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On the Potential for Bioenergy and Biofuels from Hydrothermal Vent Microbes

BY PETER R. GIRGUIS AND JAMES F. HOLDEN

ASTONISHING BIOLOGICAL PRODUCTIVITY AT HYDROTHERMAL VENTS

The discovery of deep-sea hydrothermal vents caused scientists to reconsider their notions about life in the deep sea. In these seemingly inhospitable environments, free-living microbes, as well as microbial-animal symbioses, thrive in the warm waters around vents. The biomass per unit area in this environment is comparable to that of rainforests. Uniquely, these highly productive ecosystems are based on microbial chemoautotrophic metabolism, wherein microbes generate metabolic energy by drawing oxygen or nitrate from surrounding seawater to oxidize reduced chemicals (e.g., sulfide) found in the vent fluids (Sievert and Vetriani, 2012, in this issue). The rapid and voluminous fluid flux through hydrothermal vents replenishes these substrates at a rate sufficient to support this substantial community. The tremendous microbial productivity observed at vents raises the question as to whether these microorganisms are also well suited for bioenergy and biofuel production. Here, we discuss the utility and issues associated with two example approaches: in situ bioelectricity generation and microbially mediated large-scale biofuel production.

IN SITU BIOENERGY GENERATION

In the early twentieth century, scientists found that electrical current could be harvested from microbes by culturing them in a reactor with an electrode and soluble compounds that could "capture" electrical charge from within the cells and conduct that charge to the electrode (Potter, 1911). It wasn't until 1988, however, that Lovley and Philips described an iron-reducing microbe in which this capacity occurred naturally (Lovley and Philips, 1988). Microbial extracellular electron transfer (EET) broadly refers to the physiological capacity of a microbe to exchange electrons to and from insoluble materials located outside of the cell. In natural settings, microbes capable of EET use solid-phase oxidants, such as iron oxides, in environments where dissolved oxidants such as nitrate or oxygen are absent. Although EET was first observed in iron-reducing microbes, it now appears that a variety of microbes with varying physiological capacities employ EET to access solid-phase minerals and other compounds.

Lately, there has been a growing interest in microbial fuel cells (MFCs)—devices that harvest electrical current from microbial cultures and

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communities—as well as bioelectrochemical systems, wherein an externally produced electrical current is provided to microbes to enable the reduction of CO_2 to biofuel precursors by "electrosynthesis" (for reviews, see Franks and Nevin, 2010; Lovley, 2010). To date, the power densities observed in MFCs for electricity generation are modest, on the order of µW–mW m^{-2} of electrode (Rabaey and Rozendal, 2011), making it difficult to conclude that they will be commercially viable in large-scale energy production in the near future. Furthermore, it is unclear whether operating MFCs at substantially greater scales than previously demonstrated will ever be feasible due to parasitic losses at the low voltage potentials generated by microbial activity (Dewan et al., 2008). Their utility in electrosynthesis remains to be fully assessed.

Recently, investigators have been developing microbial fuel cells for power production in remote environments, such as rural areas or the deep ocean (Reimers et al., 2006; Nielsen et al., 2008; White et al., 2009; Girguis et al., 2009; Gong et al., 2011). Though MFCs may not be competitive for large-scale energy generation, they do afford some distinct advantages where power is needed in harsh or remote environments. In natural settings, such as when they are deployed in sediment or soil, MFCs are fueled by natural biogeochemical cycles, operate independent of sunlight, and are well suited for use in damp environments. Such "environmental MFCs" are typically simple and robust, without any moving parts, and are fabricated from affordable materials. Despite their lower power densities, MFCs are more efficient at cold temperatures than most batteries. Based on data from previous studies (Reimers et al., 2006; Nielsen et al., 2008), an MFC equipped with 1 m^2 electrodes and deployed atop 7°C deep-sea sulfidic sediments can produce ~ 110 mW continuously (or ~ 963 W hr^{-1} per year), the equivalent energy available from more than 120 D-cell alkaline batteries at the same temperature and time.

