



Integrated electrochemical-biological process as an alternative mean for ammonia monitoring during anaerobic digestion of organic wastes

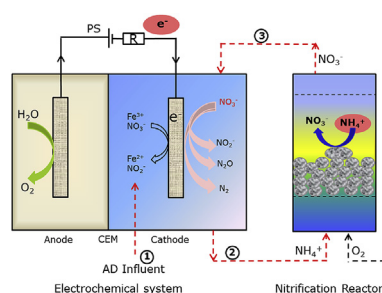
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HIGHLIGHTS

- Integrated electrochemical-biological system was developed as ammonia sensor.
- Linear correlation was established between current and ammonia levels of digestate.
- Digestate pH and external voltage affected monitoring performance.
- Biosensor could exclude the interference of other electron acceptors beforehand.
- There was no significant difference compared to conventional methods in accuracy.

GRAPHICAL ABSTRACT



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ABSTRACT

Ammonia monitoring is important to control anaerobic digestion (AD) process due to inhibition effect. Here, an electrolysis cell (EC) was integrated with a complete nitrification reactor as an alternative approach for online monitoring of ammonia during AD processes. The AD effluent was pumped into nitrification reactor to convert ammonia to nitrate, followed by the introduction of nitrate-rich effluent to EC cathode. It was first evaluated with synthetic ammonia-rich digesters and was observed that the current at 5 min were linearly corresponding to the ammonia levels (from 0 to 7.5 mM $\text{NH}_4^+\text{-N}$, $R^2 = 0.9673$). The linear relationship was always observed regardless of different wastewater pH and external voltage. Pre-removal of other electron acceptors from digestate at cathode could eliminate their disturbances to sensor performance. Finally, the accuracy of biosensor was verified with real digestate test. The simple and reliable biosensor showed great promising for online ammonia monitoring of AD processes.

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1. Introduction

Anaerobic digestion (AD), as a sustainable technology to

produce biogas from various wastes such as manure, solid waste and sludge, is becoming more and more attractive across the world (Chatterjee and Mazumder, 2016; Choong et al., 2016; Rayner et al., 2017; Senghor et al., 2017). However, ammonia coming from degradation of protein in AD substrate could be an inhibitor for AD process (Angelidaki and Ahiring, 1994; Hansen et al., 1998; Rajagopal et al., 2013). As reported before, low free ammonia

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concentrations (e.g. 100 mg-N L⁻¹) could inhibit an unadapted AD process (Hansen et al., 1998). Therefore, ammonia monitoring is significantly important to maintain stability of AD. Currently, the common methods to test the ammonia in aquaculture include colorimeter procedure (Nessler, phenate, and salicylate methods), ion-selective electrode for NH₄⁺, and test kits relying on the Nessler or salicylate methods (Krug et al., 1979; Le and Boyd, 2012; Zhou and Boyd, 2016). However, there are still some drawbacks among these methods. Firstly, the accuracy of colorimeter methods is easily disturbed by the sample color or turbidity. Secondly, the issue of disposal of hazardous waste, which increases the complexity and operation cost, becomes a severe concern of Nessler method. Thirdly, most of the colorimetric methods need extra sample preparations (e.g., membrane filtration) which are time-consuming and costly. Lastly, the ion-selective electrode is strongly affected by the sodium and potassium although with high precision and wide detection range. New conductive materials have also been developed to monitor dissolved ammonia in water, but the synthesis of materials and sensor fabrication are still complicated and the costs are relatively high (Tao et al., 2006; Huang et al., 2016). Thus, development of an alternative environmental-friendly, simple, rapid, online and reliable ammonia sensor is in urgent need for environmental monitoring and water quality control.

On the other hand, electrochemical system has been widely applied into ammonia sensor field because of their high selectivity and sensitivity (Ribeiro et al., 2012; Herzog, 2015; Zhybak et al., 2016; Ning et al., 2017). The electrochemical ammonia sensors, which are based on the ammonia ion transfer reactions across different electrolyte interfaces, mainly include potentiometric and amperometric methods (Bertocchi et al., 1996; Abass et al., 1998; Zolotov et al., 2014; Herzog, 2015). Luo and Do (2006) reported two kinds of polyaniline-poly composite films which exhibited a high sensitivity of NH₄⁺-N up to 100 mM. Ribeiro et al. (2012) also developed an amperometric sensor to detect ammonia concentrations in the range from 4.2 to 66 μM. Most of the electrochemical ammonias sensors were focused on the development of new nanomaterials or fabricating electrodes. Although with high sensitivity and selectivity, these electrochemical sensors still require tedious and complex fabrication process and high consumption of nanomaterials.

