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Light-Induced Extracellular Electron Transport by the Marine Raphidophyte *Chattonella marina*

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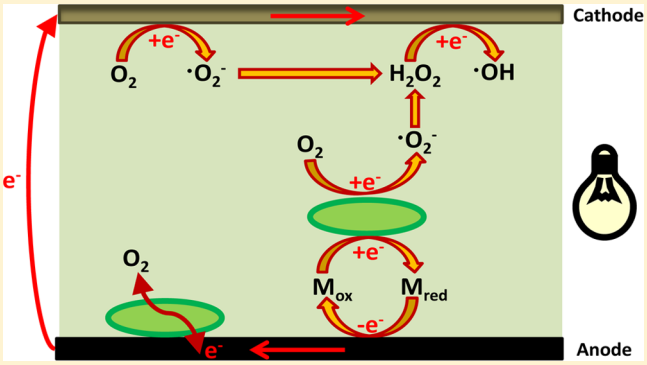
Supporting Information

ABSTRACT: There is increasing interest in extracellular electron transfer (EET) from organisms to receptors, particularly in anaerobic biofilms at mineral surfaces. Less attention has been given to EET by planktonic organisms in oxic environments where extracellular electron generation and transport might be expected to be of limited consequence. In this study, the EET activity of the photosynthetic marine raphidophyte, *Chattonella marina*, was examined using a mediatorless photosynthetic microbial fuel cell with results showing positive light response. Electron output by organisms present in cell suspension was substantially higher than those present in biofilms at the electrode surface. Indeed, current generation under light illumination of the *C. marina* suspension continued even when contact between the organisms and the electrodes was prevented by dialysis membrane, suggesting that soluble electron carriers secreted by *C. marina* were facilitating the EET process. Cyclic voltammetry measurements of the cell-free exudate showed redox peaks in the range of 0.1−0.5 V (vs Ag/AgCl), confirming that redox active species were present in the cell suspension. Facilitation of electron transfer from the planktonic organism to the anode by endogenous redox-active exudates appears to be critical to current generation. The ability of these exudates to remain in their reduced state in the presence of oxygen is possibly a function of the spin-restricted nature of oxygen-mediated exudate oxidation. Quantification of the EET processes operating in this planktonic system assists in understanding the means and extent to which *C. marina* induces redox transformations in the external medium with these transformations presumably of benefit to the survival of this organism, potentially including facilitation of iron uptake and induction of toxicity to other organisms.



INTRODUCTION

Many microorganisms are able to transport electrons from the cell to solid electron acceptors (e.g., metal oxides and electrodes) with three distinct extracellular electron transfer (EET) mechanisms proposed: (i) direct electron transfer via outer-membrane cytochromes when the cells attach to the solid electron acceptors, (ii) indirect electron transfer via soluble electron carriers, and (iii) indirect electron transfer via electrically conductive components (e.g., pili/nanowires) of the biofilm matrix.[1−3] While most studies of EET by microorganisms have been conducted under anoxic conditions using heterotrophic microbes harvesting energy from oxidation of organic substrates,[4−6] recent studies report that the diversity of microorganisms able to conduct EET from cell to solid electron acceptors appears more widespread than previously thought, and even aerobic photoautotrophs (e.g., algae and cyanobacteria) have been shown to possess such EET ability in the light.[7−12] The linking of microbial respiration to an electrode through EET has resulted in the development of microbial fuel cells (MFCs) in which electrical current can be generated by biocatalytic reactions with electrogenically active microorganisms.[4,13] These fuel cells have been developed principally as a means of quantifying energy production, though the magnitude of the charge generated is well below that considered to be of any economic value. However, the same fuel cells can also be used to probe the nature and extent of extracellular electron generation and transport.

Recently, a number of biofilm-forming prokaryotic photoautotrophs have been shown to produce electrical current in the absence of externally added mediators with photosystem II suggested to be involved in the light dependent electrogenic activity.[7−9] Only a few species of eukaryotic photoautotrophs (i.e., *Chlorella vulgaris* and *Dunaliella tertiolecta*) have been demonstrated to be able to produce current in a light-dependent manner in a mediatorless photosynthetic MFC (PMFC) using biofilm grown on indium tin oxide (ITO)-coated polyethylene terephthalate electrodes.[10] EET in

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eukaryotic photoautotrophs is expected to occur more slowly than in prokaryotic cells because the photosystems in eukaryotic photoautotrophs are embedded in organelles known as chloroplasts which are enclosed by the cell membrane and wall, while the photosystems in prokaryotic cells are covered by a smaller number of membranes.[14] While the ability of these eukaryotic photoautotrophs to capture solar energy might have little relevance to mankind's need for alternate energy supplies, the fact that this reductive capacity is actively transferred to the medium external to the organisms themselves may be of considerable relevance to the induction of reductive processes in the aquatic environment with possible implications to the bioavailability of trace nutrients such as iron, cell-to-cell signaling and, possibly, toxicity effects.

Chattonella marina, as a eukaryotic photoautotroph, is widely distributed in tropical, subtropical, and temperate coastal waters worldwide.[15,16] *C. marina* generates extracellular polymeric substances (EPS) which form a glycocalyx around the cell with this sheath composed mostly of polysaccharides, proteins, and lipids.[17] Most EPS components are considered to fall within the electrically semiconductive range in the cultivation environment of an MFC,[1] but they have generally not been considered particularly suitable electron transfer agents with exogenous electron mediators typically added to facilitate electron shuttling from the microorganisms to the anodes in photosynthetic MFCs.[18−22] It is unclear however if the soluble EPS secreted by *C. marina* can mediate EET from the cell to the solid electron acceptors. In addition, *C. marina* has been shown to produce extraordinary levels of the reactive oxygen species (ROS) superoxide ($^{\bullet}O_2^-$) and, concomitantly, hydrogen peroxide (H_2O_2) as a result of disproportionation of the biogenically generated superoxide.[23−25] The relationship between the ability of this organism to actively reduce oxygen and its extracellular electron transfer ability is unclear.

In the current study, the potential electron output activity of *C. marina* is evaluated by measuring current generation in an aerobic mediatorless single-chamber PMFC following both autotrophic biofilm cultivation on the anode and cell suspension cultivation without any acclimation. The role of exudates on the electrogenic activity of *C. marina* is investigated by electrochemical characterization of the exudates and by prevention of biofilm formation by enclosing the anode in a dialysis bag, while the role of ROS on current generation is examined by removal of ROS by addition of particular scavengers. The objectives of this study are (i) to examine the extent of electron output by *C. marina*, (ii) to ascertain the contribution of ROS produced by *C. marina* to extracellular electron transport, and (iii) to examine the redox properties of exudates secreted by *C. marina* and to ascertain whether these exudates have the ability to facilitate long-range EET processes.