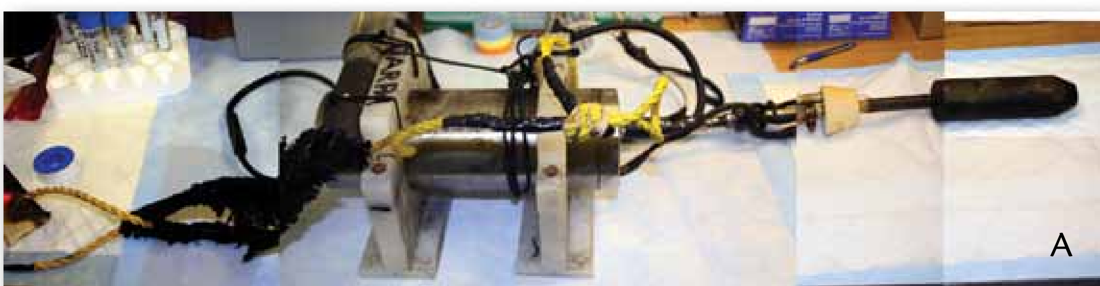
> "GIVEN THE TREMENDOUS COSTS ASSOCIATED WITH SHIPS AND SUBMERSIBLE DIVES, THE USE OF [VENT MICROBIAL FUEL CELLS] AS ALTERNATIVE POWER SOURCES...IS EXTREMELY ATTRACTIVE..."

An ongoing study by Mark Nielsen and author Girguis at Harvard University found that many hydrothermal vent microbes are capable of EET, including those living at elevated temperatures inside the walls of an active sulfide chimney. These observations, as well as the data from deployments in marine sediments, led Girguis and colleagues to develop and deploy an MFC for use at hydrothermal vents (Figure 1A). Briefly, the vent MFC (vMFC) consists of a circuit in which a chemically inert, electrically conductive graphite electrode is placed in anoxic vent fluid, such as a diffuse flow or in holes drilled into active sulfide mounds. A graphite "brush" cathode is placed in the surrounding seawater. The two are connected through a power management system, that uses the energy to drive sensors. While operating, the electrical current resulting from microbial metabolic activity is transferred to the anode, through the power management system and sensors, and ultimately to the cathode (note that a fraction of the current is also produced abiotically via sulfide oxidation at the anode). Data from two deployments at hydrothermal vents reveal current densities of 250 to 500 mA m^{-2}, suggesting that at 0.5 V potential power densities between 125 and 250 mW m^{-2} are attainable (Figure 1B). Given the tremendous costs associated with ships and submersible dives, the use of vMFCs as alternative power sources (or as chargers for existing rechargeable batteries) is extremely attractive as it could result in longer deployment times (we have run MFCs deployed in marine sediment for eight years without intervention). Equally appealing is the use of vMFCs to power both sensors and onboard telecommunication devices, such as acoustic or optical modems, to build an extensive "wireless" network for sensors around vents for research and monitoring. At vent observatories that are already equipped with high power and data cables, vMFC-powered sensor and telecommunication systems could readily extend the sensing "footprint" of the observatory, reaching locations that are impractical to access with cabled instrumentation. Recently, MFC-powered sensor and telecommunication systems, replete with an in situ oxygen sensor (optode, Aanderaa Data Instruments) and acoustic modem (Teledyne Benthos), have been operated in marine sediment for nearly two months (Gong et al., 2011). This system produced ~ 20% of the power that we generated with the vMFC, establishing that power production at vents would be sufficient to run a more substantial suite of sensors as well as the acoustic modem.

