Nitrogen Removal from Closed Aquaculture System by Bio-electrochemical System

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Abstract

Removal of nitrogen elements in culture water is one of the major concerns in recirculating aquaculture system (RAS). Maintaining a low concentration of nitrogen compounds is essential for a good quality of aquaculture production. Due to fish is very sensitive to the toxic ammonium/ammonia, nitrification biofiltration tank is often an integrate part of filtration in RAS to remove ammonium via nitrification. However, nitrate accumulation via nitrification in RAS is often observed during the operation, which is usually solved by replacing with the fresh water into the system. With the concern of water consumption, bio-electrochemical system (BES) was introduced in this study to realize simultaneous nitrate removal for the system while generating the electricity through electron transferring. A microbial fuel cell (MFC) with an anion exchange membrane (AEM) was constructed. The removal of nitrate from aquaculture water generated from RAS was achieved by nitrate migration across the AEM and heterotrophic denitrification in the anode chamber. To further investigate the potential application of BES in RAS, the cathode chamber was incubated with biofilm to do the nitrification while the denitrification processing in the anode chamber. The study gave a total inorganic nitrogen removal efficiency of 38.72% ± 4.99, and a COD removal of 86.09% ± 9.83. The average daily electricity generation was 67.98 A m⁻³ ± 13.91, and nitrate-nitrogen concentration remained at 21.02 ± 2.62 mg L⁻¹ throughout the experiment. These results of treating aquaculture water indicate that BES has a potential to install within RAS for enhanced nitrogen removal.
Nitrogen Removal from Closed Aquaculture System by Bio-electrochemical System

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General Audience Abstract

The demand of aquaculture products is continuously increasing; however, the wastewater discharges from aquaculture systems also brings the environmental concerns. Recirculating aquaculture system is one of the reliable aquaculture systems applies in fish farming, which is able to treat the culture water within the system loop. The ammonia, which is produced and released continuously from deamination of protein, is the primary concern in aquaculture system due to its toxicity. The ammonia/ammonium and can be removed by the nitrification biofiltration part in recirculating aquaculture system. Nitrification process removes ammonia or ammonium to nitrate, which is less toxic to fish. During the operation, nitrate accumulation via nitrification in recirculating aquaculture system is often observed. High level of nitrate in culture water may leads to fish health issues. To have a good quality of aquaculture production, exchanging with the fresh water into the system regularly is needed for the recirculating aquaculture system.

With the consideration of water consumption, bio-electrochemical system was brought in this study to perform simultaneous nitrogen compounds removal for the recirculating aquaculture system while generating the electricity through electron transferring. Microbial fuel cell, which is a form of bio-electrochemical system, with an anion exchange membrane was designed. The microbial fuel cell was constructed with two chambers, which are anode and cathode. The cathode chamber was incubated with biofilm to do the nitrification, whereas the denitrification was processing in the anode chamber to achieve the nitrate removal. Culture water with a certain amount of ammonia/ammonium that obtained from recirculating aquaculture system first entered the cathode chamber of microbial fuel cell, and oxidized to nitrate via nitrification. The generated nitrate in cathode chamber migrated across anion exchange membrane to the anode chamber, and removed via denitrification process to complete nitrogen compounds removal for the entire system.
The study presented a total inorganic nitrogen removal efficiency of 38.72% ± 4.99, and a chemical oxygen demand removal of 86.09% ± 9.83 from the system. The average daily electricity generation was 67.98 A m⁻³ ± 13.91, and nitrate-nitrogen concentration remained at 21.02 ± 2.62 mg L⁻¹ for the system throughout the experiment period. These results of treating aquaculture water indicate that bio-electrochemical system has a potential to apply within recirculating aquaculture system for enhanced nitrogen removal, while reducing the water consumption and generating the electricity.
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Chapter 1 - Introduction

Due to the rapid increase of aquatic product market, reliable aquaculture water treatment systems are demanding to ensure the healthy fish growth. Among them, recirculating aquaculture system (RAS) is one of the technologies that allows the aquaculture suppliers to address the world needs economically and environmentally friendly (Rijn 1996). Comparing to the other aquaculture systems, such as pond aquaculture system, RAS consumes much less water by treating water within the loop of recirculation. Ammonia, which is generated from deamination of protein in aquaculture and released through gills of the fish, is a major concern in the aquaculture due to its toxicity to fish. It can be effectively removed via biofilter, an integral part of RAS, with nitrification process (nitrifying bacteria oxidizing ammonia to nitrite then nitrate) (Nazar et al. 2015). Compared to ammonia, nitrate is significantly less toxic to fish, and can be removed by daily water replacement or denitrification process.