EXPERIMENTAL SECTION

Cultures. Cultures of *C. marina* (CMDE01) were grown in either GSe medium (Table S1) or Aquil* medium[26] at 20 °C under a 12 h/12 h light/dark cycle with light supplied at 360 μmol photons/m^2/s by cool-white fluorescent tubes. Cells in exponential growth phase were subcultured in sterilized GSe medium at regular intervals. The synthetic seawater medium Aquil* was used in some instances to avoid interference from any organic matter that may have been present in the filtered seawater used to prepare the GSe medium. Cell concentration was determined by measuring *in vivo* chl *a* fluorescence (excitation 450 nm, emission 680 nm) using a Cary fluorescence spectrometer (Varian Inc., Australia) with calibration (r^2 = 0.86, n = 12) between fluorescence and cell density obtained using a Leica DM4000 microscope (Leica Microsystems Pty Ltd., Australia) as described earlier.[25] The 12 cultures used for calibration between fluorescence and cell density were harvested at different culture age (T = 2, 7, and 12 d) growing in both GSe and Aquil* medium. Hydrogen peroxide produced in the cultures was measured by a fluorescence technique using 2.0 μM amplex red (AR) with 1 kU/L horseradish peroxidase (HRP) as the reagent as described previously.[27] Catalase (Sigma, final concentration of 500 U/mL) and superoxide dismutase (SOD, Sigma, final concentration of 30 U/mL) were prepared as previously described.[25,28]

PMFC Construction. A Nalgene Disposable Sterile Filter Unit (115 mL) was used as a single-chamber PMFC with a working solution volume of 65 mL (Figures 1a and S1a). The bottom of the filter unit (A_{an} = 36.3 cm^2) was coated with four layers of electrically conductive carbon paint (No. 05006-AB, SPI Supplies) and used as the anode.[7] For each layer of carbon paint, 0.5 mL of paint was distributed with a brush, allowed to air-dry for 10 min, then dried at 70 °C in a furnace for 5 min, and subsequently cooled to room temperature. The fourth layer was coated using carbon paint mixed with 10 mg of polypyrrole (undoped, 20 wt % on carbon black, No. 577065, Sigma-Aldrich, USA) and dried as described. The cathode (A_{ca} = 25.5 cm^2, SLGDE, 0.5 mg/cm^2 60% Platinum on Vulcan-Cloth, Fuel Cells Etc., USA) was submerged ~0.3 cm beneath the surface of the medium. Titanium wire was attached to the anode and cathode as described after Figure S1 in the Supporting Information and connected by an external resistance of 1000 Ω.

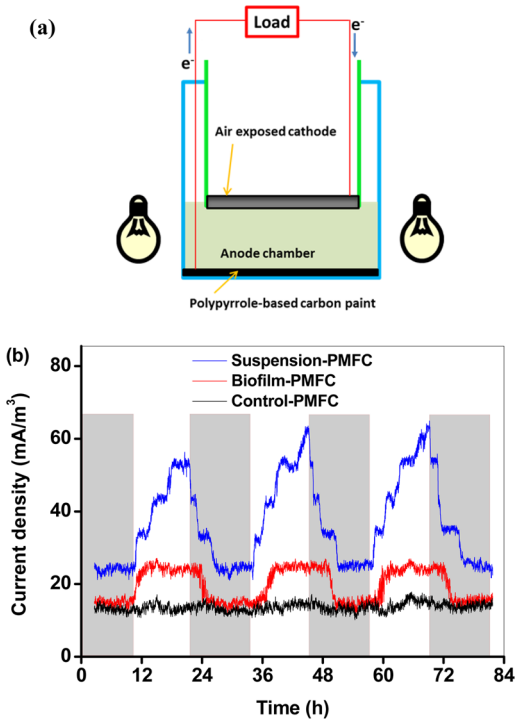

Figure 1. (a) Schematic diagram of the single-chamber PMFC and (b) current densities from the single-chamber PMFCs with biofilm and suspension of *C. marina*. Condition: GSe medium, pH 8.2; 20 °C; 12 h/12 h light/dark cycle. Dark-phases are indicated by gray rectangles.

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Two Nalgene square bottles (No. 2015-0250, Polycarbonate, 250 mL, USA) separated by a cation exchange membrane (4.4 cm × 4.4 cm, Qianqiu Group Co., Ltd., Zhejiang, China) were used as a double-chamber PMFC with a working solution volume of 150 mL in each chamber (Figure S1b and 4a). In this case, both the anode and cathode were constructed of carbon felt (4.0 cm × 4.0 cm × 0.5 cm each, Liaoyang Jingu Carbon Fiber Sci-Tech Co., Ltd., Tianjin, China) coated with 10 mg of polypyrrole using 0.5 mL of carbon paint as described. The anode was covered by a dialysis bag (MWCO: 8000−14000 D, flat width: 4.4 cm, Solarbio, China) in order to prevent the attachment of cells to the anode by inoculating cell suspension into the anodic chamber outside the dialysis bag. Titanium wire was attached to the anode and cathode and connected by an external resistance of 1000 Ω.

Three-electrode potentiostat experiments were performed using an electrochemical cell (4.8 cm × 2.3 cm × 5.4 cm) with a piece of ITO glass set on the bottom used as working electrode (Figure S2). A platinum electrode (2 mm in diameter) was used as the counter electrode with a Ag/AgCl electrode as the reference electrode (+0.222 V vs SHE). The three electrodes were connected to a CHI 650D Electrochemical Workstation (Texas, USA) with potential fixed at +0.2 V (vs Ag/AgCl).

PMFC Operation. Cultures in the single-chamber PMFC and the double-chamber PMFC were grown in GSe medium and Aquil* medium, respectively. Cells in stationary phase (12−14 days) were transferred to the single-chamber PMFC, and supplementary nutrients including major nutrients, trace metals, and vitamins were added (0.1% v/v) to prevent depletion of nutrients during the PMFC operation. The PMFCs were operated under open circuit conditions for 10 days until the open circuit voltage stabilized and under closed circuit conditions, respectively. The interior of the PMFC reactor was sterilized using 70% ethanol before adding the medium or culture. Phytoplankton in the PMFC reactors were cultivated under a 12 h/12 h (light/dark) or 16 h/8 h (light/dark) cycle (360 μmol photons/m^2/s cool-white fluorescent tubes) and supplemented daily with sterilized Milli-Q water to maintain the volume of culture at 65 or 150 mL. All treatments in the single-chamber and double-chamber PMFCs were conducted in duplicate for confirmation.

