

Substrate-Enhanced Microbial Fuel Cells for Improved Remote Power Generation from Sediment-Based Systems

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A sediment microbial fuel cell (MFC) produces electricity through the bacterial oxidation of organic matter contained in the sediment. The power density is limited, however, due in part to the low organic matter content of most marine sediments. To increase power generation from these devices, particulate substrates were added to the anode compartment. Three materials were tested: two commercially available chitin products differing in particle size and biodegradability (Chitin 20 and Chitin 80) and cellulose powder. Maximum power densities using chitin in this substrate-enhanced sediment MFC (SEM) were 76 ± 25 and 84 ± 10 mW/m² (normalized to cathode projected surface area) for Chitin 20 and Chitin 80, respectively, versus less than 2 mW/m² for an unamended control. Power generation over a 10 day period averaged 64 ± 27 mW/m² (Chitin 20) and 76 ± 15 mW/m² (Chitin 80). With cellulose, a similar maximum power was initially generated (83 ± 3 mW/m²), but power rapidly decreased after only 20 h. Maximum power densities over the next 5 days varied substantially among replicate cellulose-fed reactors, ranging from 29 ± 12 to 62 ± 23 mW/m². These results suggest a new approach to power generation in remote areas based on the use of particulate substrates. While the longevity of the SEM was relatively short in these studies, it is possible to increase operation times by controlling particle size, mass, and type of material needed to achieve desired power levels that could theoretically be sustained over periods of years or even decades.

Introduction

A microbial fuel cell (MFC) is a device that can directly produce electricity from the bacterial oxidation of organic matter such as glucose or acetate (1) or inorganic species such as sulfides (2). Reimers et al. (3) first demonstrated that microbial communities naturally present in marine sediments could produce electricity (3). Sediment MFCs consist of a noncorrosive but conductive anode such as a graphite rod embedded in an anaerobic marine sediment and a cathode made of a graphite plate or carbon fibers (4)

suspended in the overlying oxygenated seawater. The electrons released by the bacterial degradation of the organic matter flow from the anode to the cathode through an external circuit, while protons diffuse through the water between the electrodes. The electrons and protons then react at the cathode with oxygen, forming water. The microbial communities that produce power in sediment MFCs have primarily been classified as Fe(III) reducing bacteria in the *Geobacteraceae* family (5). *Desulfurumonas* spp. were found to be dominant on electrodes placed in marine sediments, while the *Geobacter* species predominated on electrodes placed in freshwater sediments (6).

The first reported sediment MFCs provided a stable power output of 10–20 mW/m² (normalized to the anode projected surface area) (3, 5). Although the power output was low, it was realized that the power output could be improved by using sediments with higher organic matter contents, improving the electrode design, and selecting optimal temperatures (3). In the first sediment MFC tests, the organic matter content was 2–6% (5), a value that is already quite high relative to typical marine sediments. Using sediments with an organic matter content of 4–6%, Lowy et al. (7) showed that power densities could be increased up to 2.5 times by modifying the anode with different metals or known mediators. The maximum power generated using AQDS (9,10-anthraquinone-2,6-disulfonic acid) bound to the anode was ~ 98 mW/m² (normalized to the anode surface area), and ~ 105 mW/m² was produced using ceramic–graphite composite anodes containing Mn²⁺ and Ni²⁺. However, the maximum voltage decreased rapidly over time (as the square root of time over several days). For example, while the AQDS-modified anode initially produced 5 times greater power than plain graphite, the power decreased to that of the unmodified graphite anode within a few days (7). Power output was also improved by modifying the cathode. Higher voltages were sustained using brush cathodes containing graphite carbon fibers as compared to spinal coated stainless steel wool cathodes due to a 50% reduction in the internal resistance (4).

One obstacle hindering widespread distribution of sediment MFCs as remote power sources is the low organic matter content of many sediments. While sediments can range in organic carbon content from 0.1 to 10% by weight (8), many sediments have organic matter contents of 0.4–2.2% (9). Thus, a low concentration of organic matter could prohibit sufficient power generation in some locations. Cold seeps have been tested as potential sites for increased power generation due to their higher organic matter content (10). Besides the limited numbers of such sites, the lifetime of sediment MFCs in these locations was reduced by anode passivation due to the buildup of sulfide oxidation products (10).

