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Detection of low concentration of assimilable organic carbon in seawater prior to reverse osmosis membrane using microbial electrolysis cell biosensor

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ABSTRACT

Biofouling of reverse osmosis (RO) membranes is one of the most serious problems encountered for seawater desalination. This problem is commonly associated with a significant decline in flux, elevated energy requirement, and increased cost of operation. As the biofouling of the membranes is due to bacteria growing on the membrane, which is supported by assimilable organic carbon (AOC), a good AOC monitoring system is essential and crucial for RO biofouling prediction. This study focuses on the development of a new biosensor that is online, robust, and allows accurate quantification of AOC concentrations in seawater based on a microbial electrolysis cell (MEC) principle. The biosensor is based on the quantification of the current generated by bacteria in the presence of AOC. The biosensor response to AOC was rapid (within 10 min) and sensitive (detection limit = 10 μM acetate) in seawater samples. The results reproducibly showed a linear relationship between trace amounts of AOC and electrochemical signals ($R^2 > 0.99$). The MEC-based biosensor developed can be effectively used as an online and rapid measure of AOC concentrations and hence as an indicator for biofouling potential of influent seawater prior to RO membrane.

Keywords: Biosensor; Microbial electrolysis cell; Seawater desalination; Biofouling; Assimilable organic carbon

1. Introduction

Membrane biofouling occurs from the accumulation of bacteria on the membrane surface, and is by far the most problematic type of fouling in seawater desalination by reverse osmosis (RO). Biofouling leads to significant decrease in permeate flux, elevated energy requirement, the need for regular chemical cleaning, membrane deterioration, decreased water production, compromised water quality, and increased cost of operation [1].

Membrane biofouling depends on many factors. These include the presence of micro-organisms, oxygen availability, water temperature, and most importantly the concentration of assimilable organic carbon (AOC) that are present in seawater [2]. In RO desalination plants, suspended solids (e.g. microorganisms)

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that are present in the feed water will be removed by ultrafiltration. AOC is one of the main food sources for bacterial communities in the biofilms and hence it is often used as an indicator of the relative biofouling potential of feed water. Numerous biochemical oxygen demand (BOD)-based techniques have been considered to enable the quantification of AOC such as BOD_5 [3], modified bioassay [4], optical fiber [5], bioluminescence [6,7], and flow cytometric enumeration [8]. However, these methods can be labor intensive, time consuming, need a pure culture, or are not sensitive enough for early detection of biofouling potential. Therefore, a critical need exists for a better AOC monitoring and measurement tools for predicting biofouling.

In the recent years, microbial fuel cell (MFC)-based biosensors, which use the principle of electron transfer caused by oxidation of organic matter by bacteria, have demonstrated great potential to determine the concentration of organic carbon [9–11]. Recent work has demonstrated this principle of AOC measurement for marine conditions at concentrations sufficiently low to detect AOC levels encountered in typical ocean water. Traditional MFCs use an external resistor in the external electrical circuit [9,10,12]. However, these MFCs are difficult to operate continuously at a constant anodic potential (AP) over a long term due to deterioration of the catholyte (e.g. ferricyanide) and the lack of control over the AP. Furthermore, ferricyanide, used as catholyte within traditional MFCs, is not only toxic but also non-renewable. An alternative is to use a microbial electrolysis cell (MEC), where a potentiostat is used to control the AP.

The aim of the present study is to develop a MEC-based biosensor that is: (a) sensitive to trace amounts of organic matter in the ocean (~100 μmol/L dissolved organic carbon); (b) rapid; and (c) online to be used as an early warning system for biofouling potential.

2. Materials and methods

2.1. Marine-MEC biosensor

2.1.1. Bacterial inoculum and growth medium

The biofilm for the biosensor in the anodic compartment originated from marine sediment at Coogee Beach, Coogee, South Fremantle, Western Australia. The sediment was mixed with real seawater (obtained from the same location) with a weight ratio of 1:5 followed by continuous stirring for 24 h. After settling for 2 h, the supernatant with OD_{600} value of about 0.2 was collected and used as inoculum for the marine anodophilic biofilm. Seawater obtained at the same location was used as anolyte and catholyte. In RO plants, suspended solids that are present in the feedwater will be removed by ultrafiltration. Therefore, this study utilized real seawater with no suspended solids ($OD_{600} < 0.01$) to demonstrate the applicability of this method in industry.

For the first 10 d, yeast extract solution was periodically added (ca. every 5 d) to the anolyte (50 mg L^{-1} final concentration) as bacterial growth supplement. The catholyte was renewed periodically.

2.1.2. MEC sensor set up

A two-chamber MFC (made of transparent Perspex) was used in the present study. The chambers (anode and cathode) of the fuel cell having equal dimension (9 cm × 6 cm × 1 cm) were physically separated by a cation selective 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) about 2–6 mm in diameter. A potentiostat was used to control the AP. To facilitate electrical connections, two graphite rods (5 mm diameter and 10 mm length) were connected by conductive wire to the potentiostat's anode and cathode, respectively. The graphite rods were then inserted into the granules of the respective anode and cathode chamber. The potentials of the electrodes were measured against a saturated Ag/AgCl reference electrode (BASi, MF-2079) placed inside the anodic chamber.

2.2. MEC sensor operation

2.2.1. Startup procedure

The anodic chamber (as described in Section 2.1.2) of the MEC sensor was inoculated with 50 mL of inoculum (prepared according to the procedure described above) and 60 mL of seawater containing 50 mg L^{-1} yeast extract and 10 mM of acetate. The cathodic chamber was filled with 110 mL of catholyte (as described in Section 2.1.1). The MEC was operated in a fed-batch mode with both catholyte and anolyte continuously recirculating via the cathodic and anodic compartments, respectively. The MEC was maintained at −300 mV (vs. Ag/AgCl) throughout this study, otherwise where stated in the experiments.

After the anodophilic biofilm had been successfully established (indicated by a steady current (>1 mA) production), the anodic chamber of the MFC was drained to remove utilized anolyte and refilled with fresh seawater.

2.2.2. Acetate detection procedure

Specific concentrations of sodium acetate (as stated in the Section 3), which represent readily AOC and are utilized by the marine anodophilic bacteria, were fed into the anolyte via a septum-sealed injection port to test for electrical signal production.

2.3. Control and monitoring

Control and monitoring of the biosensor was partly automated. The anolyte and catholyte were maintained at room temperature and ambient atmospheric pressure. The anode was kept under anaerobic conditions. The AP, cell potential (potential differences between anode and cathode) and pH were monitored continuously using LabVIEW™ 7.1 software interface with a National Instrument™ data acquisition card (DAQ). All data were logged every 30 s into an Excel spreadsheet using LabVIEW™ 7.1. The pH of the anolyte was controlled at 8 ± 0.2 manually using 1 M sodium hydroxide.

In experiments where acetate was added to test the response of the biosensor, automated acetate dosing was implemented using a computer feedback-controlled peristaltic dosing pump. The steady baseline current was used as the reference set point in the LabVIEW™ feedback control program.

2.4. Determination of current and cumulative charges

The electron (acetate addition) flow from anode to cathode in MFCs is proportional to the rate of acetate oxidation by the bacteria. The electrons obtained from acetate oxidation can be retrieved as current using the potentiostat. Cumulative charges were obtained by integrating the electrons transferred by the biofilm as current throughout the detection period [13].

The steady state was defined as no changes in current (±0.1 mA) over a period of 10 min. Recovery time was defined as the time required for the AP to return to the initial level after the depletion of acetate. The signal (current peak) obtained from acetate addition was calculated by subtracting the steady-state value from the current value after the addition of acetate.

3. Results and discussion

3.1. Marine-MEC biosensor startup

Our previous study found that a traditional MFC could be developed as a biosensor under marine conditions and responded to the addition of low concentration of organic substances (acetate). To investigate to what extent a potentiostat can be used to operate a marine-MEC, a MEC was inoculated with a marine inoculum (as described in Section 2.1.2) and operated over 14 d for the establishment of a marine biofilm that could respond to the addition of organic substances (acetate) (Fig. 1).

Over this period, the AP was maintained at −300 mV (vs. Ag/AgCl). The current generated by the bacteria in the presence of acetate increased steadily over the first 14 d after inoculation (Fig. 1) indicating the growth of anodophilic marine bacteria and suggesting that a potentiostat can be used to develop a MEC biosensor. This result is in accordance with previous studies reporting the use of potentiostats for the development of MECs (inoculated with the effluent of active acetate fed MFCs), where bacterial growth was recorded as changes in current [14].

3.2. Marine-MEC biosensor responsiveness

3.2.1. Reproducibility

The results above showed that the potentiostat-controlled marine biofilm in the MEC was successfully established. One of the main performance criteria of biosensors is the reproducibility of their response to a given concentration of substrate. Therefore, it is important to test the signal reproducibility of this MEC-biosensor. The response of the MEC-biosensor can be quantified by detecting the oxidation of AOC by anodophilic bacteria as current. The maximum current (peak height) and peak area could be used as amperometric and coulometric signals, respectively.

In order to investigate the reproducibility of the biosensor, three identical AOC spikes (39 μM of acetate) were added to the anodic compartment (Fig. 2)

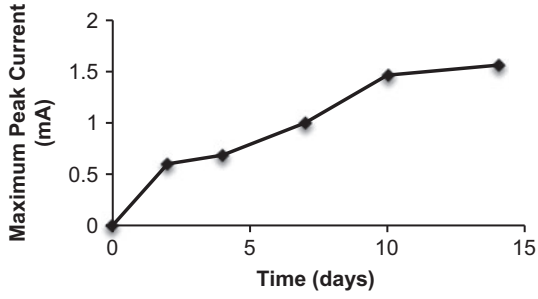

Fig. 1. Development of current generation of the anodophilic marine biofilm after inoculation over a period of 14 d. MEC biofilm was saturated with 10 mM acetate. The marine-MEC was maintained at −300 mV (vs. Ag/AgCl) and operating at room temperature and pH was maintained at 8 ± 0.2.

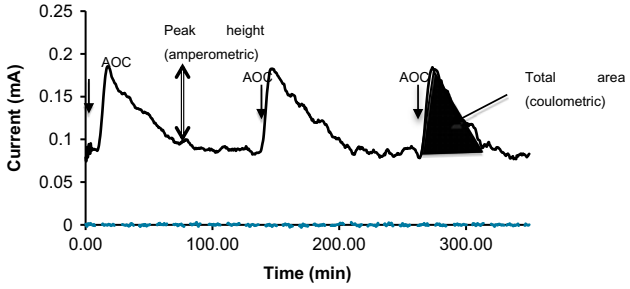

Fig. 2. The response the MEC-biosensor to three identical AOC additions (39 μM acetate). The marine-MEC was operating at AP of −300 mV, room temperature, and pH was maintained at 8 ± 0.2.

after the current had stabilized at the steady state (Fig. 2).

Over short periods of time when biomass fluctuations are negligible, the MEC-biosensor generated reproducible responses (Fig. 2). The calculated standard deviations for amperometric and coulometric signals were <5 and <3%, respectively. From the low standard deviations, one could assume that the response obtained was accurate and can be directly related to acetate concentration.

3.2.2. Standard curves, recovery time, and detection limits

The results above demonstrated that the biosensor had sufficient reproducibility to warrant further development. In order to determine the correlation between the signals (amperometric and coulombic) and acetate concentrations, a low range of acetate concentrations (0–170 μM) was introduced to the anodic compartment of the biosensor (Fig. 3). The acetate concentrations and signals generated for both amperometric and coulometric were highly correlated with R^2 values of >0.99 (Fig. 3) suggesting that the biosensor can be of high precision.

For all tested concentrations, the current peaks (amperometric peak signal) reached the maximum level within the first 10 min. By contrast, the coulometric signals could be obtained only after the peaks completed, which required between 10 min and 2 h depending on the AOC concentrations. The lowest detection limit of the described biosensor was 10 μM acetate. In comparison to other microbial organic substrate determination methods, the method in this study is more sensitive, quicker (range from 5 to 20 h [15]), and does not require pure culture (e.g. bioluminescence-based test that required *Pseudomonas fluorescens* P17 of *Spirillum* sp. Strain NOX [7]).

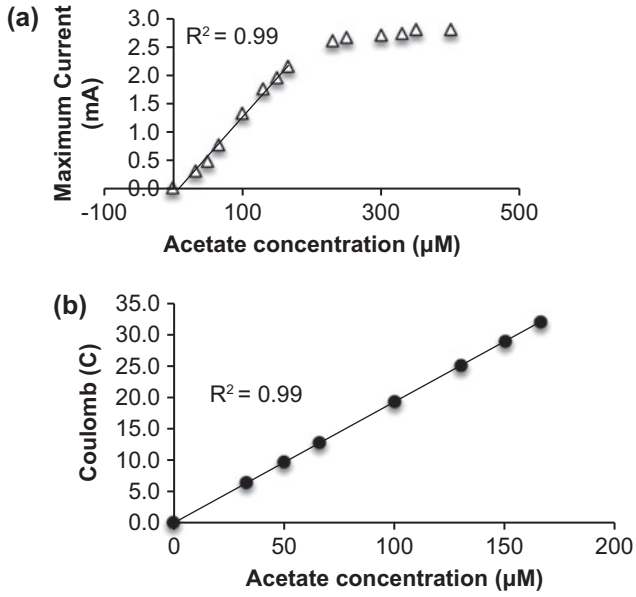

Fig. 3. The response (a) amperometric and (b) coulometric of MEC-biosensor as a function of acetate concentration (range 0–170 μM) in seawater.

