Abstracts

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PLATFORM PRESENTATIONS

Improving the anodic biocatalysis in microbial fuel cells

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The anodic biocatalyst is the driving force to liberate electrons from substrates in microbial fuel cells (MFCs). By regulating the anode potential, both the theoretical energy gain for the biocatalyst as the output of electrical energy is determined. In addition, the loading rate and external resistance are two operational parameters affecting the biocatalyst's activity. We continuously operated three acetate fed reactors at a poised anode potential of 0 (R_0), -200 (R_{-200}) and -400 (R_{-400}) mV versus Ag/AgCl. The anode potential had no influence on the start-up time of the three reactors. However, during a 31 day period, R_{-200} produced 15% more charge compared to R_0 and R_{-400}. In addition, R_{-200} generated the highest maximal power density (up to 199 $W.m^{-3}$ total anode compartment (TAC)), but the three reactors evolved to the same power density. The maximum respiration rate of the bacteria in R_{-400} was considerably lower although they had the highest specific biomass activity (6.93 g $COD.g^{-1}$biomass-$VSS.d^{-1}$). Doubling the loading rate to 3.3 g $COD.L^{-1}TAC.d^{-1}$ of 5 MFCs with different three-dimensional anodes and a non sustainable cathode, only resulted in an increased current generation when the external resistance was low (10 – 25 Ω) or during polarization. Conversely, lowering the external resistance resulted in a steady increase of both the kinetic capacities of the biocatalyst and the continuous current generation from 77 (50 Ω) up to 253 (10 Ω) $A.m^{-3}$ TAC. Operating MFCs at an anode potential of -200 mV versus Ag/AgCl or at a low external resistance close to the internal resistance, enhances the current output and kinetic performance of the biocatalyst.

Potentials and limiting factors of bio-electrochemical systems (BES)

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Bio-electrochemical systems (BESs) enable the microbial catalysis of electrochemical reactions both in the anode and in the cathode. In addition to plain electrical power production from organic substrates by microbial fuel cells (MFCs), a number of valuable oxidation or reduction reactions, demonstrating the versatility of BESs, have recently been described. Indeed, BESs can produce hydrogen and peroxide and bring about denitrification and reductive dehalogenation. Moreover, BESs also appear to be promising in the field of online biosensors. However, there is still a long way to go to obtain high throughput and economical viable reactor systems. In order to effectively apply BESs, both biological and electrochemical losses need to be minimized. Expensive materials, low conductivity, pH gradients and large overpotentials are primary constraints in BESs nowadays. During this presentation, we give an overview of the established and conceptual achievable applications of BESs. Subsequently, the limitations of BESs and how they can be surmounted are discussed both from an electrochemical and a microbial viewpoint. Finally, we discuss the roads to explore and give an outline of the future fundamental and applied research lines for BES.

Low solids production using microbial fuel cells for power generation and domestic wastewater treatment

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Conventional domestic wastewater treatment processes typically produces large amounts of residual solids and are highly energy intensive due to the use of aerobic bioreactors. Using microbial fuel cells (MFCs) for wastewater treatment can achieve energy recovery and also reduce solids production, but there is little information on energy recovery or solids production using actual wastewaters. Laboratory batch and continuous flow tests were conducted with domestic wastewater (440~490 mg COD/L) using single chamber air-cathode MFCs containing ammonia-treated brush anodes. The highest power densities of 422 mW/m^2 (12.8 W/m^3, and 1.10 A/m^2) were achieved under continuous mode mesophilic conditions, at an organic loading rate of 54 g COD/L-d, with 25.8% COD removal and 2% Coulombic efficiency respectively. Two reactors in-series, using a mesophilic (30^{o}C) MFC for the first reactor and ambient (20^{o}C) MFC for the second reactor achieved higher hydrolysis rates, power generation and effluent quality production than other configurations. The optimal loading condition was 6.4 g COD/L-d (1.8 h) for the first phase and 0.36 g COD/L-d (15.5 h) for the second phase, resulting in an overall loading rate of 0.67 g COD/L-d (17.3 h) and 85% COD removal. The energy recovery was 161 Wh/kg COD removal (65 Wh/m^3 wastewater added). Particulate COD removal in the overall system was 94% (< 13 mg pCOD/L). Solids production was so low in tests conducted over a six-month period that effluent discharge limits of <30 mg/L TS could be met without the need for a clarifier. However, an additional polishing step would still be required for the conditions examined here to meet secondary limits of < 20 mg COD/L and for nutrient control.

Electric power generation from municipal, food, and animal wastewaters using microbial fuel cells

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Researchers in the fields of Biological and Environmental Engineering have shown a real potential to apply microbial fuel cell (MFC) technology to wastewater treatment. Motivations of their work are based on the economic and social need for environmental sustainable wastewater treatment systems. In this presentation, I explore if MFC technology can replace activated sludge processes for wastewater treatment with an emphasis on the microbiology and reactor configuration for full-scale treatment. I will also discuss the present limitations and problems of electric current generation when a complex wastewater is treated with a diverse and nondefined community of microbes in large-scale systems. These limitations and problems include slow kinetic rates, thick biofilm formation, low anticipated coulombic efficiencies due to reactor configuration problems, nonlinear power density increases during scaleup, and the requirement for intricate reactor configurations with multiple MFC cells in parallel and series to boost the voltage. Finally, I will discuss that the main economic gain from treating wastewater with MFCs will be biological oxygen demand (BOD) removal efficiency without nonrenewable energy consumption rather than municipal electric power generation.

High throughput screening array for electrochemically active bacteria (EAB) using voltage detection

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The identification and characterization of potential microbes for microbial fuel cells (MFCs) utilize metal reduction as an indicator for power generation even though a connection between power and metal reduction is not well established in the literature. The thousands of species of bacteria and the infinite number of potential genetic mutations indicate that a high throughput screening (HTS) assay to determine current output from microbes is required. Here we show preliminary (four- and nine-anode well) HTS assays using microbial fuel cell engineering with a common ferricyanide cathode/reference electrode. The base of the device is fabricated from a Nafion-117 membrane hot pressed with a single Toray carbon paper cathode connected with titanium wire. Each identical anode is made from a carbon ink-coated titanium flag and resides in an isolated culture well. Several experiments were performed to probe the utility of this high throughput platform to screen electrochemically active bacteria (EABs) for current and power production. The HTS platform was able to be used to correlate growth of *S. oneidensis* MR-1 with electrochemical activity. Both MR-1 and other relatively unknown *Shewanella* species (*S. baltica*, *S. japonica*) were also screened to determine whether current can be generated from an array of carbon sources (fructose, sucrose, glucose, agar, starch, etc.). Data from these experiments suggest that *S. japonica* can be used to generate current from complex carbon under fully aerobic conditions. These preliminary studies indicate that HTS technology is a convenient and rapid method to screen unknown EABs for usefulness in MFC applications.

Novel single chamber microbial electrolysis cell (MEC) for high efficiency hydrogen gas production

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With fossil fuel energy reserves rapidly disappearing coupled with concerns over global climate change, accelerating a new hydrogen based economy is reliant on finding sustainable technologies for producing hydrogen. Microbial electrolysis cells (MECs) combine energy derived from bacterial oxidation of organic matter with a small input of electrical energy to produce hydrogen gas at up to $1/10^{th}$ the energy demand of conventional water electrolysis. Up to this point, all MECs have incorporated a membrane in order to prevent bacterial consumption of the produced hydrogen. In this study, we removed the membrane and achieved hydrogen recoveries of up to 96% and hydrogen production rates of over 3 m^3-H_2 per m^3 reactor per day. Efficiencies relative to the electrical input reached 400% at an applied voltage (E_{ap}) of 0.3 V and the overall efficiency (including the energy of the organic matter) was a maximum of 86% at an applied voltage of 0.4 V. The energy demand for this process was only 0.9 kWh/m^3-H_2 (E_{ap} = 0.4 V), which is substantially lower than the typical 5.6 kWh/m^3-H_2 required for water electrolysis. These energy demands translate into a cost of \$0.62/kg-H_2 for the MEC and \$3.80/kg-H_2 for water electrolysis, which make MECs an attractive technology for meeting the US Department of Energy's delivered hydrogen target of \$2.00 - \$3.00/kg-H_2.

Bio-electrochemically assisted reductive dechlorination of TCE: novel biotechnological approach to sustainable groundwater remediation

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The ability to engage in extracellular respirations is typically exploited by those microorganisms which use insoluble electron acceptors, such as iron- or manganese-oxides or inert electrodes in microbial fuel cells. The reverse process i.e., the use of solid surfaces or electrodes as electron donors in microbial respirations, although largely unexplored, could potentially have important environmental applications, particularly for the removal of oxidized pollutants from contaminated groundwater or waste streams. In a recent paper we described the proof-of-principle of a novel process named BEARD (i.e., Bio-Electrochemically Assisted Reductive Dechlorination) in which a polarized glassy carbon electrode, in the presence of methyl viologen (E°=-440 mV vs. SHE), as a redox mediator, is used as electron donor for the microbial reductive dechlorination of trichloroethene (TCE), a common groundwater pollutant, to harmless ethene. In the BEARD process, the mediator, either dissolved in the bulk liquid or physically "anchored" to the electrode surface, facilitated the shuttling of electrons from the carbon electrode to the dechlorinating bacteria. The proposed process potentially carries several advantages over traditional bioremediation processes for chlorinated solvents based on the use of chemicals as electron donors in the bacterial metabolism. In the present study we investigated the factors affecting the performance of the BEARD process, with main reference to the role, mode of use (dissolved in the bulk liquid or immobilized at the electrode surface), and concentration of the redox mediator. Several batch potentiostatic experiments were performed at -450 mV (vs. SHE) using a highly enriched TCE-to-ethene dechlorinating culture, containing *Desulfitobacterium* spp. and *Dehalococcoides* spp. as the putative dechlorinating microorganisms. We found that both the rate of TCE dechlorination and current efficiency were strongly influenced by the mode of use of the mediator. Notably, the highest current efficiencies (up to over 90%) were obtained when the mediator was immobilized at the electrode surface. Under such conditions, side reactions such as biocathodic H_2 evolution were minimized. On the other hand, higher TCE dechlorination rates where typically achieved when the MV was freely available to microorganisms in the bulk liquid.

Electricity generation and biofilm formation by *Geobacter sulfurreducens* stimulated by a pairing microorganism in a microbial fuel cell

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Microbial fuel cell (MFC) utilizes the catalytic activity of exoelectrogenic bacteria for efficient conversion of a variety of organic substances to electricity. Following inoculation in anode chamber, there is usually a start-up period before power production reaching the first maximum. Mixed culture with shorter start-up period than pure culture is usually observed in similar condition. In this paper, model systems comprised of a facultative anaerobe *Escherichia coli* and an obligate anaerobic exoelectrogenic bacteria *Geobacter sulfurreducens* were constructed to elucidate the possible mechanism of the start-up difference. In batch experiments using "H" typed MFCs with ferricyanide as the catholyte, the start-up period of the co-culture system was 1 day while that of the sole *G. sulfurreducens* system was 3 days. The reduction of start-up time in co-culture system was found to be reproducible in additional tests. In control experiments with heat-inactivated *G.*

sulfurreducens cells and viable *E.coli*, no current was generated. During start-up period, in situ DO and oxidation reduction potential (ORP) of anode chamber was measured by microelectrodes. The results showed that ORP and DO decreased gradually and then become stable during start-up. Comparing to the sole *G. sulfurreducens* system, the decreasing rate of ORP in co-culture system containing *E. coli* was much higher while the change of DO was not significant. At the end of the start-up, the biomass of *G. sulfurreducens* in co-culture system was found 2.5 times larger than the sole system. Fluorescent *in situ* hybridization analyses revealed that more coverage of the anode surface with *G. sulfurreducens* cells in co-culture system. The reduction of ORP was presumably the key factor that stimulated the electricity generation and biofilm formation by *G. sulfurreducens*.

Electricity generation from cellulose in a single-chamber air cathode microbial fuel cell

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Cellulose is one of the most abundant renewable resources in the world. Cellulose has previously been investigated to produce H2, ethanol and biogas mainly through biological fermentation processes. Based on recent progress in using different substrates in microbial fuel cells (MFCs), we now know that cellulose can also be used to directly generate electricity in an MFC or to generate hydrogen in a microbial electrolysis cell (MEC). However, we need to increase current densities to make this MFC technology useful for biopower production. We report here that a maximum power density of 1080 mW/m2 can be obtained using cellulose in a single-chamber MFC with a Pt-catalyzed air-cathode and carbon fiber brush anode. This power density is around 20 times higher than that previously obtained using a two-chamber bottle MFC. Further increases are possible based on additional modifications of the MFC architecture, and we are investigating increasing these power densities by reactor changes and by manipulating the feeding and microbial growth conditions in the system.

The effect of carbon metabolism on the efficiency of microbial fuel cells operating with different *Shewanella spp.* as the anode catalysts

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The efficiency of a microbial fuel cell system is dependent on, among other things, how well the microbial catalyst can convert a given substrate into energy and CO_2. Several factors may affect the success of a given organism (or community of organisms) to drive these processes efficiently, including inherent metabolic pathways, environmental conditions such as pH, and the ability to transfer electrons to a solid surface. Here we have investigated several different *Shewanella* strains and their ability to oxidize lactate to completion in an anaerobic MFC anode compartment. Additionally, we have observed their power producing abilities using different buffers and ion exchange membranes. The results indicate that those strains that are able to convert a majority of lactate to CO_2 also demonstrate higher coulombic efficiency. However, higher coulombic efficiency and power production do not

always correlate with successful biofilm growth at the anode surface, indicating that different strains of *Shewanella* may employ unique strategies for electron transfer to solid surfaces. Electrochemical evaluations of lactate oxidation rates also indicate that each strain is kinetically diverse in its ability to metabolize this organic acid in the MFC system. These results can now be applied to designing synergistic microbial communities that are predisposed to drive each step of carbon oxidation to energy that can be harnessed in MFC systems.

Cellulose Conversion to Electricity in Microbial Fuel Cells: Challenges and Constraints

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Cellulosic biomass has the largest potential to contribute to the sustainable and secure provision of energy due to economic factors, scale of available supplies, and environmental considerations. Bio-chemical approaches have been developed for energy production from cellulosic materials by converting them to intermediate biofuels such as ethanol, H_2 and methane. These approaches encounter technical and economic hurdles. An alternative strategy is direct conversion of cellulose to electrical energy in microbial fuel cells (MFCs). This conversion is, however, constrained by the lack of microorganisms capable of catalyzing both cellulose hydrolysis and anaerobic oxidization of metabolites using the anode as an electron acceptor. By employing a consortium of microorganisms indigenous to the bovine rumen as biocatalysts, we have integrated the processes of saccharification and fermentation of cellulose with electricity production in MFCs. This integrated process has produced power outputs as high as 66 mW/m^2 in two-compartment MFCs. The rate of electricity production from cellulose has been found to be limited by the rate of anodic electron transport, electron flow through the circuit, and the accumulation of metabolites in the anode compartment. Improvements in power output can be achieved as process constraints are overcome and more robust biocatalysts are developed.

Performance of the anode compartment when treating complex wastewaters

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The satisfactory treatment of domestic wastewater in microbial fuel cells (MFC) is desirable. Ideally this will be achieved within the frame work of the existing assets renewal strategies. Civil works are typically built to last ~60 to 70 years and may be refitted two or three times in that period. A number of technical issues must be resolved to realise the relatively cheap compact systems this implies. The relationship between loading rate and coulombic and treatment efficiency is likely to be an important one. In laboratory experiments with domestic wastewater treatment efficiency in a naively designed reactor with an air cathode it appeared that up to 70% of the electrons present in the wastewater could be recovered from the waste stream. This is surprising in view of the complex nature of the wastes. However, experiments with a variety of complex wastes and wastewaters suggest that MFC performance with complex wastes is strongly affected by the presence and activity of the microorganisms present. The better recovery of electrons is associated with the putative presence of a naturally occurring mediator. Intriguingly, the putative mediator producing organism appears to be transmissible from waste to waste. Anode materials also affect the performance with simple chemical treatments improving the performance with domestic

wastewater in simple bench scale reactors. We hypothesise that the anode compartment is influenced by competition between the methanogenic and electrogenic bacteria the latter being more competitive but constrained by anode area and mediator concentration. If this hypothesis proves correct will have to design retain the naturally produced mediators in the system.