To quantify the PMFC performance throughout the experiments, the potential difference between the anode and cathode (i.e., cell voltage, V) was recorded every minute using a data acquisition system (PicoLog 1216, Pico Technology, Cambridgeshire, UK) connected to a personal computer. Current density (I) and power density (P) normalized to the volume of the anodic chamber were calculated according to $I = V/R$ and $P = V^2/R$, respectively, where R is the external resistance.

Electrochemical Characterization. For recording the polarization curve, PMFCs were stabilized at open circuit potential, and then the voltage was measured by adjusting the external resistance from 100 KΩ to 600 Ω with a maximum of 15 min required for the potential between anode and cathode to stabilize at each resistance. The internal resistance of the MFC was obtained from the slope of the linear region of the polarization curve according to Logan et al.[29]

Tafel plot measurements were also performed during steady current outputs of the PMFCs in the light phase at a scan rate of 10 mV/s using the CHI 650D Electrochemical Workstation with the anode and cathode of the PMFC as working and counter electrode and the Ag/AgCl electrode used as reference electrode. Corrosion current (I_c) and polarization resistance (R_p) of the anodes were directly obtained using CHI 650D software.

Cyclic voltammetry of the solution in the dialysis bag of the double-chamber PMFC was measured using the same electrochemical cell in Figure S2 with the ITO glass as the working electrode, the platinum electrode as the counter electrode, and the Ag/AgCl electrode as the reference electrode. The measurements were carried out in a potential range of −0.4 V to +0.8 V (vs Ag/AgCl) with a scan rate of 10 mV/s.

RESULTS AND DISCUSSION

Quantification of EET Capacity of *C. marina*. The EET capacity of *C. marina* was evaluated by measuring the electricity generation in single-chamber PMFCs. The PMFC inoculated with *C. marina* culture (1.20×10^5 cells/mL) exhibited steady voltage output after 10 days of operation under open circuit conditions (Figure S3), with biofilm visibly apparent on the surface of the anode and less than 10% of the initial cell density remaining in suspension (8.7×10^3 cells/mL) (very few cells were observed to attached on the cathode). Current outputs from the PMFCs operated under different conditions are shown in Figure 1b and reveal that the PMFC containing *C. marina* biofilm (biofilm-PMFC) exhibited a positive light response over the 12 h/12 h light/dark cycles, while the PMFC control containing only GSe medium and no cells lacked any response to light or detectable electrogenic activity. When the solution in the biofilm-PMFC was replaced by fresh medium and the cathode replaced by a new electrode, the current output after replacement was similar to the current output before replacement (Figure S4), confirming that the cells attached to the cathode do not contribute to the current outputs in the biofilm-PMFC. In addition, the PMFC containing only cell-free medium from the biofilm-PMFC lacked any response to light or detectable electrogenic activity (Figure S4), suggesting that the current output is driven by the organisms present in the biofilm and not by chemicals accumulated in the medium. These results indicate that the eukaryotic photoautotroph *C. marina* possesses light-dependent electrogenic activity when attached to an anode as an extracellular electron acceptor.

The current from the *C. marina* biofilm-PMFC increased from 15.3 mA/m^3 in the dark to 24.4 mA/m^3 in the light. McCormick et al.[10] reported that the power densities produced by biofilms of eukaryotic photoautotrophs were substantially lower than those obtained with biofilms of prokaryotes when operated under identical conditions with this difference possibly a result of the fact the photosystems in prokaryotic cells are covered by a small number of membranes, while the photosystems in eukaryotic photoautotrophs are embedded in chloroplasts which are enclosed by several chloroplast enveloping membranes, the cell membrane and wall.[14]

Acclimation is typically necessary for biofilm formation in MFCs. However, the alga *C. marina* generally exists in the form of planktonic cells. Herein, another treatment was also conducted with the PMFC operated under closed circuit conditions once inoculated with cell suspension (suspension-PMFC) in order to examine whether the planktonic cells of *C. marina* also possesses electrogenic activity. As can be clearly seen in Figure 1b, the current outputs in the suspension-PMFC are substantially higher than those in the biofilm-PMFC in both dark and light phases. Interestingly, the current outputs in the

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suspension-PMFC increase in a step-by-step manner during the light-phase and exhibit a similar step-by-step decrease in the dark-phase. A maximum current of around 63.3 mA/m^3 is observed in the light with a maximum increase in current between the light- and dark-phases of 39 mA/m^3 − 4.4-times higher than that obtained in the biofilm-PMFC. Such a step-by-step behavior is also confirmed by the current outputs obtained under continuous light/dark (24 h/24 h) conditions (Figure S5). When the same treatments were performed in the potentiostat three-electrode electrochemical cell, the current output by the organisms present in cell suspension was again found to be substantially higher than that obtained when the organisms were present as a biofilm on the electrode surface (Figure S6).

In the dark, the current outputs in the suspension-PMFC were higher than those in the control PMFC. Previous studies have reported that current output was also obtained in the dark from photoautotrophs as a result of oxidation of endogenous organic materials in PMFCs involving cyanobacterial cultures.[8,19] Additionally, the current outputs in the suspension-PMFC are higher than those in the biofilm-PMFC in the dark suggesting that more endogenous organic materials may be secreted by cells in the suspension-PMFC. Steady current outputs were obtained in both the biofilm- and suspension-PMFCs during the 15 days operation (Figure S7), suggesting that nutrient depletion does not affect the electrogenic activity of cells in both PFMCs over the 2 weeks of analysis. A decrease in current output was observed after 2 weeks of operation in both the biofilm-PMFC and suspension-PMFC with this decrease possibly due to either passivation of the anode by EPS or decrease in viability of the organisms at extended times. Results in Figure S8 show that the dissolved oxygen and pH increased under light conditions and decreased under dark conditions in both the biofilm-PMFC and suspension-PMFC in accord with results obtained in previous studies.[7,30]

