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Light-Responsive Current Generation by Phototrophically Enriched Anode Biofilms Dominated by Green Sulfur Bacteria

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ABSTRACT: The objective of this study was to employ microbial electrochemical cells (MXCs) to selectively enrich and examine anoxygenic photosynthetic bacteria for potential anaerobic respiration capabilities using electrodes. In the process, we designed a novel enrichment strategy that manipulated the poised anode potential, light, nitrogen availability, and media supply to promote growth of phototrophic bacteria while minimizing co-enrichment of non-phototrophic anode-respiring bacteria (ARB). This approach resulted in light-responsive electricity generation from fresh- and saltwater inocula. Under anoxic conditions, current showed a negative light response, suggesting that the enriched phototrophic consortia shifted between phototrophic and anaerobic respiratory metabolism. Molecular, physical, and electrochemical analyses elucidated that anode biofilms were dominated by green sulfur bacteria, and biofilms exhibited anode respiration kinetics indicative of non-mediated electron transfer, but kinetic parameters differed from values previously reported for non-phototrophic ARB. These results invite the utilization of MXCs as microbiological tools for exploring anaerobic respiratory capabilities among anoxygenic photosynthetic bacteria.

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KEYWORDS: microbial fuel cell; anode-respiring bacteria; photosynthesis; biofilm; selective enrichment; green sulfur bacteria

Introduction

Photosynthetic bacteria use photochemical reaction centers to convert light energy into chemical energy (Bryant and Frigaard, 2006). Anoxygenic photosynthetic bacteria oxidize a variety of organic and inorganic compounds as electron donors, and CO_2 is the principal electron acceptor (Blankenship, 2002). All anoxygenic photosynthetic bacteria use light to drive ATP synthesis via photophosphorylation (Ehrlich and Newman, 2008; Gottschalk, 1986). In the dark, however, anoxygenic phototrophs can obtain energy for cell maintenance from the oxidation of storage polymers such as glycogen or poly(3-hydroxyalkanoates; PHA) accumulated in the light (Mas and van Gemerden, 2004). Anaerobic respiration could potentially provide an alternative strategy for energy production in the dark. Some purple bacteria perform respiration using nitrate, dimethyl sulfoxide (DMSO), or trimethylamine-*N*-oxide (TMAO) as electron acceptors, suggesting that anaerobic respiratory pathways may exist in anoxygenic photosynthetic bacteria (McEwan, 1994).

Microbial electrochemical cells (MXCs) provide useful platforms for studying several aspects of bacterial anaerobic respiration in vivo (Marsili et al., 2008b; Speers and Reguera, 2011; Torres et al., 2008). Anode-respiring bacteria (ARB) oxidize organic substrates and transfer electrons to a solid electrode acting as the terminal electron acceptor. *Geobacter sulfurreducens* expresses an extracellular network of *c*-type cytochromes for long-range electron transfer to electrodes with minimal potential losses (Strycharz-Glaven et al., 2011; Torres et al., 2010). *Shewanella oneidensis* MR-1 performs anode respiration at a rate of $1.3 \times 10^6\ e^-\ cell^{-1}\ s^{-1}$ (McLean et al., 2010), a value similar to *Escherichia coli* respiring oxygen ($4.0 \times 10^6\ e^-\ cell^{-1}\ s^{-1}$; Andersen and von Meyenburg, 1980), indicating that utilization of an insoluble electron acceptor does not inherently limit the respiration rate. Several other bacteria can respire electrodes as via direct, shuttling, or long-range electron transfer mechanisms (Logan, 2009), but only two anoxygenic phototrophs, the purple non-sulfur bacteria *Rhodobacter capsulatus* and *Rhodopseudomonas palustris* DX-1, have been shown to perform anaerobic respiration using either Fe(III) or a graphite anode (Dobbin et al., 1996; Xing et al., 2008).