Combined electrochemical and topographic characterization of *Geobacter sulfurreducens* cell surface under electrogenic conditions

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The mechanism by which electricity-producing microorganisms interact with an electrode is poorly understood. Outer membrane cytochromes and conductive pili are being considered as possible players, but the available information does not concur to a consensus mechanism yet. In this work we demonstrate that *Geobacter sulfurreducens* cells are able to change the way in which they exchange electrons with a graphite electrode as a response to changes in the applied electrode potential. After several hours of polarization at 0.1 V (Ag/AgCl – KCl sat.), the voltammetric signature of the attached cells showed a single redox pair with a formal redox potential of about -0.08 V as calculated from chronopotentiometric analysis. However, new redox couples were detected after conditioning at 0.6 V. Importantly, when polarization was lowered again to 0.1 V the signals obtained at 0.6 V were found to greatly diminish in amplitude while those previously found at a potential of 0.1 V were recovered. Results clearly show the reversibility of cell adaptation to the electrode potential and point to the polarization potential as a key variable to optimize energy production from an electricity producing population. In addition, we have analyzed the microbial cell surface by Atomic Force Microscopy (AFM) in order to identify changes in the cell topography in response to the applied electrode potential.

Quantification of the Internal Resistance Distribution of Microbial Fuel Cells and Proton Transfer Mechanisms

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Identifying the limiting factors in a microbial fuel cell (MFC) system is critical for the development of MFC technology. In this study, a model was developed to identify the limiting factors in MFCs through the quantification of the internal resistance distribution of each MFC component. For a single chamber MFC system with equal size of anode and cathode made of carbon cloth and 200 mM phosphate buffer, it is the cathode and electrolyte rather than the anode that limited power generation. The model was also successfully used to simulate the power generation of two-chamber MFCs using dissolved oxygen or ferricyanide as electron acceptors. In addition, a facilitated proton transfer mechanism was proposed for MFCs in the presence of pH buffers based on the quantitative comparison of free proton transfer rates, diffusion rates of pH buffer species, and the current generated.

Electricity Generation from corn stover using air-cathode single chamber microbial fuel cell

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Lignocellulosic materials containing cellulose, hemicellulose, and lignin are the most abundant polymer on earth. The conversion of lignocellulosic materials for diverse energy is expected to be increased in the near future. Corn stover is one kind attractive feedstock for energy generation and chemicals production with a `purpose to developing a technically and economically viable method of energy generation and corn stover utilization process. Since the cellulose is not easily freed from the cell wall's complex, rigid structure of corn stover, a pretreatment using steam-explosion is used to destroy the corn stover's structure. During this process, some toxic substances might be produced, which should be washed before enzyme liquefied or microbial fermentative for cellulose hydrolyzed. Large amounts of washing solution with high concentration of xylose and some toxic substrates will be produced. So the present research was divided into two parts: washing solution part and solid part (cellulose). A series of experiments showed that electricity could be produced from washing solution over a COD range of 140 to 1000 mg L^{-1} (50mM PBS) with power density (mWm^{-2}) increased from 300.76 to 644.07, while CE (%) decreased from 18.48 to 9.53. The maximum power density of 644.07 mWm^{-2} was gained at 1000mg-$CODL^{-1}$, which is comparable with 696.16mvm^{-2} generated by xylose in the same concentration. COD removal efficiency of 1000mg-$CODL^{-1}$ washing solution was 60%, comparable with the result reported previously. PBS concentration (50mM PBS and 200mM PBS) and solution conductivity were found to have an effect on electrode potential, which resulted in different cell voltages and power densities. Electricity could also be produced from the residual solid part when cellulose degradation bacteria noted as *H-C* conserved in our lab, co-operated with electro-genesis bacteria. Valuable electric current was not found with only adding cellulose degradation bacteria *H-C* or electrogenesis bacteria into MFCs. The maximum power density was 502 mW/m^2 , which was higher than that of 406 mW/m^2 when using raw stover as the substrate. On average, 35% of the residual solid and 25% of the raw stover could be degraded during each batch. The percentage of cellulose was reduced by 23% from 40% to 17% and by 11% from 59% to 48% in the residual solid and raw stover, respectively.

Power production by sediment-hosted microbial fuel cells: the influence of substrate availability and microbial ecology

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In recent years, microbial fuel cells (or MFCs) have been deployed in a variety of natural environments, and shown to produce electrical energy from the biocatalytic oxidation of sedimentary organic matter. However, the relationships between organic carbon decomposition, microbial diversity and density, and power production remain largely

unknown. In particular, our understanding of which metabolic processes are active within the anode-hosted community – and thereby most directly contributing to MFC power production- is limited. To examine the influence of the particular reductants on the anode-hosted microbial community and net power production, we conducted a time-series experiment in which MFC anodes were incubated in columns of coastal marine sediments for up to four months. Individual columns were irrigated with methane, sulfide or acetate at seepage rates and concentrations comparable to those *in situ*. Our data demonstrate that all three reductants stimulated power production, though stoichiometrically acetate produced the most power per mole, followed by methane and sulfide respectively. Our quantitative molecular analyses further implicate sulfate-and iron-reducing bacteria, anaerobic methanotrophs, and sulfur disproportionators in power production. These observations demonstrate that anaerobic methane oxidation coupled to sulfate reduction may be a mechanism that contributes to power production. We posit that power production results from the catabolism of a variety of reductants by the anode-hosted community, including methane, and subsequent shuttling of electrons to the anode likely occurs via both direct electron transfer and chemical mediators such as sulfide.

Low conductivity and alkalinity as major design challenges in MFC & MEC systems

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Bio-electrochemical cells can be used to transform organic material in wastewater into electricity (MFC) or hydrogen (MEC). Both the MFC and MEC system offer the potential of highly efficient energy conversion as no thermal conversion takes place. To realize this high energetic efficiency, the internal energy losses in the bio-electrochemical cell must be minimized. Internal losses are basically of two kinds; (i) reaction related (activation, metabolic) and (ii) transport related (ions, electrons, pH change and reactants). Minimal internal losses is thus one of the prime objectives of a bio-electrochemical cell design. Application of MFC and MEC in wastewater treatment poses additional constraints on the design. In general wastewater has a low conductivity and alkalinity, limiting the performance of bio-electrochemical cells. Alkalinity and conductivity levels can not be increased as wastewater discharge standards and economics make this undesirable. To improve the bio-electrochemical cell, the nature of the losses and their interplay must be better understood and to do so we will present a minimal model for the bio-electrochemical cell. The minimal model will be applied to analyze the limitations arising from low conductivity and alkalinity. The role of enhanced mass transfer and the need to use a membrane will be discussed. Minimal modeling aims at finding the smallest set of equations that still gives an adequate description of the bio-electrochemical cell. By constructing a minimal model the necessary insight is obtained. A minimal model can be more easily transformed into an equivalent circuit making validation by EIS possible.

Analysis of Bio-Anode Kinetics Using a Minimal Model

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Over the past years, Microbial Fuel Cells (MFCs) have been broadly investigated for their ability to convert a wide range of organic materials directly into electricity. The power output of the MFC is amongst others influenced by the electrochemical performance of the bio-anode. The bio-anode is defined as the assembly of the biofilm and the solid electrode

to which it is attached. The electrochemical performance of the bio-anode is determined by the microbial kinetics of substrate oxidation and the associated electron transfer from the microorganism to the anode. This performance can be expressed in a polarization curve, i.e. the relationship between the current density and the anode potential. To gain more insight in the bio-anode kinetics, we have studied the bio-anode through polarization curves and impedance spectroscopy. A minimal model was developed that combined the biochemical kinetics of substrate oxidation with the electrochemical kinetics of electron transfer. The experimental data were analyzed with the minimal model, and kinetic parameters were obtained. The model enables us to distinguish between the biochemical and electrochemical parameters influencing the bio-anode kinetics. The polarization curves showed a limiting current, which could not be attributed to mass transfer but reflected the maximum biomass activity. Care should be taken in analysis of Tafel plots as no clear exponential behavior was observed. This analysis of bio-anode kinetics can be used to further improve MFC performance.

Employing *Shewanella oneidensis* MR1 as a cathodic biocatalyst

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This work explores the unique possibility of driving both the anodic and the cathodic reactions of a microbial fuel cell (MFC) with *S. oneidensis* MR1. MR1 has been chosen for use in mediatorless MFCs due to its ability to efficiently pass electrons to the anode electrode without the introduction of an external electron shuttle/mediator such as AQDS or neutral red. In published MFC studies employing MR1 at the anode, an inorganic catalyst is usually required to drive the oxygen reduction reaction at the cathode. The use of such catalysts is costly and limits the variety of electron acceptors. To overcome this obstacle, the use of MR1 as a biocathode offers a potential alternative. This presents the possibility for its use as an inexpensive catalyst, and also allows the MFC to exploit MR1's versatile metabolism at both the anode and cathode. For these experiments, lactate was utilized as the electron donor in the anodic compartment. Different electron acceptors including oxygen were examined individually at the cathode and MFC performance was compared with regards to electron transfer efficiency and relative current output. The ability of MR1 to successfully attach and form a biofilm at the cathode in the presence of these different electron acceptors was also examined by scanning electron microscopy.

Microbial electrohydrogenesis using single-chamber membrane-free cells

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Microbial electrohydrogenesis provides a new route for producing hydrogen from renewable and carbon-neutral biomass resources. In all investigations reported up to date, hydrogen evolution via electrohydrogenesis was conducted in two-chamber systems in which membranes were applied to separate the anode and cathode chamber. However, membranes could increase internal resistance, resulting in a decrease in current density and hence hydrogen production rate. In this study, a single-chamber membrane-free microbial

electrolysis cell (MEC) was designed and its performances with a mixed culture enriched in microbial fuel cells were evaluated in terms of hydrogen production rate, current density and energy efficiency. A volumetric production rate of 0.55 $m^3/day/m^3$ was achieved at applied voltage of 0.75 V with a current density of 13.5 A/m^2 and an efficiency of 162% based on input electricity energy. Methane was detected in the process and negatively affected the hydrogen production. Various methods were, therefore, investigated to suppress the growth of methanogens and it has been found that exposure of cathodes to air could maintain methane content under an acceptable level. Results of this study indicate that this single-chamber membrane-free configuration may provide an efficient and practical design to produce hydrogen via electrohydrogenesis.

Effects of electrode potentials on the electricity generation in microbial fuel cells

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Electrode potentials will affect the electrons transfer between the bacteria and the electrodes in microbial fuel cells (MFCs). In order to have a better understanding of the effect of electrode potentials on electricity generation, the performance of MFCs with different start-up anode potentials was studied. When anode potentials ranging from -500 mV to 100 mV (vs Hg/Hg_2Cl_2) were applied, the start-up periods of the MFCs changed with the potentials. The MFC with a controlled anode potential of -300 mV was demonstrated to have the shortest start-up period. Further experiment showed that in the MFCs with start-up anode potentials of -400 mV, -300 mV and -200 mV, the characteristics of current productions at different anode potentials were different, which implied different microbial colonies on the anode surface. Besides, the MFCs applied with controlled start-up anode potentials showed much better performance on power generation. The maximum current productions were 4-6 folds higher than that in free MFCs, and the anode resistances were only about 20~25%. When constant potentials ranging from -200 mV to 300 mV (vs Hg/Hg_2Cl_2) were applied to the biocathodes in MFCs, different changes in current productions were also observed. All these results indicated that potential control of the electrodes in MFCs could be a promising method to enhance the performance of MFCs.

Microbes meet with resistance; the effect of external load on anode communities and MFC performance

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Individual based modeling of MFC anodes indicates that the external load on an MFC may influence the anodic microbial community at least at the level of biomass yield and potentially may also affect community composition. To test this, the influence of external load on the composition of the anodic community microbial and biomass yield was investigated in a microbial fuel cell fed a glucose as fuel and inoculated with wastewater as a source of electrogenic bacteria (MFC). In addition the performance of the MFC under different loads was assessed in terms of current density, Coulombic yield and removal of chemical oxygen demand (COD). Denaturing gradient gel electrophoresis (DGGE) of polymerase chain reaction (PCR) amplified 16S rRNA gene fragments revealed that the anodic bacterial communities in MFC operated with different external load were distinct. MFC operated with lower external load resulted in anode communities of lower diversity than was the case with MFC operated at higher external loads. These results indicated that

in MFC, functionally stable electrogenic bacteria are enriched under higher current densities i.e. low external load, and were able to sustain better current and effluent quality. As expected current generation, Coulombic yield, COD removal and the biomass yield were all directly affected by the external load. Significantly, when operated under lower external load, the biomass yield in the MFC was less than that in conventional anaerobic digestion.

Experiences from MFC pilot plant operation: How to get the technology market-ready?

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Microbial fuel cells (MFCs) have demonstrated increasing performance in the past few years, but unfortunately the increase in performance has not been followed by an increase in size, with many systems even downsized to achieve higher power densities. However, for practical applications, systems have to be scaled up by several orders of magnitude compared to most lab-scale experiments. Yet, the size of a single MFC module directly influences the internal resistance, which is perhaps the foremost bottleneck we know today. To test scale-up options and performance, we have constructed a pilot scale MFC at a brewery near Brisbane, Australia. Prefermented wastewater (COD ≈ 3.5 g/L) is fed to twelve MFC modules, each 100 L in volume. Electrical converters directly boost up the voltage coming from each individual module to 12 V, which creates a more manageable current flow. We have been confronted with an array of technical hurdles that were previously not known. Many key challenges faced in the scale-up are poorly addressed in MFC research at present e.g. electrode conductivity, liquid flow patterns, start-up and enrichment of large quantities of electro-active biomass and so on. Many of these challenges need to be resolved to gain any chance at market acceptance, which should be the aim of these efforts. These approaches will likely also determine which applications may be the most promising ones initially.

Alternative electron acceptors in microbial fuel cells

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Power density of microbial fuel cells (MFCs) is several orders of magnitude lower than that of a chemical fuel cell due to various constraints of the system. Low Coulomb efficiency that ranges from as low as 10% up to over 90% is one of the constraints. This low Coulomb efficiency is due to the fact that a part of electrons from the fuel oxidation are consumed reducing electron acceptors other than the anode. The Coulomb efficiency should be improved to achieve better performance of MFCs as a power source as well as a biochemical oxygen demand sensor. The cation exchange membrane is permeable to oxygen. Nitrate is a common electron acceptor found in effluent from a wastewater treatment process. Oxygen and nitrate are preferred electron acceptors over the anode. Terminal oxidase inhibitors, azide and cyanide inhibit oxygen and nitrate reduction in the anode improving Coulomb efficiency. When azide was used in the enrichment process at a concentration of 0.2mM, the Coulomb efficiency was lower than the control MFCs. Azide was completely consumed during the enrichment process, and the anode was enriched with a deltaproteobacterium. From these observations it was concluded that diverse bacterial population reported in MFCs represents various respiratory organisms including aerobic respiratory bacteria and that azide added to anode during the enrichment process is used as an electron acceptor.

Studies are being conducted to improve Coulomb efficiency through reducing the oxygen diffusion to the anode at the same time minimizing the charge transfer resistance.

Microbial fuel cell: A novel approach to convert synthesis gas to electricity

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Synthesis gas (syn-gas), mainly consisting of carbon monoxide (CO) and hydrogen (H_2), is commonly produced from the gasification of coal and a biomass. Similar to syn-gas, a huge amount of off-gas also containing CO and H_2 has been produced from the steel industry during coke making and other chemical processes. Recently, the reserves of coal and the availability of various coal processing technologies have stimulated interest in the development of the production of a coal-based alternative energy. To this extent, syn-gas production could be one of prime alternative raw materials (feedstocks) for petroleum as well as a source of chemical feedstocks. As a representative fuel cell system, hydrogen fuel cells (rather than microbial fuel cells) may use syn-gas as a fuel source. However, because of the serious poisoning effect of CO on precise metal catalysts such as platinum, sys-gas typically should be purified before its use in a conventional hydrogen fuel cell system. It has been reported that microbes can be used to convert CO and H_2 to multi-carbon compounds. The major products of the acetogenesis process are volatile fatty acids (VFAs) such as acetate and butyrate. Therefore, in this study, we attempted to combine the acetogenesis and MFC processes to convert CO to electricity. Successful operation of the combined processes confirmed that microbes can produce electric energy from CO and H_2 in both waste-gas and syn-gas. Our results imply that the microbial process does not require gas purification to eliminate CO, and is capable of direct electricity production.

Power generation by a novel tubular MFC employing a membrane electrode assembly cathode

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Membrane electrode assemblies (MEA) were investigated in tubular microbial fuel cells using either cost effective polymer based cation exchange membrane (CEM) or anion exchange membrane (AEM). MEA fabrication consisted of mechanically combining cathode electrode on the membrane by a perforated cylindrical polypropylene shell and tube. Hydrogel application between membrane and cathode increased cathode potential by *circa* 100mV in the range of current (0 to 5.5mA) in CEM-MEA. Consequently the MFC produced 6.1W/m^3 of power density as compared with 5 W/m^3 in a control (without hydrogel) in CEM-MEA. An improvement of cathode potential was also obtained using AEM-MEA when hydrogel was used as compared with that of MEA without hydrogel. These results might indicate an enlarged region of hydration and contact area within the cathode, enabled by the hydrophilic gel applied to the membrane. The coulombic efficiencies observed with MEAs were 71% with CEM and 63% with AEM respectively, which were higher than those of membrane-less MFCs. The net water loss through the membrane varied according to external resistance, indicating that total charge transfer in the MFC is related to electro-osmotic drag of water through the membrane. Fabricated MEAs using ion exchange

membranes, improved the performance of the MFCs by preventing oxygen intrusion and bioinhibition on the cathode, as well as controlling water loss. The tubular MEA MFC is feasible for scale up and continuous operation for large scale application. Performance characterization and bacterial community examination is underway.