Bio-electrochemical Systems (BES) are the systems that able to convert chemical energy from organic waste in wastewater into electricity and other value-added compounds through microorganisms oxidizing process (Kelly et al. 2014, Pant et al. 2012). Microbial fuel cell (MFC) is the most basic model among various forms of BES. MFC consists two parts, anode and cathode. Organic compounds in substrates are oxidized by microorganisms in the anode chamber, while generating the electrons to electrode and protons to the solution. The electrons released from anode transport through its external electric circuit to electron acceptors, such as oxygen and nitrate, in cathode chamber thereby generate current. The cations can be driving to the cathode chamber by generated electric field to balance the charge via ion exchange member or through the electrolyte (Liu et al. 2016, Li et al. 2015).

While nitrification is reported as one of the most common treatment approach applied in aquaculture industry, the accumulation of nitrate level in the loop of circulation is observed. Although fish is more tolerated to the nitrate than ammonia, high level of nitrate may also lead to the fish growth issues (Monsees et al. 2016). In addition to the fish health concerns, the effluent with considerable nitrate level discharges from the system should also be well taken care of to meet environmental regulations (Ghafari et al. 2008). The applications of nitrate remediation in BES systems for aquaculture water has many advantages, including removal of organic and
nitrogen compounds with a low energy consumption while generating electricity and other valuable products. Although there are limited publications on applying the BES to treat aquaculture water from RAS, combine a BES with RAS may achieve the removal of nitrate in the RAS.

In this study, the MFC equipped with an anion exchange membrane (AEM) was constructed to explore and enhance the nitrate removal in RAS (Figure 1 and Figure 2). The culture water contained a certain amount of nitrogen compounds was first fed into MFC cathode chamber, and operated in batch mode. The nitrate ions in catholyte were driven by the concentration gradient as well as the generated electric field to the anode chamber through AEM membrane. The nitrate ions in anode chamber were further reduced via heterotrophic denitrification. Meanwhile, the anode chamber was continuously fed with synthetic wastewater to provide carbon source for the denitrification process. In this MFC, oxygen and nitrate were electron acceptors, whereas the organic carbon that oxidized by bacteria acted as an electron donor.

To further examine the BES potential application for replacing the nitrification biofilters in RAS, using MFC to do nitrification and denitrification simultaneously for the system was evaluated. The cathode chamber was later inoculated with the bacteria to treat ammonium via nitrification. Ammonium in cathode was converted to nitrate at first, then nitrate migrate to the anode for the denitrification purpose. Due to culture water usually contains a low COD concentration, the COD removal for aquaculture water was not considered in this study.

The objectives of this study are to 1) investigate if MFC has the potential to apply under RAS for enhancing the nitrate removal, which has the nitrification biofilter in the system; 2) examine whether MFC has the potential to implement under RAS for nitrogen removal without the nitrification biofilter installed in the system; 3) test the removal of organic compounds with synthetic wastewater via denitrification process; 4) analyze energy consumption and generation of the MFC system treating the recirculated aquaculture water. The results will have the potential to shed light on applying MFC in the RAS.
Figure 1. Schematic of an AEM-membrane MFC used in this Study

Figure 2. Schematic of Inserting MFC in Recirculating Aquaculture System
Chapter 2 - Literature Review

2.1 Introduction to Recirculating Aquaculture System Strategy

Aquaculture industry is continuously expanding all over the world. However, due to the high nutrients and solids level in the waste discharges, the aquaculture growth is limited by the environmental challenges (Steicke et al. 2009). With the increasing aquaculture market demand, Recirculating Aquaculture Systems (RAS) is considered as one of the most feasible technologies to supply the world needs for aquatic products in an economical and low environmental impact approach (Ebeling et al. 2012). RAS is a solution to environmental issues by minimizing waste discharge and increasing the recycling and reusing of the resources (Steicke et al. 2009).