Recently, it has been demonstrated that nitrate could be successfully removed at cathode of an electrolysis cell (EC) reactor as an alternative electron acceptor (Abdallah et al., 2014; Radjenovic and Sedlak, 2015; Rajmohan et al., 2016). In biological pathway, ammonia could be aerobically oxidized to nitrate through nitrification process (Zhang et al., 2009; Samarasinghe et al., 2016; Wang et al., 2016). Therefore, coupling nitrification stage with electrochemical nitrate reduction could be a possible way to monitor ammonium during biogas production. Compared to the conventional methods and current electrochemical ammonia sensors, it may offer a fast, environmentally friendly and simple potential approach for monitoring ammonia during AD. To the best of our knowledge, such integrated system has never been explored before.

The aim of present study is to develop an alternative and potentially sustainable approach for ammonia monitoring during AD process. To achieve this goal, an integrated EC-nitrification system was constructed. In this sensor, ammonia in the AD digester was firstly oxidized to nitrate in nitrification reactor. The effluent enriched with nitrate was introduced into cathode of EC reactor for further reduction. Firstly, the sensor performance was explored with synthetic wastewater, in terms of nitrification efficiency, ammonia detection range, response time and operational stability. The effects of external power supply and pH on sensor performance were also investigated. Besides, considering other possible electron acceptors in AD digester, the digester was

pumped into cathode of EC first to eliminate effects. Finally, the new sensor was tested with real AD digesters to verify its reliability. The application of this new sensor may have the potential to provide an online and reliable approach to monitor ammonia, which could be helpful to maintain AD stability.

2. Material and methods

2.1. Reactor construction and operation

An EC reactor, made of nonconductive polycarbonate plates, was a rectangular reactor composed of anode chamber (50 mL) and cathode chamber (100 mL) (Fig. 1). The electrode materials were a titanium woven wire mesh (4 × 5 cm, 0.15 mm aperture, William Gregor Limited, London, UK) coated with 0.5 mg cm⁻² Pt for cathode, and a Titanium mesh electrode coated with Ir MMO (dimensions: 4 × 5 cm; 1 mm thickness; specific surface area 1.0 m² m⁻², Magneto Special Anodes, BA Schiedam, Netherlands) for anode. The anode chamber was filled with 50 mM PBS (pH-7) and cathode chamber was filled with synthetic wastewater. The synthetic wastewater was prepared with ammonia chloride being dissolved into tap water at different ammonia levels. Two chambers were separated by a cation exchange membrane (CEM, CMI 7000, Membrane international, NJ, USA). The rubber gaskets and screws were used to avoid leakage during the assembling. The membrane was put in 50 mM NaCl solution for 24 h, and then soaked in distilled water until use. A power supply (NEWARE Battery testing system 7.5. X, Shenzhen, China) was used to provide the external voltage to the reactor. During the study of influence of other electron acceptors on sensor performance, an anion membrane (AEM) was used to avoid NH₄⁺ migration.

A lab-scale nitrification reactor was set up to complete the oxidation from ammonia to nitrate. A glass bottle (500 mL) was used for the reactor, and the effluent of a membrane bio-reactor (MBR) was used as the inoculum to set up this nitrification stage. The characteristics of inoculum were 2.5 g L⁻¹ (total solid content) and pH 7.04. During the enrichment of nitrification bacteria, the medium, which contained 2.45 g L⁻¹ NaH₂PO₄, 4.58 g L⁻¹ Na₂HPO₄, 0.1 g L⁻¹ KCl, 0.1 g L⁻¹ MgCl₂ · 6H₂O, 0.1 g L⁻¹ CaCl₂ · 2H₂O, 12.5 mL L⁻¹ mineral solution and vitamin solution as describe before, was used. NaHCO₃ (2 g L⁻¹) was used as the carbon source and electron donor. The aeration was obtained directly from the air as it was open to air and the solution was fully mixed by magnetic stirrer.