Is Geobacteraceae the universal family of anode-respiring bacteria in the biofilm-anode of microbial electrolytic cells?

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Microbial electrolytic cells (MECs) have high potential for producing biohydrogen, since MECs can convert non-fermentable substances (e.g., acetate) into H_2. In an MEC, anode-respiring bacteria (ARB) remove electrons from their donor substrates and transfer them to the anode; the electrons are used to reduce protons and generate H_2 gas at the cathode. Geobacteraceae are commonly found in the biofilm anode of microbial fuel cells, and are true ARB. When a fermentable substance is the donor (e.g., glucose), ARB can share the biofilm with fermenters, and this also leads to the possibility of having methanogens consuming some of the fermentation products. Using the quantitative real time polymerase chain reaction (QRT-PCR) and fluorescent *in situ* hybridization (FISH)-confocal microscopy, we compared biofilm-anode communities in MECs fed glucose or acetate and with the anode potential set at -200 mV vs Ag/AgCl. According to QRT-PCR, Geobacteraceae comprised only 4.3-29% and ~ 1% of all bacteria in the glucose- and acetate-fed MECs, respectively. Methanogens were more abundant in the glucose-fed MEC, which supports the significance of methanogenesis in MECs fed by a fermentable substrate. FISH-confocal microscopy gave similar trends as QRT-PCR, but also showed that Geobacteraceae were rich inside biofilm-anode. These results demonstrate that Geobacteraceae form an important ARB group, but are not necessarily dominant when the anode potential is around -200 mV.

Energy sustainability of the water infrastructure

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Current methods of water and wastewater treatment are not sustainable due to high energy consumption by conventional treatment processes. In the USA, for example, 1.5% of the electricity produced is used for wastewater treatment, and 5% is used for the whole water infrastructure. However, by recovering energy from wastes, such as organic matter in wastewaters and agricultural cellulosic wastes, it may be possible to develop an energy-sustainable water infrastructure. Using microbial fuel cells (MFCs), we have shown that it is possible to produce electricity while simultaneously accomplishing wastewater treatment. An alternative to electricity production is to modify these systems to produce hydrogen gas by adding an additional voltage to that produced by the bacteria. These hydrogen producing devices, called microbial electrolysis cell (MECs), can be used to obtain nearly 100% of the theoretical maximum conversion of substrates such as acetate into hydrogen gas. Typically, two to four times more energy is recovered in the hydrogen gas produced than in the electrical energy used. We have found based on combustion energies, that 64-82% of the energy in organic matter (including acetate, glucose, cellulose and other biodegradable materials) and the electrical energy added could be recovered in hydrogen gas. Significant challenges remain for scaling up both MFCs and MECs. I will review in my talk recent advances for increasing power, and identify the mostly likely future routes for improving

performance and reducing costs of materials used in these systems in order to make them practical for commercial applications.

An experimental and theoretical study of the dependence of the internal resistance of a microbial fuel on cell voltage

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The internal resistance R_{int} of a microbial fuel cell (MFC) has been determined as a function of cell voltage (V) using electrochemical impedance spectroscopy (EIS) for a MFC with and without *Shewanella oneidensis* MR-1 and in a MFC containing stainless steel balls (130 cm^2) in contact with the graphite anode (20 cm^2). Since the ohmic component of R_{int} plays a very minor role for the MFCs studied here, R_{int} was calculated as the sum of the polarization resistance of the anode (R^a_p) and the cathode (R^c_p). It has been found that R_{int} decreases with decreasing cell voltage as the increasing current flow decreases R^a_p and R^c_p. Additions of MR-1 or SS balls to the buffer and lactate solution produced a very large decrease of R_{int}. The slope of the V-I curve, R_p= dV/dI, was evaluated by first numerically differentiating the current with respect to time using a second order Savitzky-Golay procedure followed by Fourier transform based smoothing. The slope of the voltage-time curve dV/dt was then divided by dI/dt to give the R_{int} – I curve. The discrete R_{int} values measured for different cell voltages by EIS were superimposed on this curve and showed good agreement. Small deviations near the open-circuit cell voltage were attributed to artifacts due to a transient phase at the beginning of the experiment, and deviations near short circuit may be attributed to numerical artifacts. For all experimental conditions, it was found that at the cell voltage V_{max}, where the power output of the MFC has its maximum value, $R_{int} = R_{ext}$, where R_{ext} is the external resistance or load that has to be placed between the two electrodes of the MFC to achieve V_{max}.

Metagenomic approaches to understanding MFC community structure and function

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The majority of prokaryotes found in microbial fuel cells (MFCs) are not cultured using traditional techniques although they might play important roles in either direct electron transfer to the anode (or from the cathode) or in indirect community function within the biofilm. Metagenomic methods potentially provide access to the entire microbial community present in different compartments of a MFC via the extraction of the DNA (and/or RNA) from the entire community. DNA extraction can be performed either directly or indirectly by separating the prokaryotic cells before DNA extraction. Subsequently, the DNA can be cloned and sequenced, amplified by specific primer sets (e.g., targeting the 16S rDNA), or sequenced directly (such as with the 454 pyrosequencer). In addition, the extracted DNA can be analyzed by phylogenetic or functional microarrays. Although, the type of data differs depending on the method, the results provide important Information concerning the

community structure and function of MFC microbial communities. Phylogenetic microarray data can compare different operating conditions easily and quickly in order to identify community members which are favored or not by changes in operating conditions. The 454 pyrosequences can provide clues to the variations in functional gene families and potentially highlight sequences involved in different processes within the MFC. The application of these approaches with wastewater degrading MFCS will b presented.

Self-constructed *Shewanella* semiconductor electrical networks

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It was first shown more than 90 years ago that microorganisms can generate electricity in a course of their metabolism. This ability has attracted recent attention for its application in biological fuel cells. However, the serious problem of low current density in these system compared to existing platinum- or enzyme-based chemical fuel cells have prevented them from being applied in energy production systems for practical use. In this work, we report drastic improvements of current generation of *Shewanella loihica* PV-4 (and also *S. oneidensis* MR-1) achieved by self-constructed bacterial semiconductor networks on an ITO electrode. Addition of nano-sized semiconductor particles into a PV-4 MFC with an ITO electrode caused the formation of dense bacterial aggregates on the electrode surface. The interconnected PV-4/semiconductor network was found to act as an efficient electron-transfer conduit, and enable the cells located at a long distance from the electrode to participate in the current generation. The effectiveness of the present method was demonstrated by a drastic (> 300-fold) enhancement of redox currents caused by electroactive bacteria, and also its applicability to a porous graphite electrode, which is the anode materials used in many MFCs.

Adaptive evolution and systems biology approaches to elucidating electron transfer mechanisms and optimizing power output of microbial fuel cells

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Even with the recent engineering innovations in fuel cell design that have substantially increased power densities, applications of microbial fuel cells are limited because of their low power outputs. One reason for this is that there are certain properties of the microorganisms that are donating electrons to anodes that are less than optimum for high-density current production. A better understanding of electron transfer mechanisms and metabolism within anode biofilms may aid the rational design of improved anode materials, other fuel cell attributes, or the organisms themselves. *Geobacter sulfurreducens* was chosen as a model organism because it: produces current densities as high as any known pure culture; completely oxidizes fuels, resulting in high columbic efficiencies; and does not use electron shuttles for electron transfer to anodes. Furthermore, a full suite of genomic and genetic tools, including a genome-scale *in silico* model of metabolism, is available. Gene expression, proteomic, and genetic studies demonstrated that, in addition to the electrically conductive pili of this organism, a novel *c*-type cytochrome is specifically required for high-density power production. Several adaptive evolution strategies yielded strains that can produce substantially higher power densities and/or have enhanced

substrate ranges. With new genome resequencing technologies it has been possible to identify the mutations associated with these improvements. The adaptive evolution approach has proven to be an important basic discovery tool for investigating the physiological factors limiting current production and is helpful for developing strategies for further enhancing the power output of microbial fuel cells.

***Shewanella loihica* PV-4 outperforms *S. oneidensis* MR-1 in an MFC using mechanisms of direct electron transfer and adaptability**

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MFCs have attracted a lot of attention recently although many questions remain as to the process by which electrons are transferred from the bacterial surface to the anode. *C*-type cytochromes (*c*-cyts) appear to be the major cellular components that play this role. We are examining two *Shewanella* species, *S. oneidensis* MR-1 and *S. loihica* PV-4, which although are closely related, show differences at the genetic level in terms of the type and organization of *c*-cyt genes. The two species, after inoculation into a single-chamber MFC, exhibited markedly different profiles for current production and cell density. While MR-1 reached its maximum potential after only a few days of operation, PV-4 did not reach its peak until after ca. 20 days. Interestingly, although MR-1 exhibited a similar profile of current production after repeated injections of lactate over time, PV-4 became more efficient at generating current and produced more electricity than MR-1. Measurements of acetate and headspace CO_2 indicated that PV-4 more efficiently oxidized lactate and transferred more electrons to the anode than MR-1. In contrast to MR-1, PV-4 was unable to sustain a large number of planktonic cells. The contribution of attached versus planktonic cells was demonstrated by transferring the anode of an MFC run for 30 days into a new MFC. PV-4 attached cells produced 9-fold more current than the remaining planktonic cells, as compared to only 1.3-fold for MR-1. Genomic analysis revealed that PV-4 contains some distinct, decaheme *c*-cyts that are currently being targeted for knockout analysis to explore their contribution to current production.

Theoretical modeling of anodic microbial fuel cell

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To realise the full potential of microbial fuel cells (MFCs) to produce reliable, sustainable electricity we need an increased understanding of the bacterial energetics and electron transfer mechanism at the anode of a fuel cell. Mathematical modelling has a role to play in synthesising our current understanding of the various processes in a fuel cell system, assessing their relative importance and ultimately optimising the design of new MFC systems. Here we develop a theoretical model to represent electron/proton transfer under isobaric and isothermal conditions. It is based on three components: a metabolic electrolyte model, a catalytic biofilm model, and a bio-electrode model. The model is solved in two spatial dimensions using finite-difference approximations to the underlying partial differential equations. Its explicit representation of electrochemical potential interactions makes it distinct from other recently published MFC models. This allows us to simulate the electrons and protons jumping through the lattice of electrochemical potentials. The model is demonstrated by simulating the voltage, current and enthalpy in a 1 litre batch MFC fed with 2 mM acetic acid solution. We simulated supplementing the feed with sodium ions at a

variety of different concentrations. The results suggest that when the biofilm biomass is in steady state then the higher concentration of sodium ion (Na^+) in anodic chamber the higher the power density. However, the MFC produced crystal sodium carbonate, which has the potential limit the diffusion of oxygen and the proton transfer at the proton exchange membrane. This effect could only be simulated in a model that explicitly the electrochemical potential interaction, including the electrochemical thermodynamics and kinetics.

Biocathodes as the new opportunity for Bio-Electrochemical Systems

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Thus far, microbial fuel cells have primarily focussed on the generation of electrical power while simultaneously treating wastewater. A biotic anode was linked to an abiotic, catalyzed cathode. Recently, we and others pursued the development of biocatalyzed cathodes. Bacteria such as *Sphingobacterium* can even as pure culture decrease the activation overpotential at the cathode. Biocatalysts come for free, have long lifetimes and are not polluted by the anodic byproducts such as sulfide. When using a loop concept, where the anode effluent is conveyed to the cathode, the pH of the cathode can be controlled, which may be one of the reasons why biocathodes operated in such fashion perform better than their non-linked counterparts. But biocathodes can take us further. The bacterial versatility drives us beyond the reduction of oxygen at the cathode. The reduction of nitrate was a first application, but now also value added products such as hydrogen and ethanol were produced with biocathodes. Organochlorines have been reductively dehalogenated. The emergence of these new biocathode based applications require us to redefine the label for this technology to Bio-Electrochemical Systems (BESs). BESs allow us to generate the added value needed to guarantee success on the market. In the coming years, a dirty wastewater stream will serve solely as electron donor for a cathode based, clean production of value added chemicals.

Effect of biofilm properties on the electrochemical performance of microbial fuel cells

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Recent studies on microbial fuel cells (MFCs) have shown that the anode limitation can play a dominant role in MFC performance for certain reactor configurations. This limitation is predominantly caused by the anode biocatalyst and is largely dependent on the properties of the microbial biofilm. Our recent work has provided conclusive evidence of the influence of initial biofilm growth on the anode impedance and power output of MFCs. In this work we studied the electrochemical performance of MFCs during biofilm growth and evaluated the correlation between biofilm properties and the power output performance in the absence of other influencing cathode parameters. A single-chamber, acetate-fed MFC was used for all the experiments, with carbon paper electrodes and a Pt/C microporous catalyst layer at the cathode. Biofilm growth was induced under a potential difference of 0.4 V between the electrodes, and the current (reaction rate) was monitored as a function of time. The continuous growth of biofilm on the anode increased the biocatalyst density and resulted in

a steady improvement in the power density, with the peak power density doubling between hours 25 and 150 during the initial growth period. Several MFCs were tested using different external loads and for different run times with multiple replacements of anode and cathode medium to obtain anode biofilms with different physical properties. The performance of these MFCs is being evaluated using polarization and electrochemical impedance spectroscopy experiments and correlated to biofilm properties such as thickness, dry density, and composition.

Community interactions with complex electron donors

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Mixed-culture microbial fuel cells (MFCs) induce an interesting microbial ecology that blends anaerobic decomposition pathways with the respiration of an extracellular electron acceptor. For particulate substrates, the ecology is further complicated by the need for access to a suspended electron donor and an insoluble electron acceptor. The communities that develop include cooperative and competitive relationships, particularly for more complex or diverse electron donors that can support a diverse community and perhaps require substrate conversions prior to exoelectrogenesis. Mixed-culture systems also often show low Coulombic efficiencies, due in part to aerobic respiration occurring at the cathode interface for aerobic cathodes, but this does not fully account for the electron losses. In addition to the expected functional groups of microbes, molecular community analyses collectively show diversity that includes unknown phenotypes and bacteria with unknown exoelectrogenic capabilities. This phylogenetic breadth of characterized exoelectrogens is expanding as new isolation and screening strategies are developed that specifically assess extracellular electron transfer. I will review some of the key cooperative and competitive microbial interactions, based on molecular community analyses and system performance data, and provide suggestions for engineering more productive communities.

New Developments in Ocean Microbial Fuel Cells

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Ocean microbial fuel cells (MFCs) are being developed in a variety of configurations to serve as long-term power sources for autonomous underwater gliders and moored sensors such as acoustic receivers. Unlike their wastewater counterparts, the electrical energy harvested from these devices is constrained primarily by environmental fluxes that fuel the metabolism of marine microorganisms and lead to a cascade of natural redox reactions. Harvesting useful amounts of energy therefore depends on targeted placement and/or design measures to direct organic matter or primary metabolites to the MFC. While we have only just begun to collect comparative data in different ocean environments, we have observed sustained daily-averaged power densities ranging from <0.01 to 0.3 W/m^2 of electrode surface when plain graphite is used as the anode material. The highest power densities have been at ocean seeps and vents and in response to high sulfide fluxes. Anodes recovered from these environments have hosted biofilms enveloping a diversity of microorganisms including *alpha-*, *delta-*, *epsilon-* and *gammaproteobacteria*, as well as the

Flavobacterium-Cytophaga-Bacteroides (FCB). These findings are guiding current efforts to optimize system designs by improving the rate and efficiency of bioelectrochemical oxidation of sulfide to sulfate.