The practicality of incorporating photosynthetic microorganisms into MXCs, referred to as either microbial

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photoelectrochemical cells (MPCs) or photo-MXCs, has been studied in multiple contexts. In these systems, microorganisms assist in the conversion of light energy into electricity, potentially improving the overall MXC energy balance (Rosenbaum and He, 2010; Rosenbaum and Schroeder, 2010). In several MPC studies, current could only be generated by addition of redox mediators (Tanaka et al., 1985; Yagishita et al., 1998) or by photosynthetic H_2 production and in situ catalyzed oxidation (Cho et al., 2008; Rosenbaum et al., 2005); thus, these systems suffered from poor current densities and the need for electrochemical catalysts. Some MPCs have shown increases in either voltage or current density in the light (Cao et al., 2008; Xing et al., 2009; Yagishita et al., 1993; Zou et al., 2009), while in other instances photosynthetic oxygen evolution by cyanobacteria or algae interfered with electricity generation during light periods (He et al., 2009; Yagishita et al., 1998), resulting in a decrease in current.

Anoxygenic phototrophs can potentially obviate the undesirable effects of photosynthetic oxygen evolution in an MXC anode. Unfortunately, only one such phototroph, *Rps. palustris* DX-1, has been shown to produce electricity in pure culture (Xing et al., 2008), a finding which provides the only evidence to date for direct electron transfer from a photosynthetic organism to the anode (Rosenbaum and He, 2010). Thus, the primary objective of this study was to use MXCs as tools for discovering phototrophic ARB since these bacteria may exist elsewhere in nature. To accomplish this, we designed a selective enrichment scheme using MXCs that promoted growth of phototrophs while minimizing enrichment of non-phototrophic ARB. Manipulation of light availability was used to survey the microbial communities for the presence of phototrophic ARB. We report electricity generation by two different (freshwater vs. saltwater) phototrophic biofilms, both of which exhibited a negative light response. Characterization of the respiration kinetics and microbial community composition revealed that dominant phototrophic bacteria were responsible for light responsiveness. Our results suggest that MXCs can be employed to evaluate alternative metabolic pathways for energy generation in anoxygenic photosynthetic bacteria.

Materials and Methods

Inocula: Freshwater inoculum was derived from the brown-green colored anode suspension of a dual-chamber MXC (anode poised at −350 mV vs. Ag/AgCl) treating fermented centrate, inoculated originally with anaerobic digested sludge from the Mesa Northwest Water Reclamation Plant (Mesa, AZ). Saltwater inoculum was taken from the top 2 cm of a shallow saline microbial mat (Cabo Rojo, Puerto Rico). Samples were collected in tightly sealed 50-mL Falcon tubes and stored at 4°C in the dark. Prior to inoculation into MXCs, samples were enriched for phototrophs as described below.

Media

Freshwater and saltwater media containing 50 mM $NaHCO_3$ were prepared as described previously (Griffin et al., 2007; Widdel and Bak, 1992), with the following adjustments: Na_2SO_4 was omitted as an alternative electron acceptor, NH_4Cl was omitted to impose N_2-fixing conditions as a layer of selective pressure against non-phototrophic ARB, 2 mL of Wolfe's vitamin mixture (ATCC, Manassas, VA) were added per liter of medium, and cyanocobalamin (vitamin B_{12}) was added to 50 μg/L. Media were made anoxic by flushing with N_2, and pH was adjusted to ~7.2 by flushing with N_2:CO_2 (80:20). Fresh- and saltwater media had conductivity of 8.3 and 49 mS cm^{-1}, and salt content of 0.46% and 2.6%, respectively, as measured with a digital conductivity meter (Oakton Instruments, Vernon Hills, IL). Sterile anoxic stock solutions of sodium acetate (1 M) and $Na_2S{\cdot}9H_2O$ were prepared separately and autoclaved sealed under N_2. Acetate and sulfide were added as electron donors to media to achieve final concentrations of 10 and 0.5 mM, respectively. Media bottles were stored in the dark.

Phototrophic Pre-Enrichment

Inocula (1 mL of a dense cell suspension for freshwater; ~1 g of the original microbial mat for saltwater) were added to 49 mL of appropriate media in sterile serum bottles in an anaerobic chamber (Coy Laboratory Products, Grass Lake, MI) and sealed with a rubber stopper. Headspaces were flushed with N_2:CO_2 (80:20). Enrichments were incubated in an enclosed box for several months at room temperature behind an RG715 optical filter (Schott Glass, Duryea, PA), which transmitted only $\lambda > 715$ nm light to select against oxygenic phototrophs (Blankenship, 2002). Illumination was provided by a 60 W incandescent bulb mounted 20 cm away from the glass filter.