A new approach for increased power generation by MFCs in sediments is proposed here based on including a particulate organic substrate within the anode matrix. Bacteria needed to act as the biocatalysts are already present in the sediment and seawater, but their growth rate can be limited by substrate availability and reducing power. We reasoned that the use of a particulate substrate could provide a source of sustained fuel for a sediment MFC as the rate of degradation of particulate substrates is slower than that of soluble materials as the breakdown is limited by particle surface area. Finkelstein et al. (11) conducted studies where acetate was added to the reactor. However, while adding a soluble substrate could increase power over the short term in a contained laboratory reactor, soluble substrates would be

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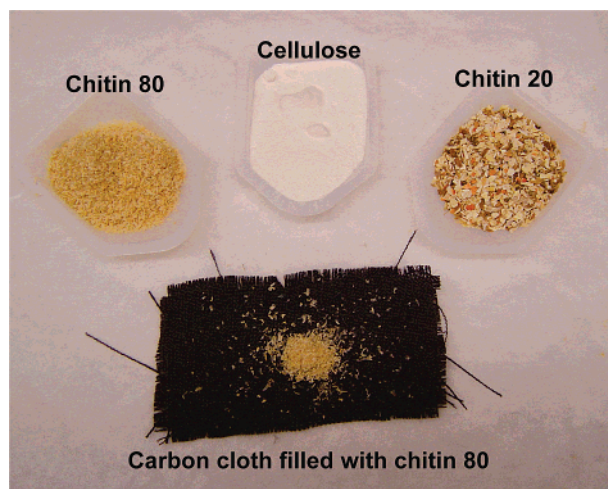


FIGURE 1. Chitin 80, cellulose, and Chitin 20 as substrates. Also shown is the carbon cloth anode filled with Chitin 80 before being sewn.

quickly lost by diffusion into the surrounding water in a system in the field. Power generation from particulate substrates has not previously been examined in a sediment MFC. To examine the idea of this new type of a substrate-enhanced microbial fuel cell (SEM), we examined power output using two different types of particulate substrates: chitin, an easily degraded material in marine environments, and cellulose.

Materials and Methods

Substrates. Chitin is a polysaccharide of *N*-acetyl- β -D-glucosamine ($C_8H_{13}NO_5$), and it is the second most abundant material in the world after lignocellulose (12). It is widely available as a seafood industry byproduct in coastal areas and is readily degraded by marine bacteria (13). Chitin has previously been used as a slowly degradable material for in situ bioremediation of tetrachloroethene (PERC) (14). Depending on the particle size, pretreatment method, and exact chemical composition (ratio of rapidly to slowly degradable fractions), different forms of chitin can be used. In this study, ChitoRem SC-20 (Chitin 20) and ChitoRem SC-80 (Chitin 80) were used as received (JRW Bioremediation, LLC). Chitin 20 consisted of crushed crab shells containing approximately 20% chitin, 25% protein, 40% calcium carbonate, and 15% water. Chitin 80 was composed of deproteinized and demineralized crab shells containing approximately 95% chitin that was sieved to a particle size less than 20 mesh ($<841 \mu\text{m}$). Chitin 20 is more easily degraded than Chitin 80 due to its higher protein content. Cellulose particles were tested as received, with a diameter of $50 \mu\text{m}$ as specified by the manufacturer (Sigmacell cellulose, type 50 particle size, Sigma Aldrich).

Electrodes. The anode was made of non-wet proofed carbon cloth (Type A, E-TEK) connected to an external circuit using a titanium wire (0.81 mm diameter, 99.7%, Sigma-Aldrich). Carbon cloth (5 cm \times 9 cm, total projected surface area of $A_{\text{an}} = 0.0045 \text{ m}^2$) was sewn together in the shape of a pillow (3 cm \times 5 cm final size) and filled with 2 g of a particulate substrate (Figure 1) or left empty (control). The pillow-shaped anode was sewn closed and wrapped with titanium wire to connect it to the external circuit. The cathode was made of carbon paper (2 cm \times 5 cm, projected surface area of $A_{\text{cat}} = 0.001 \text{ m}^2$) containing 0.35 mg/cm^2 Pt on one side (10% of Pt/C catalyst, 30% wet-proofing) (E-TEK).

