Journal of Biosensors & Bioelectronics

Quek et al., J Biosens Bioelectron 2014, 5:2
http://dx.doi.org/10.4172/2155-6210.1000152

Research Article Open Access

# Bio-Electrochemical Sensor for Fast Analysis of Assimilable Organic Carbon in Seawater

**Soon Bee Quek, Liang Cheng* and Ralf Cord-Ruwisch**

*School of Engineering and Information Technology, Murdoch University, Australia*

***Corresponding author:** Liang Cheng, School of Engineering and Information Technology, Murdoch University, Australia, Tel: +618 9360 2804; E-mail: L.Cheng@murdoch.edu.au

**Rec date:** May 09, 2014, **Acc date:** May 30, 2014, **Pub date:** June 05, 2014



## Abstract

A Microbial Fuel Cell (MFC) based biosensor for the determination of Assimilable Organic Carbon (AOC) in seawater was developed by establishing an anodophilic marine biofilm on the surface of an electrode poised at +250 mV (vs Ag/AgCl) rather than the traditionally used potentials of about -300 mV. A linear correlation ($R^2$>0.99) between electrochemical signals (peak current) and acetate concentration ranging 10 to 55 μM was achieved.Using the positive anodic potential enabled the rapid establishment of the electrochemically active anodophilic biomass within a period of less than 8 days, a higher sensitivity (0.017 mA/μM acetate added) and a lower detection limit (2.5 μM acetate, 0.16 mg $O_2$/L of Biological Oxygen Demand (BOD)) compared to the negative anodic potential. Further, it was shown that this bio-electrochemical AOC sensor could tolerate the presence of low concentrations of dissolved oxygen. The established potentiostat controlled MFC biosensor could be used for the purpose of online water quality monitoring for seawater desalination plants prone to biofouling of RO membranes.

**Keywords:** Biosensor; Microbial fuel cell; Seawater desalination; Biofouling; Potentiostat; Assimilable Organic Carbon

## Introduction

In the past few decades, clean water supplies have become a lot more critical due to the excessive usage and increasing contamination of natural water sources. In recent years, Reverse Osmosis (RO) has become an important technology to provide clean water from seawater. It is reported that membrane-based seawater desalination accounts for about 44% of the installed capacity of water desalination in the world [1].

Biofouling is referred to as the deposition and growth of bacterial biofilms on membranes, which is a universal phenomenon occurring in membrane water purification process. Biofouling has been reported as a serious problem for seawater RO systems [2], which introduces an additional hydraulic resistance, increases the feed channel pressure drop, and enhances concentration polarization. This causes significant deterioration in the performance and efficiency of the RO membranes.

The presence of organic pollutants in seawater is one of the main causes of RO membrane biofouling. The attached bacterial cells produce exopolymers and multiply by the uptake of soluble organics from the feed water. Therefore, monitoring the nutrient content, such as Assimilable Organic Carbon (AOC) one of the main food sources for the bacteria, in the feed water is important to predict the biofouling potential of particular seawater and enable possible measures to minimize biofouling.

Several methods have been reported to quantify AOC in wastewater or seawater. The techniques based on the Biochemical Oxygen Demand (BOD) detection are widely used, such as traditional BOD5 test requiring a 5 day off-line laboratory incubation making it not suitable for a fast and online monitoring system [3]. Recently, other types of BOD biosensors based on measuring the change in Dissolved Oxygen (DO) by a suspended or immobilised microbial biomass were reported [4,5]. Mediator modified BOD tests also gained interest because of the much faster analysis time [6-11]. The test bacteria would reduce the mediator, which can be detected photospectrometically or electrochemically. However, those methods are not designed for online measurement, as regular sample harvesting is required.

In a MFC, microorganisms growing on the surface of anode electrode generate an electrical current by transferring electrons from the oxidation of organic matter present in the solution through an electrical circuit to a cathode electrode [12-14]. No transducer is needed to convert the measured signal to an electrical signal because the measured signal is already an electrical current. The current study aims at developing a potentiostat-controlled MFC-based AOC biosensor and optimizing it for the detection of low AOC in seawater. The potentiostat control of the anode is expected to provide greater accuracy than the use of traditional MFC.