RAS are closed-loop facilities applied for aquaculture that recirculate and treat the water within the system. It allows water flow through the treatment processes and return to fish culture tanks (Nazar et al. 2013). Fish feed is the source of carbon and nutrients solids, whereas solid waste is mainly from fish excretions and a small portion of uneaten feed in RAS. Solids and sludge are removed by sedimentation or mechanical filtration (Yoge et al. 2013), while dissolved wastes are removed through biological filtration (Nazar et al. 2013). Those processes help maintain the water quality and conserve the water by recycling 80 to 90% of the culture water and exchanging 10 to 20% of the total system volume with fresh water per day (Steicke et al. 2009, Yoge et al. 2013). RAS use 90 to 99% less water than conventional aquaculture systems (Ebeling et al. 2012), and even achieved <1% water exchange of system’s volume per day in a novel pilot study (Yoge et al. 2013).

Ammonia is the major concern in the aquaculture, which will cause significant fish growth reduction and need to be maintained below 0.05 – 0.20 mg L\(^{-1}\) in the system (Nazar et al. 2013). Ammonia is generated from deamination of protein in aquaculture and released through gills of the fish. Biofilters are an integral part of RAS, which allows nitrifying bacteria to oxidize ammonia to nitrite then nitrate through nitrification process (Nazar et al. 2013). In contrast to open culture systems, nitrate has the potential to accumulate up to 1000 mg L\(^{-1}\) NO\(_3\)\(-\)N in RAS (Rijn 2010). Nitrate is less harmful to fish than ammonia, however the accumulation of nitrate overtime is also the concern in RAS due to the less water exchange. Nitrate is proved to have negative impact on the fish production performance even at a low concentration (Bussel et al.
Different Aquaculture species have various responses to nitrate toxicity. In general, the nitrate nitrogen is maintained between 30 to 100 mg L$^{-1}$ in RAS, and can even exceed 200 mg L$^{-1}$ in commercial RAS (Bhatnagar et al 2013, Sandu et al 2013, Davidson et al 2014). The accumulated nitrate in RAS is often removed from the system by daily water replacement or denitrification process.

![Typical Schematic of Recirculating Aquaculture System - Single Culture Tank](Ebeling et al. 2012)

Figure 3. Typical Schematic of Recirculating Aquaculture System - Single Culture Tank (Ebeling et al. 2012)
2.2 Introduction to Bioelectrochemical Systems (BES) and Microbial Fuel Cells (MFC)

Bioelectrochemical Systems (BES) are the systems that can convert chemical energy from organic waste into electricity and other value-added compounds through microorganisms oxidizing process (Kelly et al. 2014, Pant et al. 2012). Over the past decade, BES are driving attention continuously due to their various applications in the fields of wastewater treatment, sustainable energy generation, and nutrients recovery (Li et al. 2015). BES have different models which include microbial fuel cells (MFCs), microbial electrolysis cells (MECs), and microbial desalination cells (MDCs) based on the different purpose of studies and applications (Kelly et al. 2014, Pant et al. 2012). In BES systems, the current-generating microorganisms play an important role, in which *Shewanella* and *Geobacter* genera are the most incubated and used bacteria in the studies (Yang et al. 2012).

A variety of substrates have been examined such as pure organics, domestic wastewaters, and industrial wastewaters in BES for current generation and nutrients removal. However, the study of wastewater as an electron source is more attractive due to the constrains and sustainable needs on wastewater treatment (Kelly et al. 2014). The electricity generation have been studied based on the size of BES reactor from milli-liter to liter-scale and even to pilot scale. The energy recovery with a high-power density of 1000 Wm$^{-3}$ only generated from small-scale reactors (Kelly et al. 2014). Thus, the scale-up potentials, technical, and economical feasibilities for the system are needed to further investigate in the future study (Li et al. 2015).

MFC is the most basic one among various forms of BES. MFC has two parts, anode and cathode. Organic compounds in substrates are oxidized by microorganisms growing on the anode chamber, which generate the electrons and protons. The electrons from anode then transport through its external electric circuit to electron acceptors, such as oxygen and nitrate, in cathode chamber and produce current. The cations can be driving to the cathode chamber by ion exchange member or through the electrolyte (Liu et al. 2016, Li et al. 2015).
Due to the variety configurations of the reactor, operating conditions, types of electrodes, resistors, surface area and volume involved, as well as different microorganisms applied for diverse study aims, it is difficult to compare the performance of MFC from literatures. However, the electricity generation is a major factor to indicate the MFC performance and cannot be ignored. The common unit to present electricity generation is current density, which is either current generated per unit of anode surface area (mA cm\(^{-2}\)), or current generated per unit of cell volume (mA m\(^{-3}\) or A m\(^{-3}\)). From the reviewed articles, the current density ranged from 0.0004 to 2.05 mA cm\(^{-2}\) was found (Pant et al 2010).

