Illinois-Indiana Sea Grant Program
Completion project

DRAFT
Final Project Report

ENERGY EFFICIENT AND SUSTAINABLE AQUACULTURE WATER TREATMENT USING MICROBIAL FUEL CELLS AND MEMBRANE-SUPPORTED BIOFILMS

Principal Investigator: Robert Nerenberg, Ph.D., P.E.
Associate Investigator: Juan P. Pavissich, Graduate Research Assistant

Affiliation: University of Notre Dame, Civil Engineering and Geological Sciences

December, 2011

Introduction

This study investigated the feasibility of a new treatment process, based on microbial fuel cells (MFCs) and membrane-supported biofilms reactors (MBfRs), for removing nitrogen and sulfide in simulated recirculating aquaculture systems (RAS) wastewater. The main objectives were to determine the nitrogen and sulfide removal efficiencies, and the levels of electric power production, and test different reactor configurations under variable loading conditions. A complementary proposal was approved through the University of Notre Dame Center for Aquatic Conservation, which resulted in further research funding, for a combined total of one year of research support.

Background

RAS systems are becoming increasingly popular in the Midwestern United States, since they are more environmentally protective and sustainable than pen or pond-based systems. However, RAS-based aquaculture producers in the Midwest face stiff competition from international sources, which rely on less sustainable fisheries. Therefore, it is critical for local RAS plants to maximize the cost-effectiveness of all operations in order to remain competitive. A significant cost for RAS-based producers is waste management (Mook et al., 2012). RAS typically includes two waste streams: a nitrification loop to maintain ammonia concentration below toxic levels, and a solids removal system, eliminating unused food and fish feces. While biological removal of ammonia in RAS is well established, the solids are usually not treated; rather, they are discharged to a municipal wastewater treatment system. Critical contaminants in the solids stream include organic matter, nitrogen (mainly ammonia, but also some nitrate), and sulfide. The disposal of the solids results in additional expenses for the plant, a loss of water, and potential problems with sewer corrosion and odors. Also, these costs may further increase due to increasing energy costs and stricter regulations on nutrient discharges.

An attractive biological treatment technology for RAS are MFCs. MFCs have the potential to convert biodegradable wastes directly into electrical energy (Mook et al., 2012). MFC systems have shown to be effective for concurrent removal of organic matter and nitrate (Clauwaert et al. 2009), and sulfide removal (Rabaey et al. 2006). MBfRs are based on biofilms growing on gas-permeable hollow-fiber membranes (HFMs) that provide passive aeration and allow high specific surface areas. MBfRs are very effective for nitrification (Downing and Nerenberg, 2008), and have low energy requirements compared to bubbled aeration, with energy savings up to 70% (Semmens, 2005). When combined, MFCs and MBfRs may make RAS waste removal more cost-effective and allow net production of energy.

Total nitrogen removal can be accomplished through nitrification/denitrification. Nitrification is driven by ammonium-oxidizing microorganisms that oxidize ammonium to nitrite, and nitrite oxidizing microorganisms that oxidize nitrite to nitrate, both using oxygen as an electron acceptor. Denitrification is an anaerobic process that reduces nitrate to nitrogen gas. Sulfide removal can be accomplished via oxidation processes under aerobic or anaerobic conditions using oxygen or nitrate as electron acceptors respectively. Biological oxidation is driven by sulfide-oxidizing bacteria that generate sulfate as a complete oxidation product.

The development of a reactor based on the above biological processes to remove total nitrogen and sulfide, while producing energy, would build on preliminary tests in our lab where we explored organic matter and total nitrogen removal from municipal wastewater using an MFC/MBfR system (Shea et al. 2010). For sulfide removal, we propose using single chamber MFCs with different conditions to find a suitable configuration. For a sulfide-nitrogen removal process, we propose developing a combined sulfide/nitrification/denitrification reactor, incorporating a membrane supported biofilm step to enable nitrification.

Materials and Methods

Sulfide removal MFC experiments

Sulfide removal from RAS synthetic wastewater was tested using a single chamber air-cathode MFC configuration. In this system the cathode compartment was open to air and biologically catalyzed sulfide oxidation was expected in the anode compartment (Fig. 1).