## Material and Methods

### Marine Microbial Fuel Cell Biosensor

### Bacterial Inoculum and Growth Medium

The bacterial inoculum was extracted from ocean sediment, collected from Coogee Beach, Coogee, South Fremantle, Western Australia. The sediment was mixed with seawater with a weight ratio of 1 to 5 followed by continuously stirring for 24 hours. After settling for 2 hours the supernatant with $OD_{600nm}$ value of about 0.2 was collected and used as inoculum for the establishment of the marine anodophilic biofilm. Seawater was used as anolyte (working electrode compartment) and catholyte (counter electrode compartment). In RO plants, suspended solids that are present in the feed-water will be removed by ultra-filtration. Therefore, this study utilised real seawater

with no suspended solids ($OD_{600nm}$<0.01) to demonstrate the applicability of this method in industry. Yeast extract solution was periodically added (ca. every 2-5 days) to the anolyte (50 $mgL^{-1}$ final concentration) as bacterial growth supplement.

### Microbial Fuel Cell Sensor Set up

A two-chamber MFC (made of transparent Perspex) was used in the present study. The compartments of the fuel cell (anode and cathode) having equal dimension of (9 cmx6 cmx1 cm) were physically separated by a cation selectively exchange membrane (CMI-6000, Membrane International INC.) with a size of 59.4$cm^2$. Both chambers were filled with conductive graphite granules (EI Carb 1000, Graphites Sales, Inc., Chagrin Falls, OH, USA) of about 2-6 mm in diameter. As current collectors, two graphite rods (3 mm diameter and 10 cm length) were inserted into the anodic and cathodic chambers, which acted as the working and counter electrodes, respectively. A potentiostat was connected to the electrodes and was used to maintain the anodic potential. The potentials of the electrodes were measured and controlled against a saturated Ag/AgCl reference electrode (BASi, MF-2079) placed inside the anodic chamber.

### Microbial Fuel Cell Sensor Operation

### Start-up Procedure

The MFC was operated in a fed-batch mode with both catholyte and anolyte continuously re-circulating over the cathodic and anodic compartments, respectively. The anodic chamber (as described in Section 2.1.2) was inoculated with 100 mL of seawater containing 50 mL of the inoculum (prepared according to the procedure described above), 50 $mgL^{-1}$ yeast extract and 10 mM acetate. A 10 mL bottle was connected in the anolyte-circulating loop for pre-mixing of acetate addition and the anolyte prior to introducing to the anodic compartment. The cathodic compartment was filled with 50 mL seawater as the catholyte (Section 2.1.1).

After the anodophilic biofilm had been successfully established (indicated by a steady current production), the anodic compartment was flushed with fresh seawater to remove all suspended biomass.

### Acetate Detection Procedure

For calibration purposes specified concentrations of sodium acetate, which represents readily assimilable organic carbon, were added into the anolyte-circulating loop via a septum-sealed injection port. The anode was controlled at different potentials ranging from -250 mV to +600 mV (vs Ag/AgCl) using the potentiostat.

### Control and Monitoring

The anolyte and catholyte were maintained at room temperature. The anodic compartment was kept under anaerobic conditions unless stated elsewhere. The anodic potential, current and pH were monitored continuously using LabVIEW™ 7.1 software interfaced with a National Instrument™ Data Acquisition Card (DAQ).

Control and monitoring of the biosensor was automated and online. In the experiments of testing the response of the biosensor to acetate additions, automated acetate dosing was implemented using a computer feedback-controlled peristaltic dosing pump. The online interpretations of a "steady anodic potential" and/or baseline current were used as the reference set point in the LabBIEW™ feedback control program.

### Analytic Methods

The current production, which is with mA level and proportional to the rate of acetate oxidation by the anodophilic bacteria, can be retrieved by the potentiostat. Cumulative charges were calculated by integrating the electrons transferred by the biofilm as current throughout the detection period [15]. The signals (current peak/cumulative charges) obtained from the acetate addition were calculated by subtracting the background values. Steady state was defined as no changes in current (± 0.1 mA). The recovery time was defined as the time required for the current returns to the original level after the depletion of acetate.