Medium. Natural seawater (Real Ocean Pure Seawater, PETCO.com) was amended with a phosphate buffer and nutrients (NH_4Cl , 0.31 g/L; $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$, 0.75 g/L) to ensure

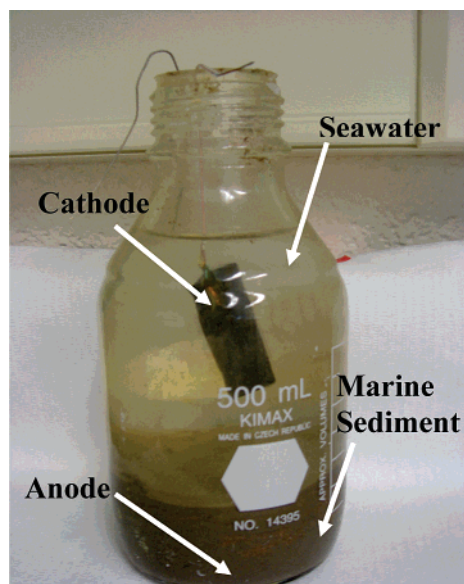


FIGURE 2. Small-scale sediment fuel cell with substrate (SEM). Anode is embedded inside the marine sediment at the bottom (anaerobic phase), and the cathode is suspended in the overlaid seawater (aerobic phase).

that nutrient limitations or pH changes did not affect power generation. Other nutrients and trace elements were assumed to be available in the sediment or seawater. An anaerobic sediment from the Delaware Bay (kindly provided by David Kirchman, University of Delaware) with an organic matter content of $4.8 \pm 0.42\%$ was combined with standard sand, sieved to a uniform size ($\sim 0.5 \text{ mm}$), in a 1:1 ratio.

SEM Construction and Operation. SEM reactors (Kimax*GL 45 media/storage bottle; 500 mL capacity) were filled with 250 mL of a 50:50 mixture of sediment and sand and 250 mL of seawater (Figure 2). The anode was placed at the bottom of the bottle and connected to the cathode using a copper wire sealed with plastic tubes. The circuit was completed using a 1000Ω resistor as a load, with the voltage monitored across the resistor every 30 min using a data logger (ADC-16, Pico Technology Ltd.). Tests were run in triplicate with a single, non-amended control reactor. Deionized water was added to the reactors to replace water lost to evaporation.

Two-Chamber MFC Tests. Additional tests were performed using two-chambered MFCs to examine the performance of these particulate substrates under conditions typically used for conventional (soluble) substrates. Particulate substrates (1 g/L) were added to media containing the following (g/L): NH_4Cl , 0.31; KCl, 0.13; $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$, 4.97; and $\text{Na}_2\text{HPO}_4 \cdot \text{H}_2\text{O}$, 2.75 and a mineral (12.5 mL) and vitamin (12.5 mL) solution as reported previously (15). Anaerobic sludge (secondary clarifier) obtained from the Pennsylvania State University Wastewater Treatment Plant was used to inoculate these reactors. The two-chamber reactors were constructed as previously described using two media bottles (200 mL capacity) with side arms containing a Nafion membrane (Nafion 117, Dupont Co.; projected surface area of 0.001 m^2) clamped between the tube ends (16). A graphite brush was used as the anode (25 mm diameter \times 25 mm length, $A_{\text{an}} = 0.22 \text{ m}^2$) (PANEX 33 160K, Zoltek) (17). The cathode was 1 cm \times 9.5 cm carbon paper containing 0.35 mg/cm^2 Pt (10% of Pt/C catalyst, 30% wet-proofing; E-TEK) coated on one side ($A_{\text{cat}} = 0.00095 \text{ m}^2$).