MXC Construction, Inoculation, and Operation

Dual chamber "H-type" MECs were constructed as described previously (Parameswaran et al., 2009) and autoclaved. A detailed description is provided in the Supplementary Information. In an anaerobic chamber, 3.5 mL of pre-enriched inoculum was added to phototrophic media described above to achieve a final anode chamber volume of 350 mL. The cathode was filled with a sterile, anoxic solution of NaOH, pH 12.5 to maintain charge neutrality without basic forms of cathode buffer ions such as phosphate or bicarbonate being transported into the anode. Graphite rod anodes (18 cm^2 total surface area) were poised using a potentiostat (VSP3; Bio-Logic USA, Knoxville, TN) versus an Ag/AgCl reference electrode (BASi, West Lafayette, IN) at the working potentials described below. All anode potentials are reported versus standard hydrogen electrode (SHE), making the appropriate conversion as described in the Supplementary Information.

The anode pH was maintained with a humidified stream of N_2:CO_2 (80:20) delivered through Viton tubing (Cole-Parmer, Vernon Hills, IL). All MEC experiments were conducted at ambient temperature ($24 \pm 2^{\circ}C$).

Phototrophic Enrichment and Chronoamperometry

Reactors were initially illuminated with $\lambda > 715$ nm light as described above and anodes were poised at either −245 or −284 mV for fresh- and saltwater reactors, respectively. After 9 days of photoenrichment, continuous media flow for 12 days at a flow rate of 0.3 mL/min (1 day hydraulic retention time) was used to washout non-phototrophic ARB. Media was supplied through Viton tubing using a peristaltic pump (Cole-Parmer). The headspace in the media bottles was replaced daily with filtered N_2:CO_2 (80:20) delivered via a Tedlar bag (SKC Inc., Eighty Four, PA). Reactors were then placed in the dark to force anode respiration. Light responses were conducted by exposing reactors to full incandescent light (1,000 lux; Extech Instruments, Nashua, NH).

Chemical Analyses

Acetate was measured by high-pressure liquid chromatography (HPLC) as described previously (Parameswaran et al., 2009). pH measurements were performed immediately after sampling using a microelectrode pH probe (Cole-Parmer) and digital pH meter (Thermo Scientific). Bacteriochlorophyll content was determined by resuspension of dried cell pellets in 1:1 (v/v) acetone:methanol and absorption spectra were read from 1,000 to 600 nm using a Cary WinUV-Vis spectrophotometer.

Electrochemical Analyses

Cyclic voltammograms (CV) were generated at a scan rate of 1 mV s^{-1} starting from the open circuit potential (−209 and −230 mV for fresh- and saltwater, respectively) and scanning forward and backward across a 170-mV window to vertex potentials of −75 and −245 mV (freshwater) or −114 and −284 mV (saltwater). Voltage sweeps were repeated once with the second sweep reported. Anode potentials in the CVs were corrected for Ohmic losses between the anode and reference electrode by electrochemical impedance spectroscopy (EIS; Torres et al., 2008). We used the Nernst–Monod equation (Kato Marcus et al., 2007),

$$j = j_{\max}\left[1 + \exp\left[\left(\frac{-nF}{RT}\right)(E - E_{\mathrm{ka}})\right]\right]^{-1} \quad (1)$$

which was fit by least-square analysis (Sáez and Rittmann, 1992) using $j_{\max}$ and E_{ka} as fitting parameters.

DNA Extraction and Pyrosequencing

DNA was extracted as described previously (Sheng et al., 2011) from a 1-cm length of scraped biofilm using a DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA). Pyrosequencing was performed by Research and Testing Laboratories LLC (Lubbock, TX) using primers 104F and 530R (Li et al., 2011) targeting the V2 and V3 regions of bacterial 16S rDNA. Raw sequences (3,301 and 3,686 for the fresh- and saltwater, respectively) were trimmed, aligned, clustered, and classified as described previously (Garcia-Peña et al., 2011).