2.2. Experimental design

In the investigation of linear relationship between current and ammonia concentration, the synthetic wastewater with different ammonia levels (0–7.5 mM NH₄⁺-N) was pumped into nitrification reactor first and then the effluent was purged with nitrogen and introduced into cathode chamber of EC. The current in circuit was recorded by NEWARE Battery testing system 7.5. X (NEWARE, Shenzhen, China). In one set of tests, the external voltage was set at 1.5 V, 1.8 V, 2 V and 2.3 V to elucidate the effect of external power on sensor performance. Then the effect of nitrification effluent pH on sensor performance was studied at four different pH levels (pH 4, 6, 7 and 8). Subsequently, the pretreatment of sensor was evaluated by adding 21.4 μM Fe³⁺ and 714.3 μM NO₃⁻ to the synthetic wastewater, respectively. To verify the accuracy of sensor, it was tested with two real AD effluents. One was collected from a lab-scale AD reactor for biogas upgrading which was operated at 37 °C (noted as AD effluent 1). The other one was taken from a thermophilic lab-scale AD reactor fed with manure (55 °C) (noted as AD effluent 2). All experiments were carried out in duplicate at room temperature.

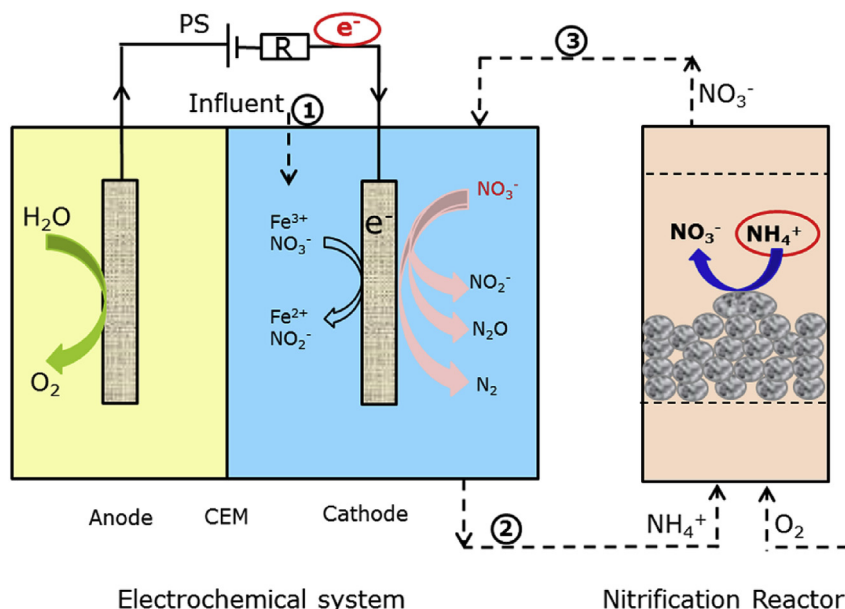


Fig. 1. Schematic diagram of the integrated EC-nitrification system. CEM: the cation exchange membrane.

2.3. Analytic methods

The pH and conductivity of tested solution were measured by a PHM 210 pH meter (Radiometer) and a CDM 83 conductivity meter (Radiometer), respectively. The concentrations of ammonia, nitrate and Fe^{3+} were determined by colorimetric test kits (Spectroquant 00683, 09713, Merck, Germany). The current in EC circuit was recorded by NEWARE Battery testing system 7.5. X every 1 min. In the statistical analysis, the student's t-test was applied and P-values < 0.05 were considered to have significance effect on the response, while values > 0.1 indicate the variables are not significant.

