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Analysis of chitin particle size on maximum power generation, power longevity, and Coulombic efficiency in solid–substrate microbial fuel cells

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ABSTRACT

Microbial fuel cells (MFCs) produce bioelectricity from a wide variety of organic and inorganic substrates. Chitin can be used as a slowly degrading substrate in MFCs and thus as a long-term fuel to sustain power by these devices in remote locations. However, little is known about the effects of particle size on power density and length of the power cycle (longevity). We therefore examined power generation from chitin particles sieved to produce three average particle sizes (0.28, 0.46 and 0.78 mm). The longevity increased from 9 to 33 days with an increase in the particle diameter from 0.28 to 0.78 mm. Coulombic efficiency also increased with particle size from 18% to 56%. The maximum power density was lower for the largest (0.78 mm) particles (176 mW m^{-2}), with higher power densities for the 0.28 mm (272 mW m^{-2}) and 0.46 mm (252 mW m^{-2}) particle sizes. The measured lifetimes of these particles scaled with particle diameter to the 1.3 power. Application of a fractal dissolution model indicates chitin particles had a three-dimensional fractal dimension between 2 and 2.3. These results demonstrate particles can be used as a sustainable fuel in MFCs, but that particle sizes will need to be controlled to achieve desired power levels.

1. Introduction

A microbial fuel cell (MFC) is a device that generates electricity through microbial oxidation of organic or inorganic substrates. Each MFC consists of two electrodes (anode and cathode) connected to each other via an external circuit with a load (usually a resistor in laboratory experiments). The electrodes can be separated into separate chambers using an ion exchange membrane (two-chamber MFC), or kept in the same chamber (single-chamber MFC). Electricity is generated through the release of electrons to the anode by bacteria from substrate oxidation. These electrons flow across a circuit to the cathode where oxygen is reduced to form water from the electrons in the circuit and protons in the water.

The use of renewable fuels and their degradation rates are important factors for producing electricity with MFCs. In many studies, simple carbohydrates such as acetate or glucose are used, or reactors are fed soluble but relatively easily degraded complex carbohydrates such as starch [1–4]. While these soluble substrates work well as fuels in an MFC, they are relatively scarce and expensive relative to the value of the electricity produced. Particulate substrates, such as cellulose and chitin, are among the most abundant biopolymeric materials in the world and thus are a readily available, often inexpensive, and renewable substrate for electricity generation. Particulate materials also compose a large portion of organic matter in industrial and municipal wastewaters. However, there are only a few studies in which particulate substrates have been used in MFCs [1,2,4,5].

A sediment MFC is a special type of MFC that can be used for long-term power generation, especially in remote applications where there is a difficulty in replacing conventional power sources such as batteries. Sediment MFCs use organic matter in the sediment as a fuel for generating power, but the low concentration of organic matter in the sediment can limit power production [5,6]. For example, it was recently shown that a sediment MFC could be used as a power source for a wireless device, but the power output was only 12 mW m^{-2} [7]. The addition of particulate substrates has been proposed as a method to increase the power density of sediment MFCs [5]. By adding particulate biodegradable fuels to the sediment, such as chitin or cellulose, substrate concentrations are increased resulting in higher power densities. Using this type of substrate-enhanced microbial fuel cell (SEMFC) [5], power output was 33 times higher than that produced in reactors without addition of substrate, reaching maximum power densities of 76 ± 15 mW m^{-2} for chitin and 83 ± 3 mW m^{-2} for cellulose.

Most MFC research has focused on improving maximum power densities using soluble substrates [8–13], but none have specifically addressed methods to increase power longevity (duration of power

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generation) using particulate substrates. In a previous study, we proposed that the length of power generation in a SEMFC using a particulate fuel could be improved by increasing the particle size [5]. In this study, we tested this hypothesis by examining the effect of particle size on maximum power, longevity of a power cycle, and Coulombic efficiencies using different sized chitin particles. In order to better understand how longevity was related to particle size, we compared our data to a model developed for predicting power generation longevity based on mass transfer limited particle dissolution.

