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Microbial Fuel Cell Biosensor for Rapid Assessment of Assimilable Organic Carbon under Marine Conditions

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Abstract

The development of an assimilable organic carbon (AOC) detecting marine microbial fuel cell (MFC) biosensor inoculated with microorganisms from marine sediment was successful within 36 days. This established marine MFC was tested as an AOC biosensor and reproducible microbiologically produced electrical signals in response to defined acetate concentration were achieved. The dependency of the biosensor sensitivity on the potential of the electron-accepting electrode (anode) was investigated. A linear correlation ($R^2 > 0.98$) between electrochemical signals (change in anodic potential and peak current) and acetate concentration ranging from 0 to 150 µM (0 – 3600 µg/L of AOC) was achieved. However, the present biosensor indicated a different-linear relation at somewhat elevated acetate concentration ranging from 150 to 450 µM (3600 – 10800 µg/L of AOC). This high concentration of acetate addition could be measured by coulombic measurement (cumulative charges) with a linear correlation. For the acetate concentration detected in this study, the sensor recovery time could be controlled within 100 minutes.

Keywords: Biosensor; Microbial Fuel Cell; Seawater Desalination; Biofouling; Marine; AOC
1 Introduction

Biofouling of reverse osmosis (RO) membranes used for seawater desalination is one of the most serious problems in seawater desalination. It causes significant decline of flux, requires frequent cleaning, and leads to elevated energy requirement. As a result, membranes are frequently replaced (Abdul-Azis et al., 2001). At present, the industry is subject to biofouling occurring without being able to monitor or predict the tendency of intake ocean water that causes biofouling.

A good biofouling monitoring/prediction system is necessary and crucial for the development and optimization of efficient anti-biofouling strategies. The information acquired from the biofouling monitoring system should be ideally on-line, and automatic (Klahre and Flemming, 2000). Current biofouling monitoring systems are acting as early warning tools by measurement of intake water quality, including total direct cell counts (TDC) (Jeong et al., 2012), silt density index (SDI) (Alhadidi et al., 2011; Hammes and Egli, 2005) and the biofilm formation rate (BFR) (Van der Kooij, 1992) prior to RO. Another patent (Ho et al., 2004) introduced methods for monitoring and controlling biofouling in membrane separation systems using fluorogenic agents, which react with bacteria to give fluorescence signal. By using fluorometers to measure the amount of fluorescence signal in the feed stream, the retentate and permeate, changes in the fluorescence signal can represent the extent of biofouling (Lee and Kim, 2011). These methods are time-consuming (may take an hour to several days), offline and typically manual laboratory testing, and therefore they are not suitable for early detection of biofouling potential of feed water.
As the biofouling of the membranes is commonly due to bacterial growth, which is supported by organics in feed water (seawater), biofouling can also be predicted by monitoring low levels of organic substrates in feed water that can serve as a bacterial food source termed “assimilable organic carbon” (AOC) (Hambsch and Werner, 2000). Therefore, AOC monitoring in the feedwater of RO plants is a suitable method for the prediction of its biofouling potential.

Methods for determining AOC exist in the fresh water and drinking water industry as biochemical oxygen demand (BOD) measurement. The BOD is a measure of the quantity of oxygen consumed by microorganisms to oxidize the organic matter in a sample of water during a period of 5 or 7 day incubation at 20 °C (BOD) (Bourgeois et al., 2001; Greenberg et al., 1992). The method is not suitable for rapid or online testing. In recent year, biosensors have demonstrated great potential to be an alternative to the conventional analytical method for BOD measurement. The main advantages of biosensors are the potential for rapid or online results, the portability and miniaturization (Rodriguez-Mozaz et al., 2006). Various BOD biosensors have been reported based on different mechanisms including monitoring dissolved oxygen (Jia et al., 2003; Trosok et al., 2001; Wen et al., 2008), measuring intensity of luminescence of luminescent bacteria (Borisov and Wolfbeis, 2008; Hambsch and Werner, 2000; Van der Kooij, 1992), and photo-catalysis of sample (Elman et al., 2008).