Analytics and Calculations. Volatile fatty acids (VFAs) were measured using a gas chromatograph (GC) (Agilent, 6890) as described previously (18). The concentration of organic matter in the marine sediment was measured as volatile solids (VS), based on differences in dry weight

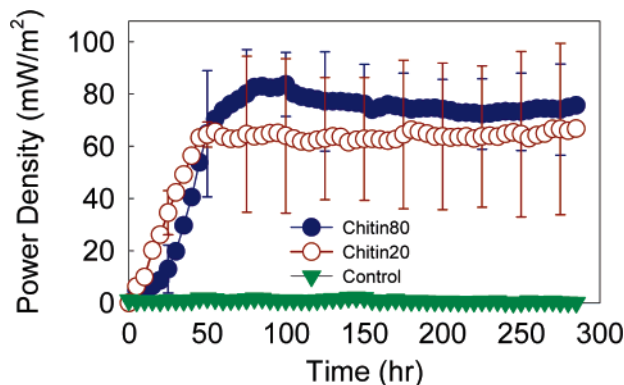


FIGURE 3. Power generation from SEM with Chitin 20 and Chitin 80 as substrate as well as a control (without substrate). Each line represents the average of three reactors (error bars \pm SD). Symbols are shown for each 10 data points, and standard deviations are shown for each 50 data points.

(110 °C for 48 h) and combusted weight (550 °C for 8 h) for 10 samples (10 g each). Solution conductivity was measured using a conductivity meter (OAKTON, CON6, Acron series) and kept between 20 and 30 mS/cm (except as noted) as the solution conductivity affects power density (19).

Cell voltages (V) were measured using a data acquisition system (Pico-ADC 16, Alison Technology Corporation) connected to a computer. Anode/cathode potentials were measured using a multimeter (83 III, Fluke) and using an Ag/AgCl reference electrode (RE-5B, Bioanalytical systems). Current (i) was calculated as $i = V/R$, where R is the external circuit resistance. Power (P) was calculated as $P = iV$ and normalized by the cathode projected cathode area (A_{cat}), as was done in previous studies (17, 20). The maximum power density was measured by varying the external resistance between 100 Ω and 200 k Ω and waiting until voltage was stable (\sim 30 min).

The total remaining substrate at the end of a test could not be directly measured due to the organic matter content of the inoculum. Therefore, Coulombic efficiencies (CEs) were estimated by assuming that all substrates were completely degraded when the voltage was reduced to a low value (around 10 mV). CEs were calculated as previously described (18) assuming that 32 mol of electrons was produced per mol of chitin and that 24 mol of electrons was produced per mol of cellulose.

Results

Power Generation with Chitin. Power generation was observed with little lag with both chitin substrates, reaching a maximum stable power density within 80 h (Figure 3). Average power densities during maximum power production (from 54 to 290 h) in three reactors with Chitin 80 were 90 ± 4 , 75 ± 7 , and 60 ± 5 mW/m² ($n = 470$ for each reactor). Power densities from the three reactors were significantly different ($p = 0.001$, 95% CI, t -test) during maximum power production, for reasons not well-understood. While distances between electrodes were set to be the same in all reactors, the motion of the water due to aeration may have moved the cathode or affected localized dissolved oxygen concentrations near the cathode. Replacing the cathodes with new cathodes, or switching them between reactors in this case, did not affect power. Repeated tests with additional reactors also resulted in differences between duplicate reactors, suggesting that bacterial distribution and decomposition rates of the substrate were in fact controlling the maximum power densities and producing different results. To provide an assessment of the range of data in the different reactors, averages are shown with associated standard deviations

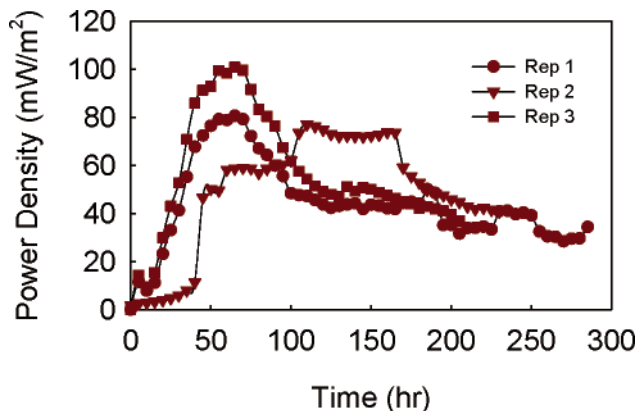


FIGURE 4. Power generation from three SEMs with cellulose as substrate.