2. Materials and methods

2.1. MFC construction and operation

A single-chamber bottle MFC (320 mL capacity, Corning Inc., NY, USA) with a brush anode and an air cathode was assembled as described previously [12]. The anode was made of large carbon fiber brush 5 cm diameter, 7 cm length and total surface area A_{An} = 1.06 m^2 [10] which was treated by ammonia gas with a high temperature process (700 °C) [12]. The air cathode (3 cm diameter) was made of 30 wt% wet proofed carbon cloth (type B-1B, E-TEK, NJ, USA), and was coated with 10% Pt (corresponding to 0.5 mg Pt cm^{-2} of cathode surface area) and four diffusion layers [14].

Each reactor was filled with 300 mL of aqueous medium, containing 50 mM phosphate buffer (PBS; 2.45 g L^{-1} $NaH_2PO_4{\cdot}H_2O$ and 4.576 g L^{-1} Na_2HPO_4), and 12.5 ml L^{-1} of a mineral solution, and 5 ml L^{-1} of a vitamin solution [15]. Reactors were inoculated (10%, v/v) once by wastewater from a secondary clarifier collected from Penn State Wastewater Treatment Plant.

2.2. Substrate

ChitoRem™ SC-20 (Chitin 20) that was used in previous MFC tests [5] was sieved and certain fractions used as substrates in the reactors (JRW Bioremediation, LLC, from Lenexa, KS, USA). To separate different particle sizes, Chitin 20 was ground and sieved (US standard sieve series, Fisher Scientific Co., TX, USA; sieve numbers (S) #20, 25, 30, 35, 40, 45, 50, 60, and 70). Particles remaining on sieves 25, 40 and 60 were used for tests here, representing large, medium, and small particles. The average selected particle sizes ($\bar{d}$, diameter) were calculated to be 0.78, 0.46, and 0.28 mm, respectively based on a number distribution assumption [16] using:

$$\bar{d} = \frac{\sum nd}{\sum n} \quad (1)$$

where n is the number of particles of size d defined as distance between the mesh fibers in the sieve. For each selected sieve, n, was calculated by dividing the total mass of particles remaining on the sieve, m_T, by the weight of a single particle (assuming spheres, with a particle mass of $m = \rho_c \pi d^3/6$), for a particle density of 1.425 g cm^{-3} [17].

Two alternatives were considered for adding substrate: (1) adding a fixed mass to each reactor to determine how longevity was affected by the same mass; or (2), adding different masses based on a fixed surface area. Since the available surface area of particles was assumed to affect the rate of biodegradation of the substrate, we selected option (2). Thus the mass of Chitin 20 added to each MFC was based on equal initial surface areas, calculated from an average particle size. Based on the surface area, the mass of Chitin 20 added to the reactors was therefore 0.5, 0.82, and 1.4 g L^{-1} for small, medium, and large particles, respectively (Table 1). Tests were conducted in duplicate for each particle size. In all tests, the anode solution was changed when the reactor voltage was <150 mV. Longevity of the reactor was therefore defined as the time before voltage decreased to this value. Before conducting tests, all reactors were examined for similar anode and cathode performance based on power generation and longevity using identical mass concentrations and chitin particle size (mesh size S#70).

2.3. Community analysis

Samples were collected from the brush anode and the bulk solution from one reactor of each different particle sizes. Bacterial DNA was extracted using PowerSoil™ DNA isolation kit (MO BIO Laboratories, CA, USA) according to the manufacturer's instructions. Polymerase chain reaction (PCR) was used to amplify the V6–V8 region of the 16S rDNA gene using an iCycler iQ™ thermocycler (Bio-Rad Laboratories, US). Forward (GC968F) and reverse (1401R) primers [18], GC968F (5′-CGCCCGCCGCGCCCC-GCGCCCGGCCCGCCGCCCCCGCCCCAACGCGAAGAACCTTAC-3′) and 1401R (5′-CGGTGTGTACAAGACCC-3′), were used in the PCR as described previously [19]. PCR products were then separated using denaturing gradient gel electrophoresis (DGGE). Using a DCode universal mutation detection system (Bio-Rad Laboratories, US), the DGGE then was used to investigate the bacterial diversity in each sample [19,20]. Intense bands in each sample were excised from the gel and the DNA then extracted by suspending the excised gel fragment in DI water overnight at 4 °C. DNA samples were re-amplified by PCR using the same primers except for the forward primer that lacked the GC clamp, using the following PCR conditions: 95 °C for 5 min; 35 cycles of 95 °C for 1 min, 60 °C for 30 s, and 72 °C for 1.5 min; and finally 72 °C for 7 min as described elsewhere [21]. PCR products were purified using QIAquick PCR purification kit (QIAGEN, CA, USA) according to manufacturer's instructions and sequenced by an ABI 3730XL DNA sequencing machine (Applied Biosystems, US). Sequenced fragments were then identified using the BLAST program to search for the closest reported strains.

