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Power generation from a hybrid biological fuel cell in seawater

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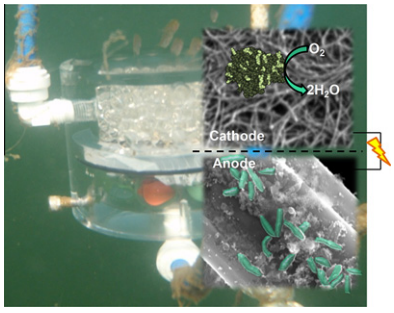
HIGHLIGHTS

- A true hybrid biological fuel cell was tested in laboratory and open environments.
- Robust anodic and cathodic bioelectrocatalysis was maintained in seawater tests.
- The hybrid fuel cell is distinct from marine sediment-based microbial fuel cells.
- The open design allows integration of alternative catalysts and electrode materials.
- System may be scaled and designed for environmental monitor and sensor applications.

GRAPHICAL ABSTRACT



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ABSTRACT

A hybrid biological fuel cell (HBFC) comprised of a microbial anode for lactate oxidation and an enzymatic cathode for oxygen reduction was constructed and then tested in a marine environment. *Shewanella oneidensis* DSP-10 was cultivated in laboratory medium and then fixed on a carbon felt electrode via a silica sol–gel process in order to catalyze anodic fuel cell processes. The cathode electrocatalyst was composed of bilirubin oxidase, fixed to a carbon nanotube electrode using a heterobifunctional cross linker, and then stabilized with a silica sol–gel coating. The anode and cathode half-cells provided operating potentials of −0.44 and 0.48 V, respectively (vs. Ag/AgCl). The HBFC maintained a reproducible open circuit voltage >0.7 V for 9 d in laboratory settings and sustained electrocatalytic activity for >24 h in open environment tests.

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1. Introduction

Biological fuel cells use biocatalysts for the conversion of chemical energy into electricity. These catalysts include redox enzymes that oxidize fuels in the anodic half-cell (e.g., hydrogen, alcohols, and sugars) or catalyze reduction reactions in the cathodic half-cell (e.g., oxygen reduction). Alternatively, microbes (typically bacteria) can act as anodic catalysts by breaking down fuel and transferring electrons to the anode. The current flow, in essence, represents an alternative route for cellular respiration, i.e., the cells using the anode rather than oxygen as an electron acceptor. Various applications are envisioned for enzymatic and microbial fuel cells; enzymatic fuel cells (EFCs), for example, may be used for implantable

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and *in situ* power supplies as well as powering mobile electronics (Heller, 2006). Microbial fuel cells (MFCs) typically have a lower power density than EFC, but will generally use a greater range of fuels that makes them applicable to waste treatment processes (Logan, 2005; Weld and Singh, 2011) and power sources for environmental sensors (Donovan et al., 2008). There are several possible operational and design advantages for biological fuel cell technology compared to conventional fuel cells that promote the research area (Barton et al., 2004; Heller, 2006). Current advances in the biochemistry of relevant redox processes—for example, understanding bacterial physiology, controlling materials architectures and bio-nano interfaces—place the biological fuel cell concept nearer practical utility.

For environmental devices such as sensors or monitors, conventional battery power presents the problems of limited lifespan and accessibility for maintenance and replacement. A marine environment provides a particularly challenging application. In this situation, MFCs that harvest energy from the environment would provide a significant advantage over conventional battery power (Gong et al., 2011; Tender et al., 2002). Monitoring natural waters using remote sensors is an application in which MFCs may provide a sustained power source for operation (Donovan et al., 2008). The type of environment in which MFCs can be deployed, however, remains limited by the inherent constraints of microbial physiology, e.g., growth in response to changes in oxygen level, pH, temperature, and nutrients. Previous testing of MFCs in marine environments placed experimental systems in the benthic zone. In such systems, the anode is buried in sediment and indigenous bacteria create a population that respires using the anode as an electron acceptor, thus producing current (An et al., 2011; Nielsen et al., 2007; Erable et al., 2009; Reimers et al., 2006). The organic-rich sediments provide sustenance and a diverse microbial population adapted to its environment; exploitation of naturally-occurring microbes that eventually populate the buried anode to form a biofilm will typically produce higher power densities than anodes that have been merely "seeded" with cultured isolates (Erable et al., 2010).