(Figure 3). For the combined reactors, the average maximum power density produced using Chitin 80 was 76 ± 15 mW/m². Power densities from the control reactors lacking substrate amendments were below 2 mW/m². Note that these power densities were normalized to the cathode projected surface area (one side, which contains the Pt catalyst). If the anode surface area would be used, these power densities would be multiplied by 0.66 (one side, folded anode), 0.33 (two sides, folded), or 0.11 (both sides, unfolded anode). No matter which area is used for normalization, in all cases, power production with the particulate substrate was larger than that of the control.

With Chitin 20 as the substrate, the average power densities for the three reactors were 94 ± 6 , 56 ± 4 , and 41 ± 6 mW/m² ($n = 513$, from 43–290 h). Taken together, the power generation for these three reactors averaged 64 ± 27 mW/m². Again, there were significant differences in power production among the reactors ($p = 0.001$, 95% CI). However, the higher power produced by one of the reactors (94 ± 6 mW/m²) this time was identified to be due to cathode performance. When the cathode from this reactor (at that time producing 110 mW/m²) was moved to the reactor with the lowest power (41 mW/m² on average but producing 71 mW/m² at that time), the power instantly increased from 71 to 107 mW/m². Thus, this cathode was more effective than the other two cathodes, resulting in the observed differences in power generation. The reasons as to why the cathodes differed in performance are not clear as they were all made from the same commercially prepared piece of carbon paper.

Power Generation with Cellulose. When cellulose was used as a substrate, power was produced, but levels were more erratic than was observed with chitin. For two of the reactors, the power generation increased rapidly over time following inoculation, but for the third reactor, the power increased more slowly (Figure 4). The maximum power generation for the first reactor reached 98 ± 2 mW/m² ($n = 46$, 50–73 h) and was significantly greater ($p = 0.004$, 95% CI) than power produced by the other two reactors of 73 ± 4 mW/m² ($n = 140$, 100–170 h) and 78 ± 3 mW/m² ($n = 52$, 50–76 h). The power output for one reactor was again attributed to the efficiency of the catalyst. When the cathode from the reactor that had produced a maximum of 98 mW/m² was hooked to the reactor that had produced a maximum of 73 mW/m², the power immediately increased (in this case from 9 to 42 mW/m²). A comparison of the results from the two chitin substrates and cellulose substrate showed that the maximum power densities using the different substrates were significantly different ($p = 0.001$, 95% CI).

Polarization and Power Density Curves. Polarization data were obtained by varying the circuit external resistance (Figure 5A). Data were obtained during startup when power

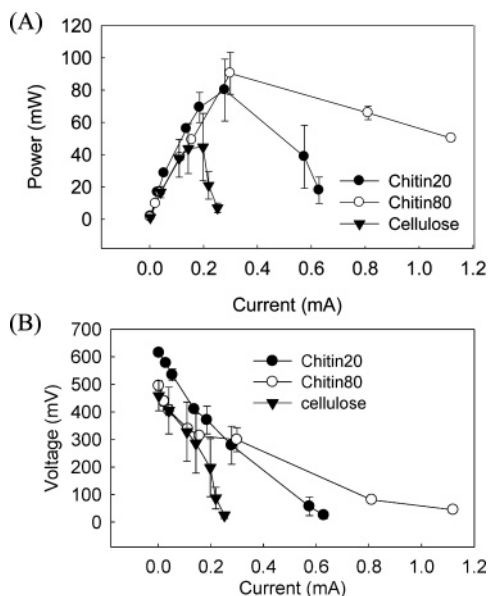


FIGURE 5. (A) Power density based on different resistances for all substrates. (Error bars are \pm SD based on duplicate measurements.) (B) Voltage based on different resistance for all substrates. (Error bars are \pm SD based on duplicate measurements.)

in the three reactors was 86 mW/m² (Chitin 20), 91 mW/m² (Chitin 80), and 50 mW/m² (cellulose) with a 1000 Ω resistor. Power density curves were then calculated based on the voltages and current. Maximum power densities reflected these general differences in power production, with 87 \pm 10 and 80 \pm 19 mW/m² for Chitin 80 and Chitin 20, respectively, and 45 \pm 21 mW/m² for cellulose (Figure 5B).