Results and Discussion

Phototrophic Enrichment

We describe a strategy for selective enrichment of anoxygenic photosynthetic bacteria using MXCs. During the initial photoenrichment period, anodes were poised at potentials (−550 mV vs. Ag/Cl, corresponding to −245 and −284 mV for fresh- and saltwater reactors, respectively) that inhibited growth of non-photosynthetic ARB by minimizing energy available from coupling acetate oxidation (−280 mV; Cao et al., 2009) to anode respiration. In addition, the ability of most anoxygenic phototrophs to fix N_2 as sole N source was exploited by omitting NH_4Cl from the media (Blankenship, 2002; Madigan, 2004). Non-phototrophic ARB lacked ATP required for N_2 fixation (Ueki and Lovley, 2010) due to the anode being poised at an unfavorably low potential. After 9 days, bacteriochlorophyll absorbance spectra showed characteristic peaks for purple bacteria (BChl *a*; 770 nm) and green sulfur bacteria (BChl *c*; 666 nm; Frigaard et al., 1996) in both reactors (Supplementary Fig. 1), indicating that the electron donors supplied (acetate and sulfide) did not preferentially select for one particular group of anoxygenic phototrophs. To evaluate whether the enriched phototrophic communities could generate current, reactors were placed in the dark on Day 14. On Day 17, anode potentials were shifted 90 mV more positive (−155 or −194 mV for fresh- and saltwater experiments, respectively), creating a potential difference from which cells could obtain energy from anode respiration. However, these potentials still limited the energy available to any remaining non-phototrophic ARB (Fig. 1A and B). To prevent washout of phototrophic ARB before having an opportunity to form an anodic biofilm and produce current, continuous media flow was stopped on Day 19, after a stable current was produced and resumed on Day 38 through the remainder of the study.

Figure 1 shows the onset of anodic current (Day 29) 12 days after adjusting poised potentials to more positive values. A prolonged dark incubation was necessary to exhaust all storage polymers accumulated by phototrophic bacteria as an energy reserve (Mas and van Gemerden, 2004) and to force cells to migrate from suspension, where light irradiance is maximized, to the electrode, where anode respiration occurs. Maximum current densities were 1 and 0.7 A m^{-2} for the freshwater and saltwater reactors,

