreased after about 10 hours. After refilled liquid (mixture of KH$_2$PO$_4$ and Na$_2$HPO$_4$ solution) in cathodic chamber, the temporary increase of voltage was mainly achieved by (i) degradation of organics (about 200 mg/L decrease of SCOD concentration), and (ii) electricity generation from salinity gradient energy due to the refilling of liquid in cathodic chamber. Salinity gradient energy has been used for powering microbial electrosynthesis systems (Li et al., 2018). Conclusively two-stage process was leading to higher ammonia-N recovery efficiency, without any significant positive effect on electricity energy production.
Figure 4. Voltage, ammonia-N concentration, and SCOD of (a) reject water and (b) digestate in two-stage recovery operation

3.3 Microbial protein production

Different nitrogen sources were used to determine the effect of extracted ammonia-N on MP process at 25 °C and 35 °C. Synthetic gas mixture was injected into the two reactors to provide the methane and oxygen for cultivation of MP. The gas consumption and biomass production, monitored as OD\textsubscript{600} and CDW during batch fermentations are presented in Figure 5. In general, no significant differences were observed after changing nitrogen source from NH\textsubscript{4}Cl (Period I) to extracted ammonia-N (Period II: reject water, Period III: digestate) in both reactors. This is consistent with previous study showing that ammonia-N recovered from different waste streams could be used as nitrogen source for cultivation of MOB (Khoshnevisan et al., 2020).

As shown in Figure 5(a), about 3000 mL and 2200 mL CH\textsubscript{4} were consumed in the reactors at 25 °C and 35 °C, respectively. Meanwhile, about 4400 mL and 3400 mL consumption of O\textsubscript{2} was detected, which is close to the theoretical based on stoichiometry according to Equation (6). The lower CO\textsubscript{2} production compared to the theoretically expected (1000 – 1400 mL) might be due to: (1) about 200 mL CO\textsubscript{2} was ionized in the system for pH variation (from 6.8 to 5.6); (2) partial CO\textsubscript{2} was dissolved according to Henry’s law, as free CO\textsubscript{2} molecules in the liquid phase; (3) CO\textsubscript{2} could be assimilated by MOB (such as \textit{Gammaproteobacteria}) for biomass accumulation (Strong et al., 2015) (e.g. \textit{Methylomonas} strains can fixate CO\textsubscript{2} for microbial biomass growth).
In addition, higher biomass was generated at 25 °C compared to that at 35 °C (Figure 5(b)-(c)). More specifically, OD\(_{600}\) was about 0.8 and 0.7 at 25 °C and 35 °C respectively, corresponding to microbial production of 0.98 and 0.61 g CDW/L.

\[
\begin{align*}
\text{CH}_4 + 0.104 \text{NH}_3 + 1.45 \text{O}_2 & \rightarrow 0.52 \text{CH}_{1.8}\text{O}_{0.5}\text{N}_{0.2} + 0.48 \text{CO}_2 + 1.69 \text{H}_2\text{O} \\
\end{align*}
\]

Figure 5. (a) Gas consumption and production, (b) OD\(_{600}\) and (c) CDW of different reactors in each period

As shown in Table 3, comparing the performance in two reactors, CDW yield on carbon (Y\(_{\text{CDW/C}}\)) was about 1.2 g CDW/g C at 25 °C, slightly higher than that at 35 °C (about 1.1 g CDW/g C). The biomass yield on carbon was in the range of 0.11 – 1.34 g CDW/g C which was in agreement with previously reported (Henard et al., 2018).

Moreover, the biomass yield on nitrogen was over 22 g CDW/g N in both reactors, which was higher than previous studies (lower than 15 g CDW/g N) (Tsapekos et al., 2019; Valverde-Pérez et al., 2020). This indicated that, except for MP, CDW produced in this study also consists of other organics with high C/N ratio (based on element composition).

<table>
<thead>
<tr>
<th>Table 3. The CDW yield on carbon and nitrogen in each period at 25 °C and 35 °C</th>
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3.4 **Amino acid profile analysis**
Since the MP production performance in all experiments (Period I – III) were similar, only samples from Period II, were collected for amino acid profile analysis. In experiments both at 25 °C and 35 °C, the MP content was approx. 420 mg/g CDW. Likewise, similar amino acids composition was observed in both reactors. Generally, asparagine, glutamine, glycine and alanine were the dominant amino acid, in samples from two reactors, obtaining the relative abundance of about 14%, 18%, 10% and 10%, respectively. Considering the CDW content, reactors operated under 25 °C could produce 421 mg/L MP, which was higher than that under 35 °C (272 mg/L).

