Intermittent contact of fluidized anode particles containing exoelectrogenic biofilms for continuous power generation in microbial fuel cells

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Highlights

- Exoelectrogenic biofilms were grown on granular activated carbon (GAC) particles.
- Particles were fluidized in the anode chamber for electricity generation in microbial fuel cells.
- GAC particles demonstrated biocapacitor-like behavior.
- High current was sustained by intermittent contact of charged particles with the anode.
- Higher power was obtained by fluidized particles compared to a packed bed control.

Abstract

Current generation in a microbial fuel cell can be limited by the amount of anode surface area available for biofilm formation, and slow substrate degradation kinetics. Increasing the anode surface area can increase the amount of biofilm, but performance will improve only if the anode material is located near the cathode to minimize solution internal resistance. Here we demonstrate that biofilms do not have to be in constant contact with the anode to produce current in an MFC. Granular activated carbon particles enriched with exoelectrogenic biofilm are fluidized (by stirring) in the anode chamber of the MFC, resulting in only intermittent contact between the particles and the anode current collector. The maximum power density generated is 951 ± 10 mW m⁻², compared to 813 ± 2 mW m⁻² for the control without stirring (packed bed), and 525 ± 1 mW m⁻² in the absence of GAC particles and without stirring. GAC-biofilm particles demonstrate capacitor-like behavior, but achieve nearly constant discharge conditions due to the large number of particles that contact the current collector. These results provide proof of concept for the development of flowable electrode reactors, where anode biofilms can be electrically charged in a separate storage tank and then rapidly discharged in compact anode chambers.

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1. Introduction

A microbial fuel cell (MFC) is an electrochemical device that converts chemical energy contained in organic matter into electricity, using the microorganisms on the anode to produce an
electrical current [1–3]. MFCs have the potential to recover energy from organic matter during wastewater treatment, compared to processes that consume energy, which could reduce net operational costs [4,5]. Although power production on the basis of electrode surface area in MFCs has substantially increased in recent years, improved volumetric power densities are needed [6]. Therefore, it is important to create reactor designs that can enable more compact configurations, in order to make MFCs more cost effective for wastewater treatment [7].

MFCs developed to date are primarily fixed biofilm reactors, where the biofilm is in constant contact with the anode and current collector. Under these conditions, the performance of the MFC can be dependent on the anode surface area per volume of reactor [8]. Increasing the anode surface area to make them more compact, while increasing power, is difficult to achieve as the anodes must be kept close to the cathode to avoid high ohmic (solution) resistances that can adversely affect power generation with low conductivity wastewaters [9]. Approaches to make MFCs more compact include: placing the anode very close to the cathode and reducing the volume of the anode chamber [10]; using air cathodes so that the cathode chamber can be very thin [11]; and placing separators between the electrodes to allow close electrode spacing while avoiding electrical short-circuiting [9,12]. Many different types of anodes have been used in MFCs, although not all materials have been tested under conditions that minimize anode chamber volume. Anodes can be made of packed beds of graphite granules [13], although not all particles in the packed bed may contribute to current generation due to high resistances for particles distant from the anode. Even with higher specific surface area materials such as porous graphite fiber brush anodes or reticulated vitreous carbon [14,15], it is still not clear how much of the anode significantly contributes to current generation. Removing graphite fibers from the anode brush distant from the cathode, for example, did not appreciably affect power generation in a single chamber, air-cathode MFC until more than half of the fibers were removed [10]. Using very thin brush anodes with small diameters, placed very close to the cathode, increased power due to reduced solution resistances [16]. However, even with these improvements a long hydraulic residence time (HRT) may be needed for power production due to slow degradation kinetics of organic matter in wastewater. In such cases where the organic matter degradation rates limit power production, biofilm activity on the anode will limit power production [17,18] and the HRT cannot be reduced without compromising the extent of total COD removal.