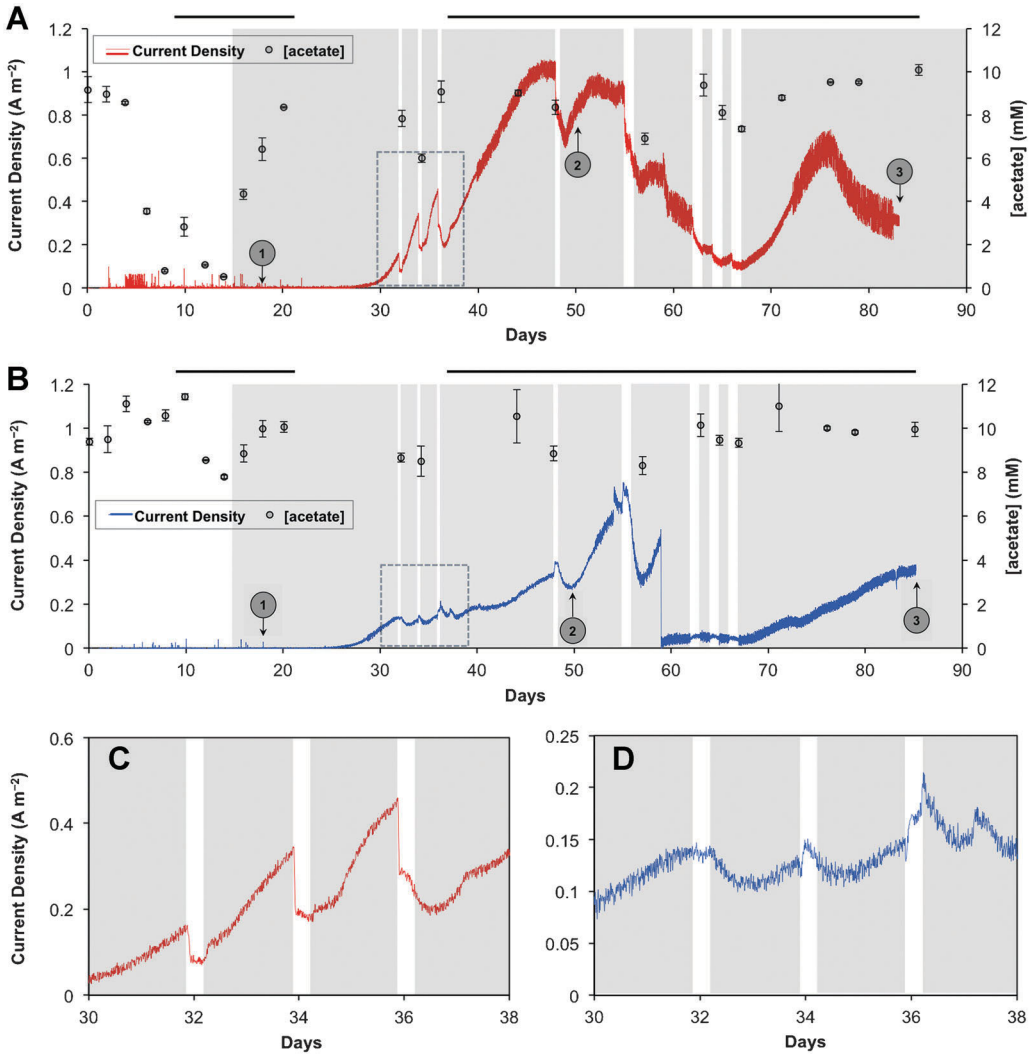

Figure 1. Chronoamperometry and light-responsive current production in freshwater (**A**) and saltwater (**B**) experiments. Shaded areas indicate periods of darkness. **Panels C** and **D** show zoomed sections of the first three light exposure periods for the fresh- and saltwater reactors, respectively (indicated by the dotted boxes in Panels A and B). During the 0–14 day initial photoenrichment, cells were incubated with $\lambda > 715$ nm light as described in Materials and Methods Section to select against oxygenic phototrophs. In all subsequent light periods biofilms were exposed to unfiltered incandescent light (1,000 lux). Solid black bars above the plots indicate periods of continuous supply of medium at 0.3 mL min^{-1} flow rate. Numbered gray circles indicate the following: 1, adjustment of poised anode potential to −155 mV (freshwater) or −194 mV (saltwater) versus SHE; 2, cyclic voltammetry (see Fig. 2); 3, sampling anode biofilms for community analysis and SEM.

respectively. These values were similar to those reported for biofilms of *G. sulfurreducens* under anode potential-limiting conditions (Marsili et al., 2010; Torres et al., 2009) and were sufficiently high to suggest extracellular electron transfer through a solid conductive matrix and not via electron shuttles (Torres et al., 2010). Scanning electron micrographs indicated that the anode biofilms were thicker than a monolayer of cells and contained several different cell morphologies (Supplementary Fig. 2).

Light-Responsive Current Generation

We exposed the anodes to three consecutive periods of illumination with direct incandescent light (1,000 lux) for 8 h followed by 40 h of darkness to investigate the phototrophic contribution to current generation. Subsequent light periods were increased to 12 and 24 h to examine the dynamics of extended light exposure. For the freshwater reactor, Figure 1C shows a sharp decrease in current within 10 min of light exposure, and current recovered only after placing the anode back in darkness. After the first two periods of light exposure (8 h each; Days 32 and 34), subsequent incubations in the light resulted in longer time needed for current to increase again in darkness. The longest light period (24 h on day 55) resulted in an unrecoverable loss of current (Fig. 1A), perhaps due to washout of a portion of the current-generating phototrophs by continuous media flow.

In the saltwater MXC, however, light exposure first resulted in a noticeable increase in current over a period of

1–4 h, followed by a similar pattern of decreased current continuing several hours into the subsequent dark period (Fig. 1D). Current density reached a maximum of 0.7 A m^{-2} before dropping below half this value in response to a 24-h light incubation period on Day 55 (Fig. 1B). On day 58, a majority of the biofilm was sloughed off the anode and current decreased significantly, demonstrating that bacteria in the biofilm were responsible for producing current. Continuous media supply ensured that decreases in current generation were not attributed to electron donor limitation (Fig. 1A and B). No sudden changes in temperature occurred during light incubations.

