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Coupling Dark Metabolism to Electricity Generation Using Photosynthetic Cocultures

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ABSTRACT: We investigated the role of green sulfur bacteria inlight-responsive electricity generation in microbial electrochemical cells (MXCs). We operated MXCs containing either monocultures or defined cocultures of previously enriched phototrophic *Chlorobium* and anode-respiring *Geobacter* under anaerobic conditions in the absence of electron donor. Monoculture control MXCs containing *Geobacter* or *Chlorobium* neither responded to light nor produced current, respectively. Instead, light-responsive current generation occurred only in coculture MXCs. Current increased above background levels only in the dark and declined slowly over 96 h. This pattern suggested that *Chlorobium* exhausted intracellular glycogen reserves via dark fermentation to supply an electron donor, presumably acetate, to *Geobacter*. With medium containing sulfide as the sole photosynthetic electron donor, current generation had a similar and reproducible negative light response. To investigate whether this metabolic interaction also occurred without an electrode, we performed coculture experiments in batch serum bottles. In this setup, sulfide served as the sole electron donor, whose oxidation by *Chlorobium* was required to provide S^0 as the electron acceptor to *Geobacter*. Copies of *Geobacter* 16S rDNA increased approximately 14-fold in batch bottle cocultures containing sulfide compared to those lacking sulfide, and did not decline after termination of sulfide feeding. These results suggest that products of both photosynthesis and dark fermentation by *Chlorobium* were sufficient both to yield an electrochemical response by *Geobacter* biofilms, and to promote *Geobacter* growthin batch cocultures. Our work expands upon the fusion of MXCs with coculture techniques and reinforces the utility of microbial electrochemistry for sensitive, real-time monitoring of microbial interactions in which a metabolic intermediate can be converted to electrical current.

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Introduction

Biogeochemical cyclesoccur via energy-conserving microbial transformations requiring cooperation among individual microbes performing specialized functions within diverse communities (Ehrlich and Newman, 2009). However, the unculturability of most microorganisms poses a major challenge in understanding the individual and synergistic roles for key microbial populations in situ. Bottom-up reconstruction of relevant biotransformations using cocultures simplifies examination of complex multispecies interactions and can even lead to laboratory culturing of previously unculturable bacteria (Stewart, 2012). Benefits of coculture-based investigations range from alleviation of product inhibition and consumption of dead-end metabolites (Jiao et al., 2012), to the exchange of beneficial substrates or growth factors (Yan et al., 2012), maintenance of energy balance (McCarty and Bae, 2011), selective coupling of redox reactions (Cord-Ruwisch et al., 1998), understanding routes of extracellular electron transfer (Stams et al., 2006; Summers et al., 2010), and applications in synthetic biology (Wintermute and Silver, 2010).

In microbial electrochemical cells (MXCs), anode-respiring bacteria (ARB) couple the oxidation of organic substrates to the transfer of electrons to an electrode, creating an electrical current (Logan and Rabaey, 2012). Interfacing bacteria with electrodes thus creates opportunities for integrating MXCs with coculture-based studies, since the anode can serve as the sole electron acceptor, and substrate oxidation can be monitored in real time. For example, a coculture of fermentative *Clostridium cellulolyticum* and anode-respiring *Geobacter sulfurreducens* cooperatively captured electricity from cellulose (Ren et al., 2007). In a separate study, a photosynthetic MXC utilizing the green alga *Chlamydomonas reinhardtii* in coculture with *G. sulfurreducens* generated light-responsive current from organic compounds

released by the phototroph in the dark (Nishio et al., 2012). These studies highlight the modularity provided by MXCs in coupling a range of upstream microbial processes to a common output, that is, electricity. Light-responsiveness is also a distinguishing feature of several mixed culture photosynthetic MXCs in which anode respiration is either spatially or temporally separated from photosynthesis (Table SI; He et al., 2009; Xing et al., 2009).

Photosynthesis plays a central role in biogeochemical sulfur cycling, in which anoxygenic photosynthetic sulfide oxidizers cooperate with anaerobic chemotrophic sulfate or sulfur reducers (Ehrlich and Newman, 2009). Sulfate reduction yields reduced sulfur compounds, whose oxidation provides reducing power for CO_2 fixation by photosynthetic purple and green sulfur bacteria. Release of organic compounds from stored photosynthate in turn can provide a carbon source for sulfate reducers in a temporally separated manner (Ehrlich and Newman, 2009; Overmann and Gemerden, 2000). This scheme involving sulfur cycling has been extensively studied in anoxic cocultures of green sulfur bacteria and sulfate or sulfur reducers, in which sulfur is cycled several times (Biebl and Pfennig, 1978) and photosynthesis becomes tightly coupled to the activity of the sulfate or sulfur reducer (Overmann and Gemerden, 2000). For example, when acetate was supplied, a coculture of sulfur-reducing *Desulfuromonas acetoxidans* and photosynthetic *Chlorobium vibrioforme* photoproduced H_2 in a light-driven sulfur cycle (Warthmann et al., 1992). Here, the electron acceptor for *Desulfuromonas* was elemental sulfur (S^0), which forms either as an intermediate or end product in both sulfate reduction and photosynthetic sulfide oxidation.

