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Microbial fuel cell biosensor for *in situ* assessment of microbial activity

J.M. Tront[a,*], J.D. Fortner[b], M. Plötze[a], J.B. Hughes[b,c], A.M. Puzrin[a]

[a] *Institute for Geotechnical Engineering, ETH Zurich, Wolfgang-Pauli Strasse 15, 8093 Zurich, Switzerland*
[b] *School of Civil and Environmental Engineering, Georgia Institute of Technology, Atlanta, GA 30332, USA*
[c] *School of Materials Science and Engineering, Georgia Institute of Technology, Atlanta, GA 30332, USA*

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ABSTRACT

Microbial fuel cell (MFC)-based sensing was explored to provide useful information for the development of an approach to *in situ* monitoring of substrate concentration and microbial respiration rate. The ability of a MFC to provide meaningful information about *in situ* microbial respiration and analyte concentration was examined in column systems, where *Geobacter sulfurreducens* used an external electron acceptor (an electrode) to metabolize acetate. Column systems inoculated with *G. sulfurreducens* were operated with influent media at varying concentrations of acetate and monitored for current generation. Current generation was mirrored by bulk phase acetate concentration, and a correlation ($R^2 = 0.92$) was developed between current values (0–0.30 mA) and acetate concentrations (0–2.3 mM). The MFC-system was also exposed to shock loading (pulses of oxygen), after which electricity production resumed immediately after media flow recommenced, underlining the resilience of the system and allowing for additional sensing capacity. Thus, the electrical signal produced by the MFC-system provided real-time data for electron donor availability and biological activity. These results have practical implications for development of a biosensor for inexpensive real-time monitoring of *in situ* bioremediation processes, where MFC technology provides information on the rate and nature of biodegradation processes.

1. Introduction

Microbial fuel cell (MFC) technology harnesses energy stored in waste streams and organic rich environments, providing electrons to an electrode from microbially catalyzed carbohydrate oxidation (Bullen et al., 2006; Logan et al., 2006; Lovley, 2006). MFC's involve the metabolic activity of microorganisms such as *Geobacter* spp., *Shewanella* spp. to either accept or donate electrons via a solid electrode in lieu of a soluble or mineral electron acceptor or donor. These organisms have the ability to donate electrons to an electrode under anoxic conditions to support oxidation of electron donors such as lactate, glucose, acetate and a number of mixed wastes (Bond and Lovley, 2003; Chaudhuri and Lovley, 2003; Rabaey et al., 2003; Liu et al., 2005; Ringeisen et al., 2006; Clauwaert et al., 2007). Depending on the organism and culture conditions, cells either use mediator compounds such as quinones and other redox active compounds to promote extracellular electron transfer or they produce electrically conductive pili which may allow for electron transfer directly to, or from, the electrode (Newman and Kolter, 2000; Gregory et al., 2004; Reguera et al., 2005; Gorby et al., 2006). A schematic of the electrodes in a MFC system is provided in Fig. 1, Supporting Information shows a pictorial representation of these currently hypothesized mechanisms of electron transfer to the anode.

MFC's have the potential to be a direct, quantitative sensor for microbial respiration. In MFC systems, microorganisms respire by transferring electrons to the anode, therefore, the electrical current produced in MFC systems is an easily measured endpoint which can be used as a direct, real time measure of metabolic rates for specific respiratory processes. Respiration involves stoichiometric coupling of electron donor oxidation with electrons transferred to the electron acceptor (*i.e.*, electrons transferred to the anode in MFC systems), therefore, the current generated is also a measure of available substrate concentration. Previous work has shown that MFC electricity production in batch and plug-flow systems can be correlated with Monod-type kinetics to the chemical demand or biochemical demand of the wastewater (Kim et al., 2003; Min and Logan, 2004; Kumlanghan et al., 2007) and related work in batch and flow through column systems has demonstrated that current generated by *Shewanella* spp. was related to lactate concentration (Kim et al., 1999). Bacteria can use a wide range of substrates as either electron donors or electron acceptors, thus MFC's have potential in a variety of substrate monitoring applications. Importantly, many organisms that act as biocatalysts in MFC's (*e.g.*, *Geobacter* spp. and *Shewanella* spp.) are also known to be involved in reduction of contaminants such as heavy metals,

* Corresponding author. Tel.: +41 44 633 25 34.
E-mail address: jacqueline.tront@igt.baug.ethz.ch (J.M. Tront).