Each biofilm exhibited different initial light responses before eventually showing a net decrease in current (Fig. 1). The freshwater biofilm consistently responded to light within 5–10 min, while the saltwater biofilm required multiple light exposures to exhibit a consistent initial light response (Fig. 1B). When placed back in darkness, both biofilms required several hours to resume the same current density as before the light cycle, possibly due to dispersion of phototrophic bacterial cells from the biofilm to the suspension during the light period in order to maximize light exposure. Changes in current were not likely due to photohydrogen production and in situ oxidation (Cho et al., 2008) since the anodes underwent continuous gas flushing.

The dynamics of these light responses raised the possibility of anode respiration by phototrophs as a pathway for energy generation in the dark. The energy available from anode respiration is determined by the difference in redox potentials between the electron-donating and -accepting half reactions, minus the potential losses associated with extracellular electron transfer (Torres et al., 2010). For phototrophic ARB in the biofilm, light serves as an additional energy source. Our data show that phototrophic ARB might perform both photophosphorylation and anode respiration to meet their ATP requirements, and that darkness is required to evaluate their anode respiration capabilities.

Several studies evaluating photosynthetic organisms in MXCs report increases in either current density or cell voltage upon illumination (Cao et al., 2008; He et al., 2009; Xing et al., 2009). A mutant of *S. oneidensis* expressing proteorhodopsin, a light-driven proton pump (Bryant and Frigaard, 2006), showed a similar increase in current generation in the light; this increase was attributed to an increased uptake rate of lactate as electron donor (Johnson et al., 2010). Here we present electricity production by two bacterial biofilms showing the opposite light response in that current decreased considerably during periods of illumination. A similar pattern was shown for a photosynthetic sediment MFC, but this effect was indirect as phototrophs did not appear to act as ARB in this system (He et al., 2009).

Cyclic Voltammetry of Light-Responsive Biofilms

The magnitude and duration of light responses for both reactors suggested the presence of current-generating anoxygenic phototrophs on the anode. To investigate this possibility further, we studied the anode respiration kinetics of the biofilms using low scan rate cyclic voltammetry (LSCV), an electrochemical technique used extensively for studying important redox species and processes occurring in anode biofilms (LaBelle and Bond, 2009).

Figure 2A and B shows cyclic voltammograms (CVs) produced in the dark by the fresh- and saltwater biofilms, respectively. As expected with continuous feeding of medium, we did not detect reversible oxidation–reduction peaks indicative of soluble electron shuttles (Marsili et al., 2008a). Instead, we found that the biofilms exhibited electron transfer kinetics that fit the Nernst–Monod equation (Equation 1; Kato Marcus et al., 2007). Surprisingly, the model fit CVs only when we adjusted the value of n (Fig. 3), where n is the number of electrons transferred per current-generating reaction by ARB. We obtained best model fits when we used values of $n = 2$ and $n = 3$ for the freshwater and saltwater reactors, respectively (Fig. 2A and B). For *G. sulfurreducens* biofilms not limited by media conditions or anode potential, $n = 1$ (Torres et al.,

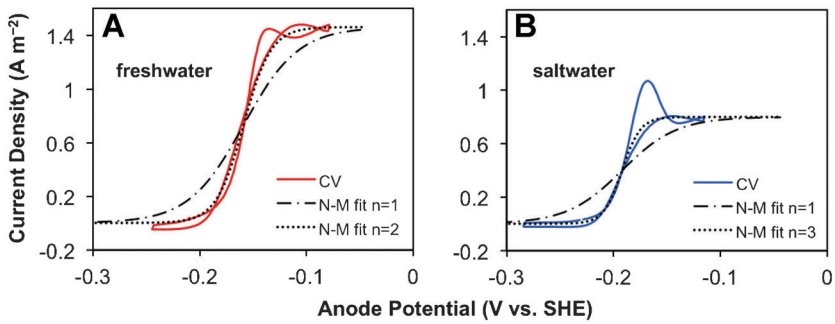

Figure 2. Cyclic voltammograms of freshwater (**A**) and saltwater biofilms (**B**) generated at 1 mV s^{-1} scan rate in the dark. Nernst–Monod plots are overlaid for $n = 1$ (dashed lines) and for best-fit n values (dotted lines). The second of two scans is shown.

