Article

Exploiting carbon and nitrogen compounds for enhanced energy and resource recovery

Bahareh Kokabian [1], Veera Gnaneswar Gude [2]*

Civil & Environmental Engineering Department, Mississippi State University, Mississippi State, MS 39762
* Correspondence: gude@cee.msstate.edu; Tel.: +1-662-325-0345

Abstract: Microbial desalination cells (MDCs), a recent technological discovery, allow for simultaneous wastewater treatment and desalination of saline water with concurrent electricity production. The premise for MDC performance is based on the principles that bioelectrochemical (BES) systems convert wastewaters into treated effluents accompanied by electricity production and the ionic species migration (i.e. protons) within the system facilitates desalination. One major drawback with microbial desalination cells (MDCs) technology is its unsustainable cathode chamber where expensive catalysts and toxic chemicals are employed for electricity generation. Introducing biological cathodes may enhance the system performance in an environmentally-sustainable manner. This study describes the use of autothrophic microorganism such as algae and Anammox bacteria as sustainable biocatalyst/biocathode in MDCs. Three different process configurations of photosynthetic MDCs (using Chlorella vulgaris) were evaluated for their performance and energy generation potentials. Static (fed-batch, SPMDC), continuous flow (CFPMDC) and a photobioreactor MDC (PBMDC, resembling lagoon type PMDCs) were developed to study the impact of process design on wastewater treatment, electricity generation, nutrient removal, and biomass production and the results indicate that PMDCs can be configured with the aim of maximizing the energy recovery through either biomass production or bioelectricity production. In addition, the microbial community analysis of seven different samples from different parts of the anode chamber, disclosed considerable spatial diversity in microbial communities which is a critical factor in sustaining the operation of MDCs. This study provides the first proof of concept that anammox mechanism can be beneficial in enhancing the sustainability of microbial desalination cells to provide simultaneous removal of ammonium from wastewater and contribute in energy generation.

Keywords: anammox bacteria, microbial desalination, microalgae, photosynthesis, nutrients, bioelectricity

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1. Introduction

The energy and water production issues are intertwined and cannot be addressed in isolation [1-4]. Wastewater treatment and desalination, in particular, are energy consuming processes which can have detrimental effects on the environment [3]. Integrated solutions that utilize waste sources to generate energy, which in turn, can be used to produce freshwater are attractive options to address current energy and water issues [5]. In this context, bioelectrochemical systems have evolved as a novel technology to convert wastes into valuable energy [6]. Bioelectrochemical systems can be employed to generate clean electricity, or high value energy or chemical products from various wastewater sources and organic or inorganic wastes that can serve as fuel feedstock for electroactive bacteria [7]. Microbial desalination cells (MDCs) are based on an integrated configuration in which, wastewater and saline water sources can be treated simultaneously without any external power input or mechanical energy or pressure application [8]. This process offers multiple benefits of energy and resource (water and nutrients) recovery while eliminating environmental pollution.

Photosynthetic microorganisms can be used in PMDCs for accomplishing proper utilization of carbon and nutrient compounds (Figure 1)[9]. Their role in PMDCs can be further controlled specifically for bioelectricity production or biomass production which depends on the process configuration. Microalgae provide in-situ oxygen production which can serve as an electron acceptor in the electron transfer process while utilizing organic carbon, nitrogen and phosphorous compounds for growth [10]. On the other hand, conventional removal of nitrogenous compounds by nitrification-denitrification process from waste water in waste water treatment plants requires considerable amount of energy and costs. Anammox process which comes from Anaerobic ammonia oxidation (anammox) is an emerging microbial process for conversion of ammonium to nitrogen gas under anaerobic condition with potential energy and cost savings [11]. Authotrophic bacteria create a bypass to oxidize ammonia to nitrogen gas by nitrite omitting the need for organic carbon source. Partially nitrification of ammonia to nitrite instead of nitrate, allows for about 40% saving on energy used for aeration. In addition, due to the authoutrophic nature of these bacteria their growth rate is slow and thus, less biosolids are produced during this process. All of these benefits, make Anammox based nitrogen removal process more cost effectiveness (cost reduction of up to 60%) and less greenhouse gas emission compared to conventional nitrification-denitrification process [12].