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radionuclides, and chlorinated solvents reduction (Anderson et al., 2003; Bencheikh-Latmani et al., 2005; Sung et al., 2006) revealing the possibility for *in situ* monitoring of microbial respiration as it relates to contaminant reduction.

Monitoring of groundwater contaminant plumes undergoing natural attenuation or bioremediation requires routine monitoring of various contaminant, biogeochemical, and water quality analytes to derive information regarding contaminant flux and the processes that are central to contaminant remediation (Semprini and McCarty, 1991; Ling et al., 2003; Wu et al., 2006). Characterization of microbial respiration rates and delivery of reducing equivalents to impacted areas would also improve our understanding of natural attenuation and bioremediation efforts. Therefore, development of innovative sensor technologies can potentially reduce the costs of site monitoring, while meeting informational needs of regulators and site managers. This work serves as a basis for development of MFC-systems into a sensing technology for *in situ* water quality monitoring applications. Although the concept is applicable to numerous systems where remote-sensing is needed, such as medical sciences, public health, bio-processing, and environmental remediation, the ultimate goal of this research is to develop a remote, *in situ* technology that can be placed within existing groundwater monitoring well networks to provide direct, real-time information regarding biological activity related to groundwater contaminants and bioremediation amendments. Here we report laboratory findings that demonstrate the ability of MFC-systems to provide feedback about substrate concentrations and microbial respiration rates in porous media systems via electrical current produced. This work examined the ability of the MFC-technology to supply useful information about delivery and microbial oxidation of reducing equivalents (*i.e.*, acetate) in flow-through porous media systems. Thus, flow-through porous media systems fitted with electrodes and inoculated with *Geobacter sulfurreducens* were used to detect and quantify electron donor availability via biological current generation. Bulk phase concentrations of a model electron donor (*i.e.*, acetate) were correlated with the current produced at discrete time points. These results serve as a basis for development of a MFC-biosensor for use in remote monitoring of analyte concentration and related microbial respiration rate.

2. Materials and methods

2.1. Bacterial strain and culture conditions

All chemicals used in media preparation were reagent grade or higher and purchased from the Sigma–Aldrich Company. A pure culture of *G. sulfurreducens*, was kindly provided by Dr. Frank Loeffler, Georgia Institute of Technology. Organisms used for inoculation were grown as described (Sung et al., 2006), except that DCB media contained 20 mM acetate, 20 mM fumarate, and 20 min of intensive N_2 gas bubbling was used in lieu of boiling prior to autoclaving.

2.2. Column systems

Upflow columns were constructed from transparent poly-vinyl chloride (Fig. 2, Supporting Information) as described previously (Tront et al., 2008). Columns were 15.0 cm long with a 2.6-cm inner-diameter and filled with 3.5 mm glass beads, densely packed, to simulate a porous media system. Pore volume (PV) was measured to be 36.4 mL, calculated by weight differential for air dried and water saturated columns. Columns were fitted with two graphite electrodes ($d = 2.5$ cm, 9.8 cm^2 accessible area; Quintech, Göppingen, Germany), where electrodes were connected to wires using silver epoxy (Loctite 3880, Distrelec, Nänikon, Switzerland) and Marine Adhesive Sealant was used to prevent corrosion of electrode connections (3M, St. Paul, MN, USA). Prior to use, electrodes were soaked overnight in 0.5N HCl, and then stored in DI water until placement in fuel cells. A graphite cloth anode placed 4.4 cm from the base served as the anode. A Pt-embedded-graphite cloth cathode (contained 0.5 mg Pt/cm^2; 32 cm^2 to enhance reduction of O_2 at the cathode) was placed at the top of the column (14.5 cm from base) where the column was open to the atmosphere to allow oxygen to reach the cathode. Columns were loosely covered with sterile aluminum foil to prevent contact of extraneous materials with the cathode. A diagram presenting the overview of the circuit in the MFC-biosensor system is presented in Fig. 1, Supporting Information. A source of 400 mV was applied to the circuit to provide the appropriately reduced conditions at the anode and reduce activation time (after Bond and Lovley, 2003). Total current flow in the circuit was monitored in real time to quantify the electron consumption by the microorganisms. The electrodes were placed in series with a 100 Ω resistor, and voltage drop across the resistor was measured every second using a Hewlett Packard 6280 M series multifunction DAQ measurement system. Voltage drop across the resistor was measured every second and each recorded data point was the average of 300 measurements. Electrodes were allowed to equilibrate for more than 24 h prior to inoculation, and background values were established (<0.01 mA).