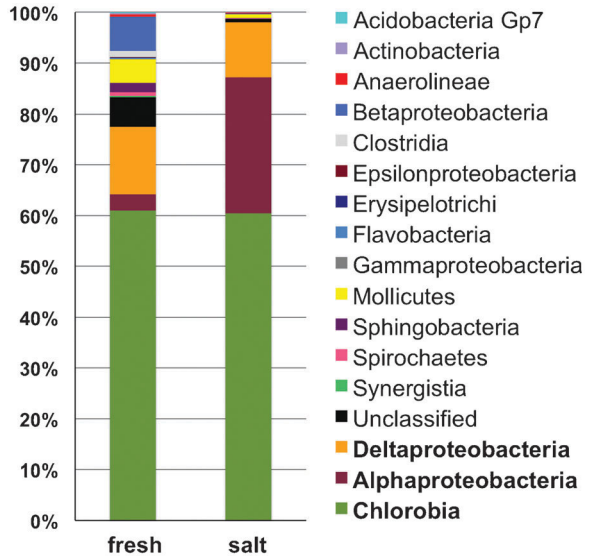

Figure 3. Bacterial relative abundance for enriched fresh- and saltwater biofilms at the class level. Classification was performed with 2,017 and 2,226 sequences for the fresh- and saltwater biofilms, respectively.

2008), while higher n values might implicate rate-limiting steps in electron transfer from the electron donor to intracellular cytochromes (Bonanni et al., 2012). CVs of *G. sulfurreducens* grown at the same limiting anode potential of −160 mV used in this study, however, produced similar sigmoidal Nernst–Monod behavior (Marsili et al., 2010), with slopes indicative of n values greater than 1. It is unclear to what extent the observed $n > 1$ behavior in both fresh- and saltwater CVs is either a signature of many ARB growing under potential-limited conditions or an electrochemical indication of phototrophic ARB possessing novel anode respiration kinetics.

Our empirical value for E_{ka} was −159 mV for the freshwater biofilm, a value in close agreement to that observed for acetate-fed *G. sulfurreducens* biofilms under well-buffered, non-limiting conditions (Marsili et al., 2008b; Torres et al., 2008). The saltwater biofilm, however, showed an E_{ka} of −192 mV, a value roughly 35 mV more negative than for *G. sulfurreducens* and the most negative E_{ka} value reported to date for any ARB, indicating that the light-responsive anode communities minimized extracellular potential losses associated with extracellular electron transfer (Torres et al., 2010).

Microbial Community Structure of Anode Biofilms

High-throughput pyrosequencing of the V2–V3 hypervariable regions of bacterial 16S rDNA from biofilm samples taken on Day 86 (48 days after resuming continuous media flow; Fig. 1A and B) revealed that anoxygenic phototrophs dominated anode biofilm communities in both the freshwater and saltwater reactors. Figure 3 shows relative abundances of operational taxonomic units (OTUs) at the class level. Genus-level classification using a 97% identity cutoff is presented in Supplementary Table I. No 16S rDNA sequences belonging to cyanobacteria were detected, indicating that photosynthetic O_2 evolution was not responsible for light-induced decreases in current.

Green sulfur bacteria belonging to the Chlorobia class (in the phylum *Chlorobi*) were the dominant phototrophs in both biofilms, comprising ~60% of the microbial sequences (Fig. 3). At the genus level, *Chlorobaculum* accounted for 7% of phylotypes in the freshwater reactor, with *Chlorobium* comprising the remainder of green sulfur bacteria detected in both MXCs (Supplementary Table S1). Green sulfur bacteria are metabolic specialists; all known species are obligate photolithoautotrophs (Blankenship, 2002) and thus are not expected to perform dark respiration. Our results, however, suggest that some green sulfur bacteria may possess novel respiratory pathways for obtaining energy in the dark, when sulfide is not used as an electron donor (Overmann, 2006), possibly via breakdown of stored glycogen to acetate and other organic acids (Mas and van Gemerden, 2004) or by reactions of a recently reported oxidative TCA cycle involved in acetate metabolism (Tang and Blankenship, 2010). To produce current, green sulfur bacteria would also require a pathway for extracellular electron transport (EET) to the anode, and the poorly understood pathway for oxidation of extracellular S^0 as photosynthetic electron donor in green sulfur bacteria is postulated to be analogous to the EET pathways for dissimilatory metal-reducing bacteria working in reverse (Frigaard and Dahl, 2009). In addition, the possibility of insoluble substrates being used directly as electron acceptors for anaerobic dark respiration by green sulfur bacteria has not been investigated, as most studies have justifiably focused on their strictly phototrophic metabolism (Feng et al., 2010; Tang and Blankenship, 2010). In this respect, the anode of an MPC could emerge as a remarkably useful microbiological tool for interrogating novel pathways for anaerobic respiration, particularly in bacteria that are not predicted to carry out such processes.

OTUs assigned to the genus *Roseospirillum* (Glaeser and Overmann, 1999) accounted for the bulk of observed phototrophic α-Proteobacteria and 22% overall relative abundance in the saltwater biofilm (Fig. 3; Supplementary Table S1). However, purple bacteria in the freshwater biofilm accounted for only ~0.6% of the community, suggesting they were not the key microorganisms contributing to current generation or light-responsiveness at the magnitude observed. If purple bacteria contributed to current production in the saltwater reactor, the differences in their relative abundance might explain why the initial light response differed between biofilms (Fig. 1). Taken together, the dramatic light-induced drop in current, the low relative abundance of purple bacteria, and the fact that *Chlorobi* were the only other phototrophs present in the

freshwater biofilm suggest that some green sulfur bacteria may be capable of conducting respiratory metabolism in the dark.

Pyrosequencing detected non-phototrophic ARB genera belonging to δ-Proteobacteria which accounted for ~6–9% of the overall relative abundance in both biofilms (Supplementary Table S1). However, it is unlikely that this small fraction, consisting mostly of phylotypes closely related to *Geobacter* and *Geoalkalibacter* in fresh- and saltwater biofilms, respectively, was solely responsible for the high respiration rates (i.e., recorded current densities) we observed, given that our reactors were operated for an extended period under acetate-fed conditions which would eventually allow these ARB to proliferate. Other possible ARB genera included *Desulfuromonas* (Bond et al., 2002), which was present at ~2% only in the saltwater biofilm. Although the contribution of non-phototrophic ARB to current generation cannot be ruled out, these ARB would not be expected to respond negatively to light. Instead, the pattern and magnitude of the light responses presented in this study indicate that phototrophic bacteria played a critical functional role in the enriched anode biofilms.

Both in the dark and in the light, acetate serves as a respiratory electron donor for non-photosynthetic ARB. In green sulfur bacteria, reductive photoassimilation of acetate leads to increased synthesis of intracellular glycogen as a storage polymer, with sulfide serving as the electron donor (Sirevåg and Ormerod, 1970). In the dark, when sulfide is not oxidized, glycogen catabolism supplies cells with energy and reducing power (Sirevåg and Ormerod, 1977; Thorud and Sirevåg, 1982) and acetate is formed as a byproduct, potentially adding to the balance of electron donors available to non-photosynthetic ARB situated in a biofilm shared with green sulfur bacteria. In addition, light-driven sulfur cycling between green sulfur bacteria and sulfur-reducing bacteria (Warthmann et al., 1992) could also lead to formation of S^0 as a competing electron sink for non-phototrophic ARB in the light. Thus acetate exchange or sulfur cycling between green sulfur bacteria and non-phototrophic ARB are possible mechanisms by which green sulfur bacteria function in phototrophic ARB biofilms. Controlled coculture experiments are warranted to further investigate these possible syntrophies and their responsiveness to light.

Implications

The data presented here establish the possibility for non-mediated electricity generation by novel phototrophic ARB. Further work is necessary to examine possible anode respiration capabilities of phototrophs in pure culture and to evaluate syntrophies in coculture with non-phototrophic ARB. Light-responsiveness of green sulfur bacteria-dominated biofilms suggests expanding the list of ARB to potentially include green sulfur bacteria. The suspected ubiquity of extracellular electron transfer in the bacterial world (Gorby et al., 2006) along with possible importance of electrochemical communication in biogeochemistry (Nielsen et al., 2010) invites further exploration of MXCs as microbiological tools for selective enrichment of phototrophic ARB. These bacteria hold several advantages in potentially providing useful couplings between photosystems and electrodes, including solar energy conversion to electricity and fuel synthesis from cathode-derived electrons, CO_2, and light.

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