These attributes notwithstanding, formidable challenges arise when using vMFCs. Environmental heterogeneity is ample at vents (as evidenced by the variations in current density seen in Figure 1B), and will likely lead to large differences in performance. Any reduction in vent flow would limit the magnitude and duration of performance. Biofouling of a cathode by animals such as vent limpets might impede performance (although we did not observe this during our deployments). Moreover, the deposition of elemental sulfur on the electrodes of environmental MFCs affects performance over time (elemental sulfur passivates the surface and reduces the available surface area for electron acceptance). While mitigating these phenomena is technically feasible, it remains to be determined how effective mitigation strategies might be in these environments. These issues are fundamental to all such bioelectrochemical systems. While vents offer some of the steepest geochemical gradients, as well as metabolically active microbes capable of EET, the relatively high performance may ultimately be offset by these or other unforeseen issues, including inefficiencies resulting from resistive losses during operation. Ongoing tests are aimed at further characterizing vMFC performance over time.

BIOFUEL GENERATION USING HYDROTHERMAL VENT MICROBIAL CULTURES

In an effort to reduce dependence on petroleum, promote economic growth and diversification, and reduce human-induced climate change, the United States has developed a strategy that includes bio-based energy production focused on the development of robust,



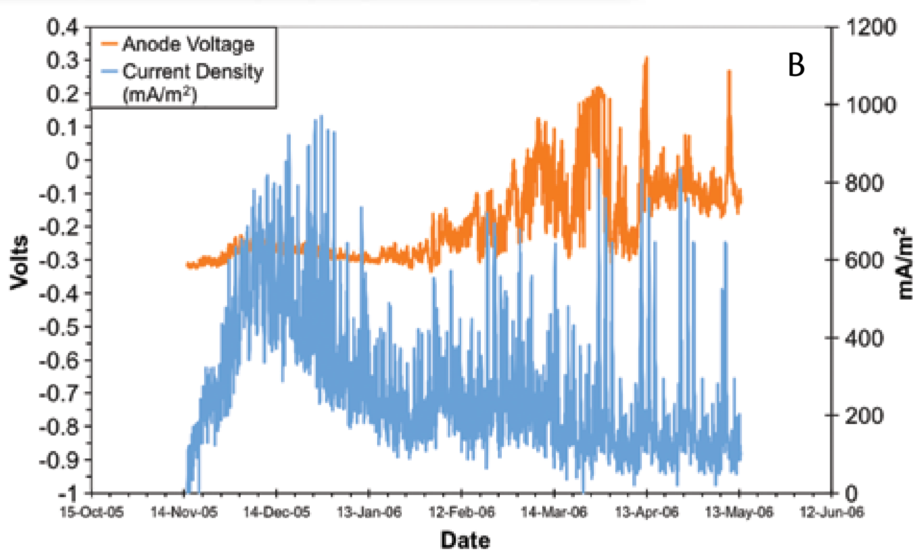


Figure 1. (A) A photomosaic of a vent microbial fuel cell (MFC). The anode (to the far right) is inserted into a drillhole in the side of a sulfide mound. The cathode (far left) remains suspended in ambient seawater. The titanium housing, which contains the power management boards and sensor suite, is placed on the substrate or atop the sulfide mound. (B) Plot of anode cell voltage and current density over time from a vent MFC deployed for six months at the Mothra hydrothermal vent field on the Juan de Fuca Ridge.

large-scale production of sustainable energy-dense biofuels. As marine hydrothermal vents harbor some of the most chemo- and thermo-tolerant microorganisms known, they have caught the attention of scientists and industrialists alike for biofuel production. While generating biofuels from vent microbes is attractive, there are, nonetheless, key issues that need to be addressed prior to commercial implementation. Here, we briefly discuss the advantages and limitations of such approaches and consider the commercial relevance of some recently proposed technologies.

In general, biofuel production depends on feedstock availability and costs, proper reactor conditions for biosynthesis, and efficient sequestration of the biomass or metabolite for biofuel production. The best-known biofuel, corn ethanol, uses starch derived from corn as feedstock for the production of ethanol via fermentation by yeast. Although the process and infrastructure for ethanol production is well developed, challenges in maintaining the supply of feedstock, the limited availability of arable land for production, and the adverse impact of corn ethanol production on food prices in the developing world have diminished the practicality of replacing existing liquid fossil fuels with corn ethanol (Singh et al., 2010).