Columns were initiated either with both *G. sulfurreducens* and *Shewanella oneidensis* MR1 (a facultative aerobe), to provide reduced conditions for growth of *G. sulfurreducens* or with *G. sulfurreducens* alone. During the initiation phase (10 d, 3 mL/h, not included in data presented), influent contained 0.5 mM lactate, 20 mM acetate. Lactate was not detected at the anode 24 h after inoculation, and after the initial growth phase, the influent did not contain lactate or any carbon source other than acetate. A peristaltic pump (Istratec, Glattbrug, Switzerland) was used to control column influent (flow rate = 4.5 mL/h). Influent was DCB media (pH 7.1) prepared with varying concentrations of sodium acetate (0–23 mM) in 0.5 L Scott Duran flasks fitted with foam stoppers to prevent contamination. Media, tubing, electrodes, and glass beads were autoclaved prior to column initiation and the column and end pieces were treated with ethanol (3×) and UV exposure (30 min, 3×). Column systems were operated at room temperature (22 ± 2 °C). All results reported were confirmed independently, however as a biofilm development was as a part of each column experiment, they were considered to be independent systems and statistical comparison was not performed.

2.3. Analytical methods

Organic acid concentrations were measured using a Dionex IC20 with an EG40 eluent generator. A 4-mm Ionpac AS11-HC anion exchange column was used in conjunction with an ASRS Ultra 11 detector. A gradient of 0.5–1 mM NaOH was used over 33 min at a flow rate of 1.5 mL/min. Injection volume was 7.5 μL.

3. Results and discussion

3.1. Column systems

Flow-through column systems fitted with electrodes were used to evaluate the relationship between electricity production and electron donor concentration under dynamic flow conditions in porous media. Control columns, operated without inoculation, showed a constant current output (<0.01 mA) regardless of influent acetate concentration, thus establishing background current level. Data shown in Fig. 1 presents results from a column inoculated

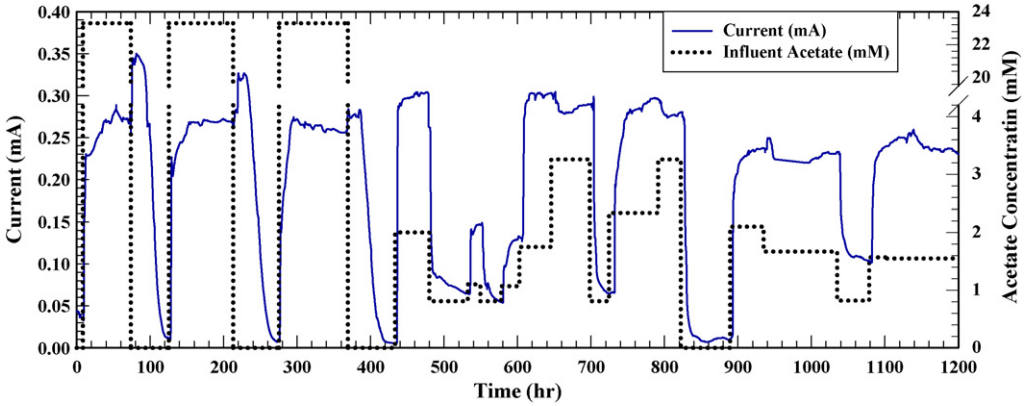

Fig. 1. Electricity output for column system with varying influent acetate concentrations, where influent acetate concentrations are indicated by a dashed line.

