Final Report

Factors Limiting Power Output of Benthic Microbial Fuel Cells: Mechanistic and Ecological Studies

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SCIENTIFIC AND TECHNICAL OBJECTIVES

The overall objective of this research was to elucidate how electrons are delivered to the anodes of benthic microbial fuel cells and to determine the factors limiting current production in order to increase the power output of benthic microbial fuel cells.
The short-term objectives of these studies were to: 1) elucidate the mechanisms for conductivity in the pili that contribute to biofilm conductivity, and thus current production; 2) determine the physiological and ecological factors controlling current production in biofilm communities in benthic microbial fuel cells; and 3) elucidate the mechanisms for long-range electron conduction through marine sediments.

APPROACH

Objective 1 was investigated by: 1) developing a method for measuring the conductivity of individual pili under physiologically relevant conditions and 2) genetically constructing new strains of *G. sulfurreducens* that produced a diversity of heterologous pili.

Objective 2 was investigated by: 1) elucidating new sources of electrons that support current production and 2) determining the role of protozoan grazing in limiting the growth and activity of current-producing anode biofilm communities.

Objective 3 was investigated by determining the influence of conductive minerals on the conductivity of marine sediments, measured with the first method capable of determining actual sediment conductivity.

RESULTS

In this project we: 1) demonstrated that cytochrome-free regions of individual *G. sulfurreducens* pili are electrically conductive under physiologically relevant conditions; 2) provided multiple additional lines of evidence demonstrating that the conductivity of *G. sulfurreducens* pili is an intrinsic property of the pilus protein filament; 3) demonstrated that minerals in anaerobic marine sediments contribute conductivity that may make a major contribution to current production in microbial fuel cells; 4) identified

a potential new role for *Geobacteraceae* in current production from sulfur in marine sediments; and 5) demonstrated that protozoan grazing substantially reduces the current output of benthic microbial fuel cells.

High Conductivity of Cytochrome-Free Regions of *G. sulfurreducens* Pili

(b) (4)

Multiple lines of Evidence for the Intrinsic Conductivity of *Geobacter* Pili

The controversy over whether c-type cytochromes associated with pili were responsible for pili conductivity led to a series of studies designed to evaluate this question. Measurements of conductivity along the cytochrome-free regions of individual pili employing the technique described above demonstrated that pili were highly conductive in the absence of pili.

As an alternative approach, a strain of *Geobacter sulfurreducens* was constructed in which the gene for the native PilA, the structural pilin protein, was replaced with the PilA gene of *Pseudomonas aeruginosa*. This strain produced abundant *P. aeruginosa* pili, highly decorated with the *c*-type cytochrome OmcS, but the pili were not conductive and the strain of *G. sulfurreducens* expressing these pili was ineffective in current production and Fe(III) oxide reduction.

In a similar manner, it was discovered that a species of *Geobacter*, *G. uraniireducens*, had a PilA gene with a sequence similar to the 'typical' PilA sequences found in many bacteria. It was much longer than the truncated *G. sulfurreducens* PilA and contained few aromatic amino acids. The *G. uraniireducens* pili were poorly conductive and further investigation revealed that *G. uraniireducens* performs long-range extracellular electron transfer via the release of an electron shuttle. Expressing the *G. uraniireducens* pili in *G. sulfurreducens* yielded a strain ineffective in current production and the pili were not conductive even though they contained abundant cytochromes.

We found that a strain of *Geobacter sulfurreducens* in which the gene for PilA was deleted adapted during long-term incubations to reduce Fe(III) oxide without pili. Further study revealed that the adaptation could be attributed to a mutation that increased the production of a *c*-type cytochrome, which was then released into the medium. Given that the possibility of long-range electron transfer without pili could have important implications for the understanding of the mechanisms of current production, we tested the ability of this strain to produce current. Current production was comparable to that of unadapted mutants deficient in pili. These results demonstrate that the alternative strategy of producing excess cytochromes as electron shuttles is not an effective method for high-density current production. Furthermore, even when Fe(III) oxide was the electron acceptor the strain releasing the cytochrome could not compete with the pili-producing strain under environmentally relevant conditions, further demonstrating the adaptive advantage of long-range electron transport via pili.