Miniature microbial fuel cells for aerobic applications

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Because most environments on Earth are exposed to significant levels of oxygen, we believe the transition from (anaerobic) sediment-based MFCs to oxygen-tolerant MFCs is necessary. This transition requires both a way to simultaneously sequester the metal-reducing microbes and reduce the overall concentration of oxygen in the anode chamber, thereby enhancing current production. By using three dimensional (3D) anodes in a miniature MFC (mini-MFC) design, electron donation to the anode competes well with oxygen scavenging, even when oxygen levels in the anolyte are nearly air-saturated. Additional results show that *Shewanella* species can be isolated around aerobic anodes by using nanoporous polymer filters in place of traditional proton exchange membranes (PEMs). We have found that mini-MFCs using polycarbonate or nylon filters generate more power than devices using Nafion™ separators. This work has been extended to show that filters enable novel pump-less MFCs to be designed for energy harvesting applications. Exposing the anode to air also creates unique growth conditions for electrochemically active bacteria (EABs). We find that significant power is generated from *S. oneidensis* by enhanced metabolism of glucose and fructose under aerobic conditions. Based on the previously published proteomic and genomic literature on *Shewanella*, this reduction in power output is most likely due to either the differential expression of proteins under oxygen-rich conditions or environmental selection of carbohydrate-metabolizing mutants. These results help to expand the role of MFCs beyond traditional anaerobic applications, and may enable microbe-based energy harvesters to power persistent surveillance sensor networks in the aerobic littoral water column.

Understanding the Biofilm Anode in MFCs

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In a microbial fuel cell (MFC), bacteria present at the fuel-cell anode catalyze the oxidation of organic fuel sources, including domestic wastewater, animal manures, plant residues, and photosynthetic microorganisms. The MFC converts the energy value stored in the organic fuel to electrical energy or to hydrogen gas (H_2). This conversion avoids combustion and combustion-associated air pollutants. An MFC is an attractive renewable energy technology, because it produces carbon-neutral electricity or H_2 from renewable biomass, including wastes. The most unique feature of an MFC is the biofilm that lives on the anode and oxidizes the fuel. This oxidation is illustrated by the simple oxidation half reaction for acetic acid: $CH_3COOH + 2H_2O \rightarrow 2CO_2 + 8H^+ + 8e^-$. The biofilm includes anode-respiring bacteria (ARB) that have the ability to transfer the electrons (e^-) to the anode, a conductive solid. The biofilm matrix that the ARB produce is conductive, which allows the electrons to move efficiently to the anode, even when the ARB are not immediately next to the anode. Being

conductive, the biofilm matrix becomes part of the anode itself; thus, we call it the *biofilm anode.* We use the Nerst-Monod expression to quantify the relationship between the rate of electron conduction and the change in electrical potential in the biofilm anode. A highly conductive biofilm anode can nearly eliminate potential losses in the biofilm. Low conductivity causes high potential losses and low current and power densities. Oxidation also produces protons (H^+), as illustrated above for acetic acid. To maintain electro-neutrality, the protons must transport out of the biofilm, usually to the bulk liquid. Proton transport is dominated by the diffusion of proton carriers, such as carbonic acid (H_2CO_3) and dihydrogen phosphate ($H_2PO_4^-$). In situations in which bicarbonate or phosphate buffering is not large, the transport of protons becomes the rate-limiting step. Thus, the rate of electron transport can be controlled by slow H^+ transport more than slow electron conduction in many practical situations.

Bioelectrochemical systems for wastewater treatment: not so elementary

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The laboratory performances of bioelectrochemical systems (BESs), such as microbial fuel cells (MFCs) and microbial electrolysis cells (MECs), on synthetic media are approaching levels that come close to the requirements for practical application. However, the performances of the laboratory systems on real wastewaters always seem to be drastically lower than the performances on synthetic media. Therefore, application of BESs for wastewater treatment does not seem to be straightforward. This has a reason: besides having to deal with complex substrates, BESs for wastewater treatment also have to cope with low conductivities of real wastewaters. At typical wastewater conductivities, the Ohmic losses can already exceed several hundreds of millivolts even if the anode to cathode distance is just a few millimeters. This means that BESs need to be very well designed to prevent high Ohmic losses. Besides that, also many other fundamental problems, such as membrane pH gradients, still have to be solved. Furthermore, due to the relatively low current densities, BESs have to apply extremely inexpensive materials to become cost-effective for wastewater treatment. This paper presents comprehensible calculations to show that practical application of BESs for wastewater treatment is not so elementary… at least not just yet.

Functionalization of electrode surfaces for more efficient microbial fuel cells

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Microbial fuel cells (MFCs) are promising alternative energy production devices. Despite recent improvement of cells designs [1] these systems are still showing rather low efficiencies regarding energetic conversion. The use of added redox mediator in the anolyte is often a way to increase the electron transfer rate between the micro-organisms and the

anode. However the reliance on soluble additives is obviously not compatible with the intended use of MFCs for wastewater treatment. In order to significantly increase the power output of these fuel cells, we propose novel approaches regarding the design of specific anodes and cathodes for MFCs. On the anodic side, we are developing electrodes functionalized with attached redox mediators, either through electropolymerization or covalent grafting. For instance, the use of electroactive porphyrins offers interesting possibilities regarding the tuning of their redox potential through metalation and/or ring functionalization. Another way to increase the power output of MFCs is to significantly decrease the oxygen reduction overpotential at the cathode. Efficient oxygen reduction by enzymes is a promising alternative to traditional air-cathode containing noble metals. Building on our expertise on enzymatic fuel cells [2] we are developping the covalent attachment of a dioxygen reducing enzyme (laccase) onto carbon electrodes (see scheme below). The use of attached redox mediators at the anode and biocatalysts at the cathode of MFCs is not only intended to increase their power output but could also allow the design of future flow-through membrane-less systems.

Approaches and challenges in studying the electron transfer in microbial fuel cells

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The performance of a microbial fuel cell (MFC) depends on a complex system of parameters. Apart from operational variables like the anode or fuel cell design, it is mainly the paths and mechanisms of the bioelectrochemical energy conversion that decisively determine the MFC power and energy output. Here, the electron transfer from the microbial cell to the fuel cell anode, as a process that links microbiology and electrochemistry, represents a key factor that defines the theoretical limits of the energy conversion. The determination of the energy efficiency of the electron transfer reactions, based on the biological redox potentials of the involved redox species in combination with the known paths (and stoichiometry) of the underlying microbial metabolism, is an important instrument for this discussion. Yet, many proposed transfer mechanisms are of putative nature and are controversially discussed. Often, the involved redox species are only insufficiently studied and electrochemical data do not exist. Some of the greatest challenges in their study are (i) the complexity of the microbial metabolism, (ii) the often extremely low concentrations of the involved redox species and (iii) the complex (electro-) chemical nature of bacterial cultures and even of microbial cell membranes, which may contain several redox active species that not necessarily contribute to the bioelectrocatalytic current flow.
In this presentation approaches and challenges for studying the anodic electron transfer will be demonstrated by means of an experimental study of anodic bacterial biofilms of metal reducing bacteria. Further, the attempt will be made to summarize known electron transfer processes in microbial fuel cells and to discuss these processes on a mechanistic and a thermodynamic basis, in order to evaluate their potential to produce electric energy.

Air-cathode microbial fuel cells with layered-electrode assemblies

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Air-cathode microbial fuel cells (MFCs) are among the most efficient MFC configurations, but they are not easily scaled up for wastewater treatment. We propose a new air-cathode

configuration, the hollow-fiber membrane MFC (HFM-MFC), obtained by layering MFC assemblies onto air-supplying, hollow-fiber membranes (HFMs). The HFMs provide air to the inner, cathode layer, while the outer, anode layer is exposed to the bulk liquid. Since HFMs are available in small diameters (200 - 300 μm), bundles with high specific surface areas can be retrofitted into an activated sludge tank. The layered configuration has minimal separation between anode and cathode, minimizing the internal resistance, but potentially increasing oxygen crossover. To test the HFM-MFC concept, we studied six flat, layered electrode assemblies with air cathodes as one-dimensional analogs. The MFC with a Nafion PEM and air cathode with diffusion layer achieved the highest power density, 20W/m^3 (380mW/m^2) and coulombic efficiency, 24%. Microelectrodes measurements of dissolved oxygen (DO) showed negligible oxygen within the anode biofilm, but up to 5 mg DO/L at the PEM in the MFC with a diffusion layer cathode, and up to 8 mg DO/L in the MFC without a diffusion layer. Oxygen crossover may have contributed to reduced coulombic efficiencies. Our air-cathode MFCs have a performance comparable to MFCs with graphite cloth electrodes. The specific surface area was 55 m^2/m^3 but specific surface areas up to 620 m^2/m^3 can be achieved, and actual power densities may be an order of magnitude higher. Ongoing work is testing actual HFM-MFC materials and configurations.

Microbial fuel cells for total nitrogen removal

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Microbial fuel cells (MFCs) with biocathodes can concurrently oxidize BOD and denitrify, achieving two important wastewater treatment objectives while producing energy. However, MFCs cannot remove ammonia, which is needed to achieve total nitrogen (TN) removal. We incorporated air-supplied, hollow-fiber membranes (HFMs) into a denitrifying biocathode to allow TN removal. We studied a plug-flow tank reactor where the influent, containing acetate and ammonium, passed sequentially through anode and cathode sections. We hypothesized that BOD would be oxidized in the anode, and a nitrifying biofilm developing on the HFMs would produce nitrite and nitrate, which would then be reduced by the biocathode. Initially, the reactor was partitioned into separate anode and cathode compartments by an Ultrex proton exchange membrane, and each section had a separate influent and effluent. This configuration achieved a power density of 296mW/m^3 (5mW/m^2 cathode) and a coulombic efficiency of 17%. The nitrification fluxes reached 1.7 gNH_4^+-N/m^2-HFM/d, which is comparable to other HFM-based processes, and denitrification fluxes were up to 1.6 gNO_3^--N/m^2-cathode/d. After 6 months, the PEM was removed and the reactor was operated in plug flow with a single influent and effluent, without a noticeable change in performance. These results suggest that TN removal may be possible in a MFC configuration. Further research is being completed to investigate the performance and the microbial ecology of the respective compartments.

Electricity generation from model organic waste in a cassette-electrode microbial fuel cell

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We have designed a detachable cassette-electrode (CE) system and applied it to construct a scalable configuration of the microbial fuel cell (MFC). One CE was composed of a flat cathode box (with two air cathodes) sandwiched in between two proton-exchange membranes and graphite-felt anodes. Air was circulated inside of the cathode box. In order to evaluate this system, an MFC reactor equipped with 12 CEs (CE-MFC) was constructed (anode volume, 1 liter; anode projection area, 1440 cm^2), inoculated with soil, and loaded with a model organic waste (containing starch, peptone, and fish extract) as a fuel. Stable performance was observed 15 days after commencing the operation, when COD-removal efficiencies were always over 90% at the COD-loading rate of as high as 5.8 kg COD m^{-3} day^{-1}. A constant electric output was observed with the maximum power densities of 129 W m^{-3} (per anode volume) and 899 mW m^{-2} (per anode projection area) and the internal resistance of 0.64 ohm. These results demonstrate a potential of the CE system for the treatment of organic wastes and the energy recovery from them.

Analyses of resistance in microbial electrolysis cells: improvement of cell design

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Microbial Electrolysis cells (MECs) are a new technology derived from Microbial Fuel cells that produce hydrogen instead of electricity by means of applying a potential to the system. The hydrogen production rate in MECs is determined by the internal resistance of different parts of the system. Changes in the design of a cell can have big influence on resistance of the system and therefore on the hydrogen production rate. We have defined five types of resistances in our system: (i) anode, (ii) cathode, (iii) pH gradient, (iv) ionic and (v) transport resistance. Anode and cathode resistances were calculated from the concentrations of reactants and products using the Nernst equation. The ionic resistance was calculated from the conductivities of the anolyte and catholyte. The transport resistance includes the transport of ions from one electrode through the membrane to the other electrode and is not influenced by the ionic strength of the anolyte and catholyte. We calculated the resistances for different cell configurations. The differences in the cell configuration involved the thickness of the anode material and the use of different buffer concentrations. These changes in cell configuration showed a decrease in anode and transport resistance. The lower resistance led to higher current densities and consequently higher hydrogen production rates. This new anode configuration is promising for future increase of current densities and hydrogen production rates.

Novel Bioprocessing Technologies Based on Microbial Consortia

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Current technologies for the conversion of biomass to ethanol incorporate a biomass pretreatment step and use genetically-engineered microbes to simultaneously hydrolyze the plant biomass and ferment the soluble sugars to ethanol and other organic acids. This is a complex process whose optimization relies on the integration of process engineering, fermentation technology, enzyme engineering and metabolic engineering. In Nature, plant biomass is efficiently degraded by the concerted action of microbes with different metabolic capabilities. Such microbial consortia cooperate to degrade the plant biomass, ferment the soluble products and completely oxidize the fermentation products to return the light-driven fixed CO_2 back to the atmosphere. Fe(III)-reducing organisms such as *Geobacter* bacteria are important components of these consortia and completely oxidize fermentation products such as H_2 and acetate to metal oxides, a process that could be harnessed to design *Geobacter*-powered fuel cells growing in consortia with biomass-degrading organisms. Our laboratory is interested in developing technologies for the conversion of biomass to ethanol using microbial fuel cells (MFCs). Here we describe the development of bioprocessing schemes using MFCs that integrate pretreated agricultural waste and biomass-degrading microbial consortia composed of a biomass-degrading and fermentative bacterium and the model electricigen *Geobacter sulfurreducens*. We show that the nature of the metabolic interaction, e.g., interspecies H_2- or organic acid- transfer, between the consortia partners as well as genetic manipulation of the metabolic capabilities of the microbial members can be used to customize the bioprocessing scheme while increasing ethanol conversion rates and the overall energetic output of the system.

Hydrogen production in a continuous flow microbial fuel cell with a gas-phase cathode

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A volumetric hydrogen production rate of 0.98 $L_{STP}\ L_A^{-1}\ d^{-1}$ (A=anodic compartment) was achieved in an electrically-assisted continuous flow microbial fuel cell (MFC). The cell was constructed with polycarbonate plates arranged to form an anodic compartment and a hydrogen-collection compartment. A 3D anode electrode with a volume of 23 mL was constructed using a 5 mm thick graphite felt, while the cathode was made of a 40% Pd/Pt coated Toray carbon fiber paper. A Nafion 117 membrane was hot-pressed onto the cathode to form the partial membrane electrode assembly. Hydrogen production was achieved by connecting the MFC to a power supply and applying voltages over a range of 0.5-1.3 V. Either acetate or glucose was used as a source of carbon. Best volumetric performance was observed on acetate at a load of 1.67 g $(L_A\ d)^{-1}$ and a voltage of 1.16 V. Under these conditions, the volumetric rate of hydrogen production approached 1 $L_{STP}\ L_A^{-1}\ d^{-1}$ (A=anodic compartment). Simultaneously a substrate removal rate of 1.6 g COD $L_A^{-1}\ d^{-1}$ with an effluent COD concentration below 100 mg L^{-1} was achieved, i.e. hydrogen production was successfully combined with COD removal. Material balance calculations highlighted the presence of a mixed microbial community in the MFC. In addition to exoelectrogenic activity, methane production was observed. Consequently, a significant amount of carbon source was used by acetoclastic methanogens, which competed with the exoelectrogenic microorganisms for a common substrate. Furthermore, hydrogenotrophic methanogens were present and converted part of the hydrogen produced at the cathode into methane.

Latest results in benthic microbial fuel cell research and development.

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The Benthic Microbial Fuels Cell (BMFC) sits on the bottom of marine environments and generates electricity from organic matter residing in anoxic sediment and oxygen in overlying water. In the 10 years since the concept was conceived, multiple fielded prototypes have demonstrated the practicality of BMFCs as alternatives to batteries in powering marine deployed sensors. Here I will describe our latest results which advance fundamental knowledge of microbe-to-anode electrode-transfer properties of BMFCs and related MFCs that utilize mineral reducing species. I will describe results of our most recent field studies.

Experimental demonstration of the Nernst-Monod equation for the biofilm anode of microbial fuel cells

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Anode-potential losses at the biofilm anode of anode-respiring bacteria (ARB) result from various microbial and transport processes. Current knowledge is scarce regarding how these losses can be measured, characterized, or described mathematically. In Kato Marcus et al (2007), we proposed the Nernst-Monod equation to describe the potential loss at an ARB anode as a function of microbial kinetics. The equation, which is derived by modifying a Monod term using the Nernst equation, describes the anode potential availability as a terminal electron acceptor for ARB. In this study, we developed experimental setups for studying two parameters that are crucial for model development: the half-maximum-rate potential (E_{KA}) and the biofilm conductivity ($\square_{bio}$). First, we grew an anode biofilm at a fixed anode potential with a limiting substrate concentration (acetate) to avoid proton-transport limitation. Under these conditions, we carried out low-scan cyclic voltammetry to develop a Nernst-Monod curve. From the results, we determined E_{KA}, as well as other important microbial kinetic parameters. Second, we grew an anode biofilm at a fixed anode potential with excess substrate concentration and tracked the shape of the Nernst-Monod curve as the biofilm grows. The observed 10-90% j_{max} range increased as the biofilm grew; this indicates potential loss due to electron-transport resistance. The increase in the 10-90% j_{max} range allows us to study the impact of $\square_{bio}$ on anode-potential loss. Our results give new insights on how an ARB biofilm anode responds to changes in potential and which processes are contributing to anode-potential loss.