With the discovery of microbes to transfer electrons to an electrode, not only can electricity be made from organic substances (Cheng et al., 2008) but also can the current be used to sense the presence of organic substances. A biosensor based on the microbial fuel cell (MFC) principle, a device that converts chemical energy to electrical energy,
has been reported (Liu et al., 2011). In general, microorganisms grown on the surface of an anode electrode oxidize organic matter in the solution under anaerobic condition and transfer electrons to the anode electrode, which eventually pass through an external circuit to a cathode electrode. The flow of electrons through the circuit is proportional to the amount of organic material oxidized and can be quantified as a current. The anode chamber of a MFC needs to be kept anaerobic as the presence of dissolved oxygen can completely suppress the metabolic activity of electrochemically active bacteria and hence the current production (Liu et al., 2005). This is due to the dissolved oxygen being the preferred electron acceptor in a typical MFC.

Biosensors based on the principal of MFCs have been applied for fast BOD detection. As the current is already a measured signal, MFC biosensors do not need a transducer to translate the signal to an electrical signal, which is commonly required by other biosensors based on photocatalysis or luminescence detection (Peixoto et al., 2011). The MFC-based sensors have long-term stability (Gil et al., 2003; Kim et al., 2003) and can be used for continuous on-line water quality monitoring (Chang et al., 2004). However, as reported by previous research works, the measuring time (i.e. response time and sensor recovery time) varies significantly from 1 hour up to several hours (Chang et al., 2004; Kang et al., 2003; Kim et al., 2003).

To date, the available methods of the MFC based biosensors could not detect very low AOC (less than 32 mg O₂/L as BOD₅ equivalent to 12 mg/L of AOC) levels (Modin and Wilén, 2012; Peixoto et al., 2011) and have not been demonstrated to be applicable under marine condition. The aim of the present study is to explore whether a marine biosensor based on MFC can be developed and gives reproducible and rapid signals to
low levels of AOC in seawater (~100 µmol/L (~1200 µg/L of AOC) of dissolved
organic carbon or less than 5 mg/L BOD) such that the foundation of a biofouling
sensing technology for seawater reverse osmosis (SWRO) plants is established.

2 Materials and methods

2.1 Marine-Microbial Fuel Cell 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
to 5 followed by continuously stirring for 24 hours. After settling for 2 hours the
supernatant with OD$_{600}$ value of about 0.2 was collected and used as inoculum for the
marine anodophilic biofilm. Seawater (salt concentration of approximately 35000 ppm)
obtained at the same location was used as anolyte. In RO plants, suspended solids that
are present in the feed-water will be removed by ultra-filtration. 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 36 days yeast extract solution was periodically added (ca. every 7 - 10
days) to the anolyte (50 mgL$^{-1}$ final concentration) as bacterial growth supplement. In
the cathodic compartment, the catholyte contained 100 mM potassium ferricyanide
(K$_3$Fe(CN)$_6$, Sigma-Aldrich, Inc., purity ca. 99%) and 150 mM sodium chloride. The
catholyte was renewed periodically to maintain a stable cathodic potential.
2.1.2 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 (14 cm x 12 cm x 1.88 cm) were physically separated by a cation selective membrane (CMI-6000, Membrane International INC.) with a size of 168 cm². 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 variable resistor box was used to set a specific desired external resistance manually. To facilitate electrical connections, the graphite granules were attached to graphite rods (5 mm diameter) by direct insertion of the rods into the granules. The potentials of the electrodes were measured against a saturated Ag/AgCl reference electrode (BASi, MF-2079), which was placed inside the anodic compartment.

2.2 Microbial Fuel Cell Sensor Operation

2.2.1 Start-up Procedure: Start-up Procedure

The anodic chamber (as described in Section 2.1.2) of the MFC sensor was inoculated with 50 mL of inoculum (prepared according to the procedure described above) and 200 mL of seawater containing 50 mg/L yeast extract and 10 mM of acetate to enhance the growth of anodophilic biofilm. The cathodic compartment was filled with 150 mL of catholyte (as described above). The MFC was operated in a fed-batch mode with both catholyte and anolyte continuously re-circulating over the cathodic and anodic compartments, respectively. A 100 mL bottle (total anolyte volume of 250 mL) was connected in the anolyte-circulating loop for pre-mixing of acetate addition and
anolyte prior to introducing to anodic compartment. During the start-up procedure the external resistor was set at 220 $\Omega$.