Our previous enrichments of photosynthetic ARB led to current generation that showed a negative light response, suggesting a role for green sulfur bacteria (Badalamenti et al., 2013b). In this study, our primary objective was to investigate the microbiological basis for this pattern of light-responsiveness by comparing monocultures with cocultures of anode-respiring *Geobacter* and photosynthetic *Chlorobium*. First, we used MXCs to study light-driven changes in metabolism in real time based on the dark release of photosynthetically accumulated organic material. Current production was light-responsive in cocultures, but not in monoculture controls, and this pattern coincided with dark fermentation of glycogen by *Chlorobium*. Next, we constructed batch bottle cocultures to investigate this metabolic interaction outside the context of an MXC. In these experiments, we examined the effect on *Geobacter* when photosynthesis and dark fermentation were required to provide its electron acceptor (S^0) and donor (acetate), respectively. This work differs from previous coculture studies in which an organic electron donor was exogenously supplied to the sulfur reducer (Biebl and Pfennig, 1978; Warthmann et al., 1992). Our work expands upon the fusion of MXCs with coculture techniques and reinforces the utility of microbial electrochemistry for sensitive, real-time monitoring of microbial interactions in which a difficult to measure metabolic intermediate can be converted to electrical current.

Materials and Methods

Isolation of Pure Cultures

A $\sim$4 mm^2 section of phototrophically enriched freshwater anode biofilm (Badalamenti et al., 2013b) was resuspended in mineral medium (described below) and split in two for isolating pure cultures of both *Geobacter* and *Chlorobium*. *Geobacter* was isolated by picking single colonies as previously described (Badalamenti et al., 2013a). *Chlorobium* was isolated as described in detail (Overmann, 2006) by serial dilution in soft agar (1% w/v) containing medium (see below) supplemented with 2 mM sulfide and 10 mM acetate. Dilution tubes were incubated at room temperature under constant illumination of 1000 lux (Extech Instruments, Nashua, NH) provided by a 60-W incandescent bulb. Individual colonies appeared within 6–9 days. Single green colonies were picked in an anaerobic glovebox (Coy Laboratory Products, Grass Lake, MI) using a sterile Pasteur pipette and resuspended into 10 mL medium amended with 2 mM sulfide. The soft agar dilution series was repeated a total of three times until a pure culture was obtained. Culture purity was verified by light microscopy and T-RFLP as previously described (Sheng et al., 2011). The *Chlorobium* isolate produced green cultures, photosynthetically oxidized sulfide to sulfur and sulfate, and incorporated acetate for photomixotrophic growth (data not shown). Cells were slightly curved rods $\sim$1–1.5 μm in length with some cells forming chains longer than 5 μm.

Identification by 16S rDNA Cloning and Sequencing

We performed PCR using primers 8F and 1525R to clone and sequence the 16S rDNA of each isolate as described previously (Torres et al., 2009). *Geobacter* had 98% sequence similarity (1,525/1,549 bases) with *Geobacter sulfurreducens* PCA (Caccavo et al., 1994). The *Chlorobium* isolate was most closely related (99%; 1,477/1,494 bases) to *Chlorobium limicola* DSM 245^T, in agreement with its size, morphology, sulfide utilization, and growth in freshwater medium (Overmann, 2006).

Media and Culture Conditions

We maintained *Chlorobium* under constant illumination (1,000 lux) at room temperature in anoxic mineral medium (pH 7.2) buffered with 50 mM, $NaHCO_3$ under an 80:20 N_2:CO_2 atmosphere (Widdel and Bak, 1992). Cultures were stored at 4°C in the dark (Overmann, 2006) until needed for inoculating fresh cultures (1:10 inoculum). To supply electron donor, we fed 2 mM sulfide every 2–3 days from an anoxic stock solution (Overmann, 2006) adjusted to pH 9 with HCl. In addition, seed cultures for MXC and batch serum bottle cocultures were amended with 1 mM acetate at each sulfide addition to promote glycogen synthesis (Sirevåg and Ormerod, 1970). *Geobacter* was maintained in the same medium lacking sulfide but containing 10 mM acetate and 40 mM ferric pyrophosphate (Caccavo et al., 1994).

MXC Construction and Operation

Dual-chamber H-type microbial electrolysis cells (330 mL anode volume) separated by anion exchange membrane (AMI 7001, Membranes International) were constructed as previously described (Parameswaran et al., 2009). Graphite counter electrodes were submerged in an anoxic NaOH solution, pH 12.5. Polished graphite anodes (5.3 cm^2) were poised at −0.25 V versus an Ag/AgCl reference electrode (+0.02 V vs. SHE; BASi, West Lafayette, IN) using a VSP potentiostat (BioLogic, Knoxville, TN). All MXC experiments were performed at room temperature (24 ± 2°C) with continuous agitation.