Combined carbon and nitrogen removal opens new perspectives on wastewater treatment with microbial fuel cells

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Wastewater contains not only carbon but also a plethora of nitrogen based compounds. Current practice for nitrogen removal typically suffers from high energy demand and the often large additional carbon supply due to the competition between different organisms.

Recently, it was shown that nitrate can be reduced at a MFC cathode. Following this discovery, we have developed a loop-based system to remove nitrogen entering the MFC. Acetate and ammonia were fed to the anode with synthetic wastewater. Most of the ammonia passed through the anode and was nitrified in an external trickling bed reactor. The effluent of the latter was then sent to the cathode for denitrification. Nitrogen removal rates up to 0.127 kg $N \cdot d^{-1} \cdot m^{-3}$ total reactor volume (0.470 $kg \cdot d^{-1} \cdot m^{-3}$ cathode liquid volume) could be reached, with coulombic efficiencies of 82% for the cathodic reaction. The MFC generated up to 9.4 $W \cdot m^{-3}$ total reactor volume. Not only nitrate could be reduced, but also nitrite appeared a suitable cathodic electron acceptor. While the nitrate based loop theoretically allows nitrogen removal with C/N ratios of 2.86 g $COD \cdot g^{-1}$ N, a loop based on nitrite could further decrease the C/N ratio to 1.72 g $COD \cdot g^{-1}$ N. Our results show that decoupling the oxidative and the reductive processes in the two MFC's compartments helps to minimize the competition between different bacterial communities. This distinguishing feature not only permits a more efficient use of the organic carbon already present in the wastewater, but it also allows the system to achieve denitrification efficiencies that are well comparable with dedicated denitrification techniques based on methanol addition.

From Waste to H_2: GeoChip-based Analysis of Microbial Community Structure and Functions in Bio-electrochemically Assisted Microbial Reactor (BEAMR)

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For the recent developed bio-electrochemically assisted microbial reactor (BEAMR), little is known about the identification and characterization of the so-called exoelectrogens (functional species) in the anode biofilm. In this study, a novel comprehensive microarray called a GeoChip, and a 16S rRNA pyrosequencing approach based on 454 sequencing (16S rRNA) were adopted to analyze the anode biofilm in a BEAMR. The experimental analysis was based on analyzing the inoculum, the community that evolved when exoelectrogenic bacteria were enriched by acclimating the community for current generation in a microbial fuel cell (Bio-E culture), followed by operation in an MEC for hydrogen production (Bio-H_2 culture). From Bio-E to Bio-H_2, the microbial community structure and functions shifted significantly based on the gene cluster and PCA analysis of whole genes and individual gene categories. According to gene intensities, cytochrom genes had the highest abundance in the Bio-H_2 microbial community. The higher the cytochrome gene abundance, the higher the H_2 recovery. *Shewanella*, *Geobacter*, *Pseudomonas*, *Desulfovibrio* and *Rhodopseudomonas* are key exoelectrogens in the Bio-H_2 microbial community based on their cytochrome gene intensities. *Shewanella* and *Geobacter* contributed more to higher H_2 recovery. Based on 454 sequencing results, *Pseudomonas* was the most frequently identified genus (~57%) in the high H_2 recovery microbial community. However, the abundance of the cytochrome gene was very low (<2%) in the detected *Pseudomonas species*, which implies that many *Pseudomonas* were involved in the system in the role of "electron transfer medium". It is presumed that cooperation of *Pseudomonas* and exoelectrogens (i.e. *Shewanella*, *Geobacter*) could increase the efficiency and to maintain the stability of the system.

Isolation and role of fermentative bacterium, *Bacteroides sp.* W7, in a microbial electrochemical assisted hydrogen production reactor

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Till now, little information presents the remarkable bacteria that function in a microbial electrochamical assisted hydrogen production reactor (MEC), especially, their characterization and motabolic behavior variation under such a power supply environment. In this study, a Gram-negative, short-rod, polar flagellum, non-spore-forming dominant bacteria, strain W7, was isolated from a MEC. Analysis of physiological-biochemical characterization and 16S rRNA gene sequence showed that W7 is a typical fermentative bacterium and most closely match to *Bacteroides* sp. Z4. Pure culture cultivation tests showed that W7 could not reduce Fe(III) if using acetate as electron donors. However, when W7 was inoculated into a mediatorless MEC fed with acetate as sole electron donor, to our surprise, W7 could utilize acetate to produce electricity, and the maximum current density is 105mA/m^2, which is about 70 percent of that preoduced by *Shewanella oneidensis* MR-1 under same conditions. This result seems indicated that fermentative bacterium, W7, could function as a exoelectrogens in MEC. In authors' view, the only electron source in this system is acetate, and W7 has to degrade acetate and donate electrons to anode electrode to get growth energy in such an oligotrophic environment. The authors also presumed that the electrons transfer from W7 to anode electrode need a "driving force" . In MEC, when the anode potential is high enough, it might form a "driving force", which could catch up the electrons and transfer to anode electrode. While, under pure culture cultivation tests, it might hard to form this kind of "driving force".

A long-tailed bacterium occurring in cellulose-fed MFC reactors and rice paddy-field MFC

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Microbiological analyses of two-chamber MFCs inoculated with rice paddy soil and fed cellulose as a fuel have suggested the possible involvement of bacteria with long tails (called "prosthecae") in the electricity generation (Ishii et al. 2008. BMC Microbiol. 8:6; Ishii et al. 2008. Biosci. Biotechnol. Biochem. in press). These bacteria were affiliated with the phylum Alphaproteobacteria, but not affiliated with the established orders. Closely related bacteria have also been detected on anode graphite felts of rice paddy-field MFC, in which microbes immediately converted photosynthesis products (organics exhausted from roots) to electricity (we termed this system an ecological solar cell) (Kaku et al. 2008. Appl. Microbiol. Biotechnol. in press). In order to reveal the niche and activities of these bacteria, the present study isolated a long-tailed Alphaproteobacterium (strain Mfc52) from the cellulose-fed MFC, and its physiologyical features were investigated. Mfc52 fermented various sugars and pyruvate, and lactate and acetate were the major fermentation products. It respired with oxygen, nitrate and ferric ion. When an MFC reactor was inoculated with Mfc52 and fed glucose, the electricity was generated after glucose fermentation was ceased; the electricity generation was coupled to lactate and acetate consumption. SEM revealed that Mfc52 had a thick extracellular filamentous appendage that was abundant when forming biofilms on anode electrodes. These observations suggest that a group of bacteria represented by Mfc52 catalyze the terminal step (conversion of organic acids to electricity) in MFC systems. Examination of roles of the long tails is underway.

High power generation by a photosynthetic bacterium in single chamber air-cathode MFCs

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Microbial communities on the anode of MFCs are diverse based on the culture-independent molecular studies. However, few microorganisms have been directly isolated from MFCs, and no isolate has yet been shown to produce more power than the original microbial community from which it was derived. Here we report that a photosynthetic purple non-sulfur bacterium *Rhodopseudomonas palustris* DX-1 isolated from an MFC produces electricity at higher power densities (2720 mW/m^2) than mixed cultures (1740 mW/m^2) in single-chamber air-cathode MFCs. While *Rhodopseudomonas* species are known for their ability to generate hydrogen, electricity was generated without the need for light or hydrogen. Strain DX-1 utilizes a wide variety of substrates (volatile acids, sugars, protein, and thiosulfate) for power production in different metabolic modes, making it highly useful for studying power generation in MFCs and generating power from a range of simple and complex sources of organic or inorganic matter. These results demonstrate that photosynthetic bacteria can efficiently generate electricity by direct electron transfer in MFCs, providing another model microorganism for future MFC investigations.

Performance Enhancement of Microbial Fuel Cell for Scalable Applications

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Microbial fuel cell (MFC) is a representation of a new and perfect biological technology for recovering electrical energy from liquid organic wastes. Its ability of generating electricity by using bacteria as catalysts suggests a green and environmental-friendly manner to make wastewater treatment more sustainable and economical. However, the power generation appears still low and the optimum design and operation of a MFC reactor seem to be inconvincible. This paper described some possible technical methods to enhance the electrical performance of a MFC for future scalable applications. It was shown here that the use of acidic permanganate catholyte could increase cell voltage and power density in the MFC. In addition, to avoid the disadvantages of liquid cathode and develop the sustainability of the MFC, the improved design of the air-cathode MFC was described. The tested results showed that the modification of design could increase power generation of an air-cathode MFC.

An exoelectrogenic bacterium *Ochrobactrum anthropi* YZ-1 isolated using a U-tube microbial fuel cell

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Microorganisms capable of transferring electrons outside the cell to insoluble electron acceptors, such as metal oxides and the anodes of microbial fuel cells (MFCs), are called exoelectrogens. These microbes have been a focus of interest recently for their potential in

many different biotechnology applications (e.g. bioremediation and MFCs). Very few exoelectrogens have been directly isolated from MFCs, and most exoelectrogens that are known to produce power in an MFC are dissimilatory metal reducing bacteria (DMRBs, e.g. *Shewanella* and *Geobacter*) initially isolated using agar plates containing metals. However, exoelectrogen isolation methods based on dissimilatory metal reduction potentially limit the diversity of possible exoelectrogenic bacteria. A special U-tube-shaped MFC was therefore developed to enrich exoelectrogenic bacteria based on electricity production, with isolation based on dilution-to-extinction methods. A pure culture was obtained and identified as *Ochrobactrum anthropi* YZ-1 based on 16S rDNA sequencing and physiological and biochemical characterization. Strain YZ-1 was unable to respire using hydrous Fe(III) oxide but produced 89 mW/m^2 using acetate as the electron donor in the U-tube MFC, demonstrating a greater diversity of exoelectrogenic bacteria than simply DMRBs. Strain YZ-1 used a much wider range of substrates as carbon sources for current production than many DMRBs, including acetate, lactate, propionate, butyrate, glucose, sucrose, cellobiose, glycerol, and ethanol, but produced lower power densities than mixed cultures in the U-tube MFC. Further applications of this new U-tube MFC system will provide a method for obtaining additional exoelectrogenic microorganisms that do not necessarily require metal oxides for cell respiration.

POSTER PRESENTATIONS

Microbial fuel cell coupling biohydrogen fermentation of food waste to energy harvesting

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Microbial hydrogen production through direct fermentation of organic waste is a promising technology for producing renewable hydrogen that couples the need for waste reduction and byproduct recovery. The fermentation approaches to biohydrogen production however, have some technical barriers such as low hydrogen yield and waste byproduct acid accumulation. Microbial fuel cells (MFCs) provide an additional opportunity for direct electricity production from readily biodegradable organic matter in the fermentation effluent. This research demonstrates the integration of MFCs with biohydrogen fermentation using food waste. The hydrolysis of cellulose in food waste is a significant rate limiting step. Anaerobic leaching bed (ALEB)-type hydrogen fermentation was therefore adopted to increase hydrolysis rates and to remove the need for pretreatment. In the first experiment using mesophilic ALEB reactors, higher hydrolysis rates were observed at pH 9 with dilution rate of 0.22 d^{-1} (VS reduction of 85%), but the optimal conditions for hydrogen production was pH 6 with dilution rate of 0.02-0.07 d^{-1} (VS reduction of 66-70%, 20 L H_2/kg VS added @STP). The hydrolysis and acidification were 0.34-0.54 g SCOD/g VS added and 0.55-0.57 g VFAs/g SCOD produced respectively. SCOD/TCOD ratio of the effluent was 0.88-0.95 and the ratio of major volatile acid was HAc : HPr : HBu : HVa = 1: 0.8-1.2 : 3-3.5 : 0.9-1.5. In the second experiment, single chamber air-cathode MFC reactors with ammonia-treated brush anodes were operated under mesophilic conditions. Based on batch mode polarization curve (1 g COD/L), the maximal power density was 1,540 mW/m^2 (48 W/m^3) at current density of 6.2 A/m^2, resulting in 80% COD removal, 72% Coulombic efficiency, and 12% energy efficiency. Continuous mode MFC tests are presently being conducted to determine optimum performance and design parameters. This research suggests that a combined fermentation+MFC system may be more useful than either system by itself.

Photoresponsive glassy carbon electrode modified with porphyrin nanostructure and anthracene-9-carboxylic acid entrapped inside nafion matrix as photocathode for proton reduction

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Recently there have been efforts to convert microbial fuel cell into bio-hydrogen producing reactor by application of small amount of voltage to supplement energy produced by microorganism immobilized on anode in order to reduce protons generated by their metabolic activities into hydrogen gas. This has been shown to have the potential of significantly reducing the amount of energy used in producing hydrogen by electrolysis. However, having a solar power driven microbial fuel cell to make hydrogen and possibly treat wastewater will make this new process greener and more energy efficient. To achieve this, there is a need to design an electrode that can act as photocathode which hopefully

can be connected to bioanode to produce hydrogen or treat waste with the aid of solar energy. We report the fabrication of photo-responsive glassy carbon electrode which on illumination with visible light can reduce proton at potentials far lower than the required thermodynamic potential. Platinized porphyrin nano-spheres acting as antenna molecules for light harvesting and anthracene-9 carboxylic acid acting as energy receptor were immobilized on glassy carbon electron with the aid of Nafion polymer. The electrode generated photocurrent when irradiated with visible light and produced hydrogen at different potential (-250mV to -350mV Vs Ag/AgCl) which are below the thermodynamic value for proton reduction at the pH (pH 3) used in the experiment. We are hoping to develop a hybrid process that combines artificial photosynthesis with bio-electrochemical techniques for hydrogen production using immobilized microorganisms as biocatalyst in a modified MFC type reactor to recombine protons and electrons produced by the respiring cells into hydrogen gas with the aid of visible light.

A Side-By-Side Comparison of Two Microbial Fuel Cells with Different Configuration at the Anode

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The objective of this investigation was to compare the performance of two laboratory scale Microbial Fuel Cell (MFC) type PEM. The MFC is a device that converts organic matter to electricity using microorganisms as the biocatalyst. The feasibility of this approach was tested in two "H" type cells made of glass and were designed to operate as batch reactors. The anode electrode introduced two different configurations: MFC1 used plain graphite mesophilic temperature and the MFC2 used granular graphite at 20 ^{O}C. Anolyte consisted of synthetic wastewater containing glucose as carbon source more inocula as biocatalyst and the catolyte was oxygen saturated aqueous solution. Anolyte and catolyte were separated by a cation permeable membrane (Nafion® 117). It was found that average power density in the CCM1 was 9 W/m^3 and 70% COD (Chemical Oxygen Demand) removal was observed, while for CCM2 average power density was about 50 W/m^3 and 95% COD removal. Both systems were evaluated during 120 days; the organic loading was 4,7 kg COD/m^3-d, HTR (Hydraulic Time of Retention) was 24 hours.

Construction of floating type of microbial fuel cell and its application for the treatment of organic contaminants in lentic environment

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We examined that floating type of microbial fuel cell (FT-MFC) could be applied to treat organic contaminants in lentic environment. The anode and cathode electrodes were connected through external circuit and cathode electrode was exposed to water surface. When four FT-MFCs were inoculated with anaerobic digest fluid (ADF) obtained from brewery waste treatment system (Gwangju, Korea), the open circuit potentials were developed to around 0.4-0.5 V. Initial COD of ADF was 1700 ppm and it decreased to 380

ppm in 2 days. Open circuits of two FT-MFCs were converted to closed circuit mode to see the current production for 5 days. The current was developed to around 0.2 mA and COD value decreased to 230 ppm. During the operation several factors such as dissolved oxygen, conductivity, and pH were monitored in the place in which anode electrode was installed. The acetate concentration used was changed from 5 to 50 mM to see if substrate was limited for the growth of bacteria involved in current generation. The current was maximized around to 0.4 mA whilst 5 mM of acetate was fed at a feeding rate of 0.08 ml/min. Acetate and COD were observed at the concentration of 3 mM and 300 ppm in anode, respectively. To authors' knowledge, up to now, MFCs with floating-body structure have not yet been developed and studied for the removal of organic waste materials in the contaminated lentic environments. These results indicate that FT-MFC could be applied to treat organic wastes concomitant with electricity production in contaminated lentic environment.