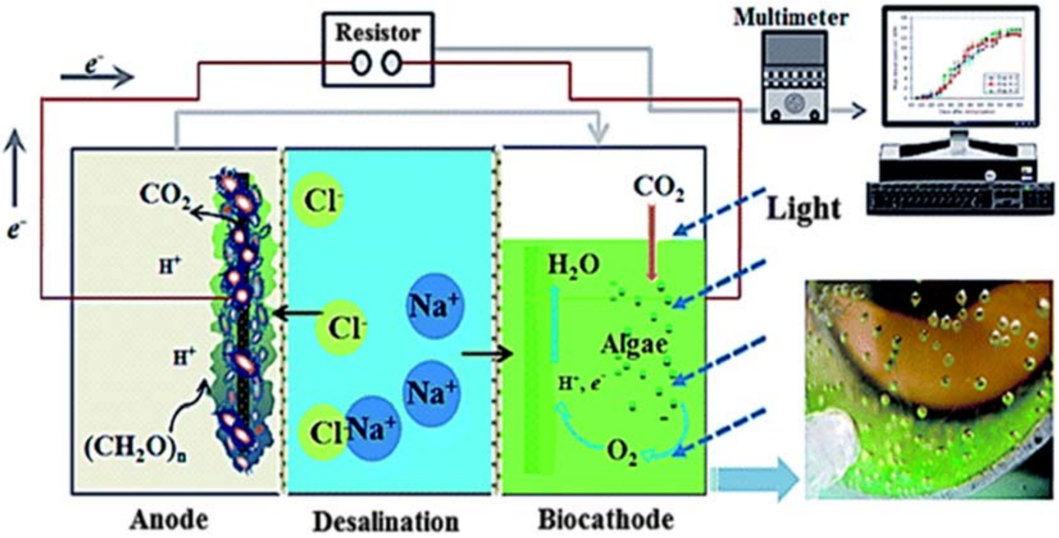

Figure 1. A photosynthetic microbial desalination cell (PMDC)

This research article presents the preliminary and proof-of-concept studies of photosynthetic (microalgal) and autotrophic (anammox bacteria) as biocathodes in microbial desalination cells. The following sections describe the experimental details and preliminary results.

2. Materials and Methods

2.1. MDC configuration and operation

Three-chamber inside circular shaped MDCs with 7.2 cm diameter were made using plexiglass. Anion exchange membrane (AEM, AMI7001, Membranes International) separated the anode and the desalination chambers, while cation exchange membrane (CEM, CMI7000, Membranes International) separated the cathode and the desalination compartments. Both membranes were preconditioned by immersing in 5% NaCl solution at 40 °C for 24 h and rinsed with distilled water (DI) water prior to use, to allow for membrane hydration and expansion as recommended by the supplier. Carbon cloth covered with stainless steel mesh were used as electrodes with 16 cm^2 surface area. Prior to use, both electrodes were washed first with 1 N HCl solution and then with 1 N NaOH and finally rinsed with deionized water. The electrodes are then soaked in DI water over a night prior to use to remove any excess residues [13]. Anode and cathode electrodes were connected through a titanium wire. The working volume of anode, desalination, and cathode chambers after inserting the electrodes were 37, 28, and 37 mL respectively. Three different operational modes, namely, static (SPMDC, Figure 2a), continuous flow (CFPMDC, Figure 2b), and photobioreactor (PBMDC, Figure 2c) were used for photosynthetic MDC to assess its performance in terms of electricity generation, biomass production and nutrient removal capacities. SPMDC was run in batch cycles. In each test, new wastewater, fresh algae medium and fresh salt solution were used in PMDCs. In the continuous mode, the algae catholyte was circulated using a peristaltic pump. Two MDC biocathodes were assembled to the large photo-bioreactor (5 liter volume). This configuration was called Photo-bioreactor MDC (PBMDC).