After the anodophilic biofilm had been successfully established (indicated by a high steady current ($> 3$ mA) production), the anodic compartment of the MFC was drained out to remove old anolyte and re-filled with fresh seawater.

### 2.2.2 Acetate Detection Procedure

A 5 mM of sodium acetate was used as a stock solution and specific amounts of acetate (as stated in the results section) were fed into the anolyte via a septum-sealed injection port to test for electrical signal production. Concentrations of acetate stated in the results section indicate the final acetate concentrations in the anolyte. In the current study acetate was only used as a preliminary substrate to establish a proof of concept. As a model substrate acetate is commonly present and also the key biological breakdown product from more complex organic substances, which represent readily assimilable AOC and can be utilized by the marine anodophilic bacteria.

### 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 anodic potential (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 was logged every 30 seconds into an Excel spreadsheet with a
computer. The pH of the anolyte was strictly controlled at 7.2 ± 0.2 by automatically
dosing NaOH (1 M).

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 external resistance was adjusted from 5 to 220 ohm as
stated in the experiments. The steady AP and/or baseline current were used as the
reference set point in the LabBIEW™ feedback control program.

2.4 Determination of Current and Cumulative Charges and Coulombic
Efficiency

The electron (acetate addition) flow, from anode to cathode in microbial fuel cells is
proportional to the rate of acetate oxidation by the bacteria and the electrons obtained
from acetate oxidation can be retrieved as current. The current was calculated from the
cell potential and external resistance using Ohm’s law (Current (mA) = Cell potential
(mV) / Resistor (Ω)). Cumulative charges were obtained by integrating the electrons
transferred by the biofilm as current throughout the detection period (Cheng et al.,
2008). The coulombic efficiency was calculated from the electrons extracted from the
substance for conversion into electricity versus that in the starting organic materials
(Logan et al., 2006).

Steady state was defined as no changes in anodic potential (AP) (± 10 mV) and
current (± 0.05 mA). The recovery time was defined as the time required for the AP
drops to the initial level after the depletion of acetate. The signals (AP and/or current
peak) obtained from acetate addition were calculated by subtracting the background/steady state value.

3 Results and Discussion

3.1 Marine-MFC Biosensor Startup

Initially, previous published MFC (Cheng et al., 2008) were exposed to increasing concentrations of sodium chloride in order to adapt the established freshwater biofilm slowly to marine conditions. The MFC responded to acetate addition until salt concentrations of about half of marine concentration, but at higher salt concentration the response became increasingly faint such that at marine salt concentrations, no signal could be obtained (data not shown). It was concluded that a marine anodophilic biofilm had to be developed from scratch using ocean water and sediment as inoculum.

A marine-MFC was set up as described above and operated over 36 days for establishment of a marine biofilm that could respond to the addition of organic substances. The lowering of potential and development of current was caused by the metabolism of the bacteria. During these period, due to the growth of anodophilic marine bacteria, the current generated by the bacteria in the presence of acetate increased steadily from day 2 (0.5 mA) and stayed steady at about 3.3 mA after 25 days of operation (at external resistor of 220 Ω) (data not shown).

The MFC was then tested for its response (change in current or AP) to low concentrations of acetate addition. Spikes of 150 μM acetate (3600 μg/L of AOC) were
added to the anodic chamber (Fig. 1) after the AP and current had stabilised at the steady state for at least one hour.

As only acetate was added, it could be assumed that the signal output was directly related to the acetate consumed. Results show that the established marine-MFC responded to 150 µM acetate addition by an increase in current and decrease in AP. Over short periods of time when biomass fluctuations are negligible, a repeat injection of acetate resulted in a reproducible response (variability less than < 5 %) in both the AP and current, suggesting a good reproducibility of acetate detection in real seawater. Apart from the change in AP and current peak, there is another electrical signal that can be derived from Fig.1 for the response of acetate addition, which is the cumulative charge (e.g. 15 and 16 C for each acetate addition respectively). It was conceivable that any of these signals obtained from MFC-biosensor could be used to detect acetate spikes reproducibly.