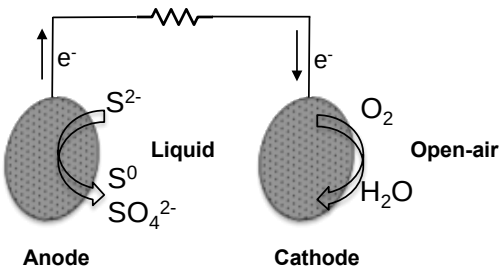


Fig. 1: Circuit schematic of the air-cathode MFC with anodic sulfide oxidation

Four MFC were constructed using 1 cm-thick acrylic frames separated by silicone gaskets and secured with screws in the corners (Fig. 2, Appendix).

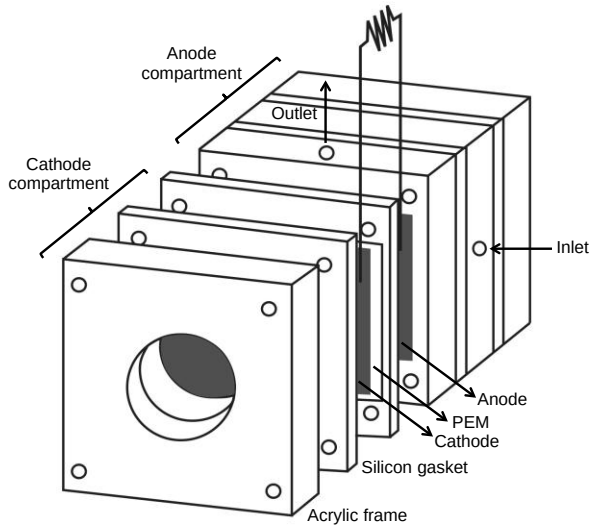


Fig. 2: Single chamber air-cathode MFC configuration

The anode compartments were cylindrical with a 3.25-cm diameter and 2-cm depth, for a total volume of 16.6 mL. Plain graphite cloth with an overall specific surface of 50 m^2/m^3 (surface to volume) or graphite granules (1.5-5 mm diameter, Le Carbone, Belgium, Appendix) with a projected average surface area of 1,769 m^2/m^3 and density of 1.83 kg/L, were used as anode material. The net volume of the anode compartment containing graphite granules was 4.5 mL. The cathode active surface was equivalent to the anode with a compartment depth of 1 cm, and was left open to the ambient air (Shea and Nerenberg, 2010). For the cathodes, graphite cloth with an optimized polytetrafluoroethylene (PTFE) diffusion layer was prepared as described to reduce the oxygen crossover (Shea and Nerenberg, 2010), including 0.1 mg/cm^2 platinum added to the internal side of the cathode as a catalyst. The MFCs included a Nafion proton exchange membrane (PEM) separating anode and cathode compartments.

Each MFC was fed with a 50 mM phosphate buffer minimal medium (pH 7.0) with the following composition per liter: 310 mg NH_4Cl, 130 mg KCl, 750 mg $NaHCO_3$, 50 mg $CaCl_2$, 100 mg $MgCl•6H_2O$, 100 mg NaCl, and 10 mL of a 100x trace mineral solution described elsewhere (Sun et al., 2009). The medium was purged with 99.99% nitrogen at 5 psi for 30 minutes and subsequently pressurized under a nitrogen atmosphere to remove oxygen in solution.

Four conditions were tested considering different inocula and anode materials for each system; mixed culture and carbon cloth (MFC1), mixed culture and graphite granules (MFC2), pure culture and carbon cloth (MFC3), and an abiotic control without inoculum and a carbon cloth electrode. For the mixed culture, the anodic compartments were inoculated with biomass from a sulfur-based denitrifying reactor and activated sludge from a local wastewater treatment plant. The strain used for the pure culture condition was *Paracoccus pantotrophus* ATCC 35512. *P. pantotrophus* is a sulfide-oxidizing organism that has been found in the anode of MFCs for sulfide removal (Rabaey et al. 2006) and a model bacterium that we are currently using in denitrification experiments. Each MFC was batch-fed with the medium dosed with 50 mg S/L of sulfide ($Na_2S•9H_2O$ from a separated feeding stock solution) as an inorganic electron donor until a measurable voltage drop was detected across an external circuit loaded with a 100 Ω resistor (R_{ext}). The experiments were then operated under continuous-flow conditions using an influent flowrate of 0.18 mL/min. For each MFC the voltage drop (V) across the external resistance was measured using a digital multimeter and polarization curves were obtained using a potentiostat in a two-electrode configuration as described (Shea and Nerenberg, 2010). The power density of each system ($P = V^2/R_{ext}$•volume) was calculated based on the maximum sustained voltage across the external resistance and using the net volume of the anodic compartment.