<u>Sediment conductivity contribution to benthic fuel cell current production</u>

We found that sediment conductivity can play an important role in the power output of benthic fuel cells. (b) (4)

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A Potential New Role for *Geobacteraceae* in Current Production in Sediments

Another factor potentially contributing to current production with benthic fuel cells is that sulfate reducers at distance from the anode can produce sulfide, which can diffuse to the anode and be abiotically oxidized to elemental sulfur. Additional electrons can be derived from the microbiological oxidation of elemental sulfur to sulfate with electron transfer to the anode. However, it has been difficult to quantify this contribution to current production and the abundance of *Geobacteraceae* (primarily *Desulfuromonas* species in marine environments) on anodes in most sediments has previously been interpreted as evidence that most of the current production is derived from the oxidation of organic compounds coupled to electron transfer to anodes. Enrichments initiated with a marine sediment inoculum, with elemental sulfur as the electron donor and an anode as the electron acceptor, yielded an anode biofilm with a diversity of microorganisms, including *Thiobacillus*, *Sulfurimonas*, *Pseudomonas*, *Clostridium* and *Desulfuromonas* species. Further enrichment of the anode biofilm inoculum in medium with elemental sulfur as the electron donor and Fe(III) oxide as the electron acceptor, followed by isolation in solidified sulfur/Fe(III) medium yielded a strain of *Desulfuromonas*, designated strain TZ1. Strain TZ1 effectively oxidized elemental sulfur to sulfate with an anode serving as the sole electron acceptor. The rates of sulfur oxidation were faster than with *Desulfobulbus propionicus*, the only other organism in pure culture previously shown to oxidize sulfur with current production. These results demonstrate that the abundance of *Desulfuromonas* species enriched on the anodes of marine benthic fuel cells can not be attributed solely to the oxidation of organic substrates and that further information on the metabolism of the *Desulfuromonas* species will be necessary in order to quantify the relative contributions of organic matter oxidation and sulfide oxidation to current production.

Demonstration that Protozoan Grazing can Limit the Current Output of Marine Benthic Fuel Cells

Analysis of 18S rRNA gene sequences associated with anodes harvesting current from marine sediments and sequences in the surrounding sediments demonstrated that there was a substantial enrichment of protozoa closely related to protozoa in the genus *Euplotes* in the anode biofilm. To determine whether inhibiting the activity of these protozoa impacted on the current output of the anode biofilm, the eukaryotic inhibitors cycloheximide and colchicine were added to marine sediments. Current output from benthic fuel cells in the presence of the inhibitors was as much as five-fold higher than current output in untreated control sediments. Analysis of 16S rRNA gene sequences demonstrated that there was a greater abundance of *Geobacteraceae* on the current-harvesting anodes in sediments that were treated with the inhibitors. These results suggested that the activity of protozoa was greatly limiting the output of marine benthic microbial fuel cells and that a likely explanation for this was protozoan grazing on the

Geobacteraceae that are primarily responsible for current production in the anode biofilm.

In order to further investigate the potential for protozoan grazing to limit current production in more detail, controlled laboratory studies were conducted with *Geobacter sulfurreducens* and pure cultures of protozoa. Four strains of protozoa were found to differ in their effectiveness in grazing on cells suspensions of *G. sulfurreducens*

When these protozoa were added to fuel cells in which *G. sulfurreducens* was the current-producing microorganism there was a decrease in current output that corresponded to the effectiveness of each protozoan species in grazing on *G. sulfurreducens*. The better the grazer, the lower the current. The impact of protozoan grazing on anode biofilm development was very apparent. In the presence of an effective protozoan grazer anode biofilms were much more sparse.

These results suggest that protozoan grazing may be greatly reducing the current output of benthic microbial fuel cells. Material coatings on anodes that permit entry of microbial cells, but screen out protozoa might dramatically increase the current output of marine benthic microbial fuel cells.

IMPACT/APPLICATIONS/TRANSISTIONS

The confirmation that the conductivity of *Geobacter sulfurreducens* pili is an intrinsic property of the protein filament, rather than associated cytochromes, has important implications for approaches to increase the current output of microbial fuel cells and opens a new field of bioelectronics materials. (b) (4)

The new information on the influence of sediment conductivity; the potential role of sulfur as an electron donor for current production; and the detrimental impact of protozoan grazing on current output of benthic microbial fuel cells will aid in the design of improved systems for harvesting electrical energy from marine sediments.

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