with *G. sulfurreducens*, where the influent acetate concentration was varied between 0 and 23 mM (flow rate = 4.5 mL/h). Electricity production was a function of acetate concentration. As shown in Fig. 1 between 0 and 400 h, increased quantities of current were observed when influent acetate concentrations were elevated (*i.e.*, 0.27–0.35 mA, ≥2.3 mM acetate). When the influent contained no acetate, current levels similar to background were observed (<0.01 mA, 0 mM acetate). After 400 h (Fig. 1), the influent acetate concentration was varied between 0 and 3.2 mM in order to find the range of concentrations over which the microorganisms were most responsive to variations in acetate concentration. In this lower-concentration exposure, the current produced paralleled the acetate concentration (*i.e.*, when concentration increased, current increased and vice versa). Current production observed for acetate concentration of 2.3 mM and above were the same as current values observed for 23 mM acetate in the influent (*i.e.*, from high concentration exposure between 0 and 400 h). These data established 2.3 mM acetate as the maximum concentration which provided the maximum current output in this system (0.35 mA), and no increase in current output was observed when acetate concentration was greater than 2.3 mM. The limitation was attributed to system design parameters, such as electrode surface area, microbial efficiency, mass transfer, or cathode design. At acetate concentrations below 2.3 mM, current values corresponded to the relative magnitude of acetate concentrations. Intermediate current values (*i.e.*, 0.05–0.31 mA) were observed for acetate concentrations of 0.5–2.1 mM, and the same current output for a given acetate concentration was repeatedly observed when that concentration of acetate was in the influent. For example, influent acetate concentration of 0.5 mM produced a current value of ~0.05 mA at 500, 575 and 725 h (Fig. 1). Therefore, data in Fig. 1 demonstrate that there was a responsive range of influent acetate concentrations (*i.e.*, 0–2.3 mM) where current values emulate the magnitude of substrate in the aqueous phase and that no additional increase in current production as observed for increasing influent acetate concentration above the value 2.3 mM.

Within the responsive range of 0–2.3 mM acetate, the response of electricity production was observed 1–4 h (0.13–0.52 PV) after the acetate concentration was observed. Using a plug-flow model, solutes require 2.3 h to travel from the media reservoir to the anode, thus, microorganisms at the anode responded immediately to changes in acetate concentrations. The rapid response of the organisms to changing concentrations of acetate was indicative of an active-biofilm on the electrode, and thus growth was not necessary for electricity production after the biofilm was established. Previous work with *G. sulfurreducens* in MFC systems (Bond and Lovley, 2003) demonstrated that a biofilm attached to the electrode was primarily responsible for electricity production. Further, additional work has demonstrated that the presence of a biofilm and associated nanowires in *G. sulfurreducens* MFC systems enhanced electricity production (Reguera et al., 2006). When acetate was at elevated concentrations in the influent (*i.e.*, 23 mM from 0 to 400 h), there was a delay (19 h, 2.5 PV) in the decrease in electricity production after the acetate concentration was decreased. The delay in current attenuation was possibly a result of biofilm-diffusion-limitations, non-ideal-plug flow conditions, or temporary carbon storage within cells for use under starvation conditions (Freguia et al., 2007), among other factors. During the delay period, an initial increase in electricity production was observed prior to attenuation of respiratory activity. Results reported by DiDonato et al. (2006) indicated that the stringent response in *G. sulfurreducens* (*i.e.*, the response to starvation) resulted in an overproduction of genes involved in respiration. Therefore, the increase in electricity production (*i.e.*, an increase in respiration rate) is possibly attributable to a starvation stress response. The delay in current attenuation and the associated initial increase in electricity production were repeatedly observed when the organisms were exposed to relatively high concentrations (*i.e.*, 23 mM) and was not observed for concentrations in the responsive range (*i.e.*, 0.5–2.3 mM) or when acetate concentration was increased.