Electrochemical Characterization. The polarization behavior of different PMFCs, which was determined during the light-phase on the 14th day of operation, provides a means of comparing the PMFCs. Results in Figure 2a show that the open circuit voltage of the biofilm-PMFC was only 97.9 mV which is similar to that reported in a cyanobacterial biofilm PMFC of similar construction,[7] while the open circuit voltage of the suspension-PMFC was substantially higher at 196 mV. As the slope reflects the rate of voltage decline as a function of current production, the biofilm-PMFC revealed a fast voltage drop with an internal resistance of R_{int} = 7.27 KΩ suggesting that it was more susceptible to polarization, whereas the suspension-PMFC displayed relatively slow voltage decline with R_{int} = 2.61 KΩ. Power density curves reveal that the maximum power densities (P_{max}) of the biofilm- and suspension-PMFCs were 5.85 mW/m^3 and 44.1 mW/m^3, respectively. The polarization behavior of these two PMFCs clearly demonstrates that the suspension-PMFC performed much better than the biofilm-PMFC. Results in Figure 2b reveal that the cathode potentials are similar for both the biofilm-PMFC and suspension-PMFC, but the anode potential of the suspension-PMFC was substantially lower than that of the biofilm-PMFC, which resulted in a higher power output in the suspension-PMFC. Such decrease in anode potential in the suspension-PMFC is likely due to the presence of redox active components in the exudate of *C. marina*.[31] Since the reaction at the cathode was catalyzed by the platinum on the cathode surface with high efficiency and the cathode potentials of both biofilm-PMFC

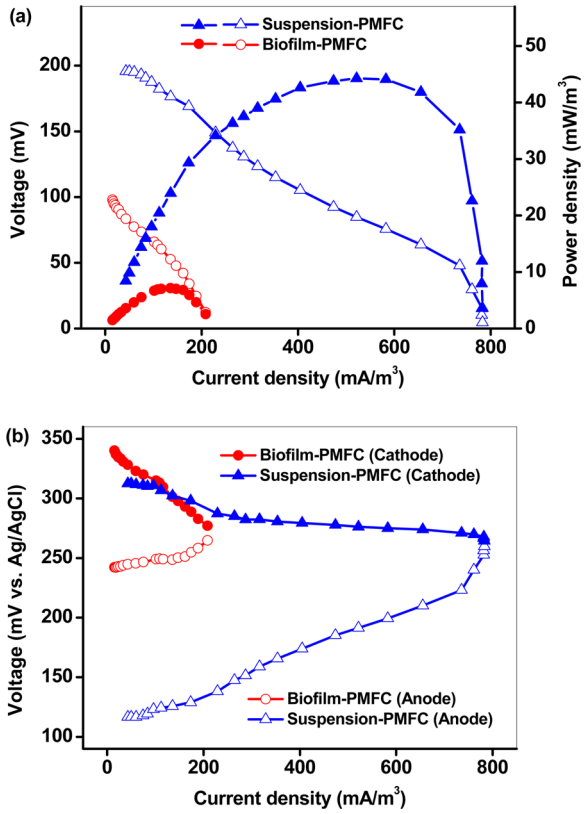

Figure 2. (a) Voltage and power density and (b) anode and cathode potentials (vs Ag/AgCl) as a function of current density of the single-chamber PMFCs with biofilm and suspension of *C. marina*. Condition: GSe medium, pH 8.2; 20 °C; under light illumination.

and suspension-PMFC were similar, it is clear that the cathode was not the limiting factor in the tested PMFCs.

The Tafel plots (Figure S9) are used to characterize the conductivities of the anodes in the different PMFCs. The corrosion current (I_c) of the anode in the control PMFC was 519 μA, whereas that in the biofilm- and suspension-PMFCs decreased to 423 μA and 450 μA, respectively (Table S2). The polarization resistance (R_p) of the anode in the control PMFC was 82.6 Ω, while that in the biofilm- and suspension-PMFCs increased to 101 Ω and 93.7 Ω, respectively. The decrease of I_c and increase of R_p of the anode in the suspension-PMFC suggest that some *C. marina* cells in the suspension study also attached or deposited on the surface of the anode with biofilm-incorporated and planktonic cells coexisting in the suspension-PMFC. Similar behavior has been reported for *Shewanella* spp. which can coexist in biofilm and planktonic forms and synergistically produce power via both direct contact with the electrode and secretion of soluble electron carriers.[32] Based on the above results (Figure S4), it is clear that direct contact of *C. marina* biofilm with the anode can drive EET from the cells to the electrode resulting in electrogenic activity in the PMFC on illumination. While it is also clear that planktonic *C. marina* are also able to generate current in the suspension-PMFC, the precise mode of electron transport from the cells to/from the electrodes remains unclear.

Role of Hydrogen Peroxide. There have been recent reports that some photoautotrophs, when placed in the cathodic chamber of PMFCs, are able to catalyze the cathodic oxygen reduction reaction (ORR) upon illumination by generating H_2O_2 which acts as an electron acceptor.[33−35] *C.*

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marina has long been known to produce extraordinary levels of ROS including $^{\bullet}O_2^-$ and H_2O_2.[23−25] As the PMFC used in this study is a single-chamber air-cathode reactor, $^{\bullet}O_2^-$ and H_2O_2 generated within the reactor by *C. marina* might affect the performance of the whole PMFC. As such, particular consideration is given below to the potential involvement of ROS in the electrogenic activity of *C. marina*.

The concentration of H_2O_2 in the solution within the PMFC was determined at the end of the light- and dark-phases of the 12 h/12 h light/dark cycles. Results in Figure 3a reveal that the

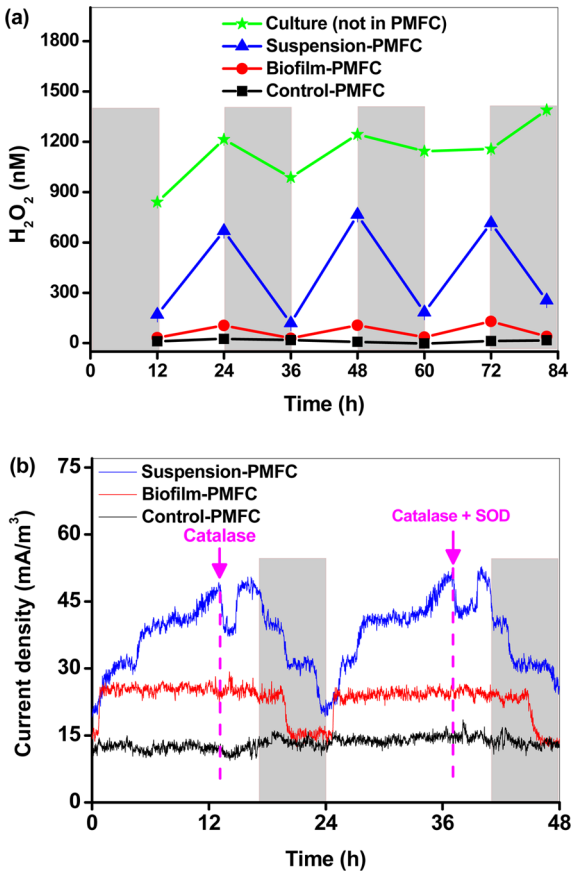

Figure 3. (a) Concentration of H_2O_2 in the solution of single-chamber PMFCs with biofilm and suspension of *C. marina* under a 12 h/12 h light/dark cycle and (b) current densities with addition of catalase (500 U/mL) and SOD (30 U/mL) from the single-chamber PMFCs with biofilm and suspension of *C. marina* under a 18 h/6 h light/dark cycle. Condition: GSe medium, pH 8.2; 20 °C. Dark-phases are indicated by gray rectangles.