Electricity-mediated methane production using methanogens: a natural gas substitute

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Methane is the major flammable component in natural gas, produced in nature by methanogens. As a fuel, methane has some advantages: infrastructure already exists for transport and utilisation, it is not spontaneously explosive in air, and it is easily stored compared to hydrogen or electricity. Methanogens are microbes belonging to the domain Archaea and produce methane via methanogenesis. Methanogenesis is essential for energy production and growth. Currently, methane-containing biogas is produced by the anaerobic digestion of organic waste by consortia of microbes. Due to unknown interactions among the species in these microbial communities, it is difficult to determine conditions that will increase the efficiency of methane production. We are working with *Methanothermobacter thermoautotrophicus*, a thermophilic methanogen that utilises carbon dioxide and hydrogen for growth. Hydrogen has a key role as the electron donor for ATP production in the electron transport chain of this organism. Our work focuses on the use of alternative electron sources for methanogenesis. Previous studies have shown that hydrogen can be replaced by using electricity as an electron source and neutral red as an electron mediator in a microbial fuel cell (MFC) (Park et al., 1999). We have designed a MFC where methanogens are grown in the cathodic compartment. Phenazines dyes including safranin-o, neutral red and phenosafranin will be tested to select the most efficient electron mediator for methane production. Reactor stability and methane production rates will be calculated to determine whether this kind of process might act as an efficient method of storing and transporting energy.

Microbial fuel cell like a new path to Brazilian renewable energy

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This work is about insertion of Microbial fuel cells (MFCs) technology to enhance the use of renewable energy in Brazil. The Brazil has achieved an international importance position of

among the industrialized economies by the high participation of renewable sources in its energy matrix. Part of this is due to the participation of biofuels market, like ethanol and biodiesel. The introduction of ethanol in the Brazilian energy matrix, enabled the generation of background that transformed the Brazilian ethanol productive chain one of the most efficient in the world. To continue in the vanguard of the production of ethanol is necessary to develop modern technology, which applied in the process of obtaining the ethanol, would guide to advances in the use of bioenergy of sugarcane. One way to increase the power energy of sugar cane is the use of by-products and waste as substrate in MFCs for direct generation of electricity. In addition, this type of device allows that the residue, after being used in the cell, return to the production cycle as fertilizer, increasing the profits of the sugar alcohol plants. For example, MFC can operate the employing up waste industrial products as substrate. In particular waste from sucroalcohol industries are suitable for this purpose, since the production of ethanol from sugarcane generates large volumes of waste rich in organic matter, with particular reference to vinasse. Normally each liter of alcohol produces about 12 liters of vinasse. Although the vinasse be used as fertilizer and as a raw material for the production of methane gas in biodigestors, its power energy can be improved using it as substrate in the MFCs. This procedure takes the full energy content of sugarcane, without serious loss of fertilization.

Delineating MFC internal resistances using electrochemical impedance spectroscopy

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Internal resistances dictate the overall power density in microbial fuel cells (MFC). The resistances in an MFC with improved biocatalyst and anode performance were determined using electrochemical impedance spectroscopy. A single-chamber MFC with a flow-through anode and an air-only cathode or a ferricyanide cathode were used in this study. The biocatalyst was an electrogenic biofilm-forming consortium. The individual components contributing to the internal resistance of the MFC with an anode capable of supporting power densities higher than 60 W/m^3 are discussed. The anode and cathode charge transfer resistances ranged between 10 to 18 ohms and 0.7 to 4.8 ohms, respectively. The total solution resistance including the PEM resistance was about 5.5 ohms. The effect of the cathode on the internal resistance was studied demonstrating an inverse relationship between voltage output and the total internal resistance. In addition, quantitation of the change in the cathode and anode resistances as a result of the use of an air-cathode vs. a ferricyanide-based cathode was demonstrated and will be discussed.

Generation of Electricity from Lignocellulosic Biomass Using Microbial Fuel Cells

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The production of fuel and energy from lignocellulosic biomass such as agricultural wastes has drawn great attention because of the abundance, ready availability and renewable nature of these resources. In this study, 12 monosaccharides and 6 sugar alcohols derived from lignocellulosic biomass were investigated as carbon sources for power generation in single chamber microbial fuel cells (MFCs). The effects of potential inhibitors generated in the pretreatment and hydrolysis of biomass, including 2 furan derivatives and 8 phenolic

compounds on MFC performance were also evaluated. Results from this study indicated that electricity can be generated from all the tested monosaccharides and sugar alcohols using a mixed bacterial culture enriched from wastewater. However, effective electricity generation from the hydrolysates of lignocellulosic biomass may require the employment of hydrolysis methods with low furan derivatives and phenolic compounds production, the removal of some strong inhibitors prior to the MFC operation, or the improvement of bacterial tolerance against these compounds.

Denitrification at the bio-cathode in the two chambered MFC

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Microbial fuel cells (MFCs) can function as an advanced treatment unit by reducing nitrate as an electron acceptor in a bio-cathode. MFC coupled with an aeration chamber has been proposed by the researcher at the AWMC, University of Queensland (patent pending). This study was to evaluate denitrification rate and electricity production of MFC with and without aeration chamber treating the synthetic wastewater. The synthetic wastewater was fed into the anode chamber of MFC coupled with the aeration chamber. The SCOD and NH_4-N concentration in the influent was 200 $mg \cdot L^{-1}$ and 40 $mg \cdot L^{-1}$, respectively. The volumetric loading rate was 1.6 kg $SCOD \cdot m^{-3}$ net anodic compartment (NAC)$\cdot d^{-1}$. Without any catalyst and buffer solution, average power density was increased to 1.2 $W \cdot m^{-3}$ NAC. In the aeration chamber, 75.9% of ammonium was transferred to nitrate. And nitrate was removed in the cathode chamber by denitrification rate of 0.07 kg of NO_3-$N \cdot m^{-3}$ net cathodic compartment (NCC)$\cdot d^{-1}$. But the overall nitrogen removal efficiency was only 12.3%. Because 11.2% of ammonium in the anode chamber was permeated into the cathode chamber through the membrane, and was released in the effluent. The aeration chamber was removed, and the synthetic wastewater was fed separately into the anode and the cathode chamber with 400 mg $SCOD \cdot L^{-1}$ and 80 mg NO_3-$N \cdot L^{-1}$, respectively. The power density was increased up to 3.3 $W \cdot m^{-3}$, while nitrate was denitrified in the cathode chamber as 0.3 kg of NO_3-$N \cdot m^{-3}$ NCC$\cdot d^{-1}$. When recirculation rate was increased from 2 $ml \cdot min^{-1}$ to 10 $ml \cdot min^{-1}$, the power density reached up to 33.9 $W \cdot m^{-3}$. The loading rate and the mixing intensity affect increasing the power production.

Comparisons of the anodic bacterial community structures and metabolic viabilities between electricity-generating microbial fuel cells and biohydrogen-producing bioelectrochemical cells

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Biohydrogen was effectively produced via acetate oxidation using a two-chambered bioelectrochemical cell (BEC), a modified microbial fuel cell (MFC) with an artificial external voltage supply to overcome thermodynamic barriers of biohydrogen evolution. The bacterial community and their metabolic viability of anode biofilms were compared between the

normal MFC and BEC, based on the concern that the artificial augmentations of the circuit by external power could generally result in changes or damage to the bacteria. The microbial communities in the anode biofilms of the MFC were dominated by the *Proteobacteria*, especially the β-subclasses. In the acetate-enriched MFC, 51.2% of the sequences obtained in the 16S rDNA clone library were β-*Proteobacteria*, and 66.8% of these were in the genus *Thauera* with a > 95% similarity to *Thauera aromatica* LG356. The next most frequently detected bacteria was δ-*Proteobacteria* (34.1%), with a predominance of *Geobacter* related species, followed by others (12.2%) and α-*Proteobacteria* (2.4%). For the biohydrogen production, the continuous augmentations of the MFC circuit by external voltage caused notable changes in the bacterial community. When the MFC was switched to hydrogen producing BEC, the bacterial diversity decreased substantially with the extreme dominance of δ-*Proteobacteria* (72% of the total clones) occurring. All of these δ-*Proteobacteria* were *Pelobacter* with a > 97% similarity to *Pelobacter propionicus* DSM 2379, which can oxidize organic acids with Fe(III) serving as the sole electron acceptor in anaerobic sediments. *Geobacter* sp., well-known iron reducing bacteria, was determined to be an integral member (16%) of the bacterial community in the BECs fed with acetate, similarly to those in the MFC. In contrast, no *Shewanella*-like sequences were retrieved from the BEC.

Effect of mediator (AQDS) and Fe(III)-citrate (FeC) on current production and biofilm formation in *Shewanella oneidensis* MR-1 and mutants

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Under anaerobic conditions electrochemically active bacteria (EAB) use an efficient way of exocellular electron transfer to/from solid substrates such as graphite electrodes in a microbial fuel cell (MFC). Mediators such as anthra-quinone-2-6-disulfonic acid (AQDS), resazurin, and humic acids have been shown to have varying effect on bacterial metabolism in MFCs. For the work reported here, we utilized the wild type and a series of single gene deletion mutants of *Shewanella oneidensis* MR-1 to compare current production and biofilms formation under a variety of different conditions. Observation via scanning electron microscopy (SEM) revealed that mutants altered in current production were often altered with regard to their ability to form biofilms, and that biofilms formation was not always an indicator of good current production. For instance, ΔSO2930, a cytochrome c deletion mutant, which produces higher current than the wild type is deficient in biofilm formation in comparison to the wild type, which forms a good biofilm. Furthermore, the addition of iron citrate (FeC) or AQDS to MFCs (for both wild type and mutants) increased the current production by one fold and two fold, respectively. Overall, results indicate either there is a bottleneck in electron transfer or availability of the substrate to both planktonic and biofilm bacterial population. We are currently analyzing the nature and levels of metabolites produced during current production for both wild type and mutants in an effort to understand the relationship between microbial physiology and current production in the MFCs.

Application of biocathode in microbial fuel cells: cell performance and microbial community

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In order to reduce the effect of chemicals on the environment and decrease the cost for noble catalyst, biocathode has been applied in a two-chamber microbial fuel cell in this study, and the cell performance and microbial community were analyzed. The MFC was provided by the AWMC at University of Queensland. Without the aid of any artificial redox mediator or other catalysts, after 2-month startup, the microorganisms of each compartments in microbial fuel cell was well developed, and the output of microbial fuel cell increased and became stable gradually, in terms of electricity generation. Anodic compartment were fed with acetate at a loading rate of 1.88 g/d; while denitrification was expected as terminal reaction in cathodic compartment. At 20 ml/min flowrate of the cathodic influent, the maximum power density reached 19.53 W/m^3, while the corresponding current and cell voltage were 15.36 mA and 223 mV, respectively. With the development of microorganisms in both compartments, the internal resistor decreased from initial 40.2 to 14.0 ohm, too. It demonstrated that the enrichment of microorganisms in cathode compartment was helpful to enhance the overall performance of microbial fuel cell. Microbial community analysis demonstrated that five major groups of the clones were categorized among those 26 clone types derived from the cathode microorganisms. *Betaproteobacteria* was the most abundant division with 50.0% (37 of 74) of the sequenced clones in the cathode compartment, followed by 21.6% (16 of 74) *Bacteroidetes*, 9.5% (7 of 74) *Alphaproteobacteria*, 8.1% (6 of 74) *Chlorobi*, 4.1% (3 of 74) *Deltaproteobacteria*, 4.1% (3 of 74) *Actinobacteria*, 2.6% (2 of 74) *Gammaproteobacteria*. The abundance of *Betaproteobacteria* indicated the ability of oxygen or nitrate utilization in cathodic compartment without the presence of artificial redox mediators or other catalysts.

A mixed anodophilic biofilm exhibits saturation behavior with anodic potential in a microbial fuel cell

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Microbial fuel cell (MFC) anodes are insoluble conductive media serving as both the terminal electron acceptor and the physical support for the anodophilic biofilm. Bacterial activity and the power output of a MFC are therefore largely depending on the capacity of the anode to accept electrons from the biofilm. However, our understanding on the bacterial electron transfers in MFC anode is still unclear. Therefore, the aim of this study was to investigate the dependency of the activity of an anodophilic biofilm on the anodic potential in a fed-batch two-chamber computer-controlled MFC. The MFC was operated for over 200 days with acetate as the sole electron donor and ferricyanide as the terminal electron acceptor to establish a highly effective anodophilic biofilm. By controlling the external resistance, steady state microbial activities at various anodic potentials were established at different conditions (e.g. substrate saturating/ starving). Our results suggest the existence of:

1) a critical anodic potential ($AP_{crit.}$) of ~ -420 mV/AgAgCl, beyond which a more positive anodic potential no longer stimulates bacterial activity and electricity flow; and
2) a half saturation value of anodic potential (~ -455 mV/AgAgCl) at which the anodophilic bacteria produce current at half maximum rate.

With the new insight about the relationship between microbial activity and anode potential, operating conditions of MFC could be optimised by controlling or maintaining the anodic potential at the $AP_{crit.}$ level. Further, knowing the actual affinity for the anode and maximum

microbial activity in a MFC is expected to help with comparing different biofilms, modeling of MFC processes, or diagnosing MFC malfunctions.

Catholyte pH effect on the performances of microbial fuel cells and biohydrogen-producing bioelectrochemical cells

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Microbial fuel cells (MFCs) purpose electricity generation, whereas bioelectrochemical cells (BECs) which are modified MFCs target on hydrogen evolution. In order to produce hydrogen using BECs, a cathode is prevented from oxygen, and a thermodynamic barrier must be overcome by assisting external power. A 140mV of external potential is theoretically required to produce biohydrogen using BECs (e.g., E_{cell} = -420 - (-280) = -140 mV vs. NHE) as acetate was a substrate. The aims of this study are to investigate catholyte effects on the performances of MFCs and biohydrogen-producing BECs. In our two-chambered systems, the anode chamber was filled with an anaerobic nutrient mineral buffer (pH7.0), while the cathode chamber was filled with 50 mM phosphate buffer. To evaluate the effects of catholyte pH, three different pH values were tested (2.0, 7.0, and 12.0). Theoretically, cathode potential ($E_{Cathode}$) increases by a 180 mV as a 3 pH units was decreased (e.g., from pH 7 to 4) at the cathode according to the Nernst equation. Consequently, this cathodic potential increase results in the magnification of overall cell potential (E_{cell} = $E_{Cathode}$ - E_{Anode}) because anodic potential maintains constantly. This cell potential increase indicates much more affinity for the current generation. As results, power density of MFCs increased from 40.2 mW/m^2 to 89.1 mW/m^2 when pH changed from 12.0 to 2.0.
In the same manner, a reduced potential difference by increasing cathodic potential indicates that the amount of required external power could be decreased. At the applied voltage of 800mV in BECs, the current generation increased from 412.6mA/m^2 to 2067.6 mA/m^2 as cathode pH was changed 12.0 to 2.0.

Development of a microfabricated toxicity biosensor based on microbial fuel cells

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Microbial fuel cells (MFCs) have been used for several years as biosensors for measuring environmental parameters such as biochemical oxygen demand and water toxicity. The present study is focused in the detection of toxic matter by using a novel silicon-based MFC. As the actual toxicity sensors based on MFCs, this device is capable of detecting the variation on the current produced by the cell when toxic compounds are present in the medium. The MFC approach presented in this work is intended to obtain a simple, compact and planar device for its further application in the design and fabrication of equipment for

toxicity monitoring. It consists on a proton exchange membrane placed between two microfabricated silicon plates that act as current collectors. An array of vertical channels of 40 x 40 µm have been defined trough the plates over an area of 6 x 6 mm. The final testing assembly incorporates two methacrylate pieces positioned onto the plates as reservoirs with a working volume of 144 µL per compartment. The operation of the microdevice as mediated electron transfer MFC has been validated by comparing its performance against a larger MFC, both tested under the same conditions. The device has been tested as a toxicity sensor by setting it at a fixed current while monitoring changes in the output power. A drop in the power production is observed when a toxic compound is added to the anode compartment. The compact design of the device makes it suitable for its incorporation into measurement equipment either as an individual device or as an array of sensors for high throughput processing.

Adaptation of bacteria to mediator-based microbial fuel cells: a source of error and variability

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In many studies, the characterization of MFCs is done by connecting an arbitrary load to the fuel cell while current is monitored through time to observe the evolution of the system. In other studies this measured current is allowed to stabilize before obtaining an I-V characteristic curve. As our results show, under certain experimental conditions, these approaches can lead to considerable errors and to large variability in power output measurements. Opposite to electrochemical fuel cells, the performance of MFCs evolves with time and so does their maximum output power. This is probably related to the fact that aerobically grown bacteria cannot transfer electrons to an anode or to a redox mediator right away, but need a certain period of adaptation. We have measured the time required for this adaptation. In our experiments we inoculated mediated electron transfer MFCs with grown *E. coli* cultures and monitored the resulting I-V curves every two hours for a period of several days. In this way, we obtained complete information about the characteristics of the cell and its efficiency. Our results show that the MFCs need up to 24 hours of operation before reaching their maximum performance and then stabilizing. Thus, short-time studies attempting to analyze the effect of external factors on MFC operation will be heavily influenced by the adaptive evolution of the system.

