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Fully reversible current driven by a dual marine photosynthetic microbial community

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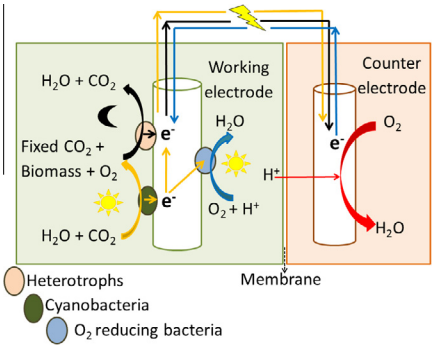
HIGHLIGHTS

- Fully reversible currents were observed only with a dual microbial community.
- Cathodic oxygen reduction occurs at high working electrode potential.
- Cathodic current is likely associated with γ-proteobacterium *Congregibacter*.
- Anodic current reflects biofilm capability to indirectly convert light to electricity.
- The highest solar bioanode (>100 mA m^{-2})/oxygen biocathode currents (>1000 mA m^{-2}).

GRAPHICAL ABSTRACT



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ABSTRACT

The electrochemical activity of two seawater microbial consortia were investigated in three-electrode bioelectrochemical cells. Two seawater inocula – from the Sunshine Coast (SC) and Gold Coast (GC) shores of Australia – were enriched at +0.6 V vs. SHE using 12/12 h day/night cycles. After re-inoculation, the SC consortium developed a fully-reversible cathodic/anodic current, with a max. of −62 mA m^{-2} during the day and +110 mA m^{-2} at night, while the GC exhibited negligible daytime output but +98 mA m^{-2} at night. Community analysis revealed that both enrichments were dominated by cyanobacteria, indicating their potential as biocatalysts for indirect light conversion to electricity. Moreover, the presence of γ-proteobacterium *Congregibacter* in SC biofilm was likely related to the cathodic reductive current, indicating its effectiveness at catalysing cathodic oxygen reduction at a surprisingly high potential. For the first time a correlation between a dual microbial community and fully reversible current is reported.

1. Introduction

Microbial solar cells (MSCs), also known as biophotovoltaics (McCormick et al., 2015), are bio-electrochemical systems (BESs) that convert solar energy into electrical energy through bio-electrocatalytic reactions driven by photosynthetic microorganisms. During oxygenic photosynthesis, solar energy is utilised to split water to oxygen, protons and electrons. These electrons are used by microorganisms for CO_2 fixation to form organic compounds (excreted and/or stored) and new cell material. Alternatively, they may be transferred extracellularly to a polarised anode (McCormick et al., 2011; Pisciotta et al., 2011). In MSC-type devices, the electrons then flow from anode to cathode where they reduce an electron acceptor, typically oxygen (Mateo et al., 2014).

Two-chamber mediatorless MSCs have been previously shown to exploit photosynthesis to generate anodic current catalysed by single species (Madiraju et al., 2012; Raman and Lan, 2012) or

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photosynthetic microbial consortia (Cao et al., 2008) without added organic carbon sources. Other studies found instead that some photosynthetic microbial consortia in mediatorless MSCs were able to generate cathodic current during the day and anodic current during the night *i.e.* reversible current (He et al., 2009; Strik et al., 2010). This puzzling phenomenon may be used to overcome the problematic formation of a pH gradient across the membrane between anode and cathode (Blanchet et al., 2014; Strik et al., 2010). Moreover, polarity reversal of MFC-electrodes enhanced the anodic and cathodic power densities by 58% and 36%, respectively, when compared to non-reversing systems and led to a reduction in the use of a chemical buffering (Li et al., 2014).

This study aims to correlate the generation of reversible current with microbial community composition. For this purpose, two seawater biofilms enriched from different inocula were utilised in working electrode chambers for approximately 2 months at a poised electrode potential of +0.6 V vs. standard hydrogen electrode (SHE). We show that cathodic current during the day and anodic current during the night were biologically generated at the same electrode without input of exogenous organic compounds or redox mediators. Two distinct microbial consortia performed differently in terms of electrochemical activities, which appear to be related to the presence of distinct functionalities in the related biofilms. Furthermore, we show that with a dual microbial community comprising cyanobacteria and strong cathodic oxygen reducers (such as γ-proteobacteria), fully reversible currents are observed at a high working electrode potential of +0.6 V vs. SHE. The results provide more fundamental information to understand the phenomenon of reversible currents, which will be useful towards future designs of microbial solar cells.