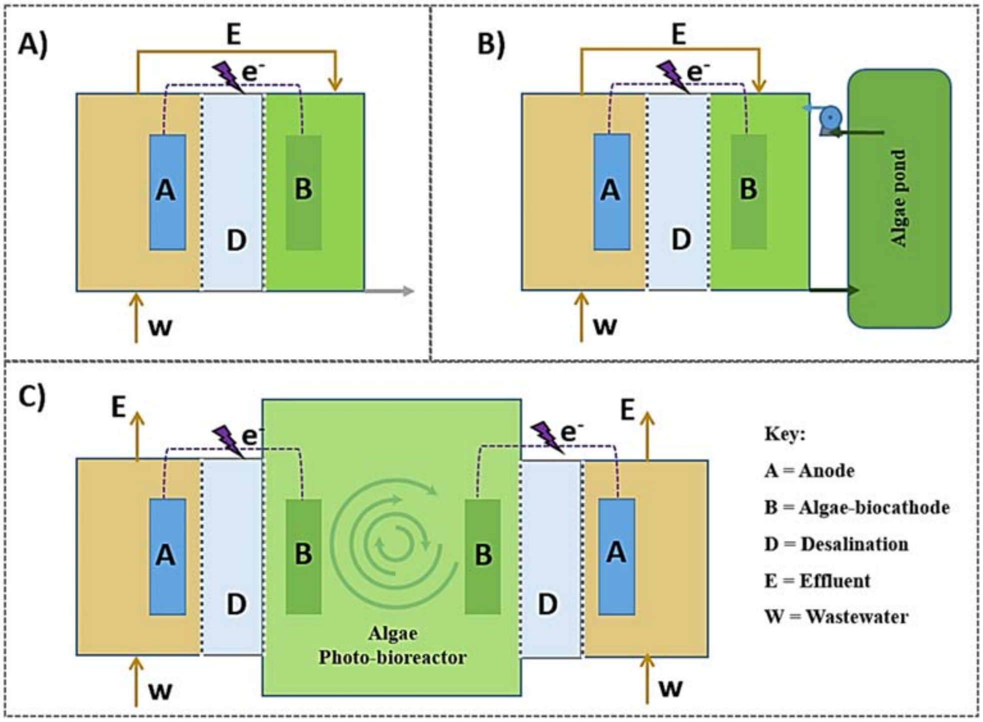

Figure 2. PMDC process configurations: a) PMDC with an algae biocathode under fed batch (static) operational mode (SPMDC); b) PMDC with an algae biocathode under continuous flow operational mode (CPMDC); c) PMDC with an algae biocathode connected to a photo-bioreactor (PBMDC)

Anammox biomass was provided by Hampton Roads Sanitation District in Virginia and was divided in three bottles under anaerobic condition in the shaker incubator at 35ºC and 150 rpm. The culture contained NH_4Cl, 382 mg L^{-1}; $NaNO_2$, 493 mg L^{-1}; $KHCO_3$, 200 mg L^{-1}; KH_2PO_4, 27 mg L^{-1}; $FeSO_4 \times 7H_2O$, 9.0 mg L^{-1}; EDTA, 5.0 mg L^{-1}; $MgSO_4 \times 7H_2O$, 240 mg L–1; $CaCl_2 \times 2H_2O$, 143 mg L^{-1} and 300 µl of trace metal solution. The trace solution contained $ZnSO_4 \times 7H_2O$, 1,247 mg L^{-1}; $MnSO_4 \times H_2O$, 1,119 mg L^{-1}; $CuSO_4 \times$5H2O, 44 mg L^{-1}; $Al_2(SO_4)_3 \times 14H_2O$, 201.5 mg L^{-1}; $Na_2MoO_4 \times 2H_2O$, 129 mg L^{-1}; $CoCl_2 \times 6H_2O$, 30 mg L^{-1}; KCl, 100 mg L^{-1}; EDTA,975 mg L^{-1} that provides micronutrients needed for microbial growth of anammox bacteria [14]. After about two months reactivation process, this sludge was transferred to the cathode chamber of MDC. The anode chamber of MDC was inoculated with 30 ml of acclimatized anaerobic sludge.