3.2. Current values correlated to acetate concentrations

Influent acetate concentrations were compared with current values after equilibrium was reached in the column system (Fig. 2). Data used in the comparison in Fig. 2 were taken from the experiment presented in Fig. 1. Based on examination of data in Fig. 1, current production after each variation of influent acetate concentration was considered to reach a stable value after a given amount of flow through the column. Therefore current values used in the comparison in Fig. 2 are an average of data points recorded from the time equilibrium was reached (3 PV for concentrations >20 mM, 1.5 PV for all other concentrations) until the next change of influent concentration; error bars represent one standard deviation for each equilibrium value in Fig. 2. Comparison of acetate concentrations and equilibrium current values revealed a linear relationship between electricity production and electron donor concentration within the responsive range of 0–2.3 mM acetate. A linear regression was applied to the responsive range of data shown in Fig. 2 (*i.e.*, 0–2.3 mM acetate, 0–0.30 mA), demonstrating a high degree of correlation between current produced and bulk phase acetate concentration ($y = 0.001 + 0.132x$; $R^2 = 0.92$; y = current (mA), x = acetate concentration (mM)). Data for influent

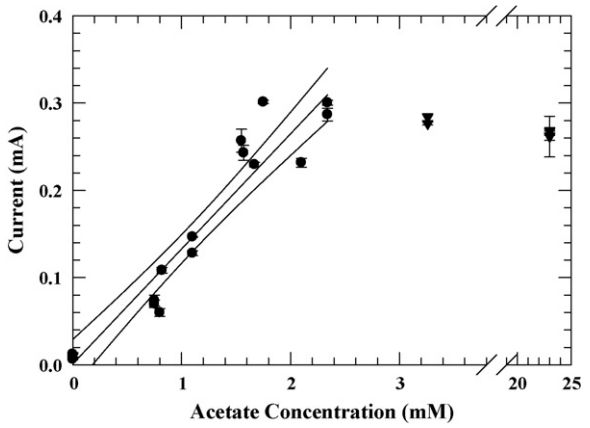

Fig. 2. Average current values are compared with influent acetate concentration for column data presented in Fig. 1. Error bars associated with data points represent one standard deviation. A linear regression is provided with 95% confidence interval ($R^2 = 0.92$; $y = 0.132x + 0.001$). Data for acetate concentrations outside of the responsive range (*i.e.*, data for influent at a concentration greater than 2.3 mM) were not included in the correlation, and are represented by triangles.

acetate concentrations greater than 2.3 mM are also presented in Fig. 2 to emphasize the responsive range, however as data outside of this range were not included in the linear regression, they are represented as triangles. Therefore, it is possible to develop a correlation between electricity produced and substrate concentration for a specific MFC-system. Based on these results, it is likely that correlations developed for related systems can be used in a sensing capacity; however it is clear that additional research is needed to examine the influence of parameters such as temperature, flow rate, microbial ecology, non-specific electron donors, long-term biomass stability and system conductivity, among other factors.

3.3. MFC-biosensor exposure to shock loading

The robust nature of the MFC-biosensor was explored by observation after exposure to shock loading. Columns running in parallel to that presented in Fig. 1 (*i.e.*, fitted with electrodes, inoculated with *G. sulfurreducens*, influent of acetate-containing minimal salts media) were periodically exposed to oxygen pulses to examine recovery rate (oxygen pulses were 10 or 20 mL of air injected into the column in lieu of media). Microorganisms reacted immediately to oxygen pulses by ceasing electricity production (Fig. 3) although the anode remained partially covered in acetate containing media. Electricity production resumed immediately after media flow recommenced, underlining the resilience of the system and, as a corollary, allowing for additional sensing capacity (*i.e.*, toxic species).