MXC Coculture Experiments

We set up a total of three MXCs consisting of one coculture and two monoculture controls. We grew duplicate 100-mL cultures of *Chlorobium* fed periodically with sulfide and acetate as described above. During the final sulfide addition, acetate was not added to ensure complete consumption before coculture inoculation. Over the same time interval (10 days), we grew *Geobacter* in duplicate MXCs fed with 10 mM acetate to a current density of ~2 A m^{-2}, and then we replaced the anode solution with donor-free medium. After 1 h, we prepared the coculture MXC by removing 100 mL of medium from one of the *Geobacter* MXCs and adding 100 mL from one of the duplicate *Chlorobium* cultures. In addition, we prepared the *Chlorobium* monoculture control MXC by adding100 mL from the second *Chlorobium* culture to a reactor containing acetate-free medium. After introduction of the *Chlorobium* culture, the reactors were allowed to stabilize to the same current in the light for 30 min before being placed in darkness. We then subjected the MXCs to light-dark cycles (1,000 lux; 8–16 h, respectively), based on the duration of light cycles in previous experiments (Badalamenti et al., 2013b), for 4 days until current dropped to zero. All experiments were repeated once. After the second run we began 12-h diurnal light cycles to emulate environmental conditions. To supply electrons to *Chlorobium*, we flowed medium, in the light only, containing 1 mM sulfide as the sole electron donor at a 0.3 mL min^{-1} flow rate as previously described (Badalamenti et al., 2013b). For sulfide-fed experiments, we lowered the anode potential to −0.35 V versus Ag/AgCl (−0.08 V vs. SHE) to eliminate the background current (~5–10 μA) observed in sulfide-containing abiotic controls poised at −0.25 V versus Ag/AgCl.

Chemical Analyses

We collected glycogen from 0.5 mL samples of anode suspension by cell lysis in 30% (w/v) KOH and extraction in ethanol as previously described (Ernst et al., 1984). We measured acetate and glycogen with a fluorimetric enzyme-based assay kit (BioAssay Systems, Hayward, CA) according to the manufacturer's instructions using a Synergy H4 fluorescence plate reader (BioTek, Winooski, VT).

Batch Serum Bottle Cocultures

We set up a total of four culture conditions: monocultures of either *Geobacter* or *Chlorobium*, as well as cocultures with or without sulfide. For *Chlorobium* only, coculture + sulfide, and coculture−sulfide conditions, we inoculated (1:10) triplicate 100-mL cultures in 125-mL serum bottles (9 bottles total) with *Chlorobium* grown to produce glycogen as described above. Then, we resuspended *Geobacter* cells from an anode biofilm producing >6 A m^{-2} in sterile medium (OD_{600} 1.0) and injected 0.05 mL (1:2,000 inoculum) of this suspension to the cocultures. In addition, we prepared *Geobacter*-only control cultures using the same *Geobacter* suspension and 1:2,000 dilution factor in 1-L stoppered media bottles. All culture bottles were incubated at room temperature with 12-h diurnal cycles. We amended *Chlorobium*-only and coculture + S cultures with 1 mM sulfide at inoculation and again every 48 h with additions occurring halfway through a light cycle (seven additions total). *Geobacter* and coculture−S cultures received only one sulfide addition at inoculation.

DNA Extraction and Quantitative PCR (qPCR)

We extracted DNA at inoculation and every 4 days thereafter following dark periods (through Day 12, then every 12 days for 48 days total) from cell pellets taken from 1 mL of serum bottle cultures and 50 mL of *Geobacter* cultures as previously described (Sheng et al., 2011). DNA was diluted 1:100 and amplified by qPCR using 16S rDNA-directed primer sets Geo564f and Geo840r (targeting *Geobacteraceae*) (Cummings et al., 2003) or GSB532f and GSB832r (targeting green sulfur bacteria) (Overmann et al., 1999). Ten microliter of qPCRs contained 0.25 μM each primer and 1X SYBR Green Mastermix (5PRIME). Amplification used a thermal profile of 95°C for 2 min, followed by 40 cycles of 95°C for 15 s, 55°C for 30 s, and 68°C for 30 s. Melting curves showed no nonspecific or cross-target amplification. We generated linear calibration curves ($R^2 = 0.999$) for both targets by serially diluting plasmids containing cloned 16S rDNA (see above) with amplification efficiencies of 92–98%.

Results

To reconstruct light-responsive current production (Badalamenti et al., 2013b), we first studied monocultures of *Geobacter* and *Chlorobium* isolates using an MXC setup, and we compared their electrochemical behavior against the coculture. In this setup, no exogenous electron donors were supplied, and the anode was the sole electron acceptor. Monocultures thus provided background controls for current generation and light-responsiveness. In the coculture, however, *Geobacter* cells attached to the electrode served as living biosensors of upstream carbon metabolism by *Chlorobium*. Green sulfur bacteria synthesize glycogen (polyglucose) in the light as an intracellular storage polymer derived from CO_2 fixation via the reductive tricarboxylic acid (TCA) cycle, with reducing power supplied from

photosynthetic sulfide oxidation (Sirevåg and Ormerod, 1970, 1977). Acetate can also be reductively photoassimilated via a pathway that augments glycogen synthesis (Sirevåg, 1975). Glycogen reserves are fermented in the dark primarily to acetate and other organic acids (Sirevåg and Ormerod, 1977). In the coculture MXC, we hypothesized that *Geobacter* cells would utilize any acetate released as an electron donor, resulting in more current being produced in the dark than in the light as shown previously (Badalamenti et al., 2013b).