2. Methods

2.1. Electrochemical cells setup

The experiments hereby presented were carried out using two dual-chamber bottle-type reactors. The reactors were constructed using 250 cm^3 borosilicate bottles as main chambers (200 cm^3 effective volume) each hosting a working electrode and a pipette tip as counter electrode chamber (6 cm^3 effective volume), immersed in the working electrode solution (Fig. S1, Supplementary material), and separated from the latter by a 4.9 cm^2 cation exchange membrane (CEM) (Ultrex CMI-7000, Membrane International). A 24 cm^2 rectangular piece of graphite felt was used as the working electrode (dimensions: 60 × 40 × 1 mm, Morgan Industrial Carbons, Australia) and pressed against a Titanium mesh (mesh size 1 mm × 2 mm, thickness 0.15 mm – Kaian Metal Wire Mesh Co. Ltd, China) that covered the whole working electrode surface for current collection. Both membrane and graphite felt were used as provided by the suppliers. Furthermore, each working electrode chamber also had an Ag/AgCl reference electrode (sat. KCl, +0.197 V vs. SHE), a pH probe (miniCHEM-pH, Australia) and a dissolved oxygen (DO) probe (O2-4100-e, Mettler Toledo, Germany) immersed in the medium. Titanium wires (length 4 cm, diameter 0.5 mm, Advent Research Materials, UK) were employed as counter electrodes. The reactors were placed in a custom made dark box with two built-in ventilation fans to maintain room temperature (24 ± 1 °C) and two white fluorescent lamps to illuminate the working electrode side of the reactors at 29 W m^{-2}.

2.2. Photosynthetic culture enrichment

Two seawater inocula at pH 7.7–8.2 were taken from the Sunshine Coast (SC) and Gold Coast (GC) shores (eastern coast of Queensland, Australia), collected in depth of 5–30 cm below water surfaces using 15-L sterilised plastic container and stored at 4 °C before use. Both inocula were separately added to the working electrode chambers of two previously-sterilised electrochemical cells (described above) by mixing 50% of each sea water sample with filtered modified F2 medium (Pisciotta et al., 2010). To enable capturing all possible electron transfer pathways, the working electrode was poised at +0.6 V vs. SHE. Approximately 2 months after inoculation, the developed photosynthetic microbial biofilms were individually scraped from the whole surface of SC and GC working electrodes and used as sole sources for the re-inoculation of two new reactors (hereafter referred to as "re-inoculated reactors"), which were operated at the same conditions for 25 days. Throughout operation, the filtered modified F2 medium was continuously supplied to the working electrode side of both reactors using a syringe pump at a flow rate of 1.04 mL h^{-1}, equivalent to a hydraulic retention time (HRT) of 8 days. This medium contained 37.6 g Sea salt (Ocean Nature, Aquasonic Pty Ltd, Australia); 75 mg $NaNO_3$; 5 mg $NaH_2PO_4{\cdot}H_2O$; 0.046 mg $ZnSO_4{\cdot}7H_2O$; 0.304 mg $MnSO_4{\cdot}H_2O$; 0.015 mg $Na_2MoO_4{\cdot}2H_2O$; 0.028 mg $CoSO_4{\cdot}7H_2O$; 0.014 mg $CuCl_2{\cdot}2H_2O$; 9.2 mg $Fe(NH_4)_2(SO_4)_2{\cdot}6H_2O$; 8.8 mg $Na_2EDTA{\cdot}2H_2O$ and 3.36 g $NaHCO_3$ as a buffering agent per litre at pH 7.5 ± 0.2. The counter electrode solution consisted of phosphate buffer (6 g Na_2HPO_4; 3 g KH_2PO_4) and 6 g NaCl per litre at pH 8.0 and was replaced on a daily basis to prevent excessively-high pH. A magnetic stirrer was used to continuously mix the working electrode medium at 500 rpm (magnetic bar diameter 0.5 cm and length 2 cm). Illumination (measured using an IR day sensor PS-2148, PASPort™) was maintained at 12 h/12 h day/night pattern and each working electrode potential was poised at +0.6 V vs. SHE with a multichannel potentiostat (CHI-1000B, CH Instruments). Unless otherwise specified, a humidified 97% N_2/3% CO_2 gas mix (BOC, Australia) was used at a rate 0.2 L min^{-1} to strip out photosynthetic DO and to provide a carbon source for photoautotrophic microorganisms in the working chamber solution.

2.3. Electrochemical analysis

The electrochemical performance of the working electrodes was tested by two methods, potential-step chronoamperometry and cyclic voltammetry (CV) using the multichannel potentiostat. Multi-step chronoamperometry was performed at a potential range of −0.4 to +0.6 V vs. SHE; each step was held for 2 h. Cyclic voltammetry was run at a scan rate of 1 mV s^{-1} (Harnisch and Freguia, 2012) with a scan range of −0.4 to +0.6 V vs. SHE and the voltammogram was compared to that recorded on an abiotic control reactor running separately in identical configuration and operational conditions. The current densities are reported normalised to the projected surface area of the working electrode (24 cm^2).

2.4. DNA extraction and amplicon sequencing

Inoculum samples were collected by centrifuging 14 L sea water samples at the time of inoculation. Biofilm samples were collected from the re-inoculated reactors by scraping the whole working electrode surfaces on the day phase (day 25) after CV and step-chronoamperometry were conducted. Genomic DNA was extracted from 25 mg of either the collected inoculum and biofilm samples by FastSpin for Soil Kit (MP-Biomedicals, USA) according to the manufacturer's protocol. 300 ng DNA of each sample were provided to the Australian Centre for Ecogenomics (ACE) at the University of Queensland for 16S amplicon pyrosequencing by Illumina Miseq Platform using 926F and 1392wR primer sets (Engelbrektson et al., 2010).