3.2 The Effect of Different Steady AP on Acetate Detection

In order to achieve a sensitive and quick response from the MFC-biosensor, the electron transfer from the bacterial cells to the anode must be very efficient, which requires a suitable anodic potential (Cheng et al., 2008). Therefore, it is worthwhile to test which steady APs allow the established MFC-biosensor to give the strongest response to low acetate concentrations. Steady APs between -200 and +200 mV (vs Ag/AgCl) were used by adjusting the external resistance between 5 and 220 Ω. As expected the use of larger resistors resulted in lower steady APs. The MFC-biosensor
was initially operated in the absence of acetate (i.e. starvation) for 1 hour before the
addition of 33 µM of acetate (792 µg/L of AOC) (Fig. A.1).

In general, a low steady AP (e.g. 0 mV (Ag/AgCl) with 150 Ω) gave a greater
response (change in AP) to the addition of acetate (Fig. 2). However, if the potential
was less than -220 mV (vs Ag/AgCl), the response was reduced and not detectable (Fig.
A.1). Although a significant change in signal (i.e. AP) is preferable, a shorter recovery
time is also important to enable frequent online testing. Other than the change of AP
(potentiometric), the MFC-biosensor can generate other types of electrochemical signals
such as current (amperometric or coulometric) (Fig. 2). At a higher AP (+220 mV), with
a smaller resistor the size of the current signal (current peak) was typically higher
resulting in a faster depletion of the spiked organic carbon (acetate) and shorter
recovery time (Fig. 2), which will be beneficial for frequent and rapid online testing.

It has been shown that additions of acetate to MFC result in a reproducible amount
of coulombs produced by the bacteria (Cheng et al., 2008). In order to obtain the total
amount of coulombs produced here the current peak was integrated. As a result, the
response only becomes available when all acetate is used. This coulometric signal
(cumulative charges) analysis is reproducible as long as the coulometric efficiency for
different samples is constant. Arguably it would be the optimum method of
quantification if all of the reducing power of the AOC were quantitatively used in
producing the signal. The coulometric signal analysis indicated that the value of
cumulative charges increases with decrease in the steady Aps (Fig. 2). For the same
amount of acetate added, the difference in the cumulative charges reflected variable
coulombic efficiency, which was increased with decrease in APs (Fig. A.2).
With the same amount of acetate addition, all the electrochemical signals are varied at different steady APs suggesting that for each steady AP, a specific correlation curve between signal and acetate concentration is required.

### 3.3 Signal Production at Various Acetate Concentrations

The results above demonstrated that steady AP between 0 and +220 mV (Ag/AgCl) enabled clear detection of acetate concentrations down to level of 33 µM. To investigate which starting steady AP is suitable for low acetate detection, acetate concentration ranging from 0 to 110 µM (0 to 2640 µg/L of AOC) were added to the MFC-biosensor at 3 different steady APs of +200, +50, and 0 mV controlled by an external resistor of 10, 56 and 150 Ω, respectively (Fig. 3).

Overall, both electrochemical signals, change of AP and peak current, increased linearly with acetate concentrations with a reasonable high coefficient of determination ($R^2 > 0.98$), irrespective of the steady APs (Fig. 3a and 3b). This indicated that for the detection of low levels of acetate the APs between 0 and +200 mV are suitable.

The coulometric signal was poorly related to the acetate concentration (Fig. 3c). A constant stoichiometric relationship would be expected between acetate used and total current produced, which is widely accepted as coulometric efficiency in MFCs (Chang et al., 2004; Cheng et al., 2008). However, in the current study, the coulometric efficiency varied relating to the added acetate concentration (Fig. 3d), and where extremely low concentrations of acetate were tested the coulometric efficiency was very low. These low coulometric efficiencies obtained are inline with previously reported...
From fundamental considerations, the correlation between peak current and acetate concentration only holds true if the reaction was of first order. However, this is known to be not the case for microbial or enzyme based reactions when the substrate concentration is elevated and saturation behavior sets in. To evaluate this expected deviation from linearity higher doses of acetate were tested (Fig. 4 and Fig. A.3). Results showed that between 150 to 450 µM (3600 to 10800 µg/L of AOC) (high concentration) of acetate the amperometric and potential measurement were indeed no longer linear with respect to acetate concentration.