2.4. Analytics and calculations

Voltage (V) was measured across an external resistor (R) (1000 Ω, unless stated otherwise) using a data logger (Keithley Instruments, OH, USA). Current was calculated as $I = V/R$, and power as $P = IV$. Power was normalized to the cathode projected surface area (A_{Cat} = 7.06 cm^2) as the cathode has been shown to be limiting factor in most MFC studies [22,23].

To test the maximum achievable power from the reactor and its corresponding resistance, a polarization curve was obtained by varying the external resistance (4 Ω to 400 kΩ) and recording the voltage after 1 h when the voltage was stable. Reactors were run under open circuit conditions for 2 h prior to obtaining polarization data.

There is no standard method to measure concentrations of chitin. The approach used here was to measure the dry matter content of the substrate and subtract the bacteria weight from the results. At the end of each batch, the anode solution was homogenized using Branson Sonifier 450 (Branson ultrasonic, CT, USA). Next, 1 mL of the solution was filtered (in triplicate) using pre-weighed 0.2 μm pore-diameter polycarbonate filters, and dry weight was measured based on standard procedures [24]. Assuming bacterial dry weight is approximately 50% protein [25], another 1 mL of homogenized solution was used to estimate bacterial dry weight based on protein concentrations measured using the Bradford Quick Start Protein [26] assay at 595 nm absorbance (BioRad Laboratories, CA, USA).

Coulombic efficiency was calculated for a batch cycle as [9,27]

$$C_E = \frac{M_s \int_0^{t_s} I dt}{F b_{es} \nu_{an} \Delta C} \quad (2)$$

Table 1
Initial chitin concentration compared to the measured final concentrations of chitin, biomass, and end product.

Average particle size (dimater, cm)	Initial substrate concentration (mg L^{-1})	Chitin consumed (mg L^{-1})	Biomass (mg L^{-1})	Methanol (mg L^{-1})	Acetone (mg L^{-1})
0.028	500	300	115	121	4.6
0.046	820	340	127	87	3.6
0.078	1400	370	112	78	3.3

where M_s is the molecular weight of substrate, ΔC is the change of substrate concentration over the batch cycle over time = t_b, F = Faraday's constant, V_{An} is the volume of liquid in the anode, and b_{es} is moles of electrons defined for the substrate, or 32e$^-$ for chitin as shown by:

$$C_8H_{13}NO_5 + 11H_2O \rightarrow 8CO_2 + NH_3 + 32H^+ + 32e^- \quad (3)$$

The Coulombic efficiency was calculated using two different approaches. The first method was to use the measured mass of chitin removed by the end of the batch cycle, or $\Delta C = C_i - C_f(CE_{rem})$. The second method assumed that all the chitin was degraded (since the power density was low), and thus the CE was based on complete removal of the added chitin (CE_{add}).

The concentrations of volatile fatty acids (acetate, butyrate, formate, propionate) and solvents (acetone, methanol, ethanol, n-propanol and butanol) were determined by gas chromatography as previously described [28].

2.5. Modeling particle degradation

The lifetime for complete dissolution of a particle into water [16,29] can be estimated from:

$$t_s = \frac{1}{K_{wc}C_s}\int_0^{m_0}\frac{dm_c}{A(m_c)} \quad (4)$$

where K is the mass transfer coefficient (cm s^{-1}), m_c the particle mass (mg), A the particle surface area, and C_s the surface concentration. Assuming a spherical particle with an area of $A = \pi(\bar{d})^2$, the area of a particle based on its mass is $A(m_c) = \pi(6m/\rho\pi)^{2/3}$. Assuming no fluid motion, the mass transfer coefficient $K = D/\bar{d}$, where D is the diffusion coefficient. The longevity of the sphere particle can then be estimated from:

$$t_s = \frac{1}{K_{wc}C_s}\left(\frac{\pi\rho_c}{6}\right)^{2/3}\int_0^{m0}\frac{dm_c}{m_c^{2/3}} \quad (5)$$