The internal resistance calculated based on using the slope of the polarization curve indicates that power generation was limited by internal resistance (20, 21). Over a range of 0.002–0.6 mA (Figure 5B), the internal resistances from two reactors of each treatment were calculated as 646 \pm 134 and 1297 \pm 442 Ω for the Chitin 80 and Chitin 20 substrates, respectively, and 1762 \pm 901 Ω for the cellulose substrate. Differences in cathode potentials in the reactors containing Chitin 20 and cellulose likely account for the large standard deviation among reactors in internal resistance. The average power production in these reactors was in general inversely correlated with internal resistance ($p = 0.01$ (slope), $n = 5$; data not shown), showing that internal resistance variations among reactors produced differences in power densities. Except for the situations noted previously for the cathode, where power was increased when cathodes were switched, these differences were a consequence of the biological development of power generation with the particulate substrates.

Volatile Fatty Acids (VFAs). The degradation of chitin and cellulose requires hydrolysis of the particulate substrate, and this degradation step could result in diffusion of the substrate out of the sediment and its accumulation in the overlying water. However, VFAs measured in the overlying water of the anode chamber in the SEM reactors were all less than 10 ppm (data not shown), indicating little accumulation of these components in the overlying water.

Power Generation in a Two-Chambered MFC. The power generated using two of the particulate substrates (Chitin 80 and cellulose) was further examined in a two-chamber MFC that has previously been tested with soluble substrates. The time to peak power production for the Chitin 80 and cellulose required a substantially longer time for the two-chamber system (320 or 577 h) than with the SEMs (57 or 60 h). Power production with a 1000 Ω resistor reached a maximum of \sim 35 mW/m² for both substrates (Figure 6). These maximum

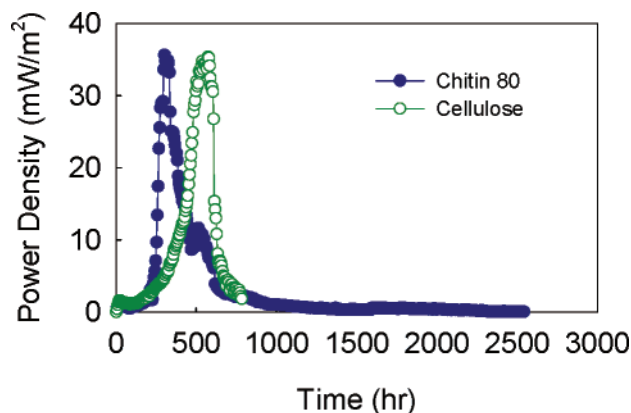


FIGURE 6. Power generation in a two-chamber MFC with Chitin 80 or cellulose (1 g/L).

power densities are slightly lower than that achieved in this system using acetate (45 mW/m²) (16), but similar to that obtained with glucose (37 mW/m²) (unpublished data), suggesting that the rate of power generation with these substrates was limited more by internal resistance of the MFC than by substrate degradation kinetics. The CE for the two substrates was 10% for cellulose and 13% for Chitin 80. However, we cannot be certain that all the particulate substrates were fully degraded. These CEs are substantially lower than those measured using soluble substrates of 22% for glucose (unpublished data) and 53% using acetate (16).

Discussion

Power generation by sediment MFCs was substantially increased by the addition of particulate substrates into the anode material. Maximum power densities for SEMs ranged from 54 to 112 mW/m² for Chitin 20 and 80, respectively, while it varied from 78 to 101 mW/m² for cellulose. In contrast, the unamended control (4.8% organic matter) produced $<$ 2 mW/m². In MFC tests with soluble substrates, power production at a fixed external resistance was reproducible between reactors but only after several repeated feeding cycles when reactors were operated in a fed batch mode (22). Here, we observed significant variability in reactor performance that was likely due to a combination of several different factors, including variations between reactors typical of startup conditions such as bacterial access to the substrate, substrate particle size distributions between reactors, orientation of the cathode relative to the anode with water sparging, and local differences in dissolved oxygen concentrations. Differences in power output by the cellulose-fed reactors were more apparent than with chitin-fed reactors. Part of this difference could be due to the nature of the cellulose versus that of the chitin. Cellulose powder used here contained both amorphous (8%) and crystalline (92%) regions, making it possible that initial and higher power production was associated with the more easily degradable amorphous portions (23), followed by lower power production associated with the more recalcitrant crystalline regions. In contrast, chitin is a polysaccharide that exhibits rapid and sustained degradation and therefore is associated with more constant power production.