Cocultures Were Required for Light-Responsive Current Production

Figure 1A shows the dynamics of current production for the coculture MXC compared to monoculture MXC controls. In acetate-free media, the coculture and *Geobacter*-only reactors stabilized to the same current (~80 μA) before being placed in darkness. As expected, the coculture MXC showed a 2–2.5-fold increase in current in the subsequent dark period compared to the *Geobacter* control. Within 10 min of light exposure, the coculture displayed a dramatic negative light response, which was signficant even in the microamp range, as current dropped from 118 ± 16 μA in the dark to 61 ± 11 μA in the light. This pattern was similar to the light response previously observed in phototrophic enrichments (Badalamenti et al., 2013b). Light responses did not result from sudden fluctuations in temperature. Repeated light-dark incubations showed light-responsiveness only for the coculture, but the magnitude decreased over the 4-day incubation (Fig. 1A), suggesting eventual exhaustion of available electron donors. Current eventually fell to near zero in all three reactors by 96 h, which was consistent with batch mode operation without further addition of electron donor leading to irreversible exit of electrons from the system once consumed by *Geobacter* and collected by the anode. In addition, we ruled out direct current production by *Chlorobium* as a possible explanation for light-responsive current production, as shown in Figure 1A. These results supported the hypothesis that *Geobacter* cells could report, in real time, the kinetics of dark metabolism occurring in *Chlorobium*.

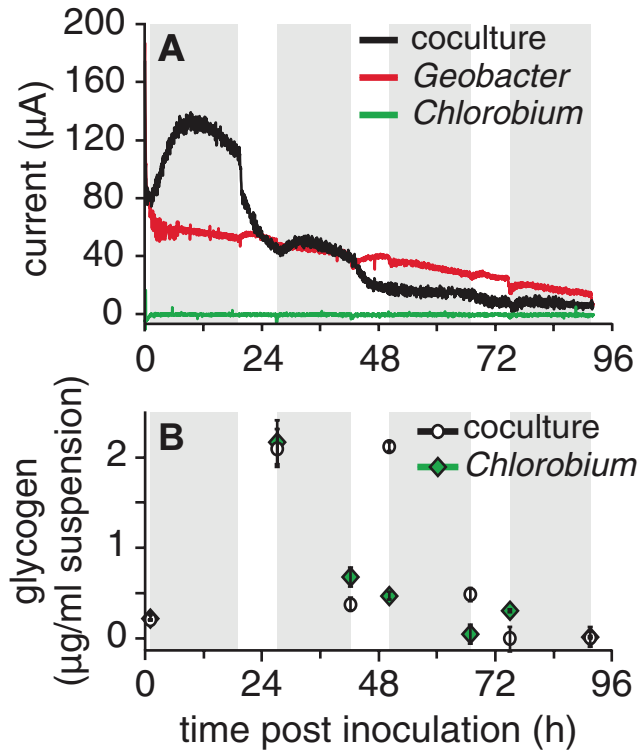

Figure 1. **A**: Dynamics of light-responsive current generation in sulfide-free batch MXCs containing monocultures of *Chlorobium* (green), *Geobacter* (red), and cocultures of these bacteria (black). Gray areas indicate periods of darkness. **B**: Production and consumption of glycogen in MXC cocultures (black) and *Chlorobium*-only control (green). Error bars represent standard deviation of triplicate measurements. Gray areas indicate periods of darkness.

Fluctuations in glycogen concentrations generally coincided with light-responsive current generation by the coculture MXC in the dark. Figure 1B shows a pattern of glycogen depletion in the dark as expected for *Chlorobium*, and the eventual loss of detectable glycogen agrees with current falling to baseline levels by 96 h post inoculation (Fig. 1A). Surprisingly, current produced by the coculture in the first dark period did not seem to be coupled to glycogen fermentation (Fig. 1B). Reappearance of glycogen before the third dark cycle did not lead to an increase in current in the coculture (Fig. 1A), an observation which raises the possibility that some *Geobacter* cells became inactive in response to continued incubation under electron donor-free conditions (Marsili et al., 2010). The coculture appeared to replenish its glycogen content during light periods (Fig. 1B, 42–50 h), which would have required a source of reduced sulfur as electron donor. It is possible that carryover of residual S^0 occurred upon inoculation of the coculture, and that *Geobacter* cells may have used some of this S^0 as an alternative electron acceptor (Caccavo et al., 1994), thereby providing sulfide to *Chlorobium*. This possibility could explain why glycogen appeared to be resynthesized in the coculture, but not in the *Chlorobium*-only control (Fig. 1B), and is consistent with the coculture yielding a relatively low Coulombic efficiency (30%). However, the fact that current first increased in the coculture (Fig. 1A) may suggest either that *Geobacter* favored anode respiration over sulfur reduction during the first dark period, in agreement with the anode serving as a more favorable electron acceptor (+0.02 V) than sulfur (−0.26 V) (Brune, 1995), or that cells were more metabolically active during this period than in subsequent light-dark cycles.