3. Results and discussion

3.1. Nitrification process for ammonia-to-nitrate conversion

The performance of the lab-scale nitrification reactor for ammonia-to-nitrate conversion is shown in Fig. 2. The nitrification process was completed within 4 h. When the ammonia level was changed from 0 to 7.1 mM $\text{NH}_4^+\text{-N}$, the corresponding nitrate concentrations after nitrification varied from 0 to 6.9 mM $\text{NO}_3^-\text{-N}$, showing a stable conversion rate. The line slope is 0.9534, illustrating the highly efficient conversion of ammonia to nitrate. The high conversion efficiency (over 90%) was in agreement with the value reported previously (Tarre and Green, 2004). However, compared to the maximum nitrification rate (up to 2000 g $\text{NH}_4^+\text{-N m}^{-3} \text{ d}^{-1}$), the nitrification rate obtained here was a little lower (581.4 g $\text{NH}_4^+\text{-N m}^{-3} \text{ d}^{-1}$), which could be due to the different operational parameters such as the different attachment forms of nitrifying bacteria, pH and dissolve oxygen concentrations (Zhang et al., 2009). It should be noted that this lab-scale nitrification reactor was just used here to prove the whole biosensor concept. The influent pH has a significant impact on the performance of a membrane-aerated bioreactor with respect to nitrification rate (Shanahan and Semmens, 2015). In addition, biofilm performs better than suspended-biomass in terms of nitrification rate (Tarre and Green, 2004). Considering aerated nitrification process has already been developed commercially with a high rate (up to 2000

g $\text{NH}_4^+\text{-N m}^{-3} \text{ d}^{-1}$) (Albuquerque et al., 2012; Nourmohammadi et al., 2013), the nitrification process could be achieved by a commercialized nitrification reactor for the future industrial application. Therefore, the high and stable nitrification efficiency provided the insurance of the following investigation of relationship between current and ammonia concentration.

3.2. Nitrate reduction in EC: linking ammonia concentration to current

The effluent containing nitrate after nitrification process was firstly flushed with nitrogen and then introduced into cathode chamber of EC. Fig. 3 (A) illustrates the variation of EC current versus nitrate concentration. A fast response of current of EC to a stepwise increase of nitrate from 0 to 7.1 mM $\text{NO}_3^-\text{-N}$ was observed within 5 min (data not shown). Based on the correlation between nitrate and ammonia concentrations shown in Fig. 2, the correlation between current and ammonia concentration was established (Fig. 3B). It is obvious that the current linearly increased (5.329 ± 1.157 to 10.139 ± 1.233 mA, $R^2 = 0.9673$) with the increasing of ammonia levels ranged from 0 to 7.5 mM $\text{NH}_4^+\text{-N}$. The slopes in Fig. 3A and B were 0.6686 and 0.6389. The current, as a representative of the numbers of electron flow per second in the circuit, was closely related to external voltage and amount of electron acceptors in cathode. Considering the stable external power of 1.8 V in this case, the different current response was mainly contributed by the different concentrations of electron acceptor (i.e., nitrate) in cathode. In addition, the detection range could reach up to 7.5 mM $\text{NH}_4^+\text{-N}$, which was much higher compared to other electrochemical ammonia sensor (0.3 mM) (Ribeiro et al., 2012). The above results demonstrated a good linear relationship between current generation and initial ammonia concentrations ranging from 0 to 7.5 mM. Contrast to the conventional testing approaches (e.g. testing kits and titration), the new biosensor developed here exhibited merits of rapid and real time monitoring properties. The whole sensor system including the sampling, nitrification and EC detection could be further automatized by integrating different parts together into one unit for online monitoring. Once the AD digestate is treated after nitrification stage, it would be automatically

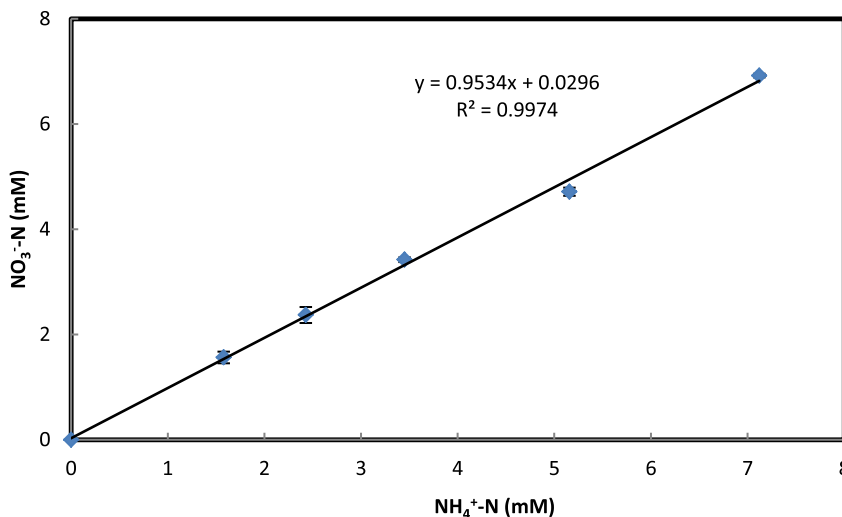


Fig. 2. The correlation between initial ammonia level and accumulated nitrate concentration in nitrification reactor.