## Results and Discussion

### The Development of Marine-MFC Biosensor and the Effect of Anodic Potentials

Ideally, a rapid start-up of MFC-biosensor is desired for practical applications. In the current study, the biosensor was ready to be used after operating the inoculated anodic compartment at a fixed potential of +250 mV (vs Ag/AgCl) for about 7 days. This start-up time was about 2 to 4 times faster compared to that needed when using negative anodic potential conditions (data not shown). This finding is in line with previous studies, which demonstrated that a higher anodic potential increased the growth rate of anodophilic bacteria, resulting in faster microbial colonisation and quicker start-up of MFCs [16-19]. The enhanced bacterial growth rate at positive anodic potential is probably due to the greater available energy [16,17,20]. Moreover, it has also been suggested that at positive anodic potentials, the positive charged electrode surfacecan enhance the adhesion of bacteria with negative charged cell membranes (e.g. Geobacter) [21]. In the current study, the MFC operated at +250 mV anodic potential resulted in an about 2-fold higher current peak and cumulative charges compared to results obtained at -250 mV anodic potential (Figure 1). This finding is consistent with previous published works, which has demonstrated that anodophilic bacteria produce higher currents at higher anodic potentials due to the faster substrate oxidation rate [22-25].

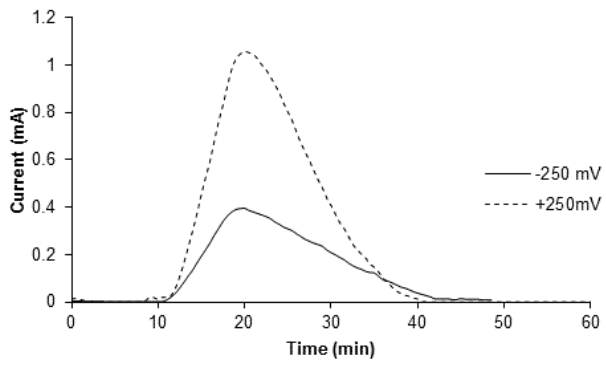


**Figure 1:** Effect of anodic potential (-250 and +250 mV (vs Ag/AgCl)) on the responses of the MFC-biosensor to the acetate addition (50 µM).

## Standard Curves and Detection Limits

The reliability of the established MFC biosensor and the correlation between the signal production and acetate concentration were tested at +250 and -250 mV anodic potentials. The peak current values (maximum current minus background current) were plotted against the acetate concentrations (Figure 2) revealing a linear relationship with high $R^2$ values (> 0.99) for both tested anodic potentials. However, the sensitivity (mA/μM acetate added) and detection limits were improved by 4 and 2 times respectively at +250mV anodic potential (Figures 2 and 3). The lowest detection limit was based on a signal-to-noise ratio of 3 [26]. The use of an even higher anodic potential up to +400 mV did not improve detection limit (2.5 μM acetate, 0.16 mg $O_2$/L BOD equivalent) (Figure 3).

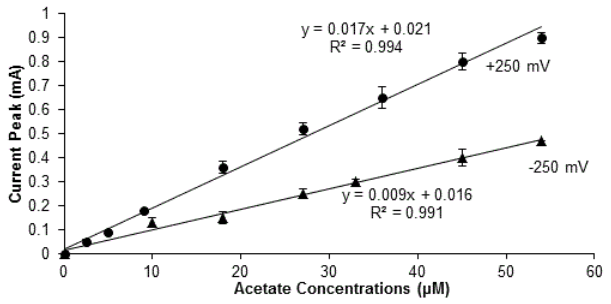


**Figure 2:** Correlation between acetate concentrations and current peak at different anodic potentials, -250 and +250 mV (vs AgAgCl).

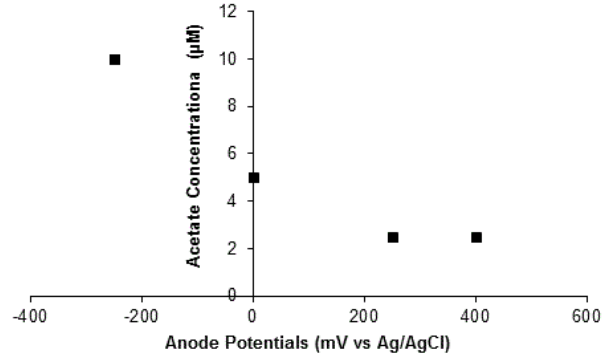


**Figure 3:** The lowest acetate concentration that can be detected by the established MFC biosensor at different anodic potentials.