As shown in Figure 6(a), 9 types of essential amino acid (EAA) were measured, with total EAA production of 166 and 83 mg/L in reactors at 25 °C and 35 °C. The composition of EAA is in agreement with previous investigations (Tsapekos et al., 2019; Valverde-Pérez et al., 2020). MP yield on nitrogen could reach 11 – 19 g/g N, similar to previous studies, which were in the range of 0.23 – 17.11 g/g N (Khoshnevisan et al., 2020; Valverde-Pérez et al., 2020). These results indicated that, although the cultivation environment of MOB was not the same, stable quality of MP could be obtained, indicating an application potential on commercial scale. Ammonia-N extracted from waste streams through bio-electrochemical systems is considered as a safe for MP production, because any pathogens and micropollutants or other unwanted compounds which might be present in the ammonia containing wastewaters, would be retained in the anode, since they cannot penetrate the CEM membrane. Furthermore, the high pH in cathode would not permit growth of pathogens that might contaminate the cathode (Khoshnevisan et al., 2020). Besides the safety issues, a good protein source should
have a EAA profile supporting food/feed requirements. Comparing with EAA profiles of meat of fish (do Nascimento et al., 2020), the MOB in this investigation presented similar EAA composition, especially in reactor at 25 °C (Figure 6(b)).

In summary, MP production at 25 °C exhibited better performance both in terms of quantity and quality than that at 35 °C. MOB could use extracted nitrogen from waste streams for their growth. Therefore, MP produced by MOB could present an attractive and safe solution for production of proteinous feed and thereby recycling nutrients from wastes back to agriculture.

The products of the integrated “MFC-MOB” system are electricity generated from MFC and MP produced by MOB. The value of electricity energy in Denmark was 0.297 €/kWh (Eurostat, 2013), and the value of crude protein for fertilization and livestock feeds was 0.454 €/kg in international market (National Bureau of Statistics, 2021).

Based on the experimental results, total values produced from 1 m³ waste streams were summarized in Table 4. Total value generation was calculated based on Equation (7).

\[
TVP (\text{€/m}^3) = EPEG (\text{kWh/m}^3) \times 0.297 (\text{€/kWh}) + MPP (\text{kg/m}^3) \times 0.454 (\text{€/kg})
\]  

Where TVP means “Total value of products”, EPEG represents “Energy produced by electricity generation”, and MPP stands for “MP production”.

Based on the above, total value of products was in the range of 1.63 – 6.54 €/m³.
waste stream. Accompanied by the high MP yield on nitrogen under 25 °C, total value of products was about 35% higher than that under 35 °C. Thus, resourceful utilization and high-value utilization of residues can be realized under ambient temperature and pressure by “MFC-MOB” system. During the feed production process, costs consist of electricity cost, annual capital costs of biotechnology process, and remaining cost components (such as labour and chemicals with nutrient inputs), giving a rough idea of the total cost of 0.40 – 0.42 €/kg feed (Solberg et al., 2021). In this “MFC-MOB” system, the cost of feed production was reduced by around 40%.

Although high total value production and low costs were obtained, further considerations on scale-up, energy consumption on ammonia-N transportation and instrument operation, and acclimation of MOB are required to determine a more accurate evaluation for practical applications.

Table 4. Economic evaluation on the products from waste streams by “MFC-MOB” system

Conclusions

Nitrogen extracted from residual resources via MFC was used for MP production. Through one-stage recovery process, 32 – 42% ammonia-N was recovered in MFC. The performance of ammonia recovery by MFC was mainly depending on initial ammonia content in the wastes, while pH and voltage performed no significant impact on recovery efficiency. 53 – 61% ammonia-N extraction was obtained by two-stage
recovery process. MP was grown in the medium with recovered ammonia-N. Amino
acid concentration was 272 – 421 mg/L. MOB had similar EAA content as fish,
indicating the potential on providing safe livestock nutrition or even safe protein food
by “MFC-MOB” system.