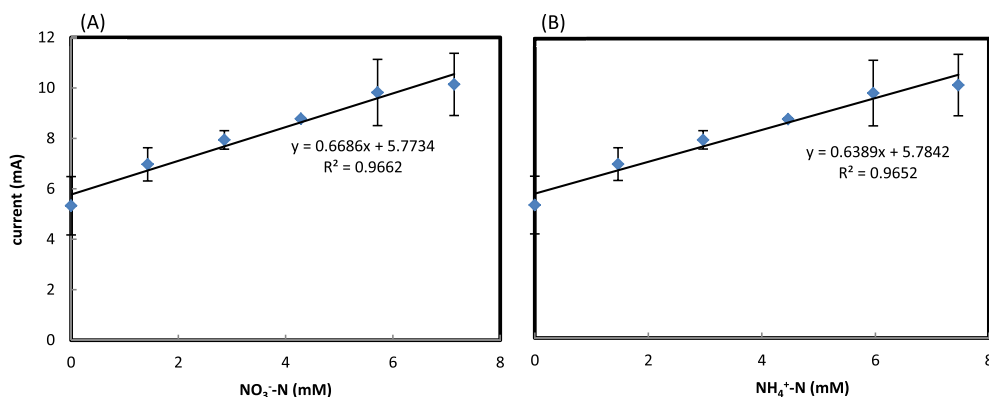


Fig. 3. The linear relationships between current and nitrate levels (A) and between current and ammonia levels (B) at external power supply of 1.8 V and pH 6. (1.4–7 mM NH₄⁺-N).

pumped into EC cathode and the current could response immediately within approx. 5 min. Considering that the nitrification could be achieved as fast as a few minutes, it is expected that the biosensor proposed in this study could be applied as real-time sensor which has response time of several minutes. Compared to other fast electrochemical ammonia sensor, this new biosensor also shows great advantages of relatively low cost and high sensitivity. Overall, it has been demonstrated that the new developed biosensor has a good performance in monitoring ammonia and especially is most suitable for high ammonia concentration detection.

3.3. Sensor performance under different external voltages

Since the current in circuit is highly related to external voltage, a series of tests were conducted at varied external applied voltages (1.5 V–2.3 V). As shown in Fig. 4, the currents always showed positive slopes with respect to ammonia levels regardless of the applied voltages. The slopes at 1.5, 1.8, 2 and 2.3 V were 0.7629, 0.6389, 1.2712 and 1.6488, respectively. The highest slope appeared at the external voltage of 2.3 V, while the slopes for 1.5 V and 1.8 V were relatively lower. The higher slope usually indicated a higher current generation. For example, at 7.5 mM NH₄⁺-N, the currents at 1.5, 1.8, 2 and 2.3 V were 8.6, 10.1, 11.2 and 15.6 mA, respectively. The higher slopes at 2 and 2.3 V were mainly contributed by the higher

external voltage. The lowest currents appeared in 1.5 V could be explained by that the amount of electrons became the limiting factor of nitrate reduction when voltage was too low as 1.5 V. Additionally, the sensor exhibited high linearity between current and ammonia concentration of more than 90% at all voltages, illustrating the good performance under different voltages.