2.2. Analyses and calculations

The voltage across a 1 kΩ external resistor was recorded every 15 min by a digital multimeter (Fluke, 287/FVF). The current was calculated using Ohm's law, $I = V/R$. The power density was calculated ($P = V/I$) as per the volumes of the anode/cathode chambers. COD tests were carried out using standard methods. The nitrogen, as nitrate (NO_3–N), and phosphorus (PO_4^{-3}–P) concentrations were measured by colorimetric methods according to the method of Hach (Methods 8039 & 8114). Electrical conductivity, total dissolved solids (TDS) removal, and salinity removal were recorded using a conductivity meter (Extech EC400 ExStik Waterproof Conductivity, TDS, Salinity, and Temperature Meter). The algae concentration was determined by measuring the absorbance of the cell suspension at a wavelength of 620 nm and then converting it to dry weight of biomass in volume by a calibration curve. pH was measured using a pH meter (Orion 720A+ advanced ISE/pH/mV/ORP). Dissolved oxygen was measured using YSI 5100 system. Continuous illumination on the algae cathode chamber was provided by CFL white light at 60W (276 mmol per m2 per second).

3. Results and Discussion

3.1. Photosynthetic microbial desalination cells

The COD removals in the anode along with pH changes in the cathode chamber for each cycle are shown in Table 1. Low COD removal rates (less than 30%) were observed in these tests. The low COD removal rates in all cycles suggest that substrate limitation was not the reason for voltage drop in the cells, but the decrease in the conductivity of the solution in the middle chamber which increases the ohmic resistance of the cells could be the reason for voltage drop [8]. The other reason could be due to the increase in pH of the cathode solution and increased pH imbalance between the anode and cathode chambers. pH is an important factor in performance of bioelectrochemical systems since it affects the biological activity of the microorganisms [15]. The increase in pH which is typically caused by consumption of the protons and photosynthetic activity of the algae, slows down the ORR rate and is often reported as the limiting factor in power production [16]. In addition to pH, ORR rate is affected by fouling and biofouling which may hinder the transfer of oxygen to the electrode surface. It can be concluded that the performance of the PMDC depends on the photosynthetic activity of the algae and the bioelectrochemical function of the biofilm on the cathode electrode which was reported in our previous study [10]. Due to the high buffer concentration in the anode chamber, the pH did not change significantly; however, the pH in the cathode chamber increased.

Table 1. Organic carbon removal and pH changes during the four batch tests of SPMDC

	Batch 1	**Batch 2**	**Batch 3**	**Batch 4**
COD removal	31% ± 3.4	22.5% ± 0.18	29% ± 1.63	28% ± 1.08
Initial Cathode pH	6.6	6.9	6.7	7.3
Final Cathode pH	10.3	11	10.6	10.83

The voltage generation profiles for continuous flow PMDC and photobioreactor PMDCs are shown in **Figure 3**.