Integrating Eq. (5) results in:

$$t_s = \frac{3m_0^{1/3}}{\pi K_{wc}C_s}\left(\frac{\pi\rho_c}{6}\right)^{2/3} \quad (6)$$

based on particle mass, or

$$t_s = \frac{\rho_c(\bar{d})^2}{2DC_s} \quad (7)$$

based on particle diameter ($\bar{d}$). Thus, we see that the lifetime of spherical particles should scale as $t_s \sim (\bar{d})^2$.

To calculate MFC longevity, the following assumptions were used: chitin density (ρ_c) of 1.425 g cm^{-3} [17], C_s the surface concentration equal to 0.00035 g cm^{-3} (estimated from Rezaei et. al. [5]) and $D = 10^{-5}$ cm^2 s^{-1}.

Many particles are known to be fractal, and therefore a second model was developed based on non-Euclidean scaling relationships. For fractal particles, the surface area of the particle was assumed to be $A = al^{D_2}$ where l is a characteristic length, and D_2 a two-dimensional fractal dimension. The volume of the fractal was scaled as $V = bl^{D_3}$, where D_3 is a three-dimensional fractal dimension. The mass of the particle is therefore proportional to particle size according to $m = \rho bl^{D_3}$. Using these relationships in Eq. (4), the lifetime for a fractal particle is:

$$t_s = \frac{D_3(\rho b)^{D_2/D_3}}{(D_3 - D_2)KC_s}m_0^{(D_3-D_2)/D_3} \quad (8)$$

Assuming $K = D/l$, we have:

$$t_s = \frac{D_3(\rho b)}{(D_3 - D_2)DC_s}l^{D_3-D_2+1} \quad (9)$$

Collapsing all constants into a single value of ξ, the scaling relationship for longevity of a single particle based on fractal geometry is:

$$t_s = \xi l^{D_3-D_2+1} \quad (10)$$

Notice that this scaling relationship is the same as that derived above for a spherical particle when $D_3 = 3$ and $D_2 = 2$.

3. Results

3.1. Effect of particle size on MFC performance

Changing the particle sizes produced variations in voltages and power densities under conditions of a fixed external resistance (1000 Ω) despite the addition of particles with the same surface areas. The maximum voltage from the MFC reactors fed the smallest particles ($\bar{d} = 0.28$ mm) over days 1–7 was 420 ± 23 mV (251 ± 27 mW m^{-2}, $n = 508$). This was slightly higher than that produced by the medium size ($\bar{d} = 0.46$ mm) particles of 394 ± 43 mV (220 ± 48 mW m^{-2}, $n = 840$) for days 1–12, and 349 ± 41 mV (173 ± 40 mW m^{-2}, $n = 1840$) for the largest chitin particles ($\bar{d} = 0.78$ mm) for days 1–25 days (Fig. 1). Results from the polarization curves show this same order of power production relative to particle size. The maximum achievable power densities were 272 ± 63 mW m^{-2} (0.28 mm), 252 ± 46 mW m^{-2} (0.46 mm), and 176 ± 55 mW m^{-2} (0.78 mm) for the three different particle sizes (Fig. 2). In all tests maximum power density was produced when reactors were connected to a 500 Ω resistor (Fig. 2).

Longevity of the MFC reactors increased as the particle sizes increased (Fig. 1). This was as expected from Eqs. (7) and (10),

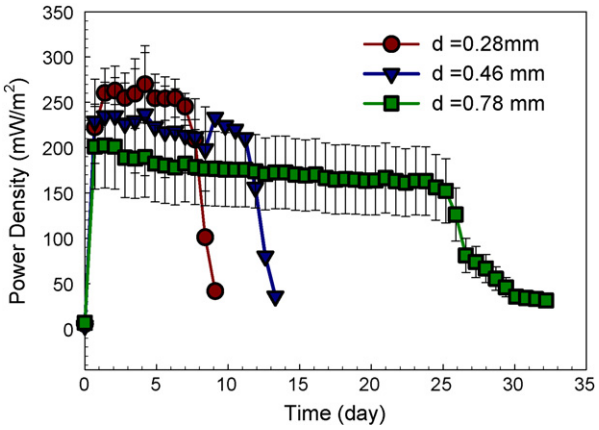

Fig. 1. Power density generation and longevity of MFC reactors fed with different particle sizes of Chitin 20 (error bars are ± S.D. based on measurements from duplicate reactors).