New fuel cell technologies which make use of low volumetric energy storage liquids are being developed. These are collectively called flowable electrode fuel cells [19]. In these systems large volumes of relatively low energy density solutions of either reduced or oxidized chemicals are stored externally in separate tanks. When power production is needed, the solutions are fed into the respective electrode chambers for current generation in the fuel cell, resulting in high power production with a short HRT in the reactor. The fluid is then regenerated externally and stored until power is needed again. The advantage of this approach is that it enables the use of low energy density fluids because the fuel cell can be much smaller than the storage tanks, allowing effective use of the relatively more expensive fuel cell compared to inexpensive storage tanks.

The known capacitive properties of exoelectrogenic bacteria, and several studies showing that granular activated carbon (GAC) can stimulate current generation by exoelectrogenic bacteria, suggested to us that it might be possible to develop a microbial flowable electrode fuel cell (MFFC) using biofilm-supported particles (Fig. 1). In an MFFC, exoelectrogenic bacteria on GAC particles could build up charge in an external tank (lacking electrodes) through substrate oxidation. The particles would then flow from this tank into a smaller and compact fuel cell reactor where they would strike the anode and discharge over a short period of time. Evidence for the feasibility of this concept of a flowable electrode is based on earlier, particle-free studies, using cell suspensions of Shewanella oneidensis MR1 that were grown in a tank and then fed into micro-sized MFC that produced high power densities [20]. The high power was likely due to a combination of the discharge of mediators produced by the cells, as well as direct electron transfer by cells. Additionally, work with Shewanella oneidensis MR-1 later showed that these microorganisms are capable of storing electrons derived from organic matter for extended periods, with subsequent discharge of these electrons during only brief encounters with manganesic particles [21]. Geobacter sulfurreducens has also been shown to effectively store electrons for several minutes, producing a spike in current when a circuit is closed to enable discharge [22]. Similarly, studies have shown that intermittent opening and closing of circuits can produce large peaks in current and power generation by exoelectrogenic biofilms [23].

The key factor in the development of an MFFC is demonstrating that current can be generated by biofilm particles that make only intermittent contact with the anode. In order to see if such a flowable electrode might in principle be possible, we examined current generation in an MFC containing GAC particles that could be fluidized (by stirring), compared to conditions where there was no stirring or where GAC particles were not used. We hypothesized that respiration and growth of exoelectrogenic biofilms on fluidized particles could be sustained even if there was only intermittent contact of the fluidized particles with the anode. This approach would allow for conditions where a larger number of particles could strike and discharge to the current collector than that possible using a fixed, packed bed, as most of a packed bed would be too distant from the anode to significantly contribute to power production.

To test the concept of intermittent discharge by suspended biofilm particles, we developed a novel reactor configuration that enabled us to quickly switch between fluidized and fixed bed conditions by using a stirrer. When the bed was fluidized, the particles could strike the anode and discharge, and when stirring was turned off the particles formed a packed bed. While this stirring approach did not create a true flowable electrode solution, as
there was no external storage tank, the operation of this system was sufficient to provide direct proof-of-concept data showing that such biofilms could develop on the particles and contribute to power production by striking the anode and producing current. The charge storage, discharge, and performance of the fluidized or packed bed of particles was examined here in terms of power densities, and through cyclic voltammetry (CV) and differential pulse voltammetry (DPV) tests.

2. Materials and methods

2.1. Reactor design

The MFC reactors used to test current generation through intermittent contact of the particles with anodes were constructed with two chambers separated by a titanium mesh that was used as the anode current collector (80 × 80 mesh, woven wire diameter 0.0055 in., McMaster-Carr, OH, anode surface area: 10 cm², anode specific surface area: 47.6 m² m⁻³ net whole cell volume) (Fig. 2). The anode chamber was on the bottom and cathode chamber on the top. The cathode chamber was 2 cm long and 3 cm in diameter (14 mL net volume). Cathodes were made of carbon cloth as previously described [24], with a diffusion layer made of PTFE on the air facing side, and a Pt catalyst layer (Pt loading of 0.5 mg cm⁻²) on the solution side.