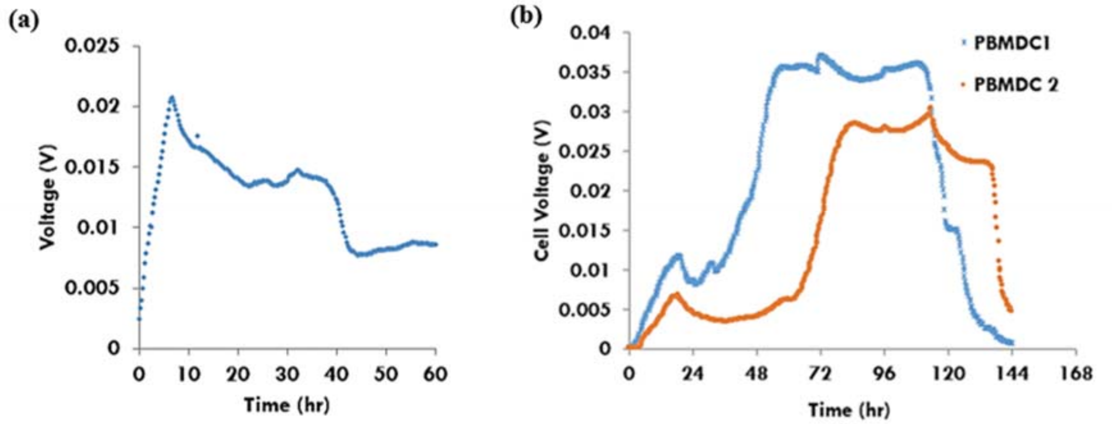

Figure 3. Voltage generation profiles in (a) PMDC with an algae biocathode under continuous flow operational mode (CPMDC); b) PMDC with an algae biocathode connected to a photo-bioreactor (PBMDC)

Due to the potential limitation for algae growth in static PMDCs, the cathode chamber of PMDCs was connected with a peristaltic pump to a 1000 mL bioreactor containing algae cells in suspension (Figure 2b). About 100 mg of sodium bicarbonate was added to the algae container with 1000 mL of growth medium to provide the inorganic carbon source for photosynthetic activity by algae cells. The electricity generation for continuous flow PMDC (CFPMDC) is shown in Figure 3a. The Maximum voltage obtained during this cycle (20 mV) was lower than SPMDC. It has been reported before that immobilized cells produce higher electricity than suspended algae [17]. This could be due to the improved oxygen reduction with easy electron transfer when algae cells deposit on the surface of the electrode whereas in the continuous mode less amount of cells settled at the surface due to the suspension. This indicates the catalytic role of algae for oxygen reduction beside its role as an oxygen supplier. The catalytic role of algae cells was reported by Cai et al. [18] in a previous study where photosynthetic biocathode generated higher electricity in comparison to the abiotic that was aerated to have the same level of dissolved oxygen (DO). Walter et al. [19] performed cyclic voltammetry analysis of a photo-biocathode and abiotic control electrode and found reduction peak for biocathode whereas no peak was observed for abiotic control electrode.

A new PMDC configuration integrating two PMDCs with one large algae biocathode chamber was developed (Figure 2c). The algae cells were maintained in suspension with a mechanical mixer. This new configuration was named as photo-bioreactor MDC (PBMDC). The advantage of this system is that two MDCs could work at the same time with one common photosynthetic biocathode chamber which may increase the overall efficiency of the system. The cell voltage for this new configuration is depicted in Figure 3b. The electricity generation profiles for the two cells followed a similar pattern. The system reached its maximum cell voltage after 50 hours and could maintain its maximum voltage for almost 50 hours. The maximum cell voltage was still lower than the SPMDC due to the aforementioned reasons in the previous section. The other reason could be due to the very large ratio of the cathode volume to electrode surface which may decrease the efficiency of this system. It has been reported before that scaled MFCs could not generate power as well as smaller scale MFCs due to the higher internal resistance that was created [20]. Our new system however, operated very well in large volume algae production. The concentration of algae cells increased from 135 mgl-1 to 362 mgL^{-1}. The system could work longer due to the better design of the system for growth of algae cells compared to CFPMDC. pH and DO profiles are similar to CPMDC.