From fundamental considerations, the linear correlation between current peak value and acetate concentration only holds true if the acetate oxidation reaction is of first order with respect to the acetate concentration. However, this is known to be not the case for microbial or enzyme based reactions when the substrate concentration is increased and saturation behavior sets in. In those higher concentrations, dilution of seawater prior to detection might be necessary.

## Sensitivity to Dissolved Oxygen

In typical MFC, the presence of oxygen completely suppresses the metabolic activity of electrochemically active anodophilic bacteria and hence signal formation [27]. This would possibly be a critical problem for the application of the biosensor in the real desalination plant as the MFC-biosensor should also be able to operate in the presence of Dissolved Oxygen (DO). In order to overcome this outcompeting effect of oxygen over the anode as electron acceptor by the bacteria, the anode potential was increased to a level that is higher than the redox potential of oxygen (about +80 mV (vs Ag/AgCl) considering the effect of overpotential) [28]. Then, the biosensor was tested for its response (current generation) to the addition of low concentration of acetate (20 μM) in the presence of DO (0 to 3 mg/L) (Figure 4).

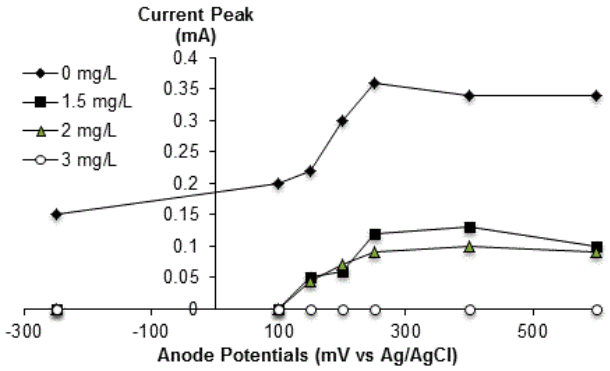


**Figure 4:** Current production from the addition of 20 μM acetate at different fixed anodic potentials (-250 to +600 mV (vs Ag/AgCl)) in absence and presence of various dissolved oxygen (0, 1.5, 2, 3 mg/L).

In the presence of low DO concentrations (< 2 mg/L), the anodic biofilm produced current production at high anodic potentials from +150 to +600 mV (vs Ag/AgCl) but not at anodic potentials lower than +100 mV (vs Ag/AgCl). This suggests that the anodophilic biofilm that had been enriched during the 7 days of operation at +250 mV was able to transfer electrons to the positive electrode in the presence of low dissolve oxygen (< 2 mg/L). However, the reduced current peak (3-fold lower) associated with the oxygen consumption (data not shown) suggested that a portion of the acetate was used for the aerobic respiration at positive anodic potentials. The observation that at low oxygen concentrations and high potentials the anode is used simultaneously with oxygen suggests that the flow of electrons to either oxygen or anode is of competitive nature. Using potentials higher than +250 mV did not further eliminate the interfering effect of oxygen. This result suggests that the anodic potential is an important factor in collecting electrons from microbial organics oxidation as it can influence the "attractiveness" of the anode compared to oxygen. The more positive redox potential of a terminal electron acceptor (i.e. higher anodic potential) the higher energy gains for a microorganism.

As the signal production from AOC was suppressed at DO concentrations higher than 2 mg/L, the elimination of DO from seawater is still necessary for the practical applications of the biosensor. The combination of an electrochemical online oxygen removal with the sensor described here is currently in progress in our laboratory.

## Conclusions

The sensitive and accurate online monitoring of low levels of organic pollutant (i.e. AOC) in seawater is essential to predict the biofouling potential of the feedwater to RO desalination plants. The use of positive anodic potentials for development of the anodophilic biofilm and operation is of advantage compared to the traditionally negative anodic potentials used as it allows a faster development of the sensor biofilm and improved signal quality.

In the current study acetate was only used as a preliminary substrate to establish a proof of concept and optimize the sensor performance under the well-controlled conditions. In the further, we plan to improve this sensor by testing complex organics and develop a disposable-type anode for the real application.

## Acknowledgement

The authors acknowledge the financial support of the National Centre of Excellence in Desalination Australia, which is funded by the Australian Government through the National Urban Water and Desalination Plan. The authors would also like to thank Murdoch University, AquaMen, Valoriza Agua (Spain) and Nanyang Technical University (Singapore) for their advice and financial support.

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