A multi-electrode microbial fuel cell for wastewater treatment with simultaneous generation of electricity with low operational costs

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A multi-electrode microbial fuel cell with maximal power density near 320 mW/l was created with open circuit voltage 0.8 V. The stability of this value was confirmed during half a year

of continues operations. The developed MFC is equipped with newly developed highly economical cathodes. The new cathodes represent an intermediate solution between immersed and air faced (dry) ones, can provide maximal current density up to 1.2 mA/cm2, and have near the same as dry cathodes operational costs. The cathodes can work as dry cathodes without any time restrictions with some loss of current density. A multi-sectional architecture of MFC gives the ability to overcome the problem of voltage increase and scaling up problem. The MFC could be reconfigured to treat wastewater and generate electricity at required rates.

Microbial fuel cell performance with a pressurized cathode chamber

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Microbial fuel cell (MFC) power is often constrained by the kinetics of the oxygen reduction reaction in the cathode. Crucial for this is the normally low solubility of oxygen in the aqueous cathode solution creating transport limitations, which hinder the oxygen reduction at the electrocatalyst (platinum). Because the solubility of air and consequent availability of oxygen are a function of their partial pressure, we theorized that a pressurized cathode chamber could significantly increase MFC power densities. With stable anode conditions, we tested both a MFC with an anion exchange membrane (AEM) and a cation exchange membrane (CEM) at 0.0, 2.5, and 5.0 psig of air pressure, respectively. Tests were conducted with a phosphate-buffered catholyte maintained at a constant pH. Anode conditions were maintained at steady state conditions with the continuous addition of a synthetic sucrose substrate. Results indicate that increasing the cathode pressure to 2.5 psig increased MFC power by 70.6% (4.26 Watts/m^3 (AEM) vs. 7.27 Watts/m^3) and the coulombic efficiency by 26% (6.8% VS. 8.6%) when compared to atmospheric pressure. The MFC produced 65 - 108% more power with the AEMs in comparison to the CEMs under the same operating conditions. We believe that the increased power results from favorable ion gradients across the exchange membranes resulting in a lower internal resistance (estimated to be 100 ohms). The open circuit voltage (OCV) at 2.5 psig with the AEM was 0.931 V, the highest OCV reported for a MFC. Results from this study demonstrate that higher MFC power densities can be realized by increasing the cathode air pressure and point forward to a MFC design that can exploit this capability.

Investigation of the biological mechanisms for electron transport in mediatorless MFC biocathodes using *Shewanella oneidensis* MR-1 cytochrome deletion mutants

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Biocathodes hold much promise for permitting the scale-up and real-world application of microbial fuel cells by replacing expensive catalysts and chemical reagents (e.g. platinum and ferricyanide) and enabling the reduction of a variety of electron acceptors in the cathode. Recent work has demonstrated the ability of certain strains of *Shewanella* to catalyze reduction reactions in batch culture and in microbial fuel cells (MFCs); however, the biological mechanisms underlying electron transport from the cathode to the terminal electron acceptors are not well understood. Recent studies focusing on the performance of MR-1 in the anodes of MFCs suggest that *Shewanella*'s ability to transport electrons from electron donors to a graphite electrode is at least partially dependent on a handful of genes

involved in electron transport and protein secretion. In this investigation of biocathodes, MR-1 deletion mutants were introduced into the cathode compartments of MFCs containing different terminal electron acceptors (fumarate, iron citrate, hexavalent chomium, and molecular oxygen) and their current production was compared to that of MR-1 wild type. Preliminary results indicate that some of the genes implicated in anode functionality are involved, to varying degrees, in electron transport from MFC cathodes to electron acceptors. In addition, different sets of genes appear to be involved in the reduction of each of the electron acceptors tested. Further study of these deletion mutants is expected to yield interesting insight into the electron transport mechanisms employed by *Shewanella* and other dissimilatory metal reducing bacteria.

Convection driven high current density for microbial fuel cells

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A systematic study using rotating disk electrodes (RDE) demonstrates that small convection rates have a profound effect on the anodic current for yeast catalyzed microbial fuel cells. The Levich equation accurately describes the anodic currents down to very small rotation rates, demonstrating that convective mass transfer easily overcomes the reaction-limited currents for microbially catalyzed reactions. In contrast, convective mass transfer is shown to be relatively less important for enzymatic catalysis and other fuel cell oxidation reactions. By maximizing convection rates, we demonstrate stable anodic current densities > 5 mA/cm^2 in poised potential half-cells using yeast catalyzed glucose oxidation. Such high current densities are unprecedented for microbial fuel cells and shed insight on methods to remarkably increase power densities of these devices. Although the study is based on yeast-catalyzed fuel cells with artificial mediators, the basic principles can be extended to microbial fuel cells using other microbes with artificial or naturally secreted soluble mediators.

Effect of operating temperature on performance of mediator-less microbial fuel cell

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Wastewater treatment using microbial fuel cell (MFC) is promising since this process can convert the major part of the chemical energy of the contaminants to electricity, thereby reducing the generation of excess sludge. Operating temperature is one among the important parameters affecting performance of the MFC. Hence, it was aimed to evaluate performance of mediator-less MFC at different temperatures to ascertain optimum operating temperature range. The performance was evaluated to optimize wastewater treatment and electricity recovery of the MFC in the temperature range of 20 to 55 ^{o}C, with the step increase in temperature of 5 ^{o}C. Synthetic wastewater, containing sucrose as a carbon source, was used in the study having chemical oxygen demand (COD) of about 500 g/L. MFC was operated under batch mode at feed cycle time of 48 hours throughout the experiments. At each temperature the MFC was allowed to acclimatize to give stable performance and after acclimation the performance was observed for a week. For the operating temperature ranging between 20 and 40 ^{o}C, increase in temperature enhanced the COD removal efficiency of the MFC and it varied from 62 % to 84 %, respectively. In

this temperature range power density increased from 2.4 mW/m^2 to 34 mW/m^2 and the maximum power per unit volume of anode chamber got increased from 27.5 mW/m^3 to 402 mW/m^3. The coulombic efficiency increased from 3.4% to 7.4 % in the operating temperature range of 20 to 40 ^oC, respectively. The MFC demonstrated an optimum performance at operating temperature of 40 ^oC. Further increase in temperature resulted in lowering of COD removal and electricity recovery. The performance at 45 ^oC was comparable with performance at 30^oC. Thus, based on the finding of this study it can be concluded that the operating temperature between 35 to 40 ^oC is optimum for the MFC operation.

Dynamic characteristics and kinetics of electron transferring processes in microbial fuel cell

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The MFC system was continuously operated with acetate (fuel), and the system showed coulombic efficiency at higher than 90% at 10 Ohm of applied resistor. The dynamic behavior of electron transferring process in this MFC was studied. The electron transferring process in MFC was divided into 3 steps: substrate oxidation by electrochemically active bacteria (EAB) to convert fuel into electron (step 1); charging of electron from EAB to anode electrode (step 2), and transfer of electron from anode to cathode accompanying with the transport of proton through electrolyte (step 3). The kinetic of each step was expressed in term of current (I) as the function of number of electron transfer through each step at certain time (I = dq/dt). Step 1 was monitored by changing of fuel concentration supplied to MFC at the range of fuel limiting domination in polarization curve. The dynamic characteristic of electron charging to electrode (step 2) was monitored by changing of MFC operation mode (CCP to OCP) in the region of ohmic loss- and fuel limiting domination. Step 3 was determined by changing the system operation from OCP to CCP mode in the region of activation loss domination. The relationship of three steps was also described to simulate the electron flow in MFC system. This study will be useful for understanding the physical and biological factors dominating the current generation in MFC system and prediction experimental results

Physicochemical selection of adhered biofilms for improving MFC performance

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The efficiency of microbial fuel cells (MFCs) depends in part on the transfer of electrons from the adhered bacteria to the anode electrode. Bacteria providing favorable electron transfer with high redox characteristics are potentially well adapted for use in a microbial fuel cell environment. However, most bacteria in biofilms might be electrochemically inactive as their cell walls and other surface structures are not electrically conductive. In order to determine conditions which enrich biofilms with bacterial strains having high redox properties, an electrochemical study of biofilm formation on different electrode surfaces under various environmental conditions was carried using the same inoculum. The

electrochemical activity of adhered bacteria was characterized by using cyclic voltammetry, an electrochemical technique optimally suited to determine redox characteristics of modified electrodes. The results show the variation of electrochemical characteristics of formed biofilms as a function of the chemical and physical properties of electrode surfaces. The variation in adhered bacterial strains provides insight to the possibility of enriching biofilms with electroactive bacteria. The performance of an electrochemically enriched biofilm was tested in a model microbial fuel cell and produced an increase of power output compared to MFC power obtained with a non-enriched biofilm.

A study of ammonia as a potential fuel in a rotating-cathode microbial fuel cell

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Nitrogen removal is an important issue during the wastewater treatment process. Traditionally, nitrogen (as ammonia/ammonium) is removed via nitrification and denitrification. As a novel process, ammonium oxidation under anaerobic conditions (anammox) makes it possible that ammonia/ammonium can potentially be used as an anodic fuel in a microbial fuel cell (MFC). However, no studies have reported that electric current can be generated using ammonium as an energy source. Here, we present a proof of concept using ammonia/ammonium as a fuel in a rotating-cathode microbial fuel cell (He et al., 2007). The MFC was inoculated with a mixture of aerobic and anaerobic sludge from a local wastewater treatment plant. The feeding solution contained no organic compounds or nitrogen sources. After several months' operation, we found that the addition of ammonium chloride stimulated an increase of electric current over the "no additions" control (from 0 to 0.06 mA, 1000 ohm), while the current was about zero when nitrate or nitrite was added. Adding more ammonium chloride resulted in a higher peak current. Nitrite was detected after an overnight operation. These results clearly show that current generation is coupled with ammonium addition. Based on the results, we hypothesized that some ammonium is oxidized on the cathode by oxygen, and that this results in the production of nitrite that functions as an electron acceptor for electricity generation. We are currently analyzing the nitrogen balance and microbial composition on the anode and cathode electrodes, respectively.

Comparison of two different types of graphite electrodes generating bioelectricity from sediment using microbial fuel cell

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By placing a pair of electrodes in sediment and overlying water, it generates electrical current for on-site use (i.e., powering electronic devices to monitor the environment) via an anaerobic oxidation process of organic matter in sediment. In this lab-scale study, two different types of electrodes, porous and non-porous electrodes, were examined using sediment collected from a contaminated site. Throughout the experimental period, sediment batteries with porous electrodes showed superior performance compared with those with non-porous electrodes in terms of current generation. More interestingly, two current peaks were observed in sediment batteries with porous electrodes, which might resulted from the enrichment of responsible bacteria. But only one current peak appeared in sediment batteries with non-porous electrodes. Other findings of this study include the direct coupling

of current generation with a decrease of the organic matter in the sediment, and a positive ORP in the vicinity of the anode in sediment batteries operated under closed-circuit conditions, thereby inhibiting methanogenesis. Conversely, the ORP value was lower than –140mV in the battery operated under open-circuit conditions, which may allow methanogenesis. Analyses of polarization and power curves and microscopic observation of the active anode indicated that the fuel availability to the anode was identified as the most serious limiting factor, which has not been commonly found in microbial fuel cells (MFCs) using water-soluble fuels. Consequently, in order to expand the benefit of MFCs in sediment, further attempts to minimize the limitation of mass transfer should be made.

Electricity production from a paper plant wastewater using microbial fuel cells

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Effluents and wastewaters from paper recycling industries contain a variety of complex molecules and can be difficult to treat. We examined power production from a paper industry, focusing on the maximum possible power production (by adding buffers) and the relative contributions of soluble and particle substrates. Power densities as high as 501 ± 20 mM/m^2 were achieved using paper wastewater by adding a 50 mM phosphate buffer solution (PBS) to the wastewater, and by using graphite fiber brush anodes and air cathode in MFCs. The coulombic efficiency (CE) was relatively low for this type of reactor (16 ± 2%). Increasing the ionic strength (100 mM PBS) improved power to 672 ± 27 mM/m^2 (reactor internal resistance of 300 Ω). The maximum power density produced without PBS addition was 144 ± 7 mM/m^2 (internal resistance of 1000 Ω). The concentrations of soluble intermediates, consisting mainly of acetate and propionate, varied in all cases and partly contributed to the different amounts of power produced. These results demonstrate that power production from these wastewaters is limited by the solution ionic strength.

Improvement of electricity production using xylose in graphite fiber brush anode and air cathode microbial fuel cells

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Improvement in electricity production from xylose was achieved using a brush anode and air cathode microbial fuel cells (MFC) with a working volume of 0.77 L. Voltage output followed saturation kinetics as a function of xylose concentration below 20 mM, with a predicted maximum of 0.13 ± 0.01 V (16.7 ± 0.8 W/m^3 or 892 ± 45 mW/m^2) and a half-saturation constant of 1.18 ± 0.01 mM. The coulombic efficiency (CE) ranged from 85 ± 4% to 61 ± 3% corresponding to xylose concentrations of 0.67 mM to 20 mM. Lower power production (0.082 ± 0.002 V) and CE (40 ± 4%) were obtained at a xylose concentration 54 mM. Increasing the solution ionic strength from 100 mM to 200 mM by adding a phosphate buffer solution (PBS) increased power output from 13 ± 1 W/m^3 (673 ± 43 mW/m^2) to 18 ± 1 W/m^3 (944 ± 32 mW/m^2). When the MFC was operated in continuous flow mode (150 mM PBS), a maximum power density of 20 ± 1 W/m^3 (1093 ± 43 mW/m^2) was generated at a hydraulic retention time (HRT) of 20 h. The CE ranged from 29 ± 3% to 52 ± 3% for HRTs

of 4.8 h to 39 h. Concentrations of soluble intermediates (acetate, ethanol, propionate, formate and lactate) at xylose concentrations 20 mM and 54 mM reflected a change in metabolism of the microbial consortium at different substrate concentrations.

Characterization of electrode-reducing rate of *Geobacter sulfurreducens* in an air-cathode microbial fuel cell

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Microbial fuel cell (MFC) systems are devices that use microbes as the catalysts to oxidize organic matter and generate electrical current. An electricity generating bacterium, Geobacter sulfurreducens PCA, was inoculated into an air-cathode single-chamber MFC in order to determine the maximum electron transfer rates from the cell to the anode. To create anodic reaction-limiting conditions, where electron transfer from cells to the anode is the rate-limiting step, six different sizes of anodes were employed. The smallest anode achieved an anodic reaction-limiting condition as a result of a limited mass of bacteria on the electrode. Under these conditions, the limiting current density reached a maximum of 1530 mA/m^2, and power density reached a maximum of 347 mW/m^2, which were higher than those previously obtained for this strain in other MFCs. The cell density on the electrode was 3.91×10^9 $cells/cm^2$ (0.53 mg-protein/cm^2). Per-cell efficiency of the electron transfer rate was calculated by using biomass on the anode and limiting current density. The electrode-reducing rate was constant at 32 fmol $cell^{-1}$ d^{-1}, a rate comparable to that with solid iron as the electron acceptor, but slower than rates achieved with fumarate or soluble iron. These results demonstrate that creating reaction-limiting conditions by bacteria on the anode and measuring the biomass amount on the anode are important to better examine the maximum efficiency of the biocatalyst for generating electricity in an MFC.

Electricity generation in microbial fuel cell using high-strength organic matters

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Microbial fuel cell (MFC), a device that uses bacteria as catalyst to generate electricity, can use various organic wastes as electron donors, even if it can use inorganic matters and toxic compounds as electron donors. The current might be proportionally generated to organic matters concentration at the condition which is not restricted by limiting factors such as oxidant supply, proton transfer, internal resistance etc. In this study, An MFC was conducted to explore whether to improve the electricity generation oxidizing organic matters of high concentration. A sodium acetate of 2000 mg/l as electron donor was used. It was fed into an anode compartment with flow rate of 0.35 ml/min. The removal of organic matter was over 30% at this point. For this study, dual cathode type MFC (DCMFC) was used to increase the cathode reaction rate by increase the contact area between anode and cathode compartment, because cathode reaction is one of the most serious limiting factors in an MFC. The current generated from the DCMFC was higher than the value produced from MFC with single cathode. It seems to be due to rise the amount of proton transfer via a membrane. These results showed that the DCMFC seemed to help the cathode

reaction rate as well as the DCMFC could be used as a device treating high-strength wastewater.

Electricity generation from microbial fuel cell using bio-cathode

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Cathode reaction is one of the most serious limiting factors in a microbial fuel cell (MFC). This constraint can be improved by increasing the affinity of cathode for oxygen. The critical dissolved oxygen (DO) concentration of platinum loaded graphite cathode was reported as 2.2 mg/l that is about 20-fold higher than an aerobic bacteria. MFCs were operated with platinum loaded or bare graphite cathode. A series of MFCs were run with cathode compartment inoculated with activated sludge, and the others as abiotic control. The anode and cathode compartments had a same volume of 3.0 ml that was filled with graphite felt as the electrodes.