H_2O_2 concentrations increase in the light-phase and decrease in the dark-phase in all PMFCs. The decrease in H_2O_2 in the dark-phase may be related to a much lower H_2O_2 production rate in the dark compared to the light as well as possible degradation of H_2O_2 by cellular peroxidases and catalases. At the end of the light-phase, the H_2O_2 concentration was 114 nM in the biofilm-PMFC with suspension cell concentration of 8.7×10^3 cells/mL, whereas the H_2O_2 concentration was 720 nM in the suspension-PMFC with suspension cell concentration of 1.20×10^5 cells/mL. Cultures of *C. marina* with the same cell concentration as that in the suspension-PMFC produced a maximum H_2O_2 concentration of 1200 nM at the end of the light-phase under the same conditions. While more detailed examination is required, it appears that electricity generation either inhibited H_2O_2 generation by *C. marina* or resulted in consumption of the H_2O_2 produced by *C. marina*.

In order to ascertain whether the *C. marina*-generated H_2O_2 was involved in the electricity generation, catalase (which induces the disproportionation of H_2O_2 to water and oxygen) was added to the PMFCs in order to eliminate the presence of H_2O_2. Results in Figure 3b show that while the current outputs were not influenced by the addition of catalase in the control and biofilm-PMFCs, the current output in the suspension-PMFC decreased from 48.5 mA/m^3 to 38.7 mA/m^3 after addition of catalase. Since *C. marina* can also produce a large amount of another ROS, $^{\bullet}O_2^-$, which can undergo disproportionation to form H_2O_2, superoxide dismutatse (SOD) was added to eliminate the presence of $^{\bullet}O_2^-$. In this case, catalase was also added because SOD catalyzes the dismutation of $^{\bullet}O_2^-$ into oxygen and H_2O_2, but the presence of H_2O_2 can inhibit the activity of SOD. The elimination of both $^{\bullet}O_2^-$ and H_2O_2 only resulted in a slight decrease in current output of the suspension-PMFC but did not display any influence on the current output of the control or biofilm-PMFC. The recovery of current in the suspension-PMFC 1.5−2 h after enzyme addition is mainly due to loss of enzyme activity. These results indicate that the limited generation of H_2O_2 did not contribute to the electricity generation by *C. marina* in the biofilm-PMFC, whereas the H_2O_2 generated by the planktonic cells was partially involved in the enhanced electricity generation in the suspension-PMFC.

Electron Outputs of Cell Suspension. Biofilm formation is generally considered critical for current generation in MFCs;[1,36] however, as the biofilm grows, more of the electrogenic cells are positioned further away from the electrode surface with some organisms apparently overcoming the inhibition to current generation associated with the lack of direct electrode contact by secreting endogenous soluble redox mediators to facilitate EET from cells to the electrodes.[2,3] The Tafel results presented above confirm that a biofilm of *C. marina* also existed in the suspension-PMFC. Since both biofilm and planktonic cells of *C. marina* coexist and may synergistically produce current in the single chamber PMFC described above, it is of interest to determine the extent of electrogenic activity in a system in which biofilm formation is absent.

Herein, a double-chamber PMFC with an anode covered by a dialysis bag was used to prevent the attachment of cells to the anode. Additionally, the presence of a cation exchange membrane between the anodic and cathodic compartments was used to prevent direct contact of the cells with the cathode and potentially microbially catalyzed cathodic ORR. As shown in Figure 4b, the current outputs in such PMFC reactor were around 0 mA/m^3 in the dark-phase and then increased immediately under light illumination at the first light/dark cycle. At the second cycle, the current output increased step-by-step during illumination and decreased step-by-step in the dark-phase at the third cycle in a manner similar to that observed in the single-chamber suspension PMFC (Figure 1b). These results indicate that the planktonic cells of *C. marina* outside the dialysis bag must secrete soluble electron carriers which can be transferred inside the dialysis bag where they facilitate the EET from the algal cells to the anode with resultant electricity generation.

The transport of soluble electron transfer mediators from the cell to the anode occurs principally by diffusion with the electricity output obtained by electrogenic microbes using

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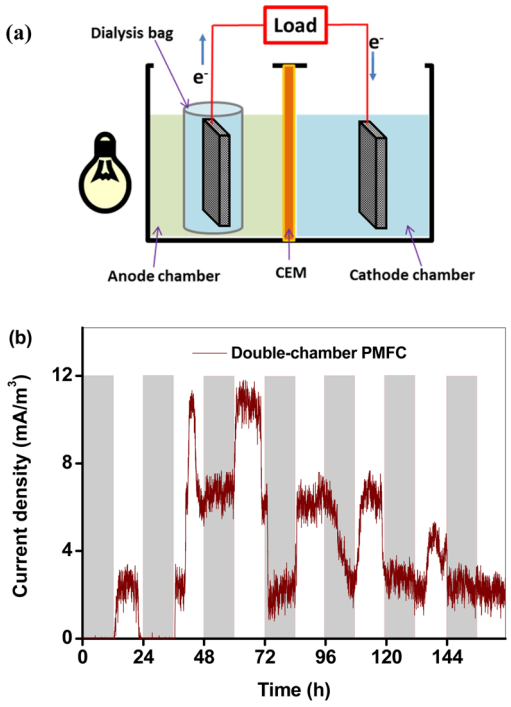

Figure 4. (a) Schematic diagram of the double-chamber PMFC and (b) current densities from the double-chamber PMFCs with suspension of *C. marina*. Condition: GSe medium, pH 8.2; 20 °C; 12 h/12 h light/dark cycle. Dark-phases are indicated by gray rectangles.