Reactor influent and effluent samples were taken several times a week. Sulfide was using the methylene blue method (Cline, 1969), sulfate using a turbidimetric method based on the precipitation of barium sulfate (Kolmert et al., 2000), nitrate and nitrite using ion chromatography as previously described (Downing and Nerenberg, 2007), and pH with a pH-probe.

Sulfide and nitrogen removal MFC-MBfR

Combined sulfide and nitrogen removal was tested using a two-chamber MFC configuration. In this system the cathode compartment was closed and biological nitrogen removal is expected to take place. Biological cathodes have been shown to denitrify (Shea et al., 2008), but removal of reduced nitrogen species requires a prior nitrification step. Treatment approaches including

nitrification and denitrification steps in the same system may be more efficient and feasible than in separate units. Air-filled MBfR supporting nitrifying biofilms within anaerobic systems for denitrification have been successfully used in our laboratory (Downing and Nerenberg, 2007) and tested in MFC systems (Shea et al. 2010). We propose incorporating HFMs in the anaerobic cathodic compartment of the sulfide-nitrogen removal MFC to support nitrification.

The MFC system was constructed using rectangular acrylic frames and the anodic and cathodic compartments were filled with graphite granules as electrode material. The use of graphite granules was selected based on the anodic results of the initial sulfide oxidation MFC experiments, described above, and the denitrifying biocathode performance from previous research (Shea et al., 2008). Each electrode compartment was made with two acrylic frames (10 x 10 x 2 cm^3 per frame) with a 400 mL total volume and a net volume of 100 mL after the granular electrode material was added. A pair of graphite rods was inserted in each compartment and used as current collector (5 mm diameter, McMaster-Carr) and an Ultrex PEM (CMI-700, Membranes International Inc.) was used for separating both compartments. HFM gassing ports were drilled for the operation of the cathode chamber as an MBfR. A rack of HFM with a total surface of 0.026 m^2 was constructed by winding up five bundles of twenty microporous polyethylene membranes (30 cm long and 280 μm outside diameter, MHF200, Mitsubishi Rayon, Japan) on a plastic grid support. The experimental set-up is shown in Fig. 3 and Appendix.

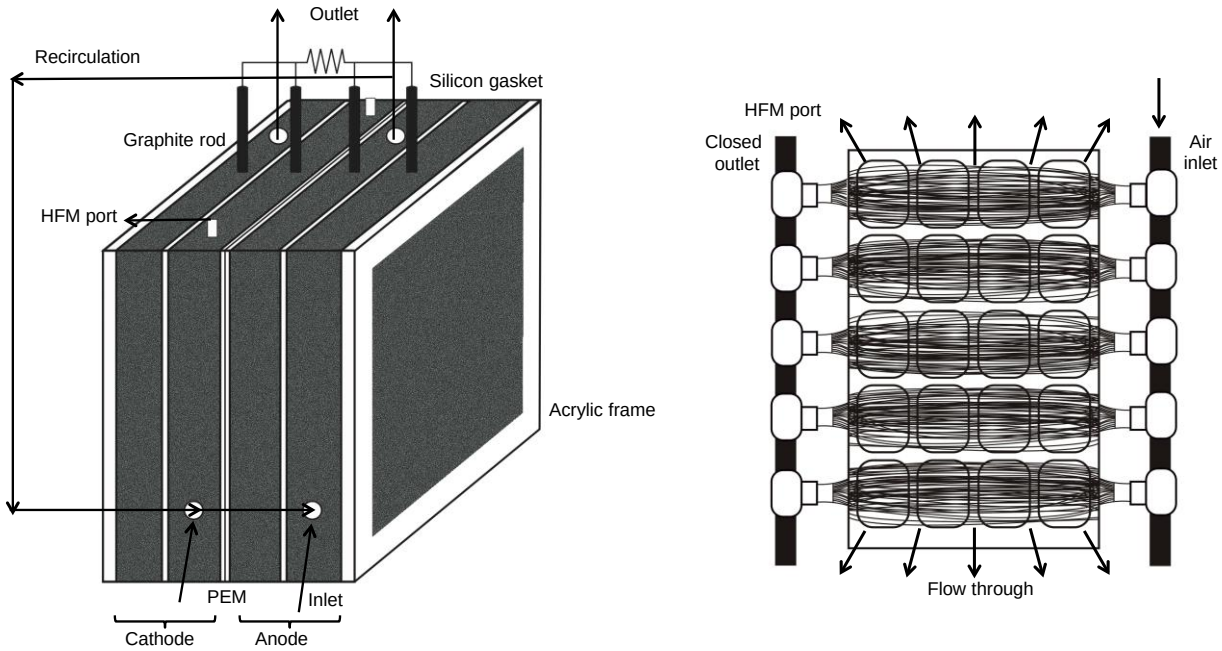


Fig. 3: Two-chamber MFC for sulfide-nitrogen removal (left) and HFM rack configuration (right)