Supplementary material

E-supplementary data for this work can be found in e-version of this paper online.

Acknowledgements

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Environmental Science & Technology, 49(7), 4001-4018.


Table Legends

Table 1. Characteristics of reject water and digestate

Table 2. Electric energy production, ammonia-N recovery efficiency and organic particle solubilization rate of different waste streams

Table 3. The CDW yield on carbon and nitrogen in each period at 25 °C and 35 °C

Table 4. Economic evaluation on the products from waste streams by “MFC-MOB” system
Figure Legends

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Figure 2. Voltage, ammonia-N concentration, and SCOD in MFC from digestate

Figure 3. (a) Average recovery rate of ammonia-N from reject water (0–30 h) and digestate (0–30 h); ammonia-N recovery rate in MFC from (b) reject water and (c) digestate

Figure 4. Voltage, ammonia-N concentration, and SCOD of (a) reject water and (b) digestate in two-stage recovery operation

Figure 5. (a) Gas consumption and production, (b) OD_{600} and (c) CDW of different reactors in each period

Figure 6. (a) MP contents, and (b) EAA profile produced by MOB in different reactors (unit: %)
<table>
<thead>
<tr>
<th></th>
<th>Reject water</th>
<th>Digestate</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>8.20 ± 0.10</td>
<td>8.90 ± 0.10</td>
</tr>
<tr>
<td>SCOD (mg/L)</td>
<td>1253 ± 65</td>
<td>2269 ± 167</td>
</tr>
<tr>
<td>TCOD (mg/L)</td>
<td>2819 ± 185</td>
<td>4209 ± 218</td>
</tr>
<tr>
<td>Ammonia-N concentration (mg/L)</td>
<td>1028 ± 13</td>
<td>1742 ± 68</td>
</tr>
<tr>
<td>TKN (mg/L)</td>
<td>1141 ± 10</td>
<td>2052 ± 71</td>
</tr>
</tbody>
</table>
Table 2. Electric energy production, ammonia-N recovery efficiency and organic particle solubilization rate of different waste streams

<table>
<thead>
<tr>
<th></th>
<th>Batch 1</th>
<th>Batch 2</th>
<th>Batch 3</th>
<th>Two-stage recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Reject water</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Electric energy production (J)</td>
<td>14.4</td>
<td>27.1</td>
<td>38.5</td>
<td>38.5</td>
</tr>
<tr>
<td>Initial ammonia-N concentration in anode (mg/L)</td>
<td>1035 ± 83</td>
<td>1035 ± 20</td>
<td>1012 ± 46</td>
<td>1012 ± 90</td>
</tr>
<tr>
<td>Ammonia-N concentration in cathode (mg/L)</td>
<td>371 ± 25</td>
<td>335.8 ± 15</td>
<td>364 ± 27</td>
<td>612 ± 21</td>
</tr>
<tr>
<td>Ammonia-N recovery efficiency in cathode* (%)</td>
<td>35.8 ± 2.3</td>
<td>32.4 ± 1.3</td>
<td>36.0 ± 2.5</td>
<td>60.5 ± 2.0</td>
</tr>
<tr>
<td>Average recovery rate of ammonia-N (r; mg/L/h)</td>
<td>5.2</td>
<td>4.7</td>
<td>5.1</td>
<td>8.5</td>
</tr>
<tr>
<td>TCOD&lt;sub&gt;in&lt;/sub&gt; concentration in anode (mg/L)</td>
<td>2742 ± 100</td>
<td>2812 ± 120</td>
<td>2875 ± 110</td>
<td>2912 ± 160</td>
</tr>
<tr>
<td>TCOD&lt;sub&gt;out&lt;/sub&gt; concentration in anode (mg/L)</td>
<td>2103 ± 95</td>
<td>2037 ± 30</td>
<td>2087 ± 60</td>
<td>1857 ± 80</td>
</tr>
<tr>
<td>Particle degradation rate in anode** (%)</td>
<td>12.4 ± 3.4</td>
<td>14.2 ± 1.1</td>
<td>15.3 ± 2.1</td>
<td>18.5 ± 2.6</td>
</tr>
<tr>
<td><strong>Digestate</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Electric energy production (J)</td>
<td>15.5</td>
<td>23.8</td>
<td>27.7</td>
<td>23.4</td>
</tr>
<tr>
<td>Initial ammonia-N concentration in anode (mg/L)</td>
<td>1674 ± 121</td>
<td>1783 ± 104</td>
<td>1761 ± 112</td>
<td>1783 ± 102</td>
</tr>
<tr>
<td>Ammonia-N concentration in cathode (mg/L)</td>
<td>686 ± 40</td>
<td>716 ± 40</td>
<td>730 ± 33</td>
<td>956 ± 43</td>
</tr>
<tr>
<td>Ammonia-N recovery efficiency in cathode* (%)</td>
<td>41.0 ± 2.4</td>
<td>40.2 ± 2.2</td>
<td>41.4 ± 1.9</td>
<td>53.6 ± 2.4</td>
</tr>
<tr>
<td>Average recovery rate of ammonia-N (r; mg/L/h)</td>
<td>11.4</td>
<td>11.9</td>
<td>12.2</td>
<td>15.9</td>
</tr>
<tr>
<td>TCOD&lt;sub&gt;in&lt;/sub&gt; concentration in anode (mg/L)</td>
<td>4186 ± 272</td>
<td>4340 ± 301</td>
<td>4021 ± 285</td>
<td>4141 ± 191</td>
</tr>
<tr>
<td>TCOD&lt;sub&gt;out&lt;/sub&gt; concentration in anode (mg/L)</td>
<td>3231 ± 183</td>
<td>3272 ± 152</td>
<td>3084 ± 176</td>
<td>2850 ± 132</td>
</tr>
<tr>
<td>Particle degradation rate in anode** (%)</td>
<td>16.9 ± 4.3</td>
<td>18.8 ± 3.5</td>
<td>23.5 ± 4.4</td>
<td>30.5 ± 3.2</td>
</tr>
</tbody>
</table>