The anode chamber was 1 cm long and 3 cm in diameter (7 mL), filled with the granular activated carbon (GAC, 0.5 g, 30 × 30 mesh, DARCO MRX M-1721, Norit Americas Inc., USA, specific surface area: 8.5 × 10³ m² m⁻³ net whole cell volume) except as noted. A magnetic stir bar (color squid IKAMAG®, white, Germany) was used to fluidize the bed and allow intermittent contact between the GAC particles and the titanium mesh current collector. In order to increase the contact area between the GAC and the current collector, titanium mesh was also placed on the inside wall of the anode chamber and joined to the upper titanium mesh current collector. Two holes (5 mm diameter) were drilled on the opposite sites of the anode chamber to allow the replacement of the medium using a syringe. The control reactor had the same configuration as the test reactors, but it lacked granular activated carbon in the anode chamber. Biofilm can form on the titanium mesh and therefore power can be produced even in the absence of the activated carbon. All the operation conditions were the same with test reactors.

2.2. Reactor operation

All reactors were inoculated with the effluent from an MFC operated for over one year, with sodium acetate as the substrate (originally inoculated with primary effluent from the Pennsylvania State University Wastewater Treatment Plant). All reactors were operated under several different conditions: with stirring and with GAC (S–G); without stirring with GAC (NS–G), and without GAC in the anode chamber or stirring (NS–NG). For the NS–G condition, the GAC particles did not touch the top current collector, but they could contact the current collector along the wall of the anode chamber, simulating a packed bed anode operation. Removing the GAC allowed examination of current generation due to biofilm growth on the titanium current collector, as it also functioned as a less effective bioanode compared to the packed bed with GAC. In one set of tests the titanium mesh on the inner face of the anode chamber was removed, resulting in greatly reduced collisions of GAC with the current collector (S–G–RC) (Fig. S1).

2.3. Calculations and measurements

The cell voltage (U) was measured across an external resistor (1000 Ω) and recorded at 10 min intervals using a data acquisition system (2700, Keithley Instrument, OH) connected to a personal computer. Electrode potentials were measured and reported versus a standard hydrogen electrode (SHE). Polarization tests were performed using the single-cycle method by varying external circuit resistances in a decreasing order with 20 min intervals at each resistance (from open circuit potential to 50 Ω) [25]. The power and current densities were normalized by the projected cathode surface area (7 cm²). Power densities were calculated as $P = U^2/(R_{dc}A_{cat})$. 

Fig. 2. (A) Schematic and (B) photograph of a novel intermittent contact of fluidized anode particles system. GAC, granular activated carbon; S–G, with GAC particles, stirring; NS–G, packed bed with GAC particles, no stirring; NS–NG, no stirring, no GAC particles; R, external resistance.
and current densities were calculated using $I = U/(R_{ex}A_{cat})$, where $A_{cat}$ the projected surface area of the cathode. Anode and cathode potentials were measured by using reference electrodes (Bio-analytical Systems, Inc., RE-5B; +0.209 V versus a standard hydrogen electrode, SHE). The total recovered coulombs were calculated by integrating the current over time for an entire batch cycle. Coulombic efficiencies (CEs) were calculated as the ratio of recovered coulombs to the theoretical amount of coulombs that can be produced from the organic matter oxidation based on the change in COD [26,27]. All error bars represent standard deviations based on experiments using duplicate reactors.

2.4. Electrochemical tests

Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were used to compare the anode biofilm activities in the presence and absence of the stirred GAC anode using a potentiostat (VMP3; Bio-Logic, Knoxville, TN). All electrochemical measurements were performed with the anode as the working electrode, the cathode as the counter electrode, and the Ag/AgCl electrode as the reference electrode. For the CV tests, the anode potentials were scanned from $-0.49 \, \text{V} \, \text{SHE}$ to $+0.31 \, \text{V} \, \text{SHE}$ at a slow scan rate of $1 \, \text{mV} \, \text{s}^{-1}$. DPV was performed over the same range of potentials as previously described [28], with a pulse height of $50 \, \text{mV}$, pulse width of $300 \, \text{ms}$, step height of $2 \, \text{mV}$, and step time of $500 \, \text{ms}$ (equivalent to scan rate of $4 \, \text{mV} \, \text{s}^{-1}$), with current averaged over the last $80\%$ of the step ($1 \, \text{s}, 12 \, \text{points}$) using an accumulation time of $5 \, \text{s}$.