3.4. Sensor performance under different wastewater pH

Considering the effluent (enriched with NO₃⁻-N) after nitrification stage has a wide range of pH (Wolfe and Lieu, 2001; Odell et al., 1996), thus it is of importance to investigate the influence of nitrification effluent pH on EC performance. In order to achieve this goal, different pH of synthetic wastewater were tested. The results are displayed in Fig. 5. It was observed that at all pH conditions, the currents always increased linearly with ammonia levels. The slopes for pH 4, pH 6, pH 7 and pH 8 were 0.6746, 0.6389, 0.9395 and 0.862, respectively. The higher slopes tended to be achieved in neutral and weak alkaline condition. However, it should be noticed that higher currents were observed in weak acid environment (pH 6). For example, when with the initial ammonia concentration was 4.5 mM NH₄⁺-N, the currents at pH 6 and pH 8 were 8.8 and 6.3 mA, respectively. As shown in Fig. 5, the current generation at weak acid condition (pH 6) actually exhibited a higher value compared to the neutral and weak alkaline condition. It has been reported that the

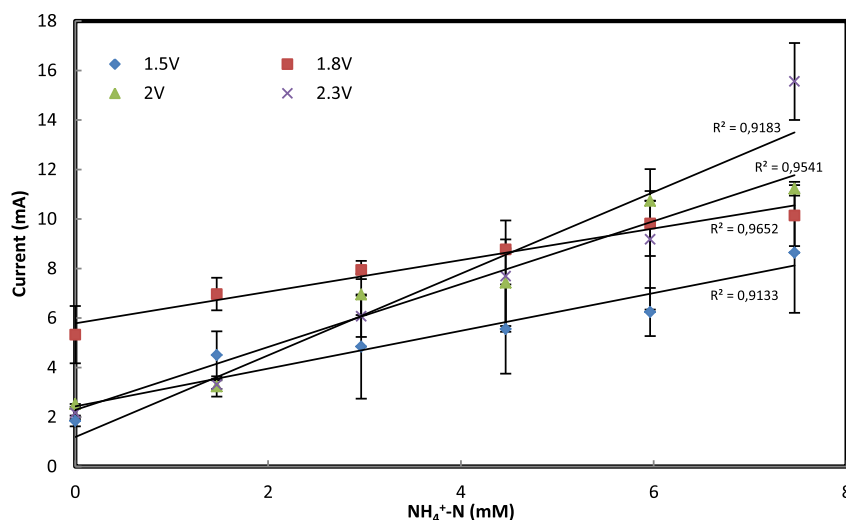


Fig. 4. Effect of external applied voltage on the performance of ammonia sensor. The initial pH of the nitrification effluent is 6 in the cathode.

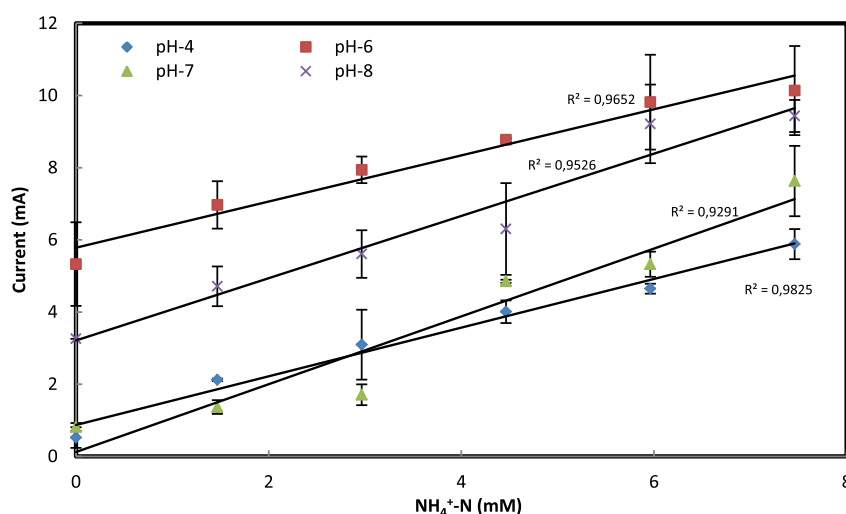


Fig. 5. Effect of pH on the performance of ammonia monitoring at external applied voltage of 1.8 V.

weak acid condition may favour electrochemical nitrate reduction (Abdallah et al., 2014). Moreover, the correlation coefficients for pH 4, pH 6, pH 7 and pH 8 were 0.9825, 0.9652, 0.9291 and 0.9526, respectively. The sensor showed high linearity at all tested pH conditions.