The development and analysis of the MFC was done in two steps. In the first start-up phase the anode, cathode and MBfR were partitioned to enrich the biofilm communities driving the specific transformation processes (sulfide-oxidation, denitrification and nitrification, respectively). The anodic chamber was inoculated with biofilm covered graphite granules from the previous anodic sulfide oxidation experiments and activated sludge, and continuously fed

100 mg S/L of sulfide in a 16 mM phosphate buffered minimal growth medium containing 1.386 g/L Na_2HPO_4, 0.849 g/L KH_2PO_4, 50 mg/L NH_4Cl, 50 mg/L $MgSO_4$, 0.1% of a trace mineral solution and 0.1% of a calcium-iron solution. The trace mineral and calcium-iron solutions were prepared as described previously (Nerenberg et al., 2002). The cathode was inoculated with microbial communities from a denitrifying biocathode and activated sludge. The cathode chamber was continuously fed 20 mg N/L of nitrate in the 16 mM phosphate buffered medium. Anode and cathode media were purged and pressurized with nitrogen as described previously, respectively fed at 0.6 mL/min and 0.3 mL/min, and recirculated with a 100 mL/min rate. The HFM system was supplied with 2 psig of air and operated in a separated 2 L completely mixed batch reactor. The reactor was inoculated with mixed liquor from a nitrifying activated sludge and fed with 16 mM phosphate buffered medium and 50 mg NH_4^+ N/L. In the second phase the HFM rack was introduced in the cathodic chamber of the MFC system keeping the anode and cathode separated by the PEM. This configuration may combine the different treatment reactions in the system (Fig. 4). All experiments were performed at room temperature (22 ± 1 ºC).

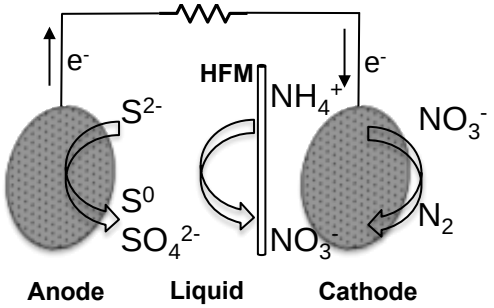


Fig. 4: Circuit schematic of the sulfide-nitrogen removal MFC-MBfR

Results and Discussion

Sulfide oxidation in air-cathode MFC with diffusion layer

Initially, all the MFCs showed a similar average voltage drop of 0.015 V, which corresponds to a power density of 0.14 W/m^3 per net anodic compartment (NAC) for the carbon cloth systems and 0.50 W/m^3 NAC for the graphite granule MFC. A significant voltage increase from this baseline was detected after four to six weeks in the biological inoculated systems. Subsequently, all systems were operated in continuous mode for other three months. During this period the abiotic control consistently maintained the initial observed voltage. This result suggested abiotic current production. Spontaneous electrochemical oxidation of sulfide with electricity generation has been observed in abiotic two chamber-fuel cells using ferricyanide as catalyst and electron acceptor in the cathode, with an average power generation of 12 W/m^3 under continuous operation (Dutta et al., 2008). A steady-state current production was observed in the three biotic MFC after five to seven weeks of continuous mode and increasing voltage drop. The average power generated was 3.17 W/m^3 for MFC1, 2.02 W/m^3 for MFC2 and 10.89 W/m^3 for MFC3. The average sulfide and sulfate effluent concentrations for the experiments after reaching steady-state are shown in Fig. 5. The difference (Δ) for sulfide corresponds to removal and for sulfate to production after sulfide oxidation.