Capacitance was measured by periodically poising the anode potential at $-0.2 \, \text{V} \, \text{SHE}$ for 20 min and open circuit for 10 min, to compare the current production of $S$–$G$ with or without stirring compared to the control reactor (no GAC, no stirring) [29].

In order to investigate the charge and discharge processes with the fluidized GAC anode, stirring was cycled from being switched on for $30 \, \text{min}$ ($100 \, \text{rpm}$) and off for $30 \, \text{min}$, while the anode was constantly held at a set potential of $-0.2 \, \text{V} \, \text{SHE}$. Control reactors were operated under the same conditions to investigate the effect of stirring (no GAC) on anode current production.

3. Results and discussion

3.1. Reactors operation

All reactors exhibited consistent and reproducible cycles of voltage generation over at least three successive batch cycles after 10 days of inoculation, indicating successful acclimation of the anodic bacteria. The reactors were then operated for another 10 days of inoculation, indicating stable reactor conditions.

The reactor with the stirred GAC particles produced a maximum power density of $951 \pm 10 \, \text{mW} \, \text{m}^{-2}$ ($S$–$G$, $2.60 \pm 0.01 \, \text{A} \, \text{m}^{-2}$). This maximum power density was $17\%$ higher than that obtained without stirring ($NS$–$G$, $813 \pm 2 \, \text{mW} \, \text{m}^{-2}$, $2.41 \pm 0.01 \, \text{A} \, \text{m}^{-2}$) due to the additional current released by the exoelectrogenic biofilm on the GAC, compared to the current collector surface alone. The control MFC without stirring or GAC had the lowest maximum power density of $525 \pm 1 \, \text{mW} \, \text{m}^{-2}$ ($NS$–$NG$, $1.9 \pm 0.01 \, \text{A} \, \text{m}^{-2}$) (Fig. 3A). The stirred reactor with GAC particles had more negative anode potentials than reactors with no stirring or GAC (Fig. 3B). The cathode potentials were similar for all the reactors (Fig. 3B), indicating that cathode performance was not a factor in the different results for the three test conditions.

The total coulombs recovered with GAC and stirring was $49.8 \pm 0.9 \, \text{C}$ (external resistance of $1000 \, \Omega$), which was higher than that without stirring ($39.1 \pm 1.7 \, \text{C}$) and also without GAC ($37.0 \pm 2.2 \, \text{C}$) as previously described [28], with a pulse height of $50 \, \text{mV}$, pulse width of $300 \, \text{ms}$, step height of $2 \, \text{mV}$, and step time of $500 \, \text{ms}$ (equivalent to scan rate of $4 \, \text{mV} \, \text{s}^{-1}$), with current averaged over the last $80\%$ of the step ($1 \, \text{s}, 12 \, \text{points}$) using an accumulation time of $5 \, \text{s}$.

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3.2. Bio-capacitor characterization of the electrodes

The capacitance of the different reactors was examined using a method developed for fixed anodes [29,30] based on periodic circuit interruption. The reactors were cycled six times between open circuit conditions (10 min) and a set anode potential of $-0.2 \, \text{V} \, \text{SHE}$ for 20 min (Fig. 5A). Reactors were examined for the three conditions identified above ($S$–$G$, $NS$–$G$ and $NS$–$NG$), but also under conditions where the Ti mesh on the reactor wall was moved to greatly reduce contact of the GAC particles with a current collector ($S$–$G$–RC).

C. As a result, the stirred reactor with GAC had a slightly higher CE of $27.7\%$ than the controls ($21.7\%$, $NS$–$G$; $20.8\%$, $NS$–$NG$) (Fig. 4). The higher CE may have resulted from a longer cycle time (27 h) compared to the other two conditions (22 h, $NS$–$G$ or $NS$–$NG$) (Fig. S2).
Under non-stirred conditions with GAC (NS–G), the anode current profiles showed a capacitive discharge effect, consistent with previous reports for biofilms growing on a fixed anode [30, 31]. When the circuit was closed after being charged under open circuit for 10 min, the peak current (1.90 ± 0.02 A m⁻²) gradually decreased to a lower and steady value of 1.71 ± 0.03 A m⁻². The decay of the current over time indicated that the GAC particles acted as capacitors, with the amount of charge stored in the anode over 10 min insufficient to maintain a high current over the next 20 min. In the absence GAC particles and stirring (NS–NG), there was much lower current (0.73 ± 0.02 A m⁻²) and no indication of a capacitive discharge when the circuit was closed.