The amperometric and potential responses followed typical Michaelis-Menten model (Eqn. 1). This model predicts that the rate of substrate (here acetate) degradation is approximately proportional to the substrate concentration. However, the linearity does not exist at high concentrations of substrates as a maximum acetate oxidation rate ($V_{\text{max}}$) is achieved at saturating substrate concentrations. As the amperometric measurement is a representation of the acetate oxidation rate (via current production) a Michaelis-Menten relationship between current and acetate concentration is expected, which has been demonstrated in a larger scale freshwater microbial fuel cell (Cheng et al., 2008).

$$V = \frac{V_{\text{max}}S}{K_s+S} \quad \text{(Eqn. 1)}$$

where $S$ is the substrate (acetate) concentration, $V$ is the rate of substrate degradation and $K_s$ is the substrate half-saturation constant.
This current finding is in line with work by Tront et al. (Tront et al., 2008) and Cheng et al. (Cheng et al., 2008), which indicated a linear correlation only applied to a certain responsive range of low acetate concentrations and a finite maximum current would be produced at high acetate concentration (saturation conditions). In contrast to potentiometric or amperometric measurements, the coulometric measurement gave more linear responses for the higher acetate concentration (150 to 450 µM) (Fig. 4c), which allowed the coulombic measurement to be a more promising signal measurement for high concentrations of acetate detection compared to using current peak as detecting signal.

To what extent a combination of different measurements can be applied over a broad range of concentrations could be the subject of further studies. Furthermore as it is of interest to the SWRO plants to determine the presence of low levels AOC the linearity of biosensor response at higher levels may not be of relevance. The possibility of dilution when the biosensor response is out of range is an option.

With continued operation of the MFC sensor, its reliability and response were improved. After 80 days of operation, the detection limit (signal to noise ratio > 5) of the described MFC sensor was improved detecting less than 10 µM acetate (less than 240 µg/L of AOC) at steady APs from about 0 to 120 mV (Fig. A.4).

### 3.4 Limitations and future work

The effect of competing electron acceptors present in the marine water, such as oxygen, on electric signal generation was tested. As expected from the fact that anodophilic bacteria of the Geobacter type are strict anaerobes (Logan et al., 2005), the
presence of oxygen completely inhibited signal production by the established marine MFC biosensor (Fig. A.5). This means that for the purpose of ocean organic pollutant detection, the de-oxygenation of the feed solution prior to measurement is necessary. In order to eliminate the effect of the deoxygenating process on the downstream MFC-biosensor measurement, oxygen removal using reducing agents (e.g. sodium sulfite, hydrazine) is not recommended. However, inert gas purging or electrochemical reduction could be utilised to remove dissolved oxygen without disturbing the AOC detection.

There are some points that must be taken into consideration when using this marine MFC biosensor to detect AOC in seawater. The biosensor in this study was developed using acetate as sole carbon source. Although the current biosensor responded (current production) to complex organic (i.e. yeast extract) and some organic acids (i.e. butyrate acid, arginine, glycine and taurine), the response was not as “strong” as that observed for acetate (data not shown). This is not surprising since the current MFC-biosensor having been adapted to acetate only. It is established that in anaerobic environments such as anaerobic digesters fermentation reactions of other organic substances such as carbohydrates, fats, and proteins lead to acetate as the predominant organic metabolite. Hence acetate was used to validate, standardize and estimate AOC in seawater.

It is clear that further work is required to evaluate the long-term stability of the described MFC-biosensor and its capacity for detecting complex organics that commonly appear in polluted marine water. Previous studies have shown that anodophilic bacteria are able to metabolize a variety of compounds if the cell is adapted to the specific compound (i.e. chitin (Rezaei et al., 2009), glucose (Kim et al., 2000),
cellulose (Catal et al., 2008), phenol (Luo et al., 2009) and landfill leachate (Gálvez et al., 2009). The adaptation of the sensor to complex biological substances will be investigated in a following paper.