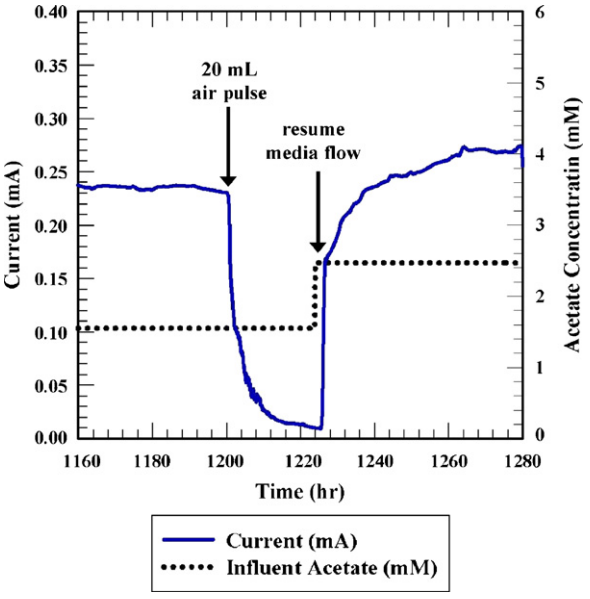

Fig. 3. Electricity output for column system exposed to an air pulse to examine response to toxic loading. Influent acetate concentrations are indicated by a dashed line.

4. Conclusions

Results presented demonstrate a reproducible biologically mediated electrical current as a function of bulk phase acetate concentration. Electric current was a measure of the respiration rate of *G. sulfurreducens* during the oxidation of acetate at varying acetate concentrations. Thus, electricity produced in well defined MFC biosensor systems can be a measure of both the rate of microbial respiration and an indirect measure of substrate concentration. The MFC-biosensor also has the potential to provide information about the presence of toxic species and poor microbial growth conditions, as indicated by the response and recovery to shock oxygen loading. This research introduces the concept of using MFC technology to monitor microbial respiration rate and bulk phase substrate concentrations. Furthermore, electricity produced during these processes could be further utilized for a variety of operations including: internal powering, other sensing application(s) or even temporarily stored, thus expanding MFC-based sensor applicability. We envision a development of the MFC biosensor as a low power, remote sensing tool for groundwater monitoring among other unique biotechnology sensing applications. These results support further research and development to explore hydrogeological parameters (*e.g.*, pH, flow rate, temperature, nutrient composition) potentially affecting the performance of this type of sensing technology and to identify requirements for the implementation of this technology for possible in-well use during groundwater monitoring (*e.g.*, during engineered bioremediation and natural attenuation).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2008.06.006.

References

Anderson, R.T., Vrionis, H.A., Ortiz-Bernad, I., Resch, C.T., Long, P.E., Dayvault, R., Karp, K., Marutzky, S., Metzler, D.R., Peacock, A., White, D.C., Lowe, M., Lovley, D.R., 2003. Appl. Environ. Microb. 69, 5884–5891.
Bencheikh-Latmani, R., Williams, S.M., Haucke, L., Criddle, C.S., Wu, L.Y., Zhou, J.Z., Tebo, B.M., 2005. Appl. Environ. Microb. 71, 7453–7460.
Bond, D.R., Lovley, D.R., 2003. Appl. Environ. Microb. 69, 1548–1555.
Bullen, R.A., Arnot, T.C., Lakeman, J.B., Walsh, F.C., 2006. Biosens. Bioelectron. 21, 2015–2045.
Chaudhuri, S.K., Lovley, D.R., 2003. Nat. Biotechnol. 21, 1229–1232.
Clauwaert, P., Rabaey, K., Aelterman, P., De Schamphelaire, L., Ham, T.H., Boeckx, P., Boon, N., Verstraete, W., 2007. Environ. Sci. Technol. 41, 3354–3360.