At the beginning of the operation currents from MFCs with bio-cathode and abiotic-cathode were 2.3±0.1 and 2.6±0.2 mA, respectively at the DO supply of 11.3 mg/l · min cathode. The current from MFCs with biotic cathode increased to 3.0mA while that from abiotic MFCs didn't change when the MFCs were operated for 8 weeks. The Coulomb efficiency was 59.6% in the MFCs with biotic cathode that was much higher than the values was 15.6%. When the DO supply was reduced the current from MFCs with abiotic cathode decreased more sharply than those with biotic cathode. When azide was added to the catholyte the current decreased in MFCs with biotic cathode, but didn't change in MFCs with abiotic cathode. The power density was higher in the MFCs with biotic cathode (430 W/m^3 cathode compartment) than the abiotic-cathode MFC (257 W/m^3 cathode compartment). Electron microscopic observation revealed nanowire structures both in biofilm developed on the anode and on the biocathode. These results show that electron consuming bacterial consortium can be used as a cathode catalyst to improve cathode reaction.

The correlation of bacterial adhesion and power generation in microbial fuel cells (MFC)

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Microbial fuel cells (MFC) depend on bacterial adhesion/biofilm growth on electrodes to transfer electrons. It has been assumed that a better extent of bacterial adhesion leads to a high power production in a MFC. For MFCs treating wastewater, a significant amount of bacteria are present in bulk solution, rather than adhering to electrodes. It is critical to understand the electron transfer of bacteria adhesion and bulk solution in order to enhance

MFC performance. Therefore, the objective of this study is to elucidate the role of bacteria growing in suspension solution and bacteria adhering to electrodes for power generation in MFCs. Two parallel tests were conducted to compare bacteria in bulk solution and bacteria adhering to electrodes. The first test used a fresh solution and a bacterial adhered electrode (referred to as old electrode in new media, OE), while the other test used a well-growing bacterial medium and a new electrode (referred to as new electrode in old media, NE). The results showed that the new electrodes (NE) in well-growing bacterial media exhibited a higher voltage generation than the bacterial adhered electrode (OE) in fresh solution. NE generated 400 mV immediately after putting in the well-growing bacterial media. This indicated that electricity-producing bacterial freely floating in the growth media can quickly generate electricity on a new electrode without the need of bacterial adhesion. In the contrast, a bacterial adhered electrode (OE) with sufficient bacteria growing on the surface did not produce sufficient electricity after being put in a fresh solution, which contradicted previous studies that the adhered bacteria on electrodes (not the freely floating bacteria in growth media) were critical in electron transfer and electricity generation. It was also found that the internal resistance (R_{in}) was not directly correlated with power generation in MFCs. The MFCs with the same R_{in} had a tremendous difference in power output due to different operation temperatures. In addition, some MFCs with high R_{in} even produced higher voltage than some MFCs with low R_{in}, proving that the R_{in} is not a deciding factor of power output, although it decides the amount of electricity to be utilized by the external circuit at the same overall power output. Novel electrode materials were developed to enhance electron transfer and bacterial adhesion. A conductive and nontoxic polymer--polypyrrole (PPy) was coated on carbon cloth. PPy-coated electrode had a higher extent of bacterial adhesion and greatly facilitated the initial voltage output of the MFCs. The voltages of the PPy-coated anodes were four times as high as that of the plain carbon cloth.

Bacterial and archaeal communities of anode biofilms in microbial fuel cells fed acetate, lactate, and glucose and anode community evolution in response to different resistances

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Methane production has been reported in microbial fuel cells (MFCs) and bio-electrochemically assisted microbial reactors (BEAMRs), decreasing Coulombic and energy efficiencies. However, methanogenic archaeal research in MFCs has not been extensively investigated. We examined manipulation of the external resistance as a potential operation strategy in order to favor exoelectrogens over methanogens. Our recent community analysis results showed that *Geobacteraceae* and *Bacteroidetes* were the major taxa in anode biofilms of MFCs fed acetate, lactate, or glucose, with *Firmicutes* only found in glucose-fed MFCs, suggesting a fermentative role of these bacteria. *Archaeal* clone library analyses revealed that the strictly acetoclastic methanogen *Methanosaetaceae* was dominant in the anode biofilms, with fewer clones derived from *Methanosarcinaceae*. Electron donors and external resistance influenced microbial ecology on anode electrodes and MFC performance. Anode biofilms acclimated under high resistance generated a higher maximum current density. The molar ratio of CH_4 to CO_2 decreased with decreasing applied external resistances, due to increasing availability of the electrode as an electron acceptor. Coulombic efficiency and energy efficiency were highly dependent on substrate. To link MFC performance with anode microbial ecology, quantification of key taxonomic groups in the anode biofilms is being measured using qPCR. This work is ongoing, and the results will be used to investigate correlations among microbial community compositions, MFC performances, and external and internal resistances. This is expected to show potential

opportunities for reducing the loss of electrons to methanogenesis through simple MFC reactor operational strategies.

Characterization of electron by-pass mechanism in mediator-based microbial fuel cells

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In microbial fuel cells employing chemical mediators to lead electron from microbial metabolic pathway to an anode electrode, it has been pointed out that the mediator molecules should have not only suitable reduction-oxidation (redox) potential values, but also good cell membrane permeability. The permeabilty can be determined by various cell stain assays, however, the electron by-pass mechanism involving redox potential values of a certain mediator was not well understood. In present study, we have examined several inhibitors for mitochondrial electron-transport chain (ETC) to clarify how mediators such as methylene blue and vitamin K_3 would interact ETC components, and transfer electron out for electricity generation using microbial fuel cell containing baker's yeast and glucose. The ETC inhibitors tested were rotenone or sersalyl acid for Complex I, Antimycin A for Complex III, potassium cyanate for Complex IV. In the presence of Antimycin A, MB-based fuel cell was found strongly inhibited but VK_3's one was not. In addition, a protonophore, carbonylcyanide *m*-chlorophenylhydrazone (CCCP) was inhibitory for the both. Neither rotenone nor cyanate were found inhibitory at all. Thus, it was likely that MB and VK3 could work at different ETC sites. Moreover, to monitor electron generation stability of the microbial fuel cell, mitochondrial activities were determined either by mitochondrial mass change with MitoTracker® Green fluorescence, or by mitochondrial potential with JC-1 fluorescence. With these results, the possible interaction of the mediators with ETC complexes and the robustness of the fuel cell are discussed.

Current Production and Microbial Diversity in Self-sustained Photosynthetic Microbial Fuel Cells

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Solar energy is a clean and infinite energy source. Previous studies have demonstrated using solar (light) energy to drive electricity generation in microbial fuel cells (MFCs). However, these studies mainly focus on hydrogen production from organic compounds via photosynthetic process and *in situ* hydrogen oxidation by catalysts to generate electric current and power. In this study, we constructed two self-sustained photosynthetic sediment MFCs. One MFC (MFC-A) contained the original sediments from Mono Lake, while the other (MFC-B) contained the autoclaved sediments. Two full-spectrum light bulbs were used to simulate the sunlight with an automatic control (turn on at 8 am and off at 4 pm everyday). In the second month of the operation, MFC-B exhibited a current variation same as what we expected, but MFC-A showed an opposite trend. After seven months' operation, both MFCs showed a similar trend that the current increased in the dark and decreased in the light, but the current production from MFC-A was higher than that from MFC-B. DGGE (denaturant gradient gel electrophoresis) analyses of microbial diversity showed that *Cyanobacteria*, *Alphaproteobacteria*, and eukaryotic algae dominated in water columns (cathodes), while *Bacteroidetes* and *Firmicutes* were major bacterial groups found in

sediments (anodes). As we proposed, self-sustained photosynthetic MFCs were likely performed with different consortia of bacteria: phototrophic bacteria produce organic compounds under the light, and heterotrophic bacteria use these products to generate electricity in the dark.

Development of a microbial fuel cell

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A microbial fuel cell (MFC) was tested in which the bacterial strain used for the catalysis reaction was Shewanella oneidensis MR-1. Experiments were performed to determine the optimal carbon source for the growth of the Shewanella oneidensis MR-1 between glucose, succinic acid, acetic acid, citric acid, sucrose, and lactic acid. It was postulated that the Shewanella cells would grow better in a minimal-media and would provide the largest consistent power production. Each medium was prepared by adding 30 mM PIPES [piperazine-N,N′-bis(2-ethanesulfonic acid)], base components (1.5 g/L of NH_4Cl, 0.1 g/L of KCl, 0.69 g/L of NaH_2PO_4, and 0.132 g/L of $CaCl_2$), 10 mL/L of mineral mix, 10 mL/L of vitamin mix, and 3M of the selected medium. Among the six media tested, glucose and succinic acid produced the highest potential. A polarization curve has been developed for glucose to determine the relationship between the obtained voltages and currents. The magnitude of the current produced is in the range of microamperes. Therefore, more tests are needed to determine which factors can be varied and their respective limitations, to increase the current output before practical applications become viable. There are two thrusts to ongoing work. The first involves improving the design of the fuel cell and fuel cell system. The second research vector is to use a microbial community rather than a single organism.

Operation of microbial fuel cells under psychrophilic- and halophilic environment

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Researches on microbial fuel cells (MFCs) have been mainly conducted under mesophilic condition in a lab-scale system. However, the temperature controlled-MFC system cannot be applied in real field which is almost lower temperature than mesophilic condition. Therefore, in this study, we operated MFC system under psychrophilic temperature (10℃) that is similar to the temperature of sampling site. Tidal-mud was used as an inoculum source from marine sediment (Yoesu, Korea). Medium contained 30 g/l of NaCl or sea water for halophilic condition. Substrate-fed MFCs were operated with three different electron donors (acetate, lactate and glucose). Mud battery type of MFCs was also constructed using marine sediment obtained from same sampling site. We also cultivated and enriched Fe(III) reducing bacteria using three substrates. Lactate fed MFC showed higher current density around 90 -130 mA/m^2 than those of acetate and glucose fed systems. This result was also supported by Fe(III) reduction activity test in vial culture. Microbial community of mud battery types was more diverse than that of MFCs. They showed similar microbial community to compare with metal reducing bacterial community and electrically active

bacterial community. This work was supported by the Korea Research Foundation Grant funded by the Korean Government (MOEHRD, Basic Research Promotion Fund) (KRF-2007-313-D00403).

Cation exchange membrane-less microbial fuel cell via convective proton transfer

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A variety of microbial fuel cells (MFC) using proton exchange membrane (PEM) have been suggested. However, the applications of PEM-based MFCs are difficult for the full scale energy harvesting and/or wastewater treatment systems, because the PEM is not only expensive, but also has proton migration problem from the anode to the cathode. Therefore, we have developed a new two-chambered MFC configuration without the PEM separation. In our membrane-less MFC, the anode and the cathode were separated completely but with a connection via narrow tubing. Substrate (acetate, 2mM) was continuously fed to the bottom of the anode chamber, and then anode effluent was directed to the bottom of the cathode chamber. This sequential connection of the anode and cathode allowed the transport of the protons produced at the anode convectively via the liquid flow itself instead of chemical migration through the PEM. Oxygen back diffusion from the cathode to the anode, a serious problem which occurred in the former similar type of membrane-less MFC, was not occurred in our system as a result of complete phase separation. The transport of the remaining organics and detached bacteria from the anode to the cathode resulted in further effluent polishing by the reaction of biocathode. In our MFC, the anode was carbon felt which was pre-enriched in the normal two-chambered MFC for over 2 months, but the cathode was Titanium plate coated by platinum. Oxygen was provided as a final electron acceptor for cathodic reduction. Electrical current was successfully generated. Coulombic efficiency (CE) was less than 25%, which is somewhat low because our system was not fully optimized as a result of relatively short operation. Further concerns are still required to balance the rate of protons production and their consumption at the cathode.

Hydrogen production from cellulose fermentation products using microbial electrolysis cells

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Hydrogen production is becoming increasingly important as a source of fuel for fuel cells. So far, most of the hydrogen produced is derived from fossil fuels. Ecologically clean and renewable methods of producing hydrogen include microbial fermentation and the use microbial electrolysis cells (MECs), also known as bioelectrochemically assisted microbial reactor or BEAMRs. Microbial fermentation using cellulose is possible, however, less than 15% of the organic matter is typically converted to hydrogen with most of the energy still contained in soluble end products such as acetate and other volatile fatty acids. In this project, we are looking at degrading cellulose fermentation end-products (acetate, succinate, formate, lactate, and ethanol) from a specific fermentation end stream to produce hydrogen in single chamber MEC reactors. We are comparing the hydrogen production by mixed

cultures to the complete mix of end products, to cultures pre-acclimated to the different substrates.

Effect of cathode area on performance of microbial fuel cell under high external resistance load

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Wastewater treatment by microbial fuel cell (MFC) represents a novel and promising green technology. This study examines the cathode area on performance of MFC having extremely high external resistance load of 10 k Ω. The employed mediator-less MFC had two chambers, separated by a proton exchange membrane of Nafion 117 (183 μm , 9.62 cm^2), where the anode was manufactured by carbon fiber (150 cm^2), and cathode by simple carbon cloth. The fuel taken from supernatant of Beisha Harbor Wastewater Treatment Plant treating fish market wastewater, and diluted with distilled water to concentration around 125-135 mg COD/l, was continuously fed into anodic chambers at 19-22 hours of hydraulic retention time. The tested cathode area cover ranges of 1, 5, 25, 150, and 500 cm^2. Results indicated that maximum power generation increases with cathode area, where the relationship between power generation and cathode area follows Monod –type kinetics, with a half saturation constant of Ka = 103 cm^2. Furthermore, the effects of cathode area on Coulombic efficiency and COD removal were insignificant. These results demonstrated that when MFC used for treating fish market wastewater under high external resistance loads, its power generation could be enhanced by increasing cathode working area. However, future research should be conducted to elucidate the improvements of Coulombic efficiency and COD removal for MFC that subject to high external resistance load.

Increasing power generation in microbial fuel cells through the use of improved cathodes

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Microbial fuel cells (MFCs) constitute an emerging technology that could potentially change the way effluents are treated in the future. However, there are still several challenges to overcome before this technology becomes viable. In particular, the amount of precious platinum (Pt) used as a catalyst at the cathode should be minimized because of its prohibitive cost and its scarcity on Earth. Sputtering is a novel deposition method that allows depositing a thin film of catalyst localized near the front surface of the electrode. This makes possible to deposit very little amount of catalyst on an electrode while retaining the same performance in conventional proton exchange membrane (PEM) chemical fuel cells. Typically, the Pt load can be reduced by a factor of 5 in a PEM fuel cell by use of sputtering. In this experiment, we compared the performance of an ultra-low sputter-deposited Pt loading cathode (0.1 mg/L of Pt) with that of a conventional low Pt loading electrode (0.5 mg/L of Pt supported on carbon black particles) in a single-chambered air-cathode MFC. Use of the sputter-deposited cathode increased the maximum power generated by our MFC by 118% from 0.14 mW to 0.31 mW, using domestic wastewater as a substrate. All other

parameters, including COD removal efficiency and reaction time, remained unchanged. A sputter-deposited cobalt electrode also performed well, generating up to 0.23 mW under the same conditions. This shows that sputtering makes it possible to reduce the cost of cathode manufacturing while improving the efficiency of a MFC, which broadens the application perspectives of the MFC technology.

Investigation of electrochemistry behavior of *Shewanella sp.* NTOU1 for electron transfer to carbon electrode

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The strain of *Shewanella sp.* NTOU 1 was screened from the pipeline sediment in a steelworks factory. The cell characteristics of this strain are gram-negative, none endospore, and rod-shaped with a single polar flagellum. The phylogenetic analysis showed that this strain is most closely related to *Shewanella decolorationis* and *Shewanella oneidensis* with 97% and 90% sequence similarity, respectively. To investigate the bacterial electrochemistry behavior, *Shewanella sp.* NTOU 1 was cultured in a 200 mL electrolysis cell with potential stat connected. The electrolysis cell is operated under anaerobic conditions, glucose-containing medium, and with carbon felt electrodes on it. Potential stat is a powerful instrument for the observation of the anodic current increased with the development of the biofilm and measurement of cyclic voltammogram. The cyclic voltammetry results could proof the presence of a redox compound(s) excreted from *Shewanella sp.* NTOU1 cells in the biofilm. The detail results of electrochemical measurement will be described in the full text.

Catalyst activity change in the air-cathode microbial fuel cell with anion membrane

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Transport of cations other than protons through proton membrane resulted in an increased pH in the cathode chamