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In-line deoxygenation for organic carbon detections in seawater using a marine microbial fuel cell-biosensor

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HIGHLIGHTS

- An electrochemical cell was developed to reductively remove oxygen from seawater.
- A MFC-biosensor could monitor assimilable organic carbon (AOC) in anoxic seawater.
- The combination of the above enabled online AOC monitoring in aerobic seawater.
- The reductive oxygen removal was specific to oxygen and caused no interference.
- Online AOC monitoring was reproducible and seems feasible for desalination plants.

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ABSTRACT

Assimilable organic carbon (AOC) is a key predictor for membrane biofouling in seawater desalination reverse osmosis (SWRO). Microbial fuel cells have been considered as biosensors for the detection of biodegradable organics. However, the presence of dissolved oxygen (DO) is known to completely suppress the signal production (i.e., current) of a typical MFC. This study describes AOC detection in normal oxygenated seawater by coupling an electrochemical cell for DO removal with a MFC-biosensor for AOC detection. The electrochemical deoxygenation for oxygen removal caused no interference in the AOC detection. A linear relationship (R^2 = 0.991) between the AOC concentration and current production from the MFC biosensor was achieved. The coupling of an electrochemical cell with a MFC-biosensor can be effectively used as an online, rapid and inexpensive measure of AOC concentrations and hence as an indicator for biofouling potential of seawater.

1. Introduction

Seawater reverse osmosis (SWRO) has been a widely applied technology for producing fresh water. One of the main challenges for successful operation in SWRO is membrane biofouling (Matin et al., 2010). Membrane biofouling involves the growth of microorganisms into a biofilm on the membrane surface, which leads to significant increase in cost of operation in SWRO treatment. Membrane flux decline, increase in pressure drops in the RO modules, increase in salt passage.

Controlling and identifying biofouling is rather challenging as it depends on various factors. There are numerous indirect measurement techniques to assess the fouling potential of feed-water. Traditional fouling potential of a membrane is measured using silt density index (SDI) and modified fouling index (MFI). These methods predict particulate fouling but are not predictive for bio/organic-fouling caused by organic adsorption or biological growth on the membrane surface. Specifically, soluble assimilable organic carbon (AOC) present in seawater is highly influential to the development of membrane biofouling (Chien et al., 2007). AOC is one of the main food sources for bacteria to proliferate and hence it is more appropriate to be used as an indicator of the relative biofouling potential of feed-water (Jeong et al., 2013). Other attempts at AOC tests such as biochemical oxygen demand BOD_5 (Bourgeois et al., 2001; Clesceri et al., 2005), modified bioassay (Lechevallier et al., 1993), optical fiber (Lin et al., 2006), bioluminescence (Weinrich et al., 2011) and flow-cytometric enumeration (Hammes and Egli, 2005) can be time consuming, need an appropriate reference organisms, and are costly with a long turn-around time for results. Hence, a critical need exists for a quick and reliable measurement of AOC in seawater.

Recent work from our research group has demonstrated that microbial fuel cell (MFC) based biosensors can detect low concentration of AOC under marine conditions in the absence of oxygen

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(Quek et al., 2014a,b). MFC-biosensor has been proven to be a convenient organic biosensor showing major advantages over many other types of biosensors given the mechanical and electronic simplicity, not only for its construction but also when signal acquisition and electronic requirements of the system are taken into consideration. MFC-biosensors have been shown to be convenient organic biosensors showing major advantages over other types of biosensors given the mechanical and electronic simplicity. Also the online signal acquisition and electronic requirements of the system are simple and rapid. In previous studies, MFC-biosensors for organic detection were developed for detecting organic substrates in anoxic waters such as wastewater (Di Lorenzo et al., 2009), groundwater (Tront et al., 2008) and anaerobic digestion liquid (Kaur et al., 2013). The use of MFC-biosensors for the detection of low concentration of AOC in marine environments is of industrial interest as it can be used in prediction and possible control strategies of bio-fouling SWRO plants. The current study focuses on the development of a MFC-biosensor for detecting low concentration of AOC under real world SWRO plant conditions, which includes the presence of oxygen, a known inhibitor of MFC sensors.

A typical MFC-biosensor consists of an anodic chamber (anaerobic) and a cathodic chamber separated by a cation exchange membrane. The active anodophilic bacteria oxidise organic carbon in the anodic chamber and generate electrons and protons. Electrons are then passed to the cathodic chamber through an external circuit and thus current is generated. However, the presence of dissolved oxygen (DO) in the anodic chamber can completely suppress the metabolic activity of electrochemically active anodophilic bacteria and hence the current productions (Liu et al., 2005; Ringeisen et al., 2007). This is quite understandable as the typical anodophilic bacteria such as *Geobacter* sp are strict anaerobes (Bond and Lovley, 2003; Lin et al., 2004). In order to obtain an accurate AOC measurement by MFC, it is mandatory to completely remove DO. It is also important to develop a DO removal method that does not interfere with the highly sensitive measurements of low concentrations of AOC measurement. Numerous physical and chemical approaches for fresh water deoxygenation have been developed, such as (a) physical methods (i.e., inert gas purging), which have inherent deficiencies of being bulky, costly and inflexible in operation (Tan et al., 2004), and (b) chemical reducing agents (i.e., hydrazine) (Moon et al., 2000), which are highly toxic and likely to modify organic species present. Therefore, these methods are not suitable for AOC analysis. The electrochemical deoxygenating method has been described as a possible method for deoxygenation of fresh water (Pedrotti et al., 1994; Tamminen et al., 1996; Vuorilehto et al., 1995). The advantages of this method are high efficiency (>99.5% DO removal) and low interference with the sample.

The aim of the current study is to develop an online low AOC detection system for oxygen-saturated seawater by combining a suitably designed electrochemical oxygen removal cell with a MFC-biosensor.

2. Methods

2.1. Marine-microbial fuel cell biosensor setup

A two-chamber MFC-biosensor physically separated by a cation exchange membrane (CMI-7000, Membrane international Inc.) filled with conductive graphite granules (EI Carb 1000, Graphites Sales Inc, Chagrin Falls, OH, USA) with internal anolyte and catholyte volumes of 100 mL was set up as described in Quek et al. (2014b). A potentiostat was connected to the biosensor was used to control the anodic potential (AP). The DO, pH, AP and cell potential were monitor continuously using LabView™ 7.1 software interface with a National Instrument™ data acquisition card (DAQ). The pH was monitored during batch and continuous operation of the MFC-biosensor and no detectable pH change was recorded from individual tests. This suggests that a pH controlling device as needed for MFC designed for electricity production (Cheng et al., 2008), will not be necessary for the real world usage of the described sensor. Marine sediment from Coogee Beach, Coogee, South Fremantle, Western Australia was used as inoculum for the biosensor. Filtered seawater obtained at the same location was used as anolyte and catholyte. Details operation and monitoring of the MFC-biosensor set up were described in our previous paper (Quek et al., 2014b).

2.2. Microbial fuel cell sensor operation

2.2.1. Operation and evaluation

The anodic chamber (as described in Section 2.1) of the MFC-biosensor was inoculated with 50 mL of the inoculum, prepared according to the procedure described in Quek et al. (2014a) and 50 mL of seawater containing 0.5 g L^{-1} yeast extract (YE). YE was added every two days. The cathodic chamber was filled with 100 mL of seawater and was replaced every 4–6 weeks. The MFC was operated in a fed-batch mode with both catholyte and anolyte continuously re-circulating at a flow rate of 5 mL/min via the cathodic and anodic compartments, respectively. Where different operation modes were used, this is specified in the results section. The biosensor-MFC was maintained at +250 mV (vs Ag/AgCl) throughout this study.

Approximately 5 days after start up, the MFC-biosensor gave responses to YE addition. The old anolyte was drained out and the anodic compartment was re-filled with fresh seawater. During batch mode, the anolyte was replaced every 3–5 days. As indicated in previous research (Cheng et al., 2014; Quek et al., 2014a), a short period (several minutes) of oxygen exposure has no effect on the sensitivity and performance of the MFC-biosensor of the MFC-biosensor operated at positive AP of +250 mV (vs Ag/AgCl).

2.2.2. Organic (YE) detection and analyses procedure

Specific concentrations of YE, which represented mixed AOC and were utilized by the marine anodophilic bacteria, were fed into the anolyte. The total COD of YE measurement was conducted according to the American Public Health Association (2005). A standard curve was drawn for different YE concentrations between 0 mg/L and 600 mg/L which resulted in a linear correlation with R^2 of 0.994 and a conversion factor of 0.64 mg COD/L per mg YE/L (data not shown).

2.2.3. Control and monitoring

The rate of electron flow from anode to cathode in microbial fuel cells is proportional to the rate of organic oxidation by the anodophilic bacterial biofilm. The electrons obtained from the organic oxidation are transferred to the anode and generate a current that is recorded by the potentiostat. To quantify the total electrons flown (charges transferred to the anode) the electron flow was integrated over the detection period. The cumulative charges were obtained by integrating the electrons transferred by the biofilm as current throughout the detection period (Cheng et al., 2014; Quek et al., 2014b,a).

The steady state was defined as no changes in current (±0.005 mA) over a period of 10 min. Recovery time was defined as the time required for the anodic potential (AP) to return to the initial level after the depletion of AOC. The signal (current peak) obtained from organic addition was calculated by subtracting the steady state value (due to maintenance respiration of the anodophilic bacteria) from the current value after the addition of AOC.

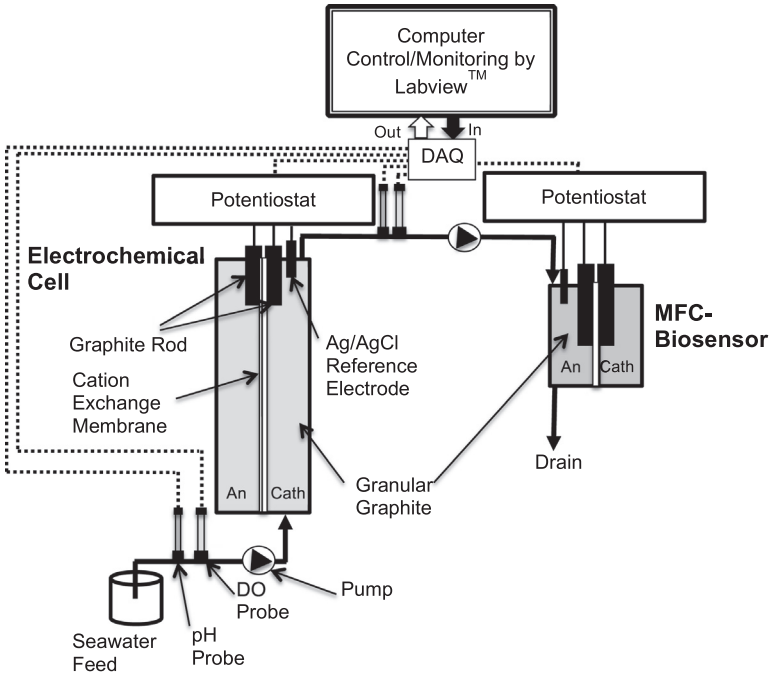

Fig. 1. Schematic diagram of the computer-feedback controlled electrochemical cell coupling with a MFC-biosensor in continuous mode in the present study.

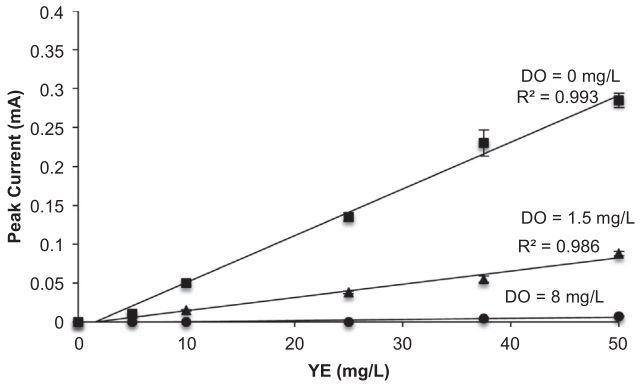

Fig. 2. Effect of dissolved oxygen on the correlation of current peak with the YE concentrations (0–50 mg/L) added in the marine MFC-biosensor at anodic potential of +250 mV (vs Ag/AgCl).

2.3. Deoxygenation methods (electrochemical cell)

Deoxygenation was carried out in a cell made of transparent Perspex, namely electrochemical cell. It was divided into two equal compartments (4.5 cm × 3 cm × 20 cm) separated by a cation exchange membrane with a surface area of 60 cm^2. Each compartment was packed with 300 g of conductive graphite granules of about 2–4 mm in diameter. The anodic compartment of the oxygen removal cell was continuously circulated with 1000 mL of seawater at a flow rate of 5 mL/min. DO and pH of the influent and effluent of the catholyte was monitored with a DO probe (Mettler Toledo) and pH probe using LabView™ 7.1 software interface with a National Instrument™ data acquisition card (DAQ). The total volume of the cathode bed of the oxygen removal cell was 200 mL. For batch experiments, the cathodic (oxygen reducing) compartment was continuously circulated with 300 mL of seawater at a flow rate of 5 mL/min. The seawater was circulated until >99.9% DO removed by the electrolysis cell. The DO removal from seawater was conducted at an electrodialysis cell and achieved by applying the oxygen reduction reaction on the electrode. Therefore, no organic matter is needed to consume the DO. In all experiments saturated oxygen (DO > 8.0 mg/L, at pH 8.2 ± 0.2) seawater flowed through the cathode compartment.

A potentiostat was used to control the cathodic potential, ranging from −800 mV to −200 mV (vs Ag/AgCl), of the cell. To facilitate electrical connections, two graphite rods (5 mm diameter and 8 mm length) were inserted into the anodic and cathodic graphite beds and connected to the counter- and working-electrodes of the potentiostat, respectively. The potentials of the electrodes were measured against a saturated Ag/AgCl standard reference electrode placed inside the cathodic chamber.

As a comparison, the DO removal from seawater was also conducted through bubbling nitrogen gas until the DO value was less than 0.1 mg/L. No stirring was used because the mixing caused by the passage of the nitrogen was considered sufficient to keep the seawater well mixed. Nitrogen was allowed to escape via a small vent hole.

A schematic diagram of the computer-feedback controlled electrochemical cell coupling with a MFC-biosensor in continuous mode in the present study is shown in Fig. 1.

3. Results and discussion

3.1. Operation of the marine MFC biosensor without oxygen removal

In early studies, MFCs were mainly focused on power generation. For maximum power production, the anodic potential of a MFC is negative and the cathode potential positive (Cheng et al., 2008). In this study, the MFC is not used for obtaining electricity output but to be used as a biosensor to detect low levels of AOC by using a potentiostat which typically keeps the cathode more negative than the anode. Our previous laboratory study found that the use of a positive anodic potential (i.e., +250 mV) for a MFC-biosensor instead of the traditional negative anodic potential of around −300 mV

(a) could shorten the start-up time (the current peak reached a steady state in the MFC during organic saturation) by 2 to 4 times (Quek et al., 2014b),

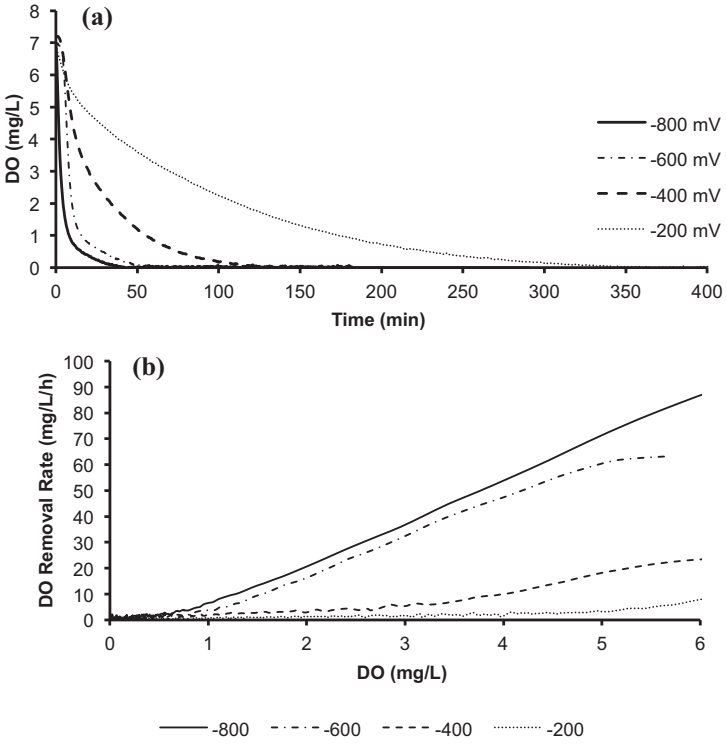

Fig. 3. (a) Time course of oxygen removal in seawater at four different cathodic potentials between −800 and −200 mV (vs Ag/AgCl) and (b) the oxygen removal rate at different cathodic potentials using the electrochemical cell under batch mode operation.

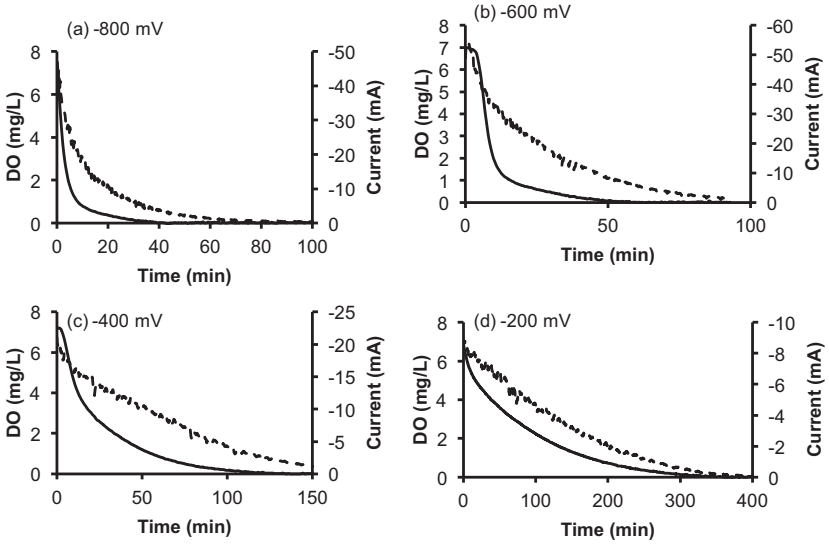

Fig. 4. Current production (dots) during the removal of dissolved oxygen (lines) at cathodic potential between −800 and −200 mV (vs Ag/AgCl) under batch mode operation.

(b) resulted in a 2-fold stronger electrical signal production (current peak and cumulative charges) from the addition of low concentrations of organic substrates (acetate) (Quek et al., 2014a) and
(c) increased the tolerance to low concentrations of DO (<2 mg/L) (Quek et al., 2014b).

In this study a MFC biosensor acclimatised to YE was set up (as described in Section 2.1) and operated for 10 weeks at AP of +250 mV (vs Ag/AgCl). When reproducible current production was obtained, the biosensor was tested for its ability to respond to changes of low YE concentrations at different DO (0, 1.5 and 8 mg/L) (Fig. 2).

The addition of small YE spikes caused increases in current leading to a current peak typically within a few minutes, which is in line with the previous results of acetate detection (Quek et al., 2014a). The current peak values were plotted against the YE concentrations under various DO (0, 1.5 and 8 mg/L). Results show that high DO (<8 mg/L) completely suppressed the signal, while at DO less than 1.5 mg/L, current signals were obtained. This result is in accordance with previously published results where anodophilic bacteria cultivated at a positive electrode potential

of about +250 mV were not completely inhibited by the presence of low DO (Quek et al., 2014b). This is in contrast to observations made with anodes operated at the typical negative potentials of −300 to −400 mV used for MFC for the purpose of power production.

The current peaks obtained in the presence of low DO (<1.5 mg/L) were about 3-fold lower than that obtained in the absence of DO, which is probably due to a portion of YE being used for aerobic respiration. This oxygen consumption by anodophilic biofilms developed at high potentials of +250 rather than −300 mV vs Ag/AgCl has been described earlier (Cheng et al., 2014; Quek et al., 2014b). Under both conditions, the correlation between the signal production and YE concentration was linear with high R^2 values of 0.99 and 0.98, respectively (Fig. 2).

The signal production was completely suppressed at DO concentrations higher than 2 mg/L indicating that for practical applications, there is a need to eliminate DO from seawater to enable accurate detection of low AOC concentration using the MFC biosensor. Previous studies showed that the signals suppression by the DO (act as alternative electron acceptors) can be overcome by adding an external redox-mediator, such as potassium hexacyanoferrate (HCF (III)) (Cheng et al., 2014), azide and cyanide (Chang et al., 2005). However, these redox-mediators would need to be maintained at constant concentrations in the MFC, which would limit the device's practical application for continuous operation.

3.2. Efficiency of dissolved oxygen removal in seawater using an electrochemical cell

Dissolved oxygen removal using electrolysis cell has been described for freshwater (Vuorilehto et al., 1995). In this study, an electrochemical cell was set up as described in Section 2.3 to test its capability to remove DO from seawater. The electrochemical reaction of oxygen reaction can be expressed as follows (Eq. (1)):

$$O_2 + 2H_2O + 4e^- \rightarrow 4OH^- \quad (E^0 = 178\ \text{mV vs Ag/AgCl}) \qquad (1)$$

3.2.1. Effect of cathodic potential

In order to achieve a fast DO removal for a frequent (or continuous) and rapid online AOC detection, the electrochemical DO removal cell must be designed to remove oxygen completely without generating other by-products such as hydrogen or modified organic or inorganic compounds. According to electrochemical theory, the kinetics of an electrochemical reaction is affected by the applied voltage. The reduction reaction proceeds faster when a more negative potential is applied (Crow, 1988). In the current study, the DO removal efficiency was tested at different cathodic potentials varying from −800 to −200 mV (vs Ag/AgCl). Cathodic potentials were chosen to be higher than −800 mV (vs Ag/AgCl) in order to avoid the undesired side reaction, such as electrochemical proton reduction to hydrogen gas (Eq. (2)) (Weast et al., 1983).

$$2H_2O + 2e^- \leftrightharpoons 2H_2 + 2OH^- \quad (E^0 = -1050\ \text{mV vs Ag/AgCl}) \qquad (2)$$

Results indicate that DO could be removed completely from seawater at all tested cathodic potentials (Fig. 3a). As expected the rate of oxygen removal was related to the cathodic potential with the fastest rate obtained at −800 mV (vs Ag/AgCl) (Fig. 3b).

3.2.2. Current as dissolved oxygen indicator

The results above demonstrated the electrochemical cell operated at cathodic potential between −800 and −200 mV (vs Ag/AgCl) enabled complete DO removal in seawater.

Theoretically, the electrochemical oxygen reduction reaction accepts electrons from the cathode of the cell enabling an actual current flow. As a consequence, oxygen depletion would be signaled by the current approaching zero. If after oxygen depletion the current approaches zero, this would suggest that no other significant reduction reaction is occurring. The current recorded during electrochemical oxygen reduction was recorded over time (Fig. 4).

In general the results showed that the current decreased with decreasing DO. Although a linear relationship has not been achieved in the current cell (data not shown) it clearly shows that the current production decreased to 0 mA when DO concentration was approaching depletion (Fig. 4). Potentially the relationship between DO and current output could be used to verify online that DO is approaching zero without requiring a dissolved oxygen probe in the system. The advantage of using a "mild reduction process" with a voltage no lower than −800 mV is that it specifically reduced oxygen only, as can be seen from the fact that the current trended towards zero as oxygen was being depleted. This suggests a low risk of reducing other species, which could otherwise lead to interference. The fact that YE detection was not altered (data not shown) by the oxygen reduction step confirms this absence of interference.

3.3. Electrochemical DO removal cell placed in-line with MFC-biosensor

3.3.1. Batch mode

The aim of this experiment is to couple the electrochemical cell with a MFC-biosensor to monitor AOC in seawater. Hence, it is crucial to test if during DO removal the electrochemical cell destructs or modifies dissolved organic matter in seawater in a way that interferes with the signal production of the biosensor. To test this, oxygen-saturated artificial seawater spiked with specific concentrations of YE was added into the cathodic compartment of the described electrochemical cell. The deoxygenated seawater was then fed into the anode compartment of the MFC-biosensor. Reproducible standard curves were obtained with linear relationships between current peak values and YE concentrations (Fig. 5). The results showed that there was no significant difference in signal production from the biosensor after DO removal using the electrochemical cell when compared to the control in which oxygen was removed by nitrogen gas purging. This indicates that DO removal using the electrochemical cell had no significant interference on AOC bio-detection and that it can be to coupled successfully with the MFC biosensor.

3.3.2. Continuous flow mode

As an alternative to the above described batch mode operation (Section 3.3.2), a continuous-flow operation mode may be more desirable and appropriate for applications of in-line AOC monitoring. Results above showed that the removal of DO is time-dependent. In a continuous flow mode, it is important to

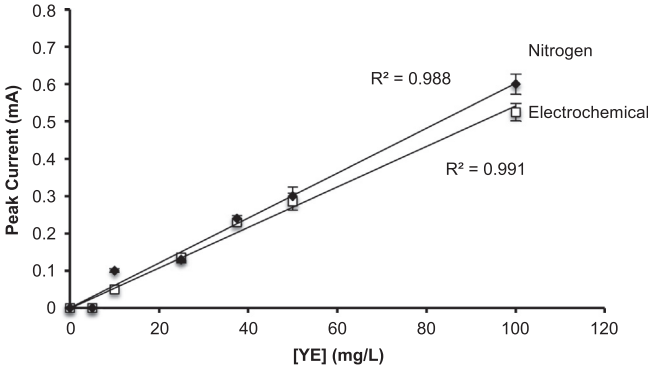

Fig. 5. Correlation between maximum peak current with the yeast extract concentration added for two different oxygen removal methods; (♦) nitrogen purging and (□) electrochemical −800 mV (vs Ag/AgCl).

Table 1
Electrochemical cell outflow dissolved oxygen in seawater coupled with biosensor at various flow rates during continuous flow mode operation. The cathodic potential of the electrochemical cell was maintained at −800 mV (vs Ag/AgCl). The inflow seawater was oxygen-saturated, contained 16 mg/L of COD at pH 8. Average of three replicates.

Flow rate (mL/min)	pH in the outflow	HRT* (min)	Current produced in the electrochemical cell (mA)	Dissolved oxygen in the outflow (mg/L)	COD signal from biosensor (mA)
4.7	8.6	42.55	−0.21	<0.00	0.2 ± 0.002
6.7	8.5	29.85	−0.46	<0.00	0.197 ± 0.015
12.5	8.5	16.00	−0.53	<0.00	0.198 ± 0.01
15	8.3	13.33	−6.07	0.12	0.17 ± 0.005
20	8.2	10.00	−10.13	0.5	0.17 ± 0.004

* HRT = hydraulic retention time.