3.5. Sensor performance under the interference from other electron acceptors

In order to avoid the interferences from other possible electron acceptors, the wastewater was firstly introduced to the cathode of EC reactor before nitrification. The synthetic wastewater with 21.4 μM Fe^{3+} was used for the test. Fig. 6 (A) shows the variations of Fe^{3+} concentration over time in the EC during pretreatment. It is clear shown that Fe^{3+} was removed totally within 90 min when external voltage was applied to 4 V. The removal mechanism was mainly electrochemical reduction in cathode. Fig. 6 (B) displays the change of biosensor current over time with and without pre-treatment in the EC reactor. For both conditions, the fast decrease of current within 20 min was observed. The stable current of biosensor with pre-treatment was 0.93 mA, while it was 0.67 mA for the biosensor

without pre-treatment. The student's test was conducted to evaluate the significant difference between two scenarios. At 99% confidence level, there was a significant difference between the current generations in two cases. The results demonstrated that EC pretreatment before nitrification could be an efficiency method to eliminate the disturbance from other electron acceptors in the AD effluent.

Likewise, the nitrate, as another important electron acceptor in water, is also not negligible. To explore if the EC pretreatment also works for the removal of NO_3^- -N before nitrification, the synthetic wastewater enriched with 714.3 μM NO_3^- -N L^{-1} was used for the test. As displayed in Fig. 7 (A), the nitrate showed a gradual decrease within 2 h owing to cathodic electrochemical reduction when 4 V was applied. Fig. 7 (B) shows the current change in the biosensor with and without EC pretreatment. Similarly, the results of student's t-test showed a significant difference between two situations at 99% confidence level, showing the importance of EC pretreatment for the accurate sensing. To conclude, the effects of Fe^{3+} and NO_3^- -N on sensor performance could be eliminated through the pre-treatment using EC, which could improve the stability during ammonia monitoring with complex waste streams.

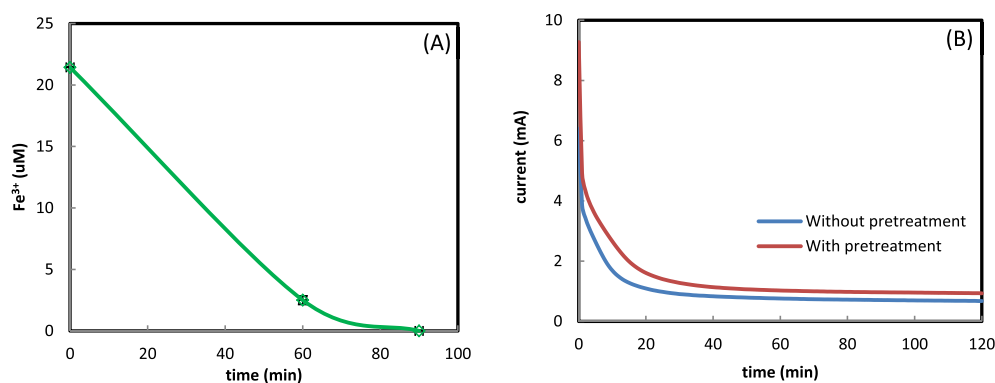


Fig. 6. The change of Fe^{3+} concentration (A) during pretreatment and current generation (B) in EC reactor. The operational parameters: external voltage of pretreatment is 4 V, initial pH is 6 and initial $\text{NH}_4^+\text{-N}$ of 6 mM in synthetic water.