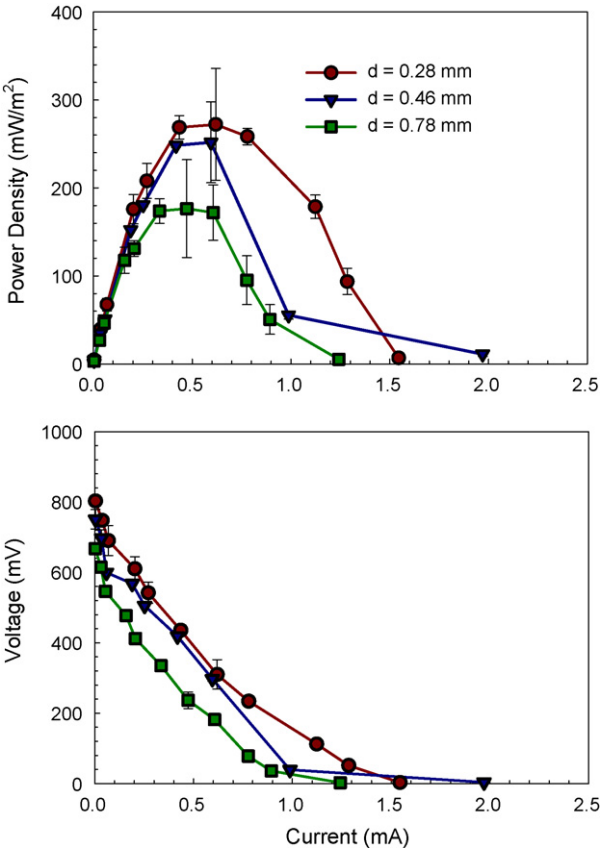

Fig. 2. Polarization curve for MFCs run on different particle sizes (error bars are ±S.D. based on duplicate measurements).

due to the increase in particle mass for larger sized particles having the same total surface area. The reactors fed with the smallest particles having the lowest initial mass concentration (0.5 g L^{-1}) produced power for the least amount of time (9 days). The reactors fed with medium size particles had intermediate results (15 days; 0.82 g L^{-1}), while the reactors treated with the largest particles lasted for 33 days (1.4 g L^{-1}) before power generation substantially declined (Fig. 1). Since MFCs fed with the largest particles had the longest cycle times they were not operated over as many cycles as the other two reactors. At the end of the batch cycle, it was calculated that the total concentrations of chitin consumed were 0.3 g L^{-1} (0.28 mm), 0.34 g L^{-1} (0.46 mm) and 0.37 g L^{-1} (0.78 mm) (Table 1). The incomplete removal of all of the chitin likely indicates that a portion of the chitin was relatively resistant to rapid biodegradation.

In all tests, there were no detectable VFAs measured in solution and there was no significant change in the pH (data not shown) at the end of any batch cycle. Methanol and a small amount of acetone (less than 5 mg L^{-1}) were the only solvents detected (Table 1). The MFC with smallest particles accumulated the most methanol (121 ± 29 ppm), followed by reactors with medium (87 ± 3 ppm) and large particle sizes (78 ± 3 ppm) (Table 1). Biomass accumulation was the highest in reactors fed with medium size chitin (127 ± 25 mg L^{-1}) with lower values of 115 ± 12 mg L^{-1} using small particles, and 112 ± 14 mg L^{-1} using larger particles (Table 1).