Cocultures Recovered Electrons From Sulfide as Current

Because we did not provide any exogenous electron donors, the electrons recovered by the coculture MXC were originally derived from sulfide fed to *Chlorobium* before inoculation into the anode. To further investigate whether electrons from

sulfide could be repeatedly captured as current via carbon cycling, we operated coculture and monoculture MXCs with 12-h diurnal cycling and continuous feeding of medium containing 1 mM sulfide during the light cycles. This arrangement ensured that glycogen-rich *Chlorobium* cells would not be diluted during dark periods.

In the coculture MXC, we observed a pattern of negative light-responsiveness in which current was detected above baseline levels only in the dark (Fig. 2). Current consistently decreased within 5–10 min of each light exposure, while increases in the dark spanned several hours (Fig. 2). This pattern was consistent with electricity generation from sulfide in two temporally separated steps: glycogen synthesis by *Chlorobium* in the light, followed by delivery of electrons to *Geobacter* via glycogen fermentation to acetate in the dark. The *Chlorobium* monoculture MXC failed to produce current (data not shown; see Fig. 1). However, current produced by the *Geobacter*-only control showed gradual decay and light-responsiveness opposite to that of the coculture (Fig. S1A). Observed changes in current did not result from abiotic sulfide oxidation on the anode, since an abiotic control poised at the same potential (−0.08 V) did not produce current (data not shown). Instead, our data raise the possibility that *Geobacter* biofilms catalyzed anodic sulfide oxidation, since current increased only during periods of continuous feeding (Fig. S1A). Whether *Geobacter* cells might obtain energy from such a reaction requires further investigation.

No obvious patterns of glycogen accumulation or fermentation were evident in the continuously fed sulfide MXCs (Fig. S1B). In the absence of other electron donors, glycogen fermentation provides both energy and reducing power for cell maintenance in the dark. This demand for reducing power can be partially met if an electron donor such as sulfide is available (Sirevåg and Ormerod, 1977). The fact that glycogen concentrations remained relatively stable when sulfide was present (Fig. S1B) but fluctuated when sulfide was absent (Fig. 1B) supports the hypothesis for selective mobilization of glycogen for reducing power based on the availability of other electron donors. However, current generation by the continuously fed coculture (Fig. 2) suggests that some glycogen was nonetheless fermented, presumably to supply ATP to *Chlorobium* in the dark, but that the amount of glycogen broken down was a small fraction of the total intracellular reserve.

Despite light responsiveness suggesting the release of an electron donor for *Geobacter* in the dark (Fig. 2), acetate concentrations were consistently below detection limits of HPLC and fluorimetric assays (data not shown). These results suggest either that the concentrations of acetate inherently produced were extremely low or that the consumption rate by *Geobacter* was greater than or equal to the production rate by *Chlorobium*. It is possible that current generation by *Geobacter* in the coculture resulted from glycogen fermentation by *Chlorobium* releasing other electron donors used by *Geobacter*, such as lactate or formate (Speers and Reguera, 2012a). However, the spectrum of organic acids released by green sulfur bacteria during dark fermentation suggests that acetate

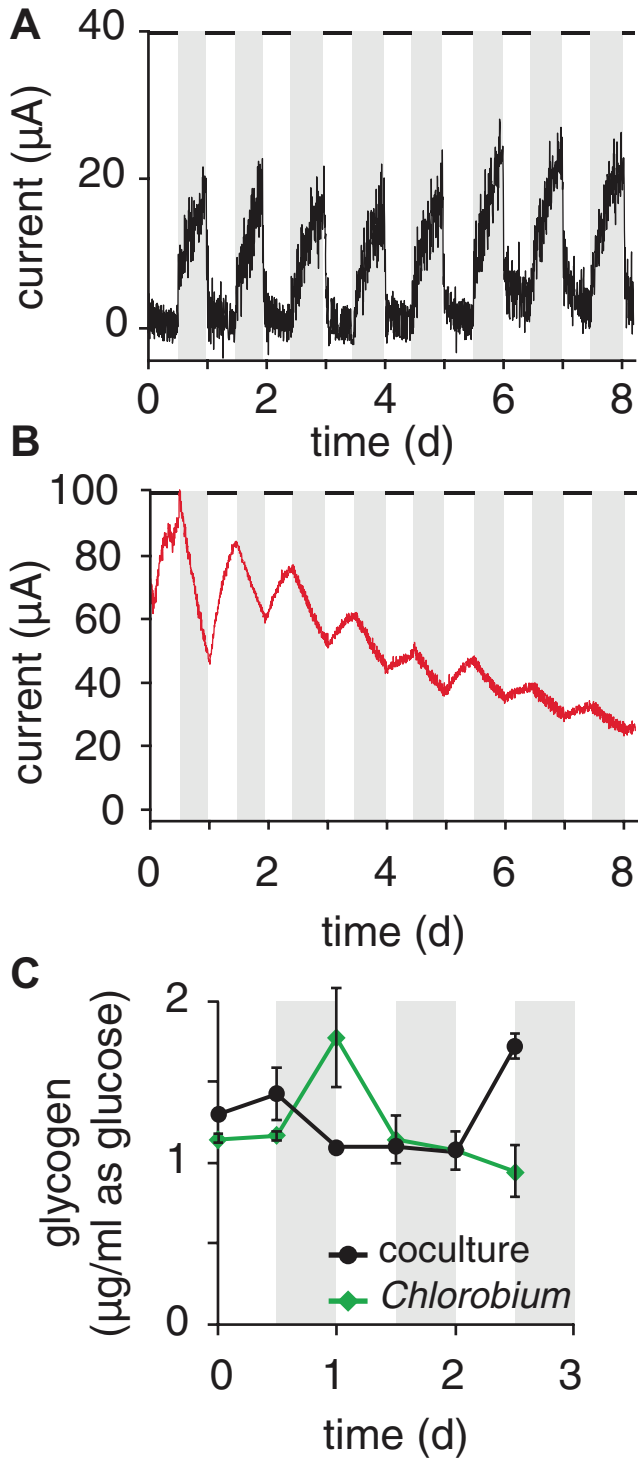

Figure 2. **A**: Light-responsive current generation in phototrophic coculture with sulfide fed as the sole electron donor. Gray areas indicate periods of darkness. Medium containing 1 mM sulfide was fed continuously in the light periods only (indicated by black bars). **B**: Light-responsive current generation by the *Geobacter*-only control fed with sulfide. Gray areas indicate periods of darkness. Medium containing 1 mM sulfide was fed continuously in the light periods only, as indicated by black bars. **C**: Concentrations of glycogen in the MXC coculture (black) and *Chlorobium* monoculture (green) over three diurnal cycles. Error bars report standard deviation of duplicate measurements. Gray bars indicate periods of darkness.

was likely the dominant fermentation product (Sirevåg and Ormerod, 1977).

Batch Cocultures Supported Growth of *Geobacter* Without Electrodes

To further investigate the interaction between *Chlorobium* and *Geobacter*, we performed experiments in batch serum bottles with *Chlorobium* and *Geobacter* such that the anode was no longer available as an electron acceptor. In this setup, *Geobacter* cells relied on *Chlorobium* to provide its electron donor (acetate) and electron acceptor (S^0). Since production of these compounds is temporally separated between light and dark periods, we maintained 12-h diurnal cycles by feeding sulfide every 48 h for 12 days in order to achieve high *Chlorobium* cell density (Overmann, 2006). We then evaluated changes in the *Chlorobium* and *Geobacter* populations over time by group-specific 16S rDNA-targeted qPCR.

We observed an approximately 14-fold increase in *Geobacteraceae* 16S rDNA copies in the sulfide-fed coculture after 40 days when compared to cocultures without sulfide feeding (Fig. 3A). These results suggested a benefit to *Geobacter* provided by *Chlorobium* in cocultures, but only when sulfide was supplied as the photosynthetic electron donor. In addition, *Geobacteraceae* 16S rDNA copies continued to increase in the sulfide-fed coculture even after the final sulfide dose on day 12 (Fig. 3A). This observation raises the possibility that a closed, light-driven sulfur cycle was active in the coculture, in which sulfur produced by *Chlorobium* was rapidly re-reduced to sulfide and sulfur was recycled several times (Biebl and Pfennig, 1978). Our results suggest that, even in the absence of exogenously supplied electron donor and acceptor, a measurable increase in *Geobacteraceae* 16S rDNA could nonetheless be achieved by supplying light as the only energy input and sulfide as the only source of electrons.

Coculture dependence on sulfur cycling was also evident when comparing 16S rDNA copies of green sulfur bacteria with and without sulfide. After 40 days of incubation, cocultures without sulfide showed a gradual loss of green sulfur bacterial 16S rDNA copies compared to monocultures and cocultures with sulfide, which showed similar relative abundance (Fig. 3B). Glycogen content increased in response to the sulfide addition on day 8 (Fig. 3C), providing evidence for glycogen being a sink for electrons derived from sulfide oxidation. Stoichiometric calculations predicted that sulfur cycling regenerated only a small fraction (~1%) of the sulfide cumulatively fed, suggesting that cocultures provided a negligible growth benefit to *Chlorobium* after sulfide feeding. Instead, results of batch serum bottle experiments agree with *Geobacter* being dependent on the products of both light and dark metabolism by *Chlorobium* in cocultures.