Alternatively, it has been suggested that microorganisms with differing physiological capacities may provide an opportunity to generate biofuels in a more sustainable, commercially viable manner (Chou et al., 2008). For example, vent hyperthermophilic microbes that grow optimally at temperatures above 80°C are known to be capable of producing hydrogen from organic matter. Recently, 19 hyperthermophilic deep-sea vent microbes were found to produce hydrogen using maltose (a breakdown product of starch) and protein as feedstocks (Oslowski et al., 2011). A closely related hyperthermophile, *Pyrococcus furiosus* isolated from a geothermally heated beach in the Mediterranean Sea (Fiala and Stetter, 1986), grew on starch, cellulose, and peptides, with the highest net hydrogen production coming from growth on starch (Oslowski et al., 2011). Because metabolic rate increases exponentially with temperature, at a rate that typically doubles with every 9°C increase (Tijhuis et al., 1993), biofuel production by a hyperthermophile growing at 95°C could be as much as 250 times higher than the same metabolic process occurring at room temperature. While hyperthermophiles may be well poised to produce biofuels at rates greater those previously observed, it is important to note that other factors, including increased energy consumption by the organism at higher temperatures and the biological regulation of metabolite flux, can influence the rate of biofuel production.

> "...MICROORGANISMS WITH DIFFERING PHYSIOLOGICAL CAPACITIES MAY PROVIDE AN OPPORTUNITY TO GENERATE BIOFUELS IN A MORE SUSTAINABLE, COMMERCIALLY VIABLE MANNER."

One goal of hyperthermophilic hydrogen production research is to determine whether hyperthermophiles could produce hydrogen using anaerobic sludge from sewage treatment plants for either hydrogen biofuel production or on-site combustion for electricity generation. This approach is attractive because hyperthermophiles can extract organics from sludge and effluent, producing hydrogen for local electricity generation while simultaneously reducing the amount of organics in the effluent stream (minimizing the potential for eutrophication downstream) and killing pathogens that may be present. The energy produced on site is distributed via the existing electrical power grid, and, as a result, feedstock production, transportation logistics, and public energy distribution concerns are minimized because the infrastructure for sludge-to-energy conversion is largely in place. To our knowledge, however, no data exist on the efficacy of this approach, though the theoretical considerations outlined above are compelling.

While it is implausible that sludge-to-energy conversion could fully replace fossil fuel use, such approaches are being successfully employed in North America and Europe for small-scale energy production. In the United Kingdom in 2005, municipal solid waste and biogas for electricity generation yielded 2,500 GWh yr^{-1}, accounting for ~ 15% of all renewable energy (see http://ec.europa.eu/energy/renewables/studies/renewables_en.htm). Although it remains to be seen whether hyperthermophile-catalyzed reactions will exhibit comparable yields, the value of such an approach resides in the promise of increased efficiency and lower environmental impact. If hyperthermophiles are capable of generating economically relevant volumes of hydrogen (or electricity from hydrogen), then subsequent research should focus on addressing the other factors that typically influence commercial relevance such as scalability and operating costs.

FROM VENT PRODUCTIVITY TO MEETING HUMANKIND'S ENERGY NEEDS

Vast amounts of energy flow through marine biogeochemical cycles, including hydrothermal vents. Research on marine microbes, in particular, in deep-sea sediment and vents, has offered a small glimpse into the variety of physiological processes by which these microbes mediate the transfer of matter and energy from the lithosphere to the biosphere. The technologies outlined herein provide a modest look at the potential role that microbes may play in energy production. The future of these particular technologies, like so many alternative energy technologies, remains uncertain. However, the lessons learned from these pursuits will certainly shed light on how we may better harness the physiological capacities of microbes to meet our growing energy demands.

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