Coulombic efficiencies were calculated on the basis of chitin removed at the end of the batch cycle (CE_{rem}), and on the basis of the total chitin added to the reactor (CE_{add}). CE_{rem} increased as the particle size increased (Fig. 3). The maximum CE_{rem} (56%) was achieved for the largest chitin particles ($\bar{d} = 0.78$ mm). CE_{rem} was 18% and 30% for particle sizes $\bar{d} = 0.28$ mm and $\bar{d} = 0.46$ mm, respectively

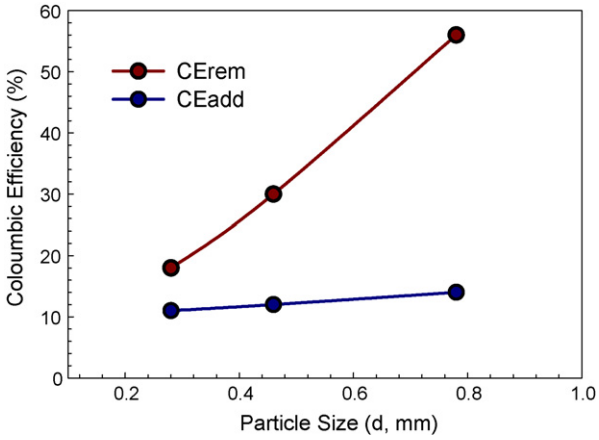

Fig. 3. Coulombic efficiency based on chitin measured at the completion of the batch cycle (CE_{rem}) and total chitin added assuming all chitin was consumed (CE_{add}) for three different initial particle sizes.

(Fig. 3). CE_{add} followed the exact same trend as CE_{rem} but with lower values (Fig. 3).

3.2. Community analysis of bacteria

Results from DGGE bands showed that several different bacteria were dominant in the reactors (Fig. 4). Bands 1–5 were excised from the gel and sequenced. Bands 1, 2, and 5 were common for all MFCs (all particle sizes); whereas bands 3 and 4 were only observed for the MFCs with small and medium particle sizes (Fig. 4). Sequencing and analysis of bands 1 and 5 using the GenBank BLAST program showed that their closest matches (percent identity) were: uncultured bacterium clone AE1_aaa03e10 (87%), *Clostridium sticklandii* (96%), *Enterobacter cloacae* strain E717 (100%), *Fusibacter paucivorans* (93%), and *Bacillus* sp. R-31029 (91%) (Table 2).

3.3. Modeling particle longevity

Using Eq. (7), assuming spherical particles and the actual added particle masses, the predicted longevities were 14-days (0.28 mm),

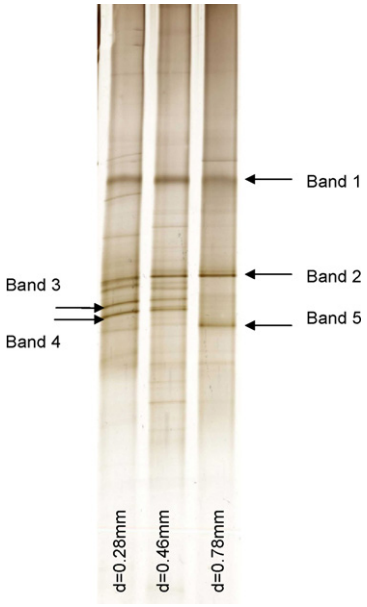

Fig. 4. DGGE analysis on the anode solution of the reactors fed with different particle sizes. Arrows shows the bands that were excised from the gel for sequencing analysis.

Table 2
Closest strains to sequenced bands from GenBank using BLAST program.

Band#	BLAST results	Identity (%)
1	Uncultured bacterium clone AE1_aaa03e10	87
2	*Clostridium sticklandii*	96
3	*Enterobacter cloacae* strain E717	100
4	*Fusibacter paucivorans*	93
5	*Bacillus* sp. R-31029	91

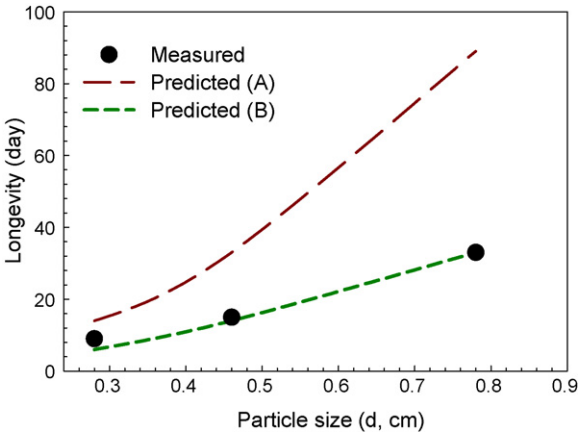

Fig. 5. Measured MFC longevity from the reactor's operational time compared to (A) that predicted using a model based on spheres and (B) a model based on fractal particles.