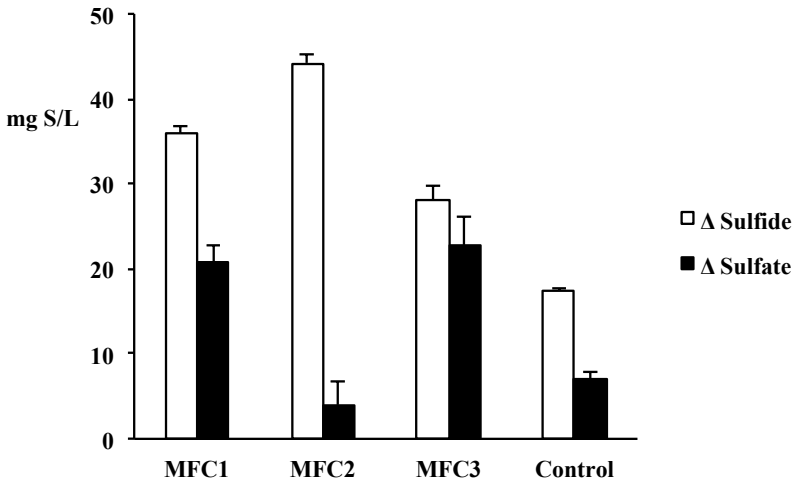


Fig. 5: Influent and effluent change in sulfide and sulfate concentrations in air-cathode MFC. Note that the change in sulfide concentration is shown as positive in the graph, for ease of comparison to the sulfate change, but actually is negative (a decrease in concentration).

Throughout the experiments the reactor influents contained an average concentration of 49.40 mg S/L sulfide and 10.33 mg S/L sulfate, the latter was presumably produced by abiotic oxidation of the stock solution due to potential oxygen diffusion through the circulation system. The Δ results showed different performances for the MFC systems. MFC2 showed the highest sulfide removal of 90% with a low net fraction of 9% being transformed to sulfate. MFC1 and MFC 3 showed also a high sulfide removal of 76% and 54%, respectively, but a significantly higher generation of sulfate. Particularly MFC3 showed an 81% of loading being transformed to sulfate. The abiotic control showed an average of 39% sulfide removal with a 40% being transformed to sulfate. The abiotic result was similar to sulfate and sulfide determinations during batch mode operation and to the initial levels observed in the biological systems (data not shown) suggesting a consistent electrochemical oxidation in the experiments and biochemical activity in the biotic conditions. In abiotic systems, elemental sulfur (S^o) has been found as main sulfide oxidation product (Dutta et al., 2008). This may explain the lack of agreement between sulfide loss and sulfate formation. In the biological systems, it seemed that this fraction of oxidation by-products is generated differently between conditions. While the mass balance in MFC3 suggested mainly a complete oxidation of sulfide to sulfate, in MFC2 the sulfate production was minimal and similar to the abiotic treatment. End point qualitative observations in the graphite showed grey to white depositions over the black electrode granules (data not shown). Microbial ecology differences between mixed culture enrichments versus a pure culture, including different metabolic requirements and pathways, may explain the observed oxidation activity. No significant pH variations were detected in the experiments.

Polarization curves analyses were used to determine the maximum attainable power of each cell during continuous operation, measuring the voltage response as a function of current with a potentiostat. The polarization curves obtained for steady-state operation are showed in Fig. 6.

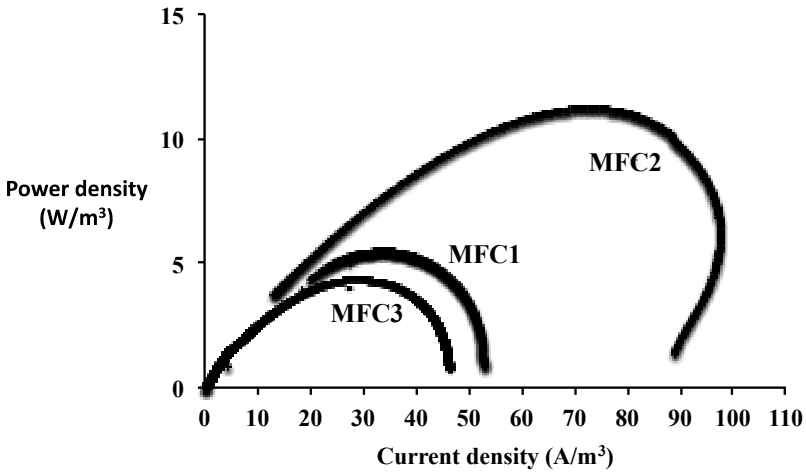


Fig. 6: Polarization curves for sulfide oxidizing MFCs operated with a 100 Ω external resistance

From the polarization curves the maximum power density obtained was 5.49 W/m^3 for MFC1, 11.22 W/m^3 for MFC2 and 4.35 W/m^3 for MFC3. For the operation conditions, the systems were close to an optimal bioelectrochemical activity. The best performing system in terms of power production and sulfide removal was MFC2 (mixed culture and graphite granules). Electrochemical (abiotic) removal of sulfide was observed, but it was significantly enhanced by biological activity.