![Figure 4. Typical Schematic of MFC, CEM - cation exchange membrane, AEM - anion exchange membrane (Li et al. 2015).](image_url)
2.3 BES and MFC Applied in Aquaculture Systems

Although treating nitrate through BES has been proved to have many advantages, there are limited publications regarding treating the real aquaculture wastewater. Synthetic aquaculture wastewater has been investigated with BES denitrification process, and several factors are being examined in different studies, such as cathodic material, initial pH, current density, and nitrate loading rate.

Graphite, carbon, platinum, and stainless steel are the common materials to use in BES abiotic electrode. However, platinum and stainless steel are relatively expensive and unsustainable when applied on bacterial solutions. One study identified that the formation of platinum oxide is formed at cathodic electrode surface, which interrupted the electrode activity (Mook et al. 2012). Biological electrodes are attractive due to less expensive and sustainable usability. The microorganisms are employed as inert electrode to accept electrode to take up electrons. Graphite and carbon are the most applied material for bio-anodes and bio-cathodes due to the inexpensive and good for active biofilms growing. However, the two materials have a relatively high electrical resistivity, in which the larger electrode ohmic losses would occur in the system. Thus, the conductive metal is often attached and supported for graphite or carbon electrodes (Mook et al. 2012).

The pH of aquaculture water is the major influencing factor on the denitrification. The optimum pH for denitrification is varied on different operating conditions and cultures used. When pH is higher than 8.6, nitrite accumulation problem is observed. For the pH lower than 7.0, the denitrification rate decreases. Hence, optimum value between 7 to 8 is concluded from several studies (Mook et al. 2012).

The nitrate removal rate is tested to have a direct proportional relationship to the initial nitrate loading rate. However, the nitrate removal efficiency is also depending on hydraulic residence time (HRT), the models of reactor, and the reactor configuration, which brings a removal efficiency between 74% to 99% (Mook et al. 2012).

Low biochemical oxygen demand (BOD) is find in most aquaculture water. Two kinds of bacteria can be applied for biological denitrification. Autotrophic bacteria are used in most of the
BES studies, which consume inorganic carbon sources such as carbon dioxide. For heterotrophic bacteria used in biological denitrification process, the external organic carbon source is needed for electron donor, whereas anaerobic bacteria use nitrate as electron acceptor under anoxic conditions (Visvanathan et al. 2008). For RAS, the organic carbon matter separate from the system are used as electron donor in one study, which gives a TN removal efficiency of 85.5% to 91.4% with a HRT of 3-hour (Visvanathan et al. 2008).

Comparing to the limited study in BES applied in RAS, more studies are found in sediment microbial fuel cells (SMFC) that applied under aquaculture pond sediment and water. The SMFC it a kind of MFC that generate electricity by electro-potential difference between oxic water and anoxic sediments in one water body. The pond sedimentation is embedded anode, where contains degrade organic compounds to generate electrons and protons. The water laying above is installed cathode. Similar to the regular MFC, the electrons are transferred from anode to cathode through its external circuit. Protons flow from sediment to cathode part and combined with oxygen to produce water. The removal of TKN efficiency is ranging from 92.6% to 95.3% (Sajana et al. 2013). However, due to the large internal resistance, the SMFC do not generate significant electrical energy (Xu et al. 2015), which is also a constrain for scaling up and applying SMFC in the real pond culture. Furthermore, the pH of water in pond, the pond configuration especially the depth, and the presence of cellulose in sediment are also needed to investigate in the future study.

![Figure 5. A Configuration of SMFC for Treating Aquaculture Pond Water (Sajana et al 2013)](image-url)
Chapter 3 - Materials and Methods

3.1 MFC Setup

The designed MFC was constructed as a tubular reactor (30 cm long and 5 cm in diameter). The anodic chamber was built by a rolled-up anion exchange membrane (AEM) with a volume of 220 mL. Carbon brush was inserted in the anodic chamber as the electrode. The carbon brush was soaked in acetone solution overnight, and then heated at 450 °C for 30-min before putting into the anode chamber (Wang et al. 2009, Li et al. 2015). The AEM was also acted as a separator between anode and cathode. The carbon cloth was used as cathode electrode, which was coated with carbon powder with loading rate of 0.2 mg of C per cm$^2$. The cathode cloth was wrapped and covered the anode membrane tube. The tube was put in a cylindrical container, which was the cathode component, and left a volume of 0.8 L for the cathode chamber. The air was pumped into the cathode chamber at the bottom of container. One gas bag was installed at the top of anode for collecting the gas that generated from denitrification process. Titanium wire was used to connect anode and cathode to the external resistor.