Discussion

We established coculture systems between *Chlorobium* and *Geobacter* with and without MXCs based on a light-driven sulfur cycle that did not require addition of an exogenous

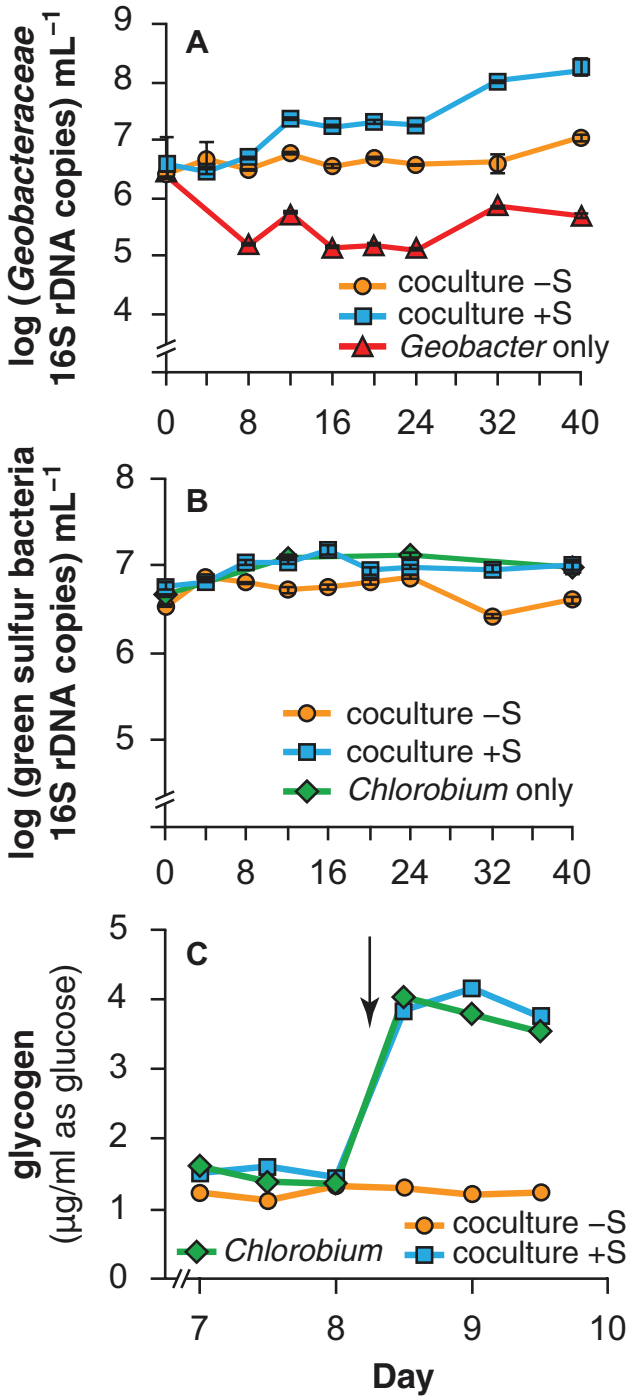

Figure 3. **A**: Progression of *Geobacteraceae* 16S rDNA copies in diurnally cycled sulfide-fed batch serum bottles containing either coculture (blue), sulfide-free coculture (orange), or *Geobacter* monoculture (red). The plot shows representative data from triplicate experiments. In the coculture with sulfide, 1mM sulfide was fed at inoculation and every 48 h thereafter through Day 12 (7 additions total). *Geobacter* monocultures were fed 1 mM sulfide at inoculation only. The *Geobacter* time zero point was calculated based on the same dilution factor of inoculum as for cocultures. Error bars indicate standard deviation of triplicate qPCRs. **B**: Progression of green sulfur bacterial 16S rDNA copies in sulfide-fed batch serum bottles containing either coculture (blue), sulfide-free coculture (orange), or *Chlorobium* monoculture (green). **C**: Light-dependent progression of glycogen concentrations surrounding the sulfide addition on Day 8, which is indicated by the black arrow. Error bars indicate standard deviation from triplicate cultures.

organic electron donor for growth of the non-photosynthetic partner. This arrangement differed from previous cocultures of green sulfur bacteria and sulfur- or sulfate-reducing bacteria in which organic electron donors such as acetate, ethanol, and propanol were supplied (Biebl and Pfennig, 1978). Photohydrogen production in a coculture of *Chlorobium* and *Desulfuromonas* required acetate as an electron donor to drive light-driven sulfur cycling and photosynthetic accumulation of glycogen by the phototroph (Warthmann et al., 1992). We hypothesized that photosynthetic cocultures could be investigated in the context of an MXC where the anode presents an alternative electron acceptor to insoluble S^0, provided the sulfur reducer is capable of anode respiration. In this respect, *G. sulfurreducens* provides an excellent model for coculture-based investigations given its robust anode respiration capabilities (Lovley et al., 2011) and utilization of S^0 as an electron acceptor (Caccavo et al., 1994). Recent work validated such a role for *Geobacter* in examining carbon utilization in *Chlamydomonas* cocultures (Nishio et al., 2010). However, sensitivity of *Geobacter* to oxygen (Qu et al., 2012) points to anoxygenic phototrophs being more suitable microbial partners in an MXC context.