When the reactor was stirred with GAC particles (S–G), the current (2.25 ± 0.04 A m⁻²) was higher than that produced in the packed bed tests, and surprisingly this high current was sustained over the full 20 min of closed circuit operation. The production of a sustained current that was 47% higher than that sustained with the packed-bed operation showed that the anode performance was improved by using the fluidized particles rather than a packed-bed. The sustained current over the full 20 min, as opposed to a drop in current expected for the capacitive particles, could have occurred for two different reasons. First, it could be that the GAC particles did not function as capacitors. However, the packed bed results indicated that these particles did have capacitive properties. Second, it could be that the fluidized bed operation enabled more efficient discharge from all GAC particles, as opposed packed bed operation, allowing operational conditions where the capacitive nature of the anode could not be observed. This latter explanation is more likely, because in the fluidized bed operation, particles are continuously cycled to strike the current collector and discharge. In addition, they can “recharge” when not in contact with the current collector and are freely suspended in the solution. However, in the packed-bed all particles are continuously in contact with the current collector and only the portion of the anode near the current collector can efficiently discharge current.

To further examine the effect of GAC particle fluidization and stirring on reactor performance, the reactor was operated so that the GAC particles had fewer contacts with the current collector (by moving the wall Ti current collector into the cathode chamber), but we maintained fully stirred conditions (S–G–RC) and thus could observe the effects of stirred conditions with the GAC particles present. Under these conditions, we observed a capacitive discharge effect, whereby there was a high initial peak current density (2.26 ± 0.03 A m⁻²) followed by a rapid decrease in current (within 5 min) to a lower and stable current (1.53 ± 0.03 A m⁻²). This initial high peak current density demonstrated that the GAC particles had typical capacitance/discharge behavior in a fluidized bed condition, but the less effective collisions of the particles with the current collector did not enable current to be sustained at a higher level.

The changes in the anode potentials (Fig. 5B) were generally consistent with the above results showing improved performance for the fluidized bed conditions compared to the other operational modes. Under open circuit conditions, the anode potentials decreased more rapidly with stirring (S–G–RC, −0.29 V), compared to −0.27 V without stirring. While the potential of the reactor without stirring or GAC particles also

![Fig. 5. (A) The current densities and (B) potential behavior with 10 min of charging (open circuit) and 20 min discharging (poised anode potential at −0.2 V vs. SHE) over 6 cycles with the S–G (with GAC particles, stirring) compared to controls (NS–G, packed bed with GAC particles, no stirring; S–G–RC, stirred GAC particles with reduced frequency of contact with the current collector; NS–NG, no stirring, no GAC).](image)

![Fig. 6. Variation in charge–discharge behavior of S–G (A) compared to controls (B) with the anode set at a potential of −0.2 V vs. SHE. The stirrer is periodically turned on and off at 30 min intervals (NS–G, packed bed with GAC particles, no stirring; NS–NG, no stirring, no GAC; S–NG, stirring, no GAC).](image)
reactor operated with stirring produced a current of 
with anode set at a potential of 3.3. Charge 
other operational conditions.

increase of 0.05 A m
2 lacking GAC particles was stirred, however, there was only a slight 
with the reactor lacking GAC (Fig. 6B). When the NS
/C6
 density gradually increased and was restored to its original value 
the NS
 bio-capacitive biofilm. The increase in current with stirring and GAC indicated that the S–G 
provided more effective conditions for current generation could be 
due to the intermittent discharge of the bio-capacitive biofilm.