Sulfide oxidation and denitrification in MFC-MBfR

The establishment of a nitrifying microbial community was observed after 7 weeks of enrichment in the 2 L reactor. After this period the biofilm of the HFM rack performed a complete nitrification of 50 mg NH_4^+/L every four days of batch cycle. This is a nitrification flux of 0.96 g N/m^2 cathode-day, similar to the rates achieved in other HFM systems for nitrification-denitrification (Downing and Nerenberg, 2007). The start-up enrichment of a sulfide-oxidizing community in the anodic chamber and a denitrifying community in the cathodic chamber coupled with electrochemical production of electricity was verified after 10 weeks of operation (Fig. 7).

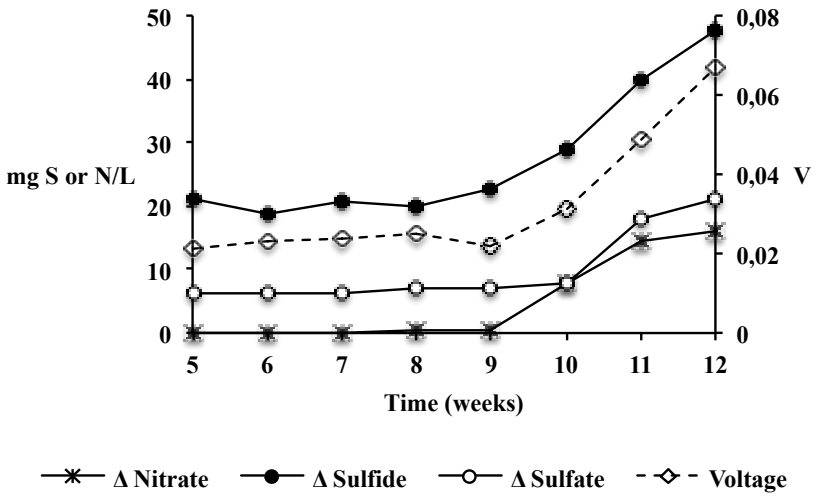


Fig. 7: Voltage drop, sulfide anodic and nitrate cathodic removal over time for the start-up phase of the MFC-MBfR system

An increasing voltage drop, sulfide removal and denitrification have been observed. The initial sulfide degradation may be attributed to electrochemical oxidation as a sustained voltage drop was observed along the experiment, and not detected when the sulfide feeding was stopped. Effluent nitrite levels remained below the detection limit. After 12 weeks, there was an average anodic removal of 47.8 mg S/L and a cathodic removal of 16.2 mg N/L, equivalent to 0.413 kg S/ m^3-day and 0.070 kg N/m^3-day based on the liquid volume. Sulfide crossover to the cathode was been detected, with concentrations of 5-6 mg S/L.

Considering continuous flow through a system with a flowrate (q), and complete oxidation of sulfide (ΔS^{2-}) to sulfate yielding 8 electrons per mole (b), the potential current generation form the amount of coulombs (C) contained in the substrate can be determined using $I = (F \cdot b \cdot q \cdot \Delta S^{2-})/M_W$ (Logan, 2008), with Faraday's constant F = 96,485 C/mol and sulfide molecular weight M_W = 32.06 g/mol. The average sulfide removed in the MFC-MBfR was not completely oxidized to sulfate biologically. Likewise the initial air-cathode experiments, there is a fraction of sulfide that may be oxidized to elemental sulfur and other sulfur species, and other abiotically oxidized. The amount of sulfate produced may be attributable to complete oxidation of sulfide coupled to electricity production (Fig. 7). The current for this amount of substrate (21.14 mg S/L) would correspond to 5.94 mA, a 594 mV potential for the external resistance used in the system. The current observed after 12 weeks was 0.67 mA, only 11% of this amount. This indicated the order of magnitude of the reactor's energy conversion efficiency assuming only complete oxidation. This may be an underestimation of the electrical efficiency because a fraction of the sulfate produced is due to chemical oxidation without electricity generation.