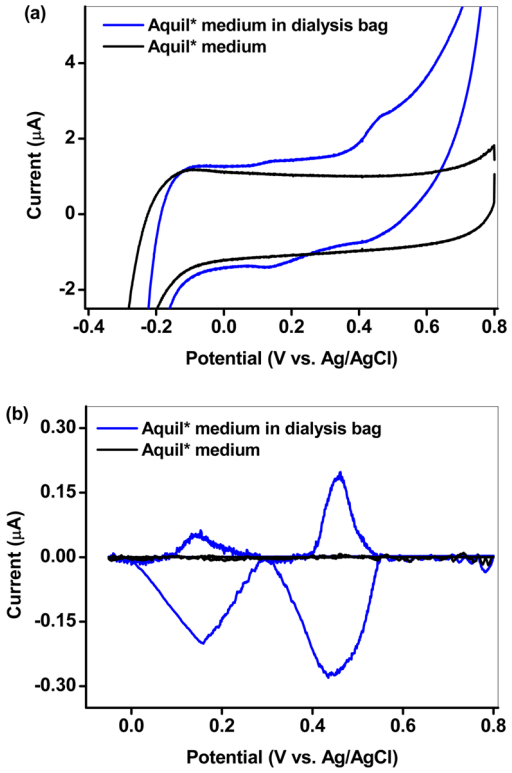

Figure 5. (a) Cyclic voltammetry and (b) background-subtracted data of the cell-free medium in the dialysis bag from the double-chamber PMFC. Condition: Aquil* medium, pH 8.2; 20 °C. Measurement was conducted in the ITO electrochemical cell under light illumination.

electron transfer mediators potentially limited by the rate and extent of diffusion of the electron transfer mediators.[1] During the fourth to sixth cycles, the current outputs were substantially lower than those at the second and third cycles and decreased gradually with time with the reduction in current possibly associated with reduced diffusion of charge carriers to the anode as a result of fouling of dialysis bag by accumulation of cells on its outer surface. It might also be attributed to a lessened extent of exudation of electroactive components as a result of the changes of the cellular metabolism on the surface of the dialysis bag.

Electrochemical Properties of Exudates. To further investigate whether any soluble electron carrier was secreted by *C. marina* into the medium, the electricity generation experiment in the double-chamber PMFC (with dialysis bag-encased anode) was repeated using the synthetic seawater medium, Aquil*, since the GSe medium is derived from filtered real seawater and may contain low molecular weight organic compounds that can pass through the dialysis membrane. The cell-free Aquil* medium in the dialysis bag was collected after 6 days of operation using 12 h/12 h light/dark cycles and then transferred to an electrochemical cell with an ITO glass working electrode.

While no significant oxidation or reduction peak was observed in the cyclic voltammetry of the control medium, the CV of the medium from the dialysis bag revealed two pairs of oxidation−reduction peaks, suggesting that two redox active compounds (or one compound with two redox active moieties) with reversible electrochemistry were released by *C. marina* into the medium over the 6 days of operation (Figure 5). The redox peaks observed are centered at around 0.15 V vs Ag/AgCl and 0.45 V vs Ag/AgCl, with these potentials substantially more positive than those of redox mediators typically observed in anaerobic MFCs.[3] In previous studies of PMFCs involving addition of exogenous redox mediators, it has been necessary that the anodic compartment be sparged with N_2, since most of the exogenous redox mediators have negative redox potentials and oxygen produced by the photoautotrophs can easily oxidize the mediators thereby limiting power production.[18−22] However, the PMFCs used in this study were operated under aerobic conditions, suggesting that only those redox mediators with positive redox potential could be effective in such a system.

Results of total organic carbon analysis showed that the organic carbon in the exudate is 3.0−3.6 mg/L higher than the cell-free medium. Results of excitation−emission matrix fluorescence measurement in Figure S11 and Table S3 showed that both humic-like and protein-like (tryptophan-like) components were present in the exudate. It is well-known that humic-like and protein-like compounds contain a variety of redox-active functional groups including quinones, phenols, and phenazines.[37] Reportedly, some quinone- or phenol-based compounds with capacity of accepting and donating electrons exhibit a positive range of redox potentials.[38] The above CV results of the medium from the dialysis bag confirm that redox active species were indeed released by *C. marina* with some of these compounds presumably playing an important role in shuttling electrons from cells to the electrode. Further effort will be given to identifying the nature of these electron carriers in future studies.

Mechanisms for EET of *C. marina*. In the present study, *C. marina*, a marine eukaryotic photoautotroph, has been shown to exhibit light-dependent electrogenic activity in pure-culture without addition of an exogenous electron mediator.

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Importantly, a PMFC to which *C. marina* cells were inoculated with no acclimation phase exhibited substantially higher current density than that of a PMFC in which a biofilm of *C. marina* cells was given time to form on the anode. These results clearly demonstrate that not only cells present within the biofilm but also planktonic cells are involved in the electrogenic activity of *C. marina* under oxic conditions. On the cathode side, the H_2O_2 generated by the planktonic cells appeared to be partially involved in the facilitated electricity generation with the H_2O_2 reduced potentially to either hydroxyl radicals or H_2O.[33,39] On the anode side, we have shown that the planktonic cells of *C. marina* secrete redox mediators into the medium that facilitate EET from the cells to the anode. Indeed, this is the first report that a eukaryotic photoautotroph releases endogenous soluble electron carriers to facilitate EET in illuminated cells without the need for direct cellular contact with the anode.

Mechanistically, it may be envisaged that, under light illumination, intracellular electron transport induces the transformation of outer membrane enzymes in their oxidized state (OME_{ox}) to reduced state enzymes (OME_{red}). The OME_{red} can directly transfer electrons to an electrode when the outer membrane is in contact with the anode (as is the case for biofilm-mediated current generation). However, for the cell suspension, the electron from OME_{red} may be captured by soluble oxidized endogenous exudates (Ex_{ox}) resulting in the generation of reduced exudates (Ex_{red}) which, in turn, can diffuse to the anode where the charge is collected. Based on the two peaks observed in the CVs (Figure 5), it appears that the exudate contains two moieties though more than two may well be present as these peaks are relatively broad and may represent composites of a number of redox active groups with closely spaced redox potential. Indeed, the stepwise increase in current observed on continuing illumination (and the stepwise decrease in current in cessation of illumination) suggests that the exudate is indeed made up of a number of redox-active moieties which are able to be reduced sequentially as the redox potential of the surface-located enzymes decrease with ongoing photosynthetic generation of reductants (e.g., reduced nicotinamide adenine dinucleotide phosphate, NADPH) in the light. Consider for example two redox active groups M-A and M-B with redox potentials $E_{M\text{-}A} > E_{M\text{-}B}$. According to the Nernst equation, the potential of the outer membrane enzyme (E_{OME}) will decrease as the proportion of OME_{red} present on continuing illumination increases (Figure S12). When $E_{OME} < E_{M\text{-}A}$, M-A will be reduced first and will enable transfer of a certain quanta of charge to the anode. With continuing decrease in E_{OME}, the point will be reached where $E_{OME} < E_{M\text{-}B}$ and M-B will be transformed into a reduced form, enabling transfer of additional charge to the anode.