3.2. Anammox microbial desalination cells

Voltage profiles generated by Anammox MDC (AnxMDC) for three batch experiments are shown in Figure 4. Since we did not provide any chemical catalyst or aeration in the cathode chamber, the production of electricity indicates the effective role of anammox bacteria as biocathode and Nitrite/Nitrate as electron acceptor. Increase of maximum power for the third batch experiment compared to the first and second test demonstrates an improvement in the catalytic activity of the biofilm. The maximum produced voltage was 0.0896 V which is equal to power density of 0.114 W/m^3. These data highlights the fact that electricity generation by these cells has the potential to improve by several batch tests and better formation of the biofilms on the electrodes.

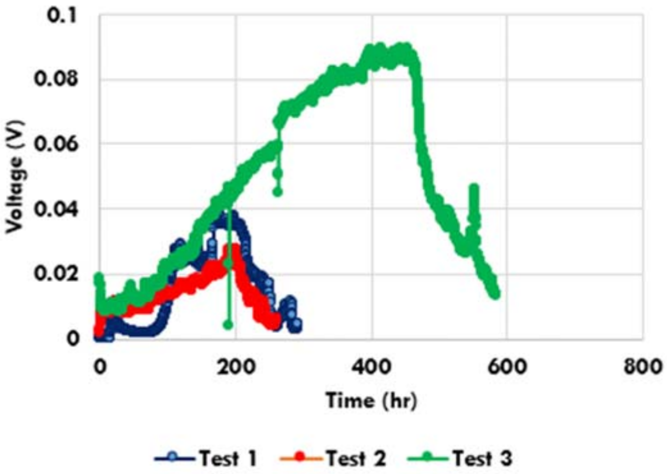

Figure 4. Voltage generation by AnxMDC during three batch tests

Only 29% of the organic carbon in the anode chamber in the third batch was removed to generate electricity. Coulombic efficiencies and salt removals increased over the three batch tests. The coulombic efficiencies for glucose oxidation were 3.4%, 6.02% and 52.7% respectively for the three batch tests while the coulombic efficiencies for nitrite/nitrate reduction were 17.5%, 35.6% and 99% respectively for the three tests. The improvement in coulombic efficiency over the three batch tests indicates the improvement of microbial growth on electrodes after several batches. Due to the higher electricity production and longer operating time, salinity removal was also higher for the third test.

4. Conclusions

Photosynthetic MDCs (PMDCs) can be operated either in fed-batch, batch or continuous flow conditions for maximizing the energy recovery from wastewater. The findings of this study demonstrate the beneficial use of photosynthetic microorganisms as biocathodes or biocatalysts in microbial desalination cells to produce oxygen, algae biomass and nutrient removal from wastewater. Different efficiencies of PMDC (Static, Continuous and Photobioreactor PMDC) observed in this study show that the design and process configuration play a critical role in the overall efficiency of the system. If harvesting higher biomass is the major target, open large scale systems are more suitable whereas for small systems, closed and static systems are more beneficial. The nutrient removal capability of PMDCs provides the opportunities to utilize agricultural, food and other industrial wastewaters that are rich in nutrients for use as catholyte medium.

The study demonstrated the feasibility of using an autotrophic microbial culture containing anammox bacteria as the biocathode of MDC to contribute in simultaneous energy generation and wastewater treatment. Batch experiments improved the coulombic efficiency of the system as well as the nitrite and ammonium removal of the wastewater. A maximum power of 0.114 W/m^3 with more than 90% removal of ammonium was achieved in this system. The finding of this research showed that this system is more useful for wastewaters with low C/N ratio to suppress the possibility for growth of heterotrophic bacteria. The proposed MDC configurations demonstrated great potential to replace conventional energy intensive nutrient removal process while at the same time generating clean energy and water and possibly valuable microalgae biomass.

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Author Contributions: Gude V.G. conceived and designed the experiments; B.K. performed the experiments; B.K analyzed the data; V.G.G and B.K. wrote the paper."

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