It was also observed that at acetate concentrations ranging from 220 to 400 μM, the amperometric signals were no longer linear to the acetate concentration. This result is not surprising as in principle, the acetate oxidation rate is expected to be correlated to the acetate concentrations via Michaelis–Menten kinetics, showing a finite maximum current would be produced at acetate saturation conditions [13,16,17].

3.3. Increasing sensitivity and reducing recovery time

Applying different APs could affect MFCs performance but this has not been widely studied. Previous studies showed that a MFC acclimated to high AP produced higher current (30% more) than a MFC acclimated to a lower AP [15]. For maximum power production, the AP should be as low and the cathode potential as high as possible of a MFC [13]. In this study, the MEC is not used for obtaining electrical energy output but to detect low levels of AOC. The AOC sensitivity of the biosensor was further investigated by setting up and acclimatize a MEC-biosensor at a high AP of +250 mV (vs. Ag/AgCl) (as described in Section 2.1.2).

At a higher AP (+250 mV), a shorter recovery time was achieved, in comparison to those obtained at lower AP (−300 mV) (Fig. 4). A shorter recovery time is important to enable online AOC monitoring. The higher AP (+250 mV) also improved the sensitivity of

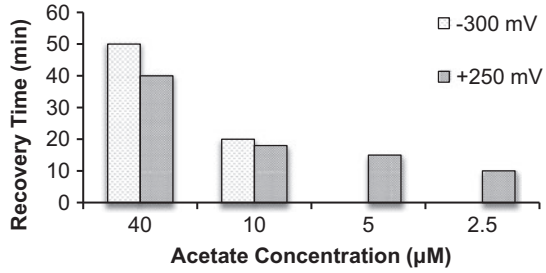

Fig. 4. Comparison of the recovery time and the sensitivity of two different AP, −300 and +250 mV (vs. Ag/AgCl), as a function of acetate concentration.

the biosensor to a level of about 2.5 μM acetate, while the linearity of the response was not compromised ($R^2 > 0.99$) (data not shown). This result indicates that higher AP could be beneficial to detect AOC using MEC-biosensor.

3.4. Limitations of the biosensor

Acetate was used in this study as the model AOC as it is often used as model organic species in the water industry [18] and it is the major breakdown product from more complex organic substances. While this biosensor had been acclimated to acetate, the response to other organic compound, such as glucose, was also tested. The signals obtained with glucose (33 μM) were lower than those obtained with acetate (33 μM) (data not shown). This is not surprising as the MEC was solely acclimated to acetate. Preliminary experiments with adaptation of the MEC to complex organic substrates (yeast extract) showed that acclimation enhanced the signals strength generation and will be described elsewhere. Other authors also demonstrated that MFC biofilms are able to metabolize a variety of complex organic substrates after an adaptation period [19–22].

There are some points that must be taken into consideration when using this biosensor to quantify AOC in seawater. In this study, seawater was deoxygenated with nitrogen gas bubbling as the dissolved oxygen present in seawater will primarily have an impact on the ability to accurately measure low AOC concentration in a MEC. The effect of dissolved oxygen on MEC should be further investigated in future studies. Parameters such as temperature and alternative organics could also be the subject of further study. The response of the biosensor should also be evaluated with real seawater instead of a model AOC.

3.5. Practical implications

The costs related to biofouling are generally comprised of: (a) additional energy costs; (b) additional chemical cleaning; and (c) decrease of membrane life due to excessive cleaning/treating the membrane. Early biofouling warning enables preventive measures to be taken, either by pretreatment optimizing or preventive membrane cleaning. The potential savings of an early warning of biofouling are estimated to be 10–20% of the annual membrane replacement, chemical costs, and energy costs [23].

The biosensor developed in this study can quantify AOC concentration in seawater rapidly. Real-time measurement of the AOC concentration in feed water with a robust MEC-biosensor as described here may provide simple early warning signals when the AOC concentration exceeds a set point, enabling corrective measures to avoid RO membranes fouling.

4. Conclusion

In comparison to previously developed methods, the MEC-biosensor developed in the current study provides real-time detection. The biosensor was in continuous operation for over three months indicating reliability. The response of the marine MEC-biosensor was rapid (within 10 min using amperometric signal), sensitive (detection limit = 10 μM of acetate), and showed a linear relationship between the trace amounts of acetate and electrochemical signals ($R^2 > 0.99$) at AP of −300 mV (vs. Ag/AgCl). A higher AP shortens the recovery time and improved the sensitivity of the biosensor.

This biosensor could, for example, be used (a) as an early warning of biofouling potential in seawater; (b) to optimize and select the appropriate pretreatment method when high AOC concentration is detected in seawater; (c) to monitor the effectiveness of pretreatment processes; and (d) change the intake water from elsewhere (e.g. beach well) if available.

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