Didonato, L.N., Sullivan, S.A., Methe, B.A., Nevin, K.P., England, R., Lovley, D.R., 2006. J. Bacteriol. 188, 8469–8478.
Freguia, S., Rabaey, K., Yuan, Z.G., Keller, J., 2007. Environ. Sci. Technol. 41, 2915–2921.
Gorby, Y.A., Yanina, S., Mclean, J.S., Rosso, K.M., Moyles, D., Dohnalkova, A., Beveridge, T.J., Chang, I.S., Kim, B.H., Kim, K.S., Culley, D.E., Reed, S.B., Romine, M.F., Saffarini, D.A., Hill, E.A., Shi, L., Elias, D.A., Kennedy, D.W., Pinchuk, G., Watanabe, K., Ishii, S., Logan, B., Nealson, K.H., Fredrickson, J.K., 2006. Proc. Natl. Acad. Sci. U.S.A. 103, 11358–11363.
Gregory, K.B., Bond, D.R., Lovley, D.R., 2004. Environ. Microbiol. 6, 596–604.
Kim, B.H., Chang, I.S., Gil, G.C., Park, H.S., Kim, H.J., 2003. Biotechnol. Lett. 25, 541–545.
Kim, H.J., Hyun, M.S., Chang, I.S., Kim, B.H., 1999. J. Microbiol. Biotechnol. 9, 365–367.
Kumlanghan, A., Liu, J., Thavarungkul, P., Kanatharana, P., Mattiasson, B., 2007. Biosens. Bioelectron. 22, 2939–2944.
Ling, M., Rifai, H.S., Newell, C.J., Aziz, J.J., Gonzales, J.R., 2003. J. Environ. Monit. 5, 126–134.
Liu, H., Cheng, S.A., Logan, B.E., 2005. Environ. Sci. Technol. 39, 658–662.
Logan, B.E., Hamelers, B., Rozendal, R., Schrorder, U., Keller, J., Freguia, S., Aelterman, P., Verstraete, W., Rabaey, K., 2006. Environ. Sci. Technol. 40, 5181–5192.
Lovley, D.R., 2006. Curr. Opin. Biotechnol. 17, 327–332.
Min, B., Logan, B.E., 2004. Environ. Sci. Technol. 38, 5809–5814.
Newman, D.K., Kolter, R., 2000. Nature 405, 94–97.
Rabaey, K., Lissens, G., Siciliano, S.D., Verstraete, W., 2003. Biotechnol. Lett. 25, 1531–1535.
Reguera, G., Mccarthy, K.D., Mehta, T., Nicoll, J.S., Tuominen, M.T., Lovley, D.R., 2005. Nature 435, 1098–1101.
Reguera, G., Nevin, K.P., Nicoll, J.S., Covalla, S.F., Woodard, T.L., Lovley, D.R., 2006. Appl. Environ. Microb. 72, 7345–7348.
Ringeisen, B.R., Henderson, E., Wu, P.K., Pietron, J., Ray, R., Little, B., Biffinger, J.C., Jones-Meehan, J.M., 2006. Environ. Sci. Technol. 40, 2629–2634.
Semprini, L., McCarty, P.L., 1991. Ground Water 29, 365–374.
Sung, Y., Fletcher, K.F., Ritalaliti, K.M., Apkarian, R.P., Ramos-Hernandez, N., Sanford, R.A., Mesbah, N.M., Loffler, F.E., 2006. Appl. Environ. Microb. 72, 2775–2782.
Tront, J.M., Fortner, J.D., Plötze, M., Hughes, J.B., Puzrin, A.M., 2008. Biotechnol. Lett. 30, 1385–1390.
Wu, W.M., Carley, J., Gentry, T., Ginder-Vogel, M.A., Fienen, M., Mehlhorn, T., Yan, H., Caroll, S., Pace, M.N., Nyman, J., Luo, J., Gentile, M.E., Fields, M.W., Hickey, R.F., Gu, B.H., Watson, D., Cirpka, O.A., Zhou, J.Z., Fendorf, S., Kitanidis, P.K., Jardine, P.M., Criddle, C.S., 2006. Environ. Sci. Technol. 40, 3986–3995.