Work in progress

The analysis and optimization of the MFC-MBfR system has not been completed yet. We plan to carry out a microbial ecology analyses of the communities present in the experiments, specially the community enriched in the graphite granules of MFC2, the best performing preliminary system, which was used for the inoculation of the MFC-MBfR reactor. Also, the MFC-MBfR

reactor has not achieved steady-state for power production and sulfide and nitrate removal yet. Once this is achieved, we plan to perform polarization curves and incorporate the nitrifying HFM.

Research significance and perspectives

The developed laboratory-scale experiments suggest, for the first time, that combined sulfide and denitrification-based nitrogen removal from synthetic RAS wastewater is possible using an MFC configuration. The removal levels led to low effluent sulfide and nitrate concentrations that may meet the aquaculture nutrient treatment objectives. The sulfide removal rate achieved was consistent with similar electrochemical systems (Dutta et al., 2008; Rabaey et al., 2006), and the denitrification performance was lower than observed in other biocathodes (Clauwaert et al., 2009). However, the experimental systems has not been optimized and reached their full potential, and to our knowledge concurrent sulfide and nitrogen removal has not been reported so far in bioelectrochemical systems.

Adding nitrification to a single unit system would also be novel. The MFC-MBfR approach may allow total nitrogen removal with the advantage of energy production and thus, the implementation of a more cost-effective and sustainable operation. The power production observed is less than in organic carbon-based anodes and oxygen-based cathodes, where power densities in the order of the several hundreds has been obtained (Logan, 2008), but has similar performance to sulfide oxidation and denitrifying counterparts (Dutta et al. 2008; Clauwaert et al., 2009).

References

Cline, J.D. Spectrophotometric determination of hydrogen sulphide in natural waters. 1969. Limnol Oceanogr 14: 454-458.

Clauwaert, P., Desloover, J., Shea, C., Nerenberg, R., Boon, N. and Verstraete, W. 2009. Enhanced nitrogen removal in bio-electrochemical systems by pH control. Biotechnology Letters 31: 1537-1543.

Downing, L. and Nerenberg, R. 2007. Performance and microbial ecology of the hybrid membrane biofilm process (HMBP) for concurrent nitrification and denitrification of wastewater. Water Sci Technol, 55: 355-362.

Dutta, P.K., Rabaey, K., Yuan, Z. and Keller, J. Spontaneous electrochemical removal of aqueous sulfide. 2008. Water Res 42: 4965-4975.

Kolmert A., Wikström P. and Hallberg K. 2000. A fast and simple turbidimetric method for the determination of sulfate in sulfate-reducing bacterial cultures. J Microbiol Methods 41: 179-184.

Logan, B. 2008. Microbial Fuel Cells. John Wiley Sons, Inc., Hoboken, NJ.

Mook, W.T., Chakrabarti, M.H., Aroua, M.K., Khan, G.M.A., Ali, B.S., Islam M.S. and Abu Hassan, M.A. 2012. Removal of total ammonia nitrogen (TAN), nitrate and total organic carbon (TOC) from aquaculture wastewater using electrochemical technology: A review. Desalination 285: 1-13.

Nerenberg, R., Rittmann, B.E. and Najm, I. 2002. Perchlorate reduction in a hydrogen-based membrane-biofilm reactor. Journal American Water Works Association 94: 103-114.