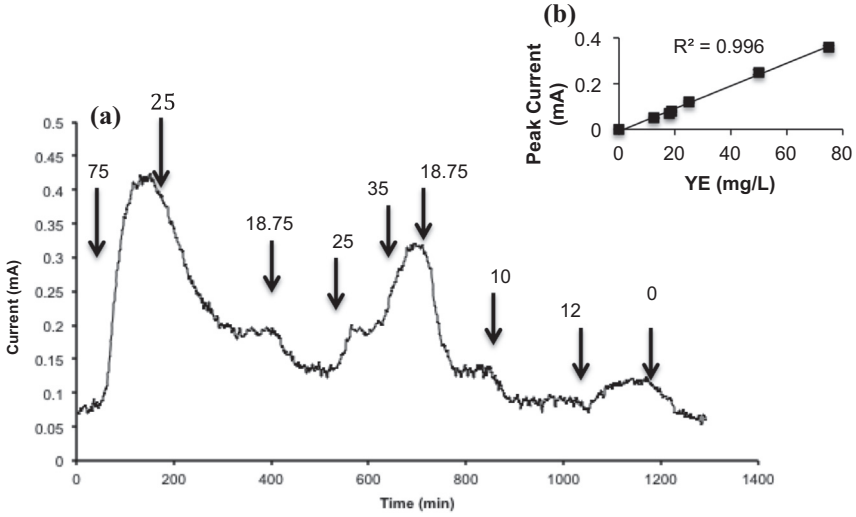

Fig. 6. (a) Responses of the marine MFC biosensor to changes in YE concentration (0–75 mg/L) of the inflow and (b) correlation between YE concentration and current peak under continuous flow mode after oxygen removal via the electrochemical cell at −800 mV (vs Ag/AgCl).

determine how fast the electrochemical cell removes oxygen from seawater without losing its deoxygenation efficiency.

To test up to what seawater flow rates the electrochemical cell could effectively deoxygenate, the effluent equilibrium DO concentration was recorded for different flow rates along with the current. When the DO at the effluent of the electrochemical cell was constant, the DO and the current obtained from the MFC-biosensor were recorded (Table 1). As expected, the DO removal efficiency and current obtained from the biosensor was dependent on the flow rate. Fig. 4 shows the results of batch mode operation, and Table 1 of continuous mode operation. During batch mode operation, the total catholyte volume was 300 mL (1.5 times more than of the cell volume) hence it took longer to get all DO removed.

This particular electrochemical cell was able to remove DO from the feed solution at flow rates up to 12.5 mL/min, which was an adequate flow to provide the sensor with deoxygenated seawater. Once the maximum flow rate (12.5 mL/min) for the electrochemical was determined, the combination of the electrochemical cell with the biosensor was tested for realistic AOC determination. For this purpose, a series of DO saturated seawater feed solution supplemented with different concentrations of YE was passed through the electrochemical cell (for DO removal) and subsequently through the anodic chamber of the MFC-biosensor (for AOC detection).

A step-wise change in YE concentrations in the inflow leads to corresponding changes in current from the MFC-biosensor (Fig. 6a). The relationship between the current peak values and YE concentrations was also linear (Fig. 6b), demonstrating the effective AOC detection by a combination of two electrochemical devices. The addition of small YE spikes caused increases in current leading to a current peak typically within a few minutes for batch operation. As expected, under continuous feeding conditions using step increases in feed composition a long lasting increase in current was obtained.

3.3.3. Limitations

Long-term stability of a biosensor is crucial for practical application, especially for an online analytical method. For the MFC-biosensor, the long-term stability was evaluated by determining the signal production of the sensor with injection of YE over a number of months without requiring replacement of the electrode material. A natural drift in the amount of active biomass on the electrode can be accounted for by using calibration solutions of known AOC (e.g., in the form of acetate).

However, for the electrochemical oxygen removal cell, the long-term operation could lead to the build-up of biomass that could affect the seawater AOC leading to potential interference in the AOC detection by the MFC-biosensor. The presence of oxygen and AOC will likely develop an aerobic biofilm on the graphite of the long term. This may need to be controlled by regular cleaning or replacement of the cathode of the oxygen removal cell.

4. Conclusion

The results of this study demonstrate that AOC detection in seawater is possible by the seamless combination of an electrochemical cell with the MFC-biosensor. The combined device could be readily mounted in line of industrial SWRO plants. The two-stage device allows AOC monitoring in practical applications for real-time monitoring. Warning signals of "high AOC" could be used for

example for redirecting the inflow of seawater to another pretreatment step.

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