Our previous phototrophic enrichment work using MXCs yielded a diverse microbial community with current generation showing a negative light response (Badalamenti et al., 2013b). Cocultures based on isolation of the two dominant bacteria predicted to be directly responsible for this behavior, that is, a phototroph (*Chlorobium*) and an anode respirer (*Geobacter*), effectively reconstructed the same pattern of light-responsiveness from the fewest possible organisms. Therefore, our results show that the addition of a single photosynthetic partner to ARB is sufficient to impart light-responsiveness to current production. The fact that electricity generation decreased in the light and increased in the dark (Figs. 1 and 2) is consistent with a model, shown in Figure 4, in which *Geobacter* requires acetate as an electron donor that is supplied by *Chlorobium* only in the dark. In the light, *Chlorobium* appears to obtain energy by rapidly (on the order of 5–10 min) shifting its metabolism away from glycogen fermentation towards either phototrophy or photosynthesis (Bryant and Frigaard, 2006), depending on the availability of sulfide as electron donor. The result is an interruption in the supply of acetate to *Geobacter*, leading to a sudden decrease in current (Fig. 4). Such a scheme agrees with the fact that significantly more ATP is available from photophosphorylation than from substrate-level phosphorylation (Gottschalk, 1986).

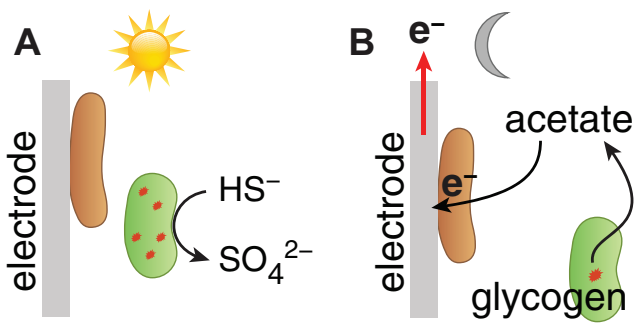

Figure 4. Coculture model for current production in the dark. **A**: In the light, *Chlorobium* photosynthetically accumulates glycogen (red particles) using electrons derived from sulfide oxidation. **B**: In the dark, *Chlorobium* ferments glycogen to acetate, which is consumed by *Geobacter* to produce electric current.

The increase in *Geobacteraceae* 16S rDNA (Fig. 3A) implies that *Geobacter* must have incorporated cell carbon in batch serum bottle cocultures, and that the necessary organic material must have been provided by *Chlorobium*, since *Geobacter* is not autotrophic. It remains unclear whether acetate was the organic compound exchanged between *Chlorobium* and *Geobacter*, since acetate escaped detection. However, acetate concentrations in the micromolar range or lower are consistent with those encountered by *Geobacter* in natural environments (Esteve-Núñez et al., 2005) and sufficient to generate an electrochemical response (Liu and Bond, 2012).The net charge collected by the potentiostat from the donor-free coculture (0.6 mA h) predicts that *Chlorobium* must have cumulatively released ~8.5 μM acetate in the dark over the 96-h incubation (Fig. 1A). This calculation was consistent with acetate concentrations being below detection limits of ~100 μM and was also in stoichiometric agreement with the range of glycogen concentrations observed (0–2.2 μg/mL as glucose), assuming acetate comprises 80% of the products released from glycogen fermentation (Sirevåg and Ormerod, 1977). Comparing the number of electrons present in glycogen at its maximum concentration (2.1 μg/mL as glucose) to the current collected by the coculture, after subtracting the electrons produced in the *Geobacter*-only control, yielded a Coulombic efficiency (CE) of 30%. The fact that glycogen transiently increased in this experiment (Fig. 1B) suggested that more electrons were initially present in the system, presumably from carryover of S^0 in the inoculum, than were originally contained in glycogen. It is possible that *Geobacter* diverted a fraction of its electrons away from anode respiration towards S^0 reduction, thereby lowering the CE. Nonetheless, these results bring attention to the sensitivity afforded by MXC-based cocultures in which measurable electrical signal is generated even if only a fraction of the available electrons are collected as current.

In this study, we report fermentation kinetics in green sulfur bacteria measured in real time by monitoring light-responsive current generation. Negative light responses were routinely detected within minutes, a pattern which reinforces the versatility of MXCs in revealing kinetic responses to physical and microbiological perturbations with high temporal resolution. In addition, MXC-based cocultures could potentially be used as biosensors for rapid detection of metabolites at extremely low concentrations with high sensitivity. To that end, our results highlight the potential opportunities for using electrodes to study

rate-limiting steps in upstream fermentations. Incorporating electrodes in coculture experiments also enables electricity production from higher substrates and dead-end fermentation products, provided the end product can serve as an electron donor to ARB. Such an approach was recently employed in an ethanol-producing MXC coculture in which *G. sulfurreducens* effectively converted undesired fermentation products to electricity (Speers and Reguera, 2012b). The work presented here supports the utility of MXCs as robust platforms for photosynthetic cocultures, both in fundamental microbiological studies and practical applications. More broadly, further investigations of photosynthetic cocultures could help elucidate key bottlenecks in the development of photo-MXCs for potential bioenergy applications (Rosenbaum and He, 2010).

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