Of particular interest is the ability of these exudates to retain their reduced state even in the presence of oxygen and, in so doing, transfer charge at a distance from its point of origin (i.e., the photosynthesizing organism). This resistance to auto-oxidation is to be expected if the redox active moieties in the exudate are quinone-like as the resistance of hydroquinones to oxygen-mediated oxidation is well recognized and understood to be a result of spin-restriction to the auto-oxidation process.[40,41] While auto-oxidation is spin-restricted, one electron redox processes such as reduction of Fe(III) to Fe(II) and reduction of Cu(II) to Cu(I) by hydroquinones are much more facile with such transformations potentially an end-result in natural environments of EET by organisms such as *C. marina*.

Implications of Findings. We have shown here that the electrogenic activity of *C. marina* occurs as a result of (i) direct electron transfer to the anode as a result of biofilm formation, (ii) indirect electron transfer to the anode facilitated by endogenous redox mediators released to solution by the organism, and, to a minor extent, (iii) acceptance of electrons from the cathode by reactive oxygen species such as H_2O_2 generated by the organism. Of these three charge transfer pathways, indirect electron transfer from the planktonic organism to the anode facilitated by endogenous redox-active exudates appears to dominate with the ability of these exudates to remain in their reduced state (and, thus, to retain charge) in the presence of oxygen possibly a function of the spin-restricted nature of the auto-oxidation process.

Quantification of the EET processes operating in this planktonic system assists in understanding the means and extent to which *C. marina* induces redox transformations in the external medium with these transformations presumably of benefit to the survival of this organism. Possible redox transformations that could be facilitated by extracellular electron transfer include reduction of ferric iron to the more bioavailable ferrous state and the reduction of oxygen to hydrogen peroxide which, in turn, may induce the formation of powerful oxidants via Fenton-like processes which render the organism toxic to predators.

■ ASSOCIATED CONTENT

Supporting Information

Additional data including Figures S1−S12 and Tables S1−S3 with illustrations. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Torres, C. I.; Marcus, A. K.; Lee, H.-S.; Parameswaran, P.; Krajmalnik-Brown, R.; Rittmann, B. E. A kinetic perspective on extracellular electron transfer by anode-respiring bacteria. *FEMS Microbiol. Rev.* **2010**, *34*, 3−17.

(2) Borole, A. P.; Reguera, G.; Ringeisen, B.; Wang, Z.-W.; Feng, Y.; Kim, B. H. Electroactive biofilms: Current status and future research needs. *Energy Environ. Sci.* **2011**, *4*, 4813−4834.

(3) Patil, S. A.; Hägerhäll, C.; Gorton, L. Electron transfer mechanisms between microorganisms and electrodes in bioelectrochemical systems. *Bioanal. Rev.* **2012**, *4*, 159−192.

(4) Logan, B. E.; Rabaey, K. Conversion of wastes into bioelectricity and chemicals by using microbial electrochemical technologies. *Science* **2012**, *337*, 686−690.