Although it was shown that adding particulate substrates improved power generation as compared to unamended controls, the longevity of the SEMs was only 30 days under laboratory conditions. However, reactor longevity should be able to be controlled by formulating particles of specific sizes and composition to extend degradation times. Experience with nonaqueous phase liquids in subsurface environments,

and globular immiscible liquids in streams and rivers, has demonstrated that the lifetime of materials is dependent on mass transfer controlled by a limited surface area (24, 25). Thus, it seems likely that we should be able to engineer a particulate substrate for an SEM through the control of particle size. For example, if we assume spherical particles, we could model the time for complete dissolution as (24)

$$t_s = \frac{3m_0^{1/3} (4\pi\rho_c)^{2/3}}{4\pi Kc_s} \left(\frac{4\pi\rho_c}{3} \right)^{2/3}$$

where ρ_c is the density of the particle (g/cm^3); K is the mass transfer coefficient (cm/s); m_0 is the initial particle mass (mg) calculated as $m = \rho_c \pi d^3/6$, where d is the diameter of the particles; and c_s is the surface concentration. For a particle in a stagnant flow field, $K = D/d$, where D is the chemical diffusivity in water, so the lifetime of the particle is proportional to d^2 . This analysis assumes complete exposure of the surface for dissolution. The analogy between dissolution and microbial degradation is not perfect because the bacteria will consume the substrate as opposed to releasing it. However, we can think of this surface flux resulting from dissolution as being analogous to the reduction of mass due to bacterial consumption. Using a lifetime for Chitin 80 of $t_s = 30$ days for $d = 0.05$ cm (average Chitin 80 particle size), we estimate from $t_s \sim d^2$ that by increasing the particle size to $d = 0.17$ cm, we could extend the lifetime of the particle to 1 year, or using $d = 0.78$ cm, we could extend the lifetime of the particle to 20 years. While further research is in progress to test this model, it provides a conceptual model for testing the scale up of the SEM to reactor sizes previously used in field tests.

These SEM reactors do not overcome a major limitation of many MFCs, which is high internal resistance. Our results indicated internal resistances ranging from 646 to 1762 Ω , which are a consequence of the electrode spacing (9 cm) and conductivity of the porous media and water. The effect of electrode spacing on power generation in MFCs is well-known (19, 20), and thus, it is a major concern for SEM design. There does not appear to be a simple solution here for decreasing the electrode spacing of a sediment fuel cell as the anode must be located in the anaerobic bottom layer, while the cathode needs to be sufficiently distant that dissolved oxygen concentrations are high. There are no other reports of internal resistances for sediment MFCs developed by others, so we do not know to what extent this may be affecting power generation in other systems. However, decreases in internal resistance for sediment MFCs can be expected to produce commensurate increases in power as demonstrated in many different MFC tests.

Although we have designed and tested the SEM with the anode imbedded in the sediment, there is no reason as to why a properly designed system could not be used with the SEM suspended in water (on a buoy) or situated on top of the sediment. When placed in the sediment, it might also be possible to achieve long term power generation by seeding the sediment with particulate substrates rather than placing them in the anode material as was done here. With proper design, the anode chamber could allow for maintaining anaerobic conditions in the anode, with the water flow enhanced past the cathode if it is off the ocean floor. In addition, it might be possible under these circumstances to achieve a lower internal resistance by decreasing the electrode spacing. A combination of reduced internal resistance, and careful design of particulate substrate size and loading, will make SEMs effective as a widely distributable power source for both freshwater and marine studies.

Acknowledgments

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