In many MFCs, the cathode reaction is a limiting factor in power output. In conventional fuel cell design, effective oxygen reduction relies on noble metal catalysts, such as platinum, that are becoming increasingly cost-prohibitive (Kjaergaard et al., 2011). Biological catalysts may offer a solution to this limitation by catalyzing cathodic oxygen reduction reactions at high onset potentials under conditions compatible with microbial activity (Harnisch and Schroder, 2010; Sakurai and Kataoka, 2007). Integrating an oxygen reduction catalyst—such as a multicopper oxidase (MCO)—to the cathode of an MFC increases the theoretical electrical potential of the system compared to metal catalysts (Biffinger et al., 2007; Bento et al., 2005; Barton et al., 2004). Combining a microbial anode with a laccase cathode, for example, provides a theoretical electromotive force of ~1.1 V but typically requires the use of mediators to shuttle electrons from the cathode, causing overpotential losses (Schaetzle et al., 2009). On the other hand, a biological fuel cell employing a microbial anode and an enzyme cathode that employs direct electron transfer (DET) offers advantages, in operating potential and in respect to simplifying design (Higgins et al., 2011).

Certain design constraints that may limit output must be overcome in order to apply non-indigenous biological processes in a marine environment. The electrode architecture and materials assembly can address some of the limiting issues. On the cathode side of the present work, MCOs, such as laccase and bilirubin oxidase (BOx), that catalyze oxygen reduction are generally inhibited by chloride which may decrease output in saltwater. Likewise, the alkaline pH of typical seawater (7.5–8.5) is above the optimal pH range for this class of enzymes. Effective DET between the MCO and the electrode surface will overcome chloride inhibition (Vaz-Dominguez et al., 2008). Several successful tethering approaches achieving DET have been reported (Blanford et al., 2009; Ghindilis et al., 1997; Ivnitski et al., 2010; Pita et al., 2006; Shleev et al., 2005). For example, laccase from *Trametes versicolor*, provides consistent electrocatalytic oxygen reduction in a fluctuating environment for >20 d when immobilized on buckypaper using a heterobifunctional cross linker (Strack et al., 2011; Ramasamy et al., 2010). Given the reported enhancement of biocatalytic current and longevity of the electrode, PBSE was selected as the tethering method applied to the MCO cathode. Other aspects can influence microbial processes at the anode. Sustaining a defined bacterial community at the anode requires both a constant supply of substrate and a design that prevents fouling from the indigenous population (Rezaei et al., 2007). For example, electrochemically active cultivated cells can be effectively immobilized using a silica sol–gel process (Luckarift et al., 2010). The deposited silica mimics an exopolysaccharide matrix that may bind cells in natural conditions. The synthetic process not only eliminates lengthy acclimation times and variability typically associated with cultivated biofilm formation, but also eliminates mixed cultures, providing a standardized anode community.

The following manuscript provides detail on characterization and design of a hybrid (microbial/enzymatic) biological fuel cell (HBFC) operating in seawater. The fuel cell electrocatalysts comprise a model organism, *Shewanella oneidensis* DSP-10, serving as a microbial anode and BOx as the enzymatic cathode.

2. Experimental

2.1. Equipment and materials

Seawater was collected from St. Andrew Sound, Florida (30° 05′ 18″ N, 85° 38′ 46″ W); filtered through Whatman filter paper (Grade 1) and autoclaved for routine laboratory experiments. Seawater experiments were positioned in St. Andrew Sound south–southwest of Tyndall Air Force Base, Florida.

Chemicals and reagents were purchased from Sigma–Aldrich (St. Louis, MO) unless otherwise noted. BOx from *Myrothecium verrucaria* was purchased from Amano Enzyme, Inc. (Nagoya, Japan) and prepared in phosphate buffer (0.1 M, pH 7.0) without further purification. Buckypaper prepared from 100% multi-walled carbon nanotubes (BuckyShield® High Conductivity, 20 μm thickness) was purchased from Buckeye® Composites (Kettering, OH). 1-pyrenebutanoic acid, succinimidyl ester (PBSE) was purchased from Anaspec, Inc., (Fremont, CA).

2.2. Preparation of silica-encapsulated S. oneidensis DSP-10 anodes

Bacteria were cultivated in 250 mL baffled shaker flasks (30 °C, 100 rpm) using 50 mL Luria–Bertani (LB) broth supplemented with rifampicin (5 μg mL^{-1}). Cultures were harvested at late stationary phase by centrifugation (8000 rpm for 4 min), washed 3× in phosphate buffered saline (PBS; composition in g L^{-1}: NaCl (8), KCl (0.2), Na_2HPO_4 (1.44), KH_2PO_4 (0.24), pH 7.0) and finally re-suspended to A_{600} of 10. (Absorbance measurements were obtained by diluting the bacterial suspension by a factor of 10.)

Four graphite felt (GF) electrodes (5 cm disks; Morgan AM&T, Inc., Greenville, SC) were sewn together by hand with titanium wire (45 cm, 0.25 mm thickness; Goodfellow, Oakdale, PA), leaving 15 cm free for electrical connections. The assembled felt electrodes were sterilized in PBS buffer at 121 °C for 15 min. GF electrodes saturated with washed cells (20 mL) were placed into a glass Petri dish with a central ring to fit the electrode dimensions. Tetramethylorthosilicate (TMOS) (2 mL) was deposited in the outer ring containing glass beads to enhance surface area for evaporation. The