3.4. Voltammetric evaluation

Cyclic voltammetry (CV) was used to further demonstrate the enhanced anode performance with GAC biofilms. The S–G reactor current increased rapidly at a potential more positive than −0.25 V, reaching a peak current of 11.6 A m
2 above a potential of 0.3 V (Fig. 7A). The NS–G system containing the packed bed of GAC produced a smaller peak current of 8.0 A m
2 at 0.15 V, which was 45% lower than that with stirring. The NS–NG control produced the smallest peak current of 4.5 A m
2 at potential of 0.29 V. The increase in current with stirring and GAC indicated that the S–G 
reactor. The increase of peak current in the DPV supported other 
results of a greater accumulation of charge that could be effectively 
transferred to anode compared to the other configurations. The control NS–NG system had a much lower current than those containing GAC, consistent with CV and power production results.

4. Conclusions

These results showed that microbial fuel cell operation could be improved through fluidization (by stirring) of exoelectrogenic biofilms on GAC particles, and therefore proved the feasibility of the MFFC concept for producing power. The enhanced performance using fluidized particles resulted from improved anode potentials and higher current densities, as fluidized particles could transfer charge to the anode more effectively than a packed bed. The GAC particles with the exoelectrogenic biofilm created an effective bio-capacitor, whereby charge was likely stored in both the bacteria and the GAC particle, and rapidly discharged when the particles made contact with the current collector. While an external tank was not used here to recycle the particles, and the fluidization was achieved using a stirrer (which consumed energy), these results indicate that it should be possible to develop true fluidized bed reactor configuration with low energy demands. The development of such MFFCs could enable the design of more effective and economical systems for wastewater treatment and energy generation.

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3.3. Charge–discharge behavior of switched flowable electrodes

To further investigate the capacitance behavior of the flowable electrodes on performance, an additional experiment was con-
ducted where the stirring was turned on and off at 30 min intervals, with anode set at a potential of −0.2 V vs. SHE (Fig. 6). The S–G 
reactor operated with stirring produced a current of 2.27 ± 0.02 A m
2, but when stirring was stopped the current output gradually decreased to 1.74 ± 0.03 A m
2 (after 30 min), similar to the current produced by the reactor maintained under the NS–G conditions. When stirring was resumed, the current density gradually increased and was restored to its original value (2.26 ± 0.03 A m
2). This demonstrated that the charge was more 
effectively conveyed from the GAC particles when they made intermittent contact with the electrode compared to the packed bed arrangement. The reactor developed under NS conditions was also examined under alternating stirring/no stirring conditions to investigate the impact of stirring on current production in these tests (Fig. 6A). Current production was initially stable at 1.75 ± 0.05 A m
2 without the stirring, but this increased rapidly to 
2 when the solution was stirred.

The same type of test with and without stirring was conducted with the reactor lacking GAC (Fig. 6B). When the NS–NG reactor lacking GAC particles was stirred, however, there was only a slight 
increase of 0.05 A m
2 in the current density (from 0.71 A m
2 to 0.76 A m
2) demonstrating only a small effect of water motion on reactor performance. This suggested that solution mass transfer to the anode had little impact on performance. Thus, the much larger change in current density with stirring (−0.5 A m
2) was due to the intermittent contact of the exoelectrogenic biofilm on the GAC that enabled significant discharge of this stored energy to the anode.

3.5. Differential pulse voltammetry (DPV) test

DPV [9,28] was used as a complementary voltammetric tech-
nique with an improved sensitivity compared to CV methods 
[31,32], to identify characteristic peaks while canceling out non-
faradic capacitive current or background current [33]. The peak 
height in the DPV is related to the abundance of the electroactive 
species in the cell. DPV on the reactors with GAC with and without 
stirring both contained one broad peak at redox potentials 
of −0.15 V (Fig. 7B). The highest peak current of 1722 ± 5 mA was 
produced by the S-G, which was 6.3% higher than that of the NS–G 
reactor. The increase of peak current in the DPV supported other 
results of a greater accumulation of charge that could be effectively 
transferred to anode compared to the other configurations. The control NS–NG system had a much lower current than those containing GAC, consistent with CV and power production results.

Fig. 7. (A) Cyclic voltammetry and (B) differential pulse voltammetry of intermittent contact of fluidized anode particles system (S–G, with GAC particles, stirring; NS–G, packed bed with GAC particles, no stirring; NS–NG, no stirring, no GAC).
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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jpowsour.2014.03.071.

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