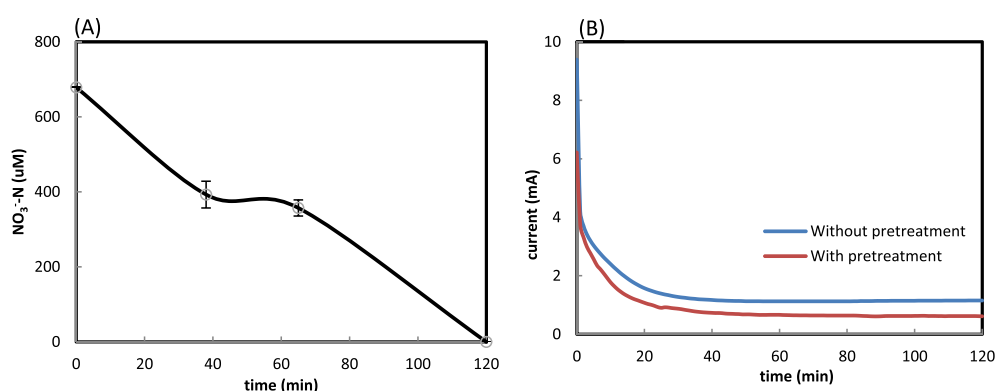


Fig. 7. The change of $\text{NO}_3^- \text{-N}$ concentration (A) during pretreatment and current generation (B) in EC reactor. The operational parameters: external voltage of pretreatment is 4 V, initial pH is 6 and initial $\text{NH}_4^+\text{-N}$ of 6 mM in synthetic water.

But for AD process, electron acceptors such as Fe^{3+} and $\text{NO}_3^- \text{-N}$ may not be existence, since they would be reduced by receiving the electrons from microbial oxidation of organics. Thus, pretreatment may not be necessary for ammonia monitoring of AD digestate. But the results offer insight into the extended application feasibility of the sensor for other water treatment processes.

3.6. Application in real AD effluent

To verify the applicability for real digestate, the biosensor was further tested with two real AD effluents. The samples were stored at 4 °C and fluxed with nitrogen before used. The ammonia levels obtained from fast kits and biosensor along with primary characteristics of two effluents were summarized in Table 1. Anova analysis was performed to investigate the difference between values tested with two methods. The results showed no significant difference between the results obtained from the two methods ($P > .05$) at 95% confidence level, which confirmed a good accuracy of the biosensor. According to the results, the sensor could monitor

the AD samples with a wide range of ammonia levels.

3.7. Significance and outlook

This study for the first time demonstrated the proof of concept of an integrated electrochemical-biological system for online ammonia monitoring during anaerobic digestion of organic wastes. Compared to traditional offline sensing technologies, the novel ammonia biosensor developed here has several advantages. First, the sensor has the potential for in-situ and online monitoring, since ammonia concentration was directly linked to current which is easy to monitor. Second, the addition of solvents is not needed which could reduce the risk of secondary pollution caused by solvents. Compared to commercial ion selective sensor, it even has its own merits. On the one hand, the ion selective sensor is easily affected by other ions while the performance of the new biosensor could withstand the interference of other ions. Furthermore, the potential interfering electron acceptor could be eliminated through the pretreatment. On the other hand, the cost for an ion selective sensor is around 200 Euro, while here the lab-scale EC reactor that can be further optimized only cost around 40–80 Euro.

Though promising, more efforts should be made before practical applications. First of all, packing different parts together into one unit is required for the system simplification. Secondly, the slow nitrification rate in this study limits its future application which could be greatly improved to the commercial rate level by employing mature nitrification systems. For instance, a biological aerated biofilter could be employed for fast nitrification process (Jenssen et al., 2010). Thirdly, in order to reduce the cost of

Table 1
Determination of $\text{NH}_4^+\text{-N}$ in real waste streams by EC-nitrification based biosensor and ammonia testing kit.

sample	$\text{NH}_4^+\text{-N}^a$	$\text{NH}_4^+\text{-N}^b$	pH	Conductivity (us/cm)
AD effluent 1	110.16 ± 0.36	117.7 ± 0.94	8.87 ± 0.10	1690 ± 2
AD effluent 2	65.52 ± 0.15	70.54 ± 1.43	8.20 ± 0.10	1329 ± 5

^a Tested by fast kits.

^b Measured by sensor.

additional energy input, the feasibility of utilizing the energy stored in organic waste instead of power supply, such as bio-electrochemical system could be further considered.

4. Conclusions

The present study for the first time demonstrated the feasibility of integrated EC-nitrification process for ammonia monitoring during AD process. It was observed that the sensor responded immediately within 5 min to a stepwise increasing of ammonia level from 0 to 7.5 mM $\text{NH}_4^+\text{-N}$. Linear relationships between current and ammonia concentration were always observed regardless of voltage and pH. Additionally, EC could pre-remove potential electron acceptors before monitoring. Finally, the sensor showed high accuracy and sensitivity to real AD effluents. Though promising, more efforts are still needed to further improve the sensor by simplifying reactor configuration, shortening aerobic process etc.

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