*Ammonia-N recovery efficiency = Ammonia-N concentration in anode / Initial ammonia-N concentration × 100%;

**Particle degradation rate = TCOD<sub>in</sub> concentration - SCOD<sub>in</sub> concentration / TCOD<sub>in</sub> concentration × 100%.
Table 3. The CDW yield on carbon and nitrogen in each period at 25 °C and 35 °C

<table>
<thead>
<tr>
<th></th>
<th>25 °C</th>
<th>35 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Y\text{CDW/C}</td>
<td>Y\text{CDW/N}</td>
</tr>
<tr>
<td>Period I</td>
<td>1.21 ± 0.07</td>
<td>34.20 ± 2.54</td>
</tr>
<tr>
<td>Period II</td>
<td>1.23 ± 0.12</td>
<td>36.46 ± 2.05</td>
</tr>
<tr>
<td>Period III</td>
<td>1.24 ± 0.08</td>
<td>34.48 ± 1.48</td>
</tr>
</tbody>
</table>
Table 4. Economic evaluation on the products from waste streams by “MFC-MOB” system

<table>
<thead>
<tr>
<th></th>
<th>Reject water</th>
<th>Digestate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>One-stage recovery</td>
<td>Two-stage recovery</td>
</tr>
<tr>
<td>Energy produced by electricity generation**</td>
<td>0.043</td>
<td>0.031</td>
</tr>
<tr>
<td>MP production*** (kg/m³)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>35 °C</td>
<td>3.55</td>
<td>5.97</td>
</tr>
<tr>
<td>Total value of products (€/m³)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25 °C</td>
<td>2.50</td>
<td>4.20</td>
</tr>
<tr>
<td>35 °C</td>
<td>1.63</td>
<td>2.72</td>
</tr>
</tbody>
</table>

*Data in third batch (Section 3.1) was used for calculating the related parameters of one-stage recovery

** According to Equation (2), energy produced from 250 mL waste stream was calculated. Based on 1 kWh = 3.6×10⁶ J, the energy produced from 1 m³ waste stream was converted into the unit of “kWh/m³”.

*** MP production (kg/m³) = MP contents (mg/g CDW) × Y_{CDW/N} (g CDW/g N) × nitrogen recovery from MFC (kg N/m³)/10³
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Notes: (i) EEA profile in (b) was calculated by $\frac{\text{Amino acid yield (mg/L)}}{\text{Total EEA yield (mg/g N)}} \times 100\%$; (ii) EEA of fish was summarized from the previous publication (do Nascimento et al., 2020).