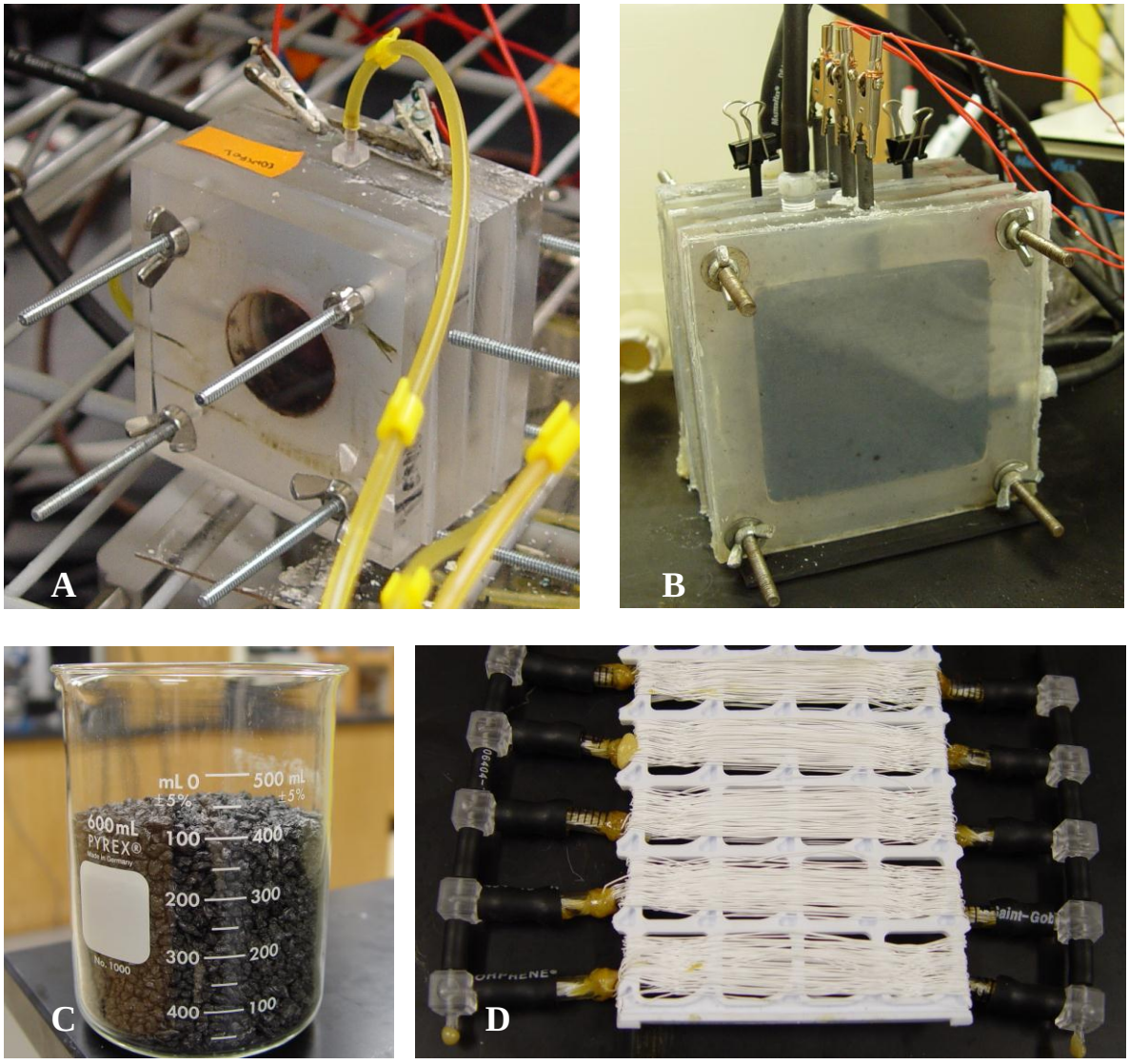
Rabaey K., Van de Sompel, K., Maignien L., Boon, N., Aelterman, P., Clauwaert, P., De Schamphelaire, L., Pham, H., Vermeulen, J., Verhaege, M., Lens, P. and Verstraete, W. 2006. Microbial fuel cells for sulfide removal. Environ Sci Technol 40: 5218-5224.

Shea, C., Clauwaert, P., Verstraete, W. and Nerenberg, R. 2008. Adapting a denitrifying biocathode for perchlorate reduction. Water Sci Technol 58: 1941-1946.

Shea, C. and Nerenberg, R. 2010. Performance and microbial ecology of air-cathode microbial fuel cells with layered electrode assemblies. Appl Microbiol Biotechnol 86: 1399-1408.
Shea, C., Pavissich, J.P. and Nerenberg, R. 2010. Total nitrogen removal in a single-chamber, plug-flow microbial fuel cell. 239th American chemical Society National Meeting, March 21-25, San Francisco, USA.
Sun, M., Mu, Z.X., Chen, Y.P., Sheng, G.P., Liu, X.W., Chen, Y.Z., Zhao, Y., Wang, H.L., Yu, H.Q., Wei, L. and Ma, F. 2009. Microbe-assisted sulfide oxidation in the anode of a microbial fuel cell. Environ Sci Technol 43: 3372-3377.

APPENDIX

EXPERIMENTAL SET-UP PICTURES



A. Air-cathode MFC. B. MFC-MBfR. C. Graphite granules. D. HFM rack.