There might be interference caused by the source of seawater, in particular if it contains contaminants that may inhibit bacterial activity. However by using interfering compounds containing seawater for the generation of standard curves of AOC, interference could possibly be limited. Further, the biosensor described may be utilized as an indicator of microbial inhibitors by comparing to the signals obtained from a defined amount of acetate in the presence and absence of microbial inhibitors.

4 Conclusions

This paper presents a MFC-biosensor established for marine conditions indicating a) a strong linear relationship between trace amounts of acetate (10 to 150 µM) and electrochemical signals, b) rapid measurement (within 5 minutes using peak current at low acetate concentration), and c) reproducible signals. This marine MFC-biosensor method can be effectively used as an indicator for biofouling potential in seawater reverse osmosis system. However, further work on dealing with the presence of oxygen in seawater and in-situ testing is needed prior to making such a sensor available for online seawater AOC monitoring.

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Fig. 1. Response of the anodic potential (---) and current flow (-----) of a freshly established (after 36 days) marine MFC biosensor to two identical acetate additions (150 µM). The marine-MFC was operating at room temperature and pH was maintained at 7.2 ± 0.2 at resistor of 56 Ω.
Fig. 2 Effect of initial anodic potential on acetate detection ( ■ = change of anodic potential, ○ = current peak, ▲ = cumulative charges, and ◆ = recovery time) after the addition of a fixed amount (33 µM) of acetate. The marine-MFC was operating at room temperature and pH was maintained at 7.2 ± 0.2. Recovery time is too long to be shown in this figure (> 18 hours) at -220 mV.
Fig. 3 Comparison of a) potentiometric, b) amperometric, c) coulometric and d) coulombic efficiency for response of the marine MFC sensor to a series of acetate concentrations. Responses were recorded after establishing a steady state AP of about +200 (●), +50 (■) and 0 (▲) mV by using an external resistor of 10, 56 and 150 Ω, respectively.
a

Change of Anodic Potential (mV) vs. Acetate concentration (µM)

V_{max}

b

Maximum Current (mA) vs. Acetate concentration (µM)

y = 0.17x - 19.92

y = 0.05x - 0.76

R² = 0.99

R² = 0.97

c

Cumulative Charge (C) vs. Acetate concentration (µM)

y = 0.17x - 19.92

y = 0.05x - 0.76

y = 0.05x - 0.76

R² = 0.99

R² = 0.97

R² = 0.97
Fig. 4 Response of the a) potentiometric, b) amperometric and c) cumulative charges to various acetate additions increasingly (0 - 500 µM). The marine-MFC was operating at room temperature and pH was maintained at 7.2 ± 0.2 at resistor of 10 Ω. The arrows indicated acetate addition.
• A marine microbial fuel cell was used as an assimilable organic carbon biosensor.
• The biosensor produced reproducible signals microbiologically and rapidly.
• The biosensor sensitivity depends on the potential of the anode.
• Good correlation between signals and acetate concentration (10-150 µM).
• Biosensor can be used to measure biofouling potential in seawater.
Appendix

Fig. A.1. Effect of different stable anodic potentials on the change of anodic potential after the addition of a fixed amount (33 µM) of acetate. The marine-MFC was operating at room temperature and pH was maintained at 7.2 ± 0.2.

Fig. A.2. Effect of anodic potentials on coulumbic efficiency of the MFC biosensor. The addition of a fixed amount (33 µM) of acetate. The marine-MFC was operating at room temperature and pH was maintained at 7.2 ± 0.2.
Fig. A.3. Response of the anodic potential (———) and current flow (--------) to various acetate additions increasingly (0 - 500 µM). The marine-MFC was operating at room temperature and pH was maintained at 7.2 ± 0.2 at resistor of 10 Ω. The arrows indicated acetate addition.

Fig. A.4. Effect of steady anodic potential on acetate detection limit by using change of anodic potential as response.
Fig. A.5. Effect of dissolved oxygen (-------) on current production (———) of response of marine MEC-biosensor.