3.2 Operating Conditions

The anode was inoculated with anaerobic sludge from Christiansburg Wastewater Treatment Plant (Christiansburg, VA, USA), and was operated under room temperature at 20 °C. A synthetic solution was feed into the anode to mimic wastewater, which contains: 1g glucose, NH$_4$Cl 0.15 g; NaCl 0.5 g; MgSO$_4$ 0.015 g; CaCl$_2$ 0.02 g; NaHCO$_3$ 1g, and 1 mL trace elements per liter of tap water. The anolyte was operated as the continuous flow mode with a hydraulic retention time (HRT) of 24-hour or 0.15 mL per minute with a pump. Aquaculture water tested in this study was obtained from recirculating aquaculture systems at the Virginia Tech Department of Food Science and Technology, and was applied under the batch mode in the cathode chamber. In order to have a relatively constant water quality, the culture water sample was collected for the study at one time.

To better mimic the RAS system with a constant nitrate accumulation rate of 50 mg L$^{-1}$ day$^{-1}$ NO$_3^-$-N, 10 ml concentrated NaNO$_3$ solution (24.28 g L$^{-1}$) was added to the cathode chamber
with a syringe. Meanwhile, 10 ml water sample was taking out of the system for analyzing each day to maintain a constant cathodic volume (0.8 L).

For the situation that nitrification tank is replaced by MFC, with a constant ammonium generation rate of 50mg L\(^{-1}\) day\(^{-1}\) NH\(_4^+\)-N, 10 ml concentrated NH\(_4\)HCO\(_3\) solution (22.57 g L\(^{-1}\)) was added to the cathode chamber with a syringe. Meanwhile, 10 ml effluent sample was taking out of the system for analyzing each day to maintain a constant cathodic volume. The samples from both chambers were collected periodically with 24-hour interval.

3.3 Data Measurements and Analysis

A voltage across an external resistor was recorded every 5-minutes by a digital multimeter for the MFC reactor. The current was calculated based on Ohm's Law. The pH value for the cathode was monitored by a pH controller. The pH for the anode and the conductivity for both chambers were measured by a benchtop pH meter and a benchtop conductivity meter, respectively. The concentration of chemical oxygen demand (COD) was tested by Hach COD Test’n’Tube. The ammonium, nitrate, and nitrite was measured by corresponding Hach powder methods. The Ion Chromatography was also used to test whether other anions or cations were generated from the system. The monitored anions including chloride, sulfate, Phosphate; whereas the cations including sodium, calcium, potassium, and magnesium (Appendix C, and Appendix D).

The coulombic efficiency was calculated based on the equation below (Yang et al. 2016):

\[
Q_{\text{eff}} = \frac{\sum I \times \Delta t}{\text{COD}_{\text{inf}} \times n \times MW \times F \times Qf \times \sum \Delta t}
\]

The estimated energy consumption was analyzed based on the pumping system. The pumping system for the continuous flow of anolyte and the aeration for the cathode were calculated based on the equation below (Li et al. 2015, Liu et al 2016):

\[
P = \frac{Q \times \gamma \times E}{1000}
\]

Where P is the power requirement in kW, Q is the flowrate (m\(^3\) s\(^{-1}\)), \(\gamma\) is 9800 (N m\(^{-3}\)), and E is the head loss per m of H\(_2\)O.
Chapter 4 - Results and Discussion

4.1 MFC for Denitrification in RAS

4.1.1 Feasibility Test

The BES has a potential to enhance nitrate removal for the RAS. To test this hypothesis, the triplicate tests of different resistors was conducted. Based on Ohm's Law, the current is inverse proportional to the resistor under a constant voltage. The voltage value was recorded across 3.3 M Ohm (very little current generation) and 1 Ohm resistor (relatively high current generation) by the digital multimeter. Both cathode and anode were operated under batch mode. The cathode was injected with 0.8 L aquaculture water with the composition in Table 1.