33-days (0.46 mm) and 89-days (0.78 mm) (Fig. 5). These values are all larger than those measured in MFC tests, of 9, 15, and 33 days for particle sizes of 0.28, 0.46, and 0.78 mm, respectively (Fig. 5).

A non-linear regression of the measured lifetime and the particle size (l, mm) produced:

$$t_s = 117 l^{1.3} \quad (11)$$

Thus we see from Eq. (11) that particle longevity was not proportional to $(\bar{d})^2$ as predicted by the model assuming spherical particles. Using the fractal lifetime model (Eq. (10)), we calculated from $D_3 - D_2 + 1 = 1.3$ that $D_3 - D_2 = 0.3$. We do not independently know the value of either D_2 or D_3. However, if D_3 was less than 2, then $D_3 = D_2$ [16] and the regression analysis would indicate $t \sim l^1$. Thus, we can conclude that the particles are fractal and that D_3 has a value $2 \leq D_3 \leq 2.3$.

4. Discussion

MFC longevity increased with particle size, but the duration of the cycle was not consistent with that expected from a dissolution model using spherical particles. We expected that for spherical particles the lifetime would be proportional to the square of particle size (i.e. the surface area of a sphere). However, the model over-predicted the longevity of larger particles, with the results showing that particle lifetime scaled as $t \sim l^{1.3}$. This difference between theory and observation is likely the fractal nature of the particles, resulting in increased exposure of surface area and thus a decreased lifetime. Through comparison of this observed scaling relationship to our data, we concluded that D_3 was in the range of 2–2.3. Additional research into the fractal nature of chitin particles will be needed to develop a better model of particle longevity.

Particle size did not affect the type of intermediate products that accumulated at the end of batch cycle, but it did affect the concentrations of those products. No volatile fatty acids were measured in the solution, likely due to their degradation and utilization for current generation. Methanol and acetone were both found to have accumulated in solution at the end of the batch cycle, but no ethanol was detected. Methanol degradation rates have been found to be slower than other alcohols, such as ethanol, under iron reducing conditions [30,31]. Thus, it may be that other alcohols were produced, but like the volatile acids they were consumed for power generation. The production of acetone and methanol both indicate fermentation of chitin particles.

DGGE analysis of the anode solution from reactors with of different particle sizes showed close similarity of the identified dominant bacteria, which was expected given that the same inoculum and substrate was used in all reactors. However, there was one difference among the treatments in that MFCs fed with the largest selected particle were not operated for as many batch cycles as MFCs with smaller particles, due to their longer longevity. Among the sequenced bands, *E. cloacae*, was recently shown to have exoelectrogenic activity and it produced electricity using N-acetyl-D-glucosamine, a monomer of chitin [21]. *Enterobacter* species have also been shown to have chitinase activity [32,33]. The absence of this bacterium from the anode solution using the largest particle sizes may have contributed to the lower power generation in that reactor.

While it has been shown in this study that larger particles increase MFC longevity, additional research is needed to improve maximum power densities and Coulombic efficiencies. For example, the Coulombic efficiencies could be increased by reducing oxygen diffusion into the reactor. Alternatively, particulate substrates could be added to anodes buried in the sediment in sediment MFCs so that the sediment effectively uses any oxygen that might reach the anode. The power densities achieved using a soluble substrate (acetic acid) in this same reactor and solution conditions produced 1430 mW m^{-2} [10]. Thus, it is clear that the amount of power production was limited even in this system by a low rate of hydrolysis of the particulate material. By increasing the total mass loading (i.e. the initial mass added to the reactor) it should be possible to increase power through a greater release of soluble substrates. Power densities have been increased by reducing electrode spacing [34] and by changing the reactor architecture. Thus, additional increases in power output may be possible in the future with new reactor designs. Such designs should consider not only the substrate present in water from soluble organics, but also organic materials released from particulate substrate sources as shown here.

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