DOI: 10.1021/es503511m
Environ. Sci. Technol. 2015, 49, 1392−1399

(5) Lovley, D. R. The microbe electric: conversion of organic matter to electricity. *Curr. Opin. Biotechnol.* **2008**, *19*, 564−571.
(6) Logan, B. E. Exoelectrogenic bacteria that power microbial fuel cells. *Nat. Rev. Microbiol.* **2009**, *7*, 375−381.
(7) Zou, Y. J.; Pisciotta, J.; Billmyre, R. B.; Baskakov, I. V. Photosynthetic microbial fuel cells with positive light response. *Biotechnol. Bioeng.* **2009**, *104*, 939−946.
(8) Pisciotta, J. M.; Zou, Y. J.; Baskakov, I. V. Light-dependent electrogenic activity of cyanobacteria. *PLoS One* **2010**, *5*, e10821.
(9) Pisciotta, J. M.; Zou, Y. J.; Baskakov, I. V. Role of the photosynthetic electron transfer chain in electrogenic activity of cyanobacteria. *Appl. Microbiol. Biotechnol.* **2011**, *91*, 377−385.
(10) McCormick, A. J.; Bombelli, P.; Scott, A. M.; Philips, A. J.; Smith, A. G.; Fisher, A. C.; Howe, C. J. Photosynthetic biofilms in pure culture harness solar energy in a mediatorless bio-photovoltaic cell (BPV) system. *Energy Environ. Sci.* **2011**, *4*, 4699−4709.
(11) Bombelli, P.; Bradley, R. W.; Scott, A. M.; Philips, A. J.; McCormick, A. J.; Cruz, S. M.; Anderson, A.; Yunus, K.; Bendall, D. S.; Cameron, P. J.; Davies, J. M.; Smith, A. G.; Howe, C. J.; Fisher, A. C. Quantitative analysis of the factors limiting solar power transduction by *Synechocystis sp*. PCC 6803 in biological photovoltaic devices. *Energy Environ. Sci.* **2011**, *4*, 4690−4698.
(12) El Mekawy, A.; Hegab, H. M.; Vanbroekhoven, K.; Pant, D. Techno-productive potential of photosynthetic microbial fuel cells through different configurations. *Renewable Sustainable Energy Rev.* **2014**, *39*, 617−627.
(13) Rosenbaum, M.; He, Z.; Angenent, L. T. Light energy to bioelectricity: photosynthetic microbial fuel cells. *Curr. Opin. Biotechnol.* **2010**, *21*, 259−264.
(14) Freguia, S.; Virdis, B.; Harnisch, F.; Keller, J. Bioelectrochemical systems: Microbial versus enzymatic catalysis. *Electrochim. Acta* **2012**, *82*, 165−174.
(15) Tiffany, M. A.; Barlow, S. B.; Matey, V. E.; Hurlbert, S. H. *Chattonella marina* (Raphidophyceae), a potentially toxic alga in the Salton Sea, California. *Hydrobiologia* **2001**, *466*, 187−194.
(16) Imai, I.; Yamaguchi, M. Life cycle, physiology, ecology and red tide occurrences of the fish-killing raphidophyte *Chattonella*. *Harmful Algae* **2012**, *14*, 46−70.
(17) Kim, D.; Nakamura, A.; Okamoto, T.; Komatsu, N.; Oda, T.; Iida, T.; Ishimatsu, A.; Muramatsu, T. Mechanism of superoxide anion generation in the toxic red tide phytoplankton *Chattonella marina*: Possible involvement of NAD(P)H oxidase. *Biochim. Biophys. Acta, Gen. Subj.* **2000**, *1524*, 220−227.
(18) Ochiai, H.; Shibata, H.; Sawa, Y.; Shoga, M.; Ohta, S. Properties of semiconductor electrodes coated with living films of cyanobacteria. *Appl. Biochem. Biotechnol.* **1983**, *8*, 289−303.
(19) Sekar, N.; Umasankar, Y.; Ramasamy, R. P. Photocurrent generation by immobilized cyanobacteria via direct electron transport in photo-bioelectrochemical cells. *Phys. Chem. Chem. Phys.* **2014**, *16*, 7862−7871.
(20) Yagishita, T.; Sawayama, S.; Tsukahara, K. I.; Ogi, T. Effects of intensity of incident light and concentrations of *Synechococcus* sp. and 2-hydroxy-1,4-naphthoquinone on the current output of photosynthetic electrochemical cell. *Sol. Energy* **1997**, *61*, 347−353.
(21) Torimura, M.; Miki, A.; Wadano, A.; Kano, K.; Ikeda, T. Electrochemical investigation of cyanobacteria *Synechococcus* sp. PCC7942-catalyzed photoreduction of exogenous quinones and photoelectrochemical oxidation of water. *J. Electroanal. Chem.* **2001**, *496*, 21−28.
(22) Tsujimura, S.; Wadano, A.; Kano, K.; Ikeda, T. Photosynthetic bioelectrochemical cell utilizing cyanobacteria and water-generating oxidase. *Enzyme Microb. Technol.* **2001**, *29*, 225−231.
(23) Oda, T.; Nakamura, A.; Shikayama, M.; Kawano, I.; Ishimatsu, A.; Muramatsu, T. Generation of reactive oxygen species by raphidophycean phytoplankton. *Biosci. Biotechnol. Biochem.* **1997**, *61*, 1658−1662.
(24) Marshall, J. A.; Hovenden, M.; Oda, T.; Hallegraeff, G. M. Photosynthesis does influence superoxide production in the ichthyotoxic alga *Chattonella marina* (Raphidophyceae). *J. Plankton Res.* **2002**, *24*, 1231−1236.
(25) Garg, S.; Rose, A. L.; Godrant, A.; Waite, T. D. Iron uptake by the ichthyotoxic *Chattonella marina* (Raphidophyceae): Impact of superoxide generation. *J. Phycol.* **2007**, *43*, 978−991.
(26) Andersen, R. A. *Algal Culturing Techniques*; Academic Press: 2005; pp 487−488.
(27) Garg, S.; Rose, A. L.; Waite, T. D. Production of reactive oxygen species on photolysis of dilute aqueous quinone solutions. *Photochem. Photobiol.* **2007**, *83*, 904−913.
(28) Kim, D.; Nakashima, T.; Matsuyama, Y.; Niwano, Y.; Yamaguchi, K.; Oda, T. Presence of the distinct systems responsible for superoxide anion and hydrogen peroxide generation in red tide phytoplankton *Chattonella marina* and *Chattonella ovata*. *J. Plankton Res.* **2007**, *29*, 241−247.
(29) Logan, B. E.; Hamelers, B.; Rozendal, R.; Schroder, U.; Keller, J.; Freguia, S.; Aelterman, P.; Verstraete, W.; Rabaey, K. Microbial fuel cells: Methodology and technology. *Environ. Sci. Technol.* **2006**, *40*, 5181−5192.
(30) Venkata, S. G.; Chandra, R.; Venkata, M. S. Microalgae mediated bio-electrocatalytic fuel cell facilitates bioelectricity generation through oxygenic photomixotrophic mechanism. *Bioresour. Technol.* **2013**, *136*, 644−653.
(31) Wu, Y.; Liu, T.; Li, X.; Li, F. Exogenous electron shuttle-mediated extracellular electron transfer of *Shewanella putrefaciens* 200: Electrochemical parameters and thermodynamics. *Environ. Sci. Technol.* **2014**, *48*, 9306−9314.
(32) Biffinger, J. C.; Pietron, J.; Ray, R.; Little, B.; Ringeisen, B. R. A biofilm enhanced miniature microbial fuel cell using *Shewanella oneidensis* DSP10 and oxygen reduction cathodes. *Biosens. Bioelectron.* **2007**, *22*, 1672−1679.
(33) Cai, P. J.; Xiao, X.; He, Y. R.; Li, W. W.; Zang, G. L.; Sheng, G. P.; Lam, M. H. W.; Yu, L.; Yu, H. Q. Reactive oxygen species (ROS) generated by cyanobacteria act as an electron acceptor in the biocathode of a bio-electrochemical system. *Biosens. Bioelectron.* **2013**, *39*, 306−310.
(34) Liu, X. W.; Sun, X. F.; Huang, Y. X.; Li, D. B.; Zeng, R. J.; Xiong, L.; Sheng, G. P.; Li, W. W.; Cheng, Y. Y.; Wang, S. G.; Yu, H. Q. Photoautotrophic cathodic oxygen reduction catalyzed by a green alga *Chlamydomonas reinhardtii*. *Biotechnol. Bioeng.* **2013**, *110*, 173−179.
(35) Erable, B.; Féron, D.; Bergel, A. Microbial catalysis of the oxygen reduction reaction for microbial fuel cells: A review. *ChemSusChem* **2012**, *5*, 975−987.
(36) Strik, D. P. B. T. B.; Hamelers, H. V. M; Buisman, C. J. N. Solar energy powered microbial fuel cell with a reversible bioelectrode. *Environ. Sci. Technol.* **2010**, *44*, 532−537.
(37) Aeschbacher, M.; Vergari, D.; Schwarzenbach, R. P.; Sander, M. Electrochemical analysis of proton and electron transfer equilibria of the reducible moieties in humic acids. *Environ. Sci. Technol.* **2011**, *45*, 8385−8394.
(38) Rao, P. S.; Hayon, E. Redox potentials of free radicals. IV. Superoxide and hydroperoxy radicals $\cdot O_2^-$ and $\cdot HO_2$. *J. Phys. Chem.* **1975**, *79*, 397−402.
(39) Wood, P. M. The potential diagram for oxygen at pH 7. *Biochem. J.* **1988**, *253*, 287−289.
(40) Song, Y.; Buettner, G. R. Thermodynamic and kinetic considerations for the reaction of semiquinone radicals to form superoxide and hydrogen peroxide. *Free Radical Biol. Med.* **2010**, *49*, 919−962.
(41) Yuan, X.; Miller, C. J.; Pham, A. N.; Waite, T. D. Kinetics and mechanism of auto- and copper-catalyzed oxidation of 1,4-naphthohydroquinone. *Free Radical Biol. Med.* **2014**, *71*, 291−302.

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Environ. Sci. Technol. 2015, 49, 1392−1399