Table 1. Composition of Aquaculture Water from RAS

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration (mg L(^{-1}))</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO(_3^--)N</td>
<td>51.8 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>NO(_2^--)N</td>
<td>2.44 ± 0.68</td>
<td></td>
</tr>
<tr>
<td>NH(_4^+)-N</td>
<td>0.33 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>COD</td>
<td>17± 3.41</td>
<td></td>
</tr>
<tr>
<td>Conductivity</td>
<td>0.76± 0.049</td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>7.79 ± 0.05</td>
<td></td>
</tr>
</tbody>
</table>

Due to the RAS has a relatively mature technique for COD removal which resulted a low COD concentration in the aquaculture recirculating water, the COD removal for aquaculture water was not considered in this study.

With a same 24-hour operation, the nitrate was first transferred from cathode chamber to anode chamber by concentration gradient. However, it was showed from Figure 6 that with 1-Ohm resistor, a higher electricity was produced which drove more nitrate to the anode for denitrification. With 1-Ohm resistor, the residual nitrate was 4.00 ± 0.88 mg L\(^{-1}\) NO\(_3^--\)N,
whereas for 3.3-M Ohm resistor, the residual nitrate was $22 \pm 1.13$ mg L$^{-1}$ NO$_3$-N. This indicated that more nitrate was removed under a higher electricity generation during a same period, and gave a better nitrate removal efficiency in the system.

![Figure 6. The Performance of MFC under Different Resistors for 50 mg L$^{-1}$ NO$_3$-N Aquaculture Water](image)

To enable a better understanding of the removal performance and potential of the MFC under RAS, the different nitrate concentrations were also tested. Although fish does not have the sensitive response for nitrate concentration in the aquaculture system, the nitrate concentration was kept around 50 mg L$^{-1}$ NO$_3$-N in most of the time. However, due to the massive fish production, the aquaculture water was also found to have the nitrate concentration up to 200 mg L$^{-1}$ NO$_3$-N (Sandu et al 2013). Two kinds of aquaculture water with different nitrate concentration were obtained from VT Department of Food Science, one with the $51.8 \pm 0.2$ mg L$^{-1}$ NO$_3$-N, the other was $210.50 \pm 16.48$ mg L$^{-1}$ NO$_3$-N. With the knowledge of electricity generation can drive more nitrate from cathode to anode, 1-Ohm external resistor was applied for MFC. Both cathode and anode were operated under batch mode with 3 cycles testing. To provide an enough carbon source (3000 mg L$^{-1}$ COD) and recirculating for the system, the anode was
built for 220 mL internal volume plus a 200 mL reservoir outside of the reactor. The cathode was injected with 0.8 L aquaculture water with different nitrate concentration.

From Figure 7, although the MFC remove more amount of nitrate aquaculture water with 200 mg L\(^{-1}\) NO\(_3\)-N under a same period of operation than that of 50 mg L\(^{-1}\) NO\(_3\)-N, the residual of nitrate was still a concern. The 50 mg L\(^{-1}\) NO\(_3\)-N brought a lower nitrate residual and a higher removal efficiency.

![Figure 7. The Performance of MFC under 1-Ohm Resistors for 50 and 200 mg L\(^{-1}\) NO\(_3\)-N Aquaculture Water](image-url)
4.1.2. Electricity Generation with MFC for Denitrification

With the feasibility study, the MFC exhibited a better performance under 1-Ohm resistor and 50 mg L\(^{-1}\) NO\(_3\)\(^{-}\)N. The anode chamber with 220 ml volume was fed with synthetic wastewater (960 mg L\(^{-1}\) COD) under continuous mode. 0.8 L aquaculture water was pumped into and remained in the cathode chamber for 15 days. 10 ml NaNO\(_3\) was injected into the system for supporting a 50 mg L\(^{-1}\) NO\(_3\)\(^{-}\)N in cathodic aquaculture water with a syringe every day. The electricity was generated in the system as showed in Figure 8 below, which brought a daily average current density of 19.87 ± 7.26 mA m\(^{-3}\). The current kept decreasing over the operational time with the accumulation of nitrate in the system. After 15 days of adding dose, the current returned to its initial value from day-16 also indicated denitrification gradually dominated the activity in the anode chamber with the limited amount of carbon source.

![Figure 8. The Current Density Generation under 1-Ohm Resistors for 50 mg L\(^{-1}\) NO\(_3\)\(^{-}\)N Aquaculture Water](image-url)
4.1.3 Removal of Nitrates and Organics with MFC for Denitrification

The removal efficiency for both nitrate and organics are the key elements for testing the system’s performance. With 24-hour HRT of the anode or an anodic recirculation rate of 0.15 mL/min, the anode removed $876.06 \pm 7.59 \text{ mg L}^{-1} \text{ COD}$, resulting in 91.26% COD removal efficiency. The immigration of nitrate in cathode was continuously moving to the anode to achieve a simultaneous nitrate removal through heterotrophic denitrification. With the daily nitrate accumulation rate of 50 mg L$^{-1}$ NO$_3^-$-N, the water should be replaced in every day. However, with the MFC installed in the system, it would consume much less fresh water due to the less accumulation of the nitrate via the denitrification process. This indicated the MFC can slow down the fresh water replacement frequency (Figure 9), plus the generated energy can further shorten the energy consumption. The other ions and cations concentrations which tested by Ion Chromatography were showed in Appendix C and Appendix D.

![Graph showing comparison between with and without MFC installation in the RAS with assumed constant nitrate accumulation rate of 50 mg L$^{-1}$ NO$_3^-$-N](image)

Figure 9. The Comparison between with and without MFC Installation in the RAS with the Assumed Constant Nitrate Accumulation Rate of 50 mg L$^{-1}$ NO$_3^-$-N
4.2 MFC for Nitrification and Denitrification in RAS

The BES can improve nitrate removal based on the installed nitrification process in RAS. However, MFC may also has a potential to achieve ammonia removal for the RAS with combined processes of nitrification and denitrification. To test this hypothesis, the biofilm was cultured on the carbon cloth for the nitrification. Aquaculture water with a same water quality and chemical composition showed in Table 1 was applied in this section.

The anode chamber with 220 ml volume was fed with synthetic wastewater (960 mg L\(^{-1}\) COD) under continuous mode. 0.8 L aquaculture water was pumped into and remained in the cathode chamber for 20 days. 10 ml concentrated NH\(_4\)HCO\(_3\) was injected into the system for supporting a 50 mg L\(^{-1}\) NH\(_4\)\(^+\)-N generation in cathodic aquaculture water with a syringe every day. With a same 24-hour operation, the ammonia was first converted to nitrate in cathode chamber, then the nitrate moved to the anode for the denitrification. The pH would drop down during the nitrification process in cathode chamber. In order to best mimic the RAS system, NH\(_4\)HCO\(_3\) was chose to provide the ammonia while adjusting the pH and supporting an appropriate alkalinity level in the water. pH and conductivity were monitored on a daily basis, and were presented in Figure 11 and Figure 12, respectively. The cathode maintained a pH of 7.78 ± 0.11, and anode of 6.78 ± 0.29. The conductivity was gradually increasing in the cathode due to the ammonia accumulation, whereas the conductivity of anode was more depending on the nitrogen removal performance of the system.

![Figure 10. Schematic of Inserting MFC in Recirculating Aquaculture System for Nitrification and Denitrification](image)
Figure 11. pH Value in Anode and Cathode

Figure 12. Conductivity Value in Anode and Cathode
4.2.2. Electricity Generation and Organics Removal with MFC for Nitrification and Denitrification

The daily averaged current density generated from the system were presented in Figure 13. With the combination of nitrification and denitrification processes, the current density was jumping between 49.36 and 88.70 A m⁻³, which gave a higher electricity production than that of only denitrification system. A possible reason for this may be the biofilm attached on the carbon cloth enhanced electron transfer. Figure 14 also indicated that electricity generation had a same pattern with coulombic efficiency. During 20 days of operation, the MFC removed 826.43 mg L⁻¹ COD in average, a COD removal of 86.09% ± 9.83, and a coulombic efficiency of 58.86 ± 8.76%. Some of the carbon source were oxidized for denitrification without involving in the electricity generation would be an appropriate reason to explain the high COD consumption and a low Coulombic efficiency in the system.

![Figure 13. The Current Density Generation under 1-Ohm Resistors](image-url)
The distribution of COD consumption was illustrated in Figure 15. The denitrification and electricity generation were considered as the major parts to consume carbon source, whereas the other factors were assumed as system loss. From the analysis, the electricity generation used almost 59% of the COD, denitrification consumed 23% of the COD, and the system loss took 18% of the consumed COD in average.

The consumed COD was calculated based on the equation below (Strohm et al 2007):

\[
5 \text{C}_6\text{H}_{12}\text{O}_6 + 24 \text{NO}_3^- + 24 \text{H}^+ \rightarrow 30 \text{CO}_2 + 12 \text{N}_2 + 42 \text{H}_2\text{O}
\]
4.2.3 Nitrogen Compounds removal with MFC for Nitrification and Denitrification

The total inorganic nitrogen balance was conducted as showed in Figure 16 under the steady state of the system with the assumption of 0 organic nitrogen compounds generation or decay. Due to the system complexity, the analysis simply tested and calculated the chemicals in and out of the MFC on a daily basis.
As illustrated in Figure 17, the ammonium was the only nitrogen source entered into the system or the cathode chamber. However, the effluent had the combination of ammonium, nitrate, and nitrite due to the system limitations on nitrification and denitrification. From Figure 18, the total nitrogen removal efficiency in the system was achieved at 38.72 ± 4.99%, with a system removal rates of 0.02 kg N m⁻³ d⁻¹. The ammonia residual in cathode chamber was 5.10 ± 0.6 mg L⁻¹ NH₄⁺-N and would cause health issue to the aquaculture products. This concern can be further investigated by changing the MFC configurations, scales, HRT, and through the fresh water replacement.

Figure 16. Influent and Effluent Nitrogen Compounds Concentrations
Figure 17. Influent and Effluent Nitrogen Compounds Concentrations in Cathode Chamber

Figure 18. Nitrogen Removal Performance of MFC
4.2.4 Energy Consumption with MFC for Nitrification and Denitrification

Energy consumption is estimated with the consideration of feeding anode chamber continuously, and pumping air for nitrification aerating in cathode chamber. The aerating took $8.82 \times 10^{-4}$ kWh m$^{-3}$, whereas feeding anode used $2.67 \times 10^{-6}$ kWh m$^{-3}$ and brought a total energy consumption of $8.85 \times 10^{-4}$ kWh m$^{-3}$. Further energy analysis is needed to conduct based on the treated water volume and the possible fish production in the system.
Chapter 5 - Perspectives

The developed MFC presented a potential to be applied in RAS with a much lower energy consumption than the conventional nitrification and denitrification processes. Further energy generation and consumption are needed to investigate with the consideration of fish production.

Although there are several advantages to insert MFC into RAS, the biofilms attaching on the MFC may also bring a concern of health issues for the fish or other aquaculture product. Hence, the water quality under inserting MFC are needed to further investigate with the real RAS system.

The ammonium level was not as low as expected, thus replacing the system with certain amount of water is an alternative for applying MFC under RAS. Manipulating the biofilm coverage, the pH, and the aeration of oxygen can be tested for further improving the nitrification performance. Looking further for the accurate DO and pH control can also be conducted in the future.

The ways to increase capacity of BES system are also encouraged. MFC is one of the form of bio-electrochemical, the other configurations of BES may provide an even better performance for RAS.

In this study, a synthetic wastewater was supplied as the carbon source for the denitrification. Although the system showed a desirable COD removal performance, other anions in wastewater may also migrate across the membrane into the RAS system (i.e. cathode chamber), which may bring a further health concern for the living fish. Inserting a digester for digesting the organic compounds and use the digested wastes to feed MFC from the system is worth to consider.

Further mathematical modelling can be performed to better evaluate the performance of MFC based on different nitrogen-compounds level, the thickness and coverage area of biofilm, and the configurations.

Finally, scaling-up is always the challenge for applying MFC in the real industry. An appropriate strategy for system scaling-up is needed to investigate from laboratory scale into a practical technology in the future study.
Chapter 6 - Conclusions

In conclusion, the study illustrated the MFC with an anion exchange membrane has the potential to apply under RAS facility, which can achieve nitrogen removal for the system. The current design approached the ammonium and nitrate removal from RAS, and organic carbon removal from synthetic wastewater. With the nitrification process integrated in MFC, a prompt energy generation with a low energy consumption was found, which also indicated a potential for biocathode applied into the RAS MFC study. Further study regarding the health concerns, improving the water quality, alternating carbon sources, are needed for applying and scaling-up MFC under RAS into the real practice in the future.
References


Appendices

Figure A. pH Value of Anode and Cathode after Adding NaNO₃ for Denitrification

Figure B. Conductivity of Anode and Cathode after Adding NaNO₃ for Denitrification
Figure C. Other Anions of Concern after Adding NaNO$_3$ for Denitrification

Figure D. Other Cations of Concern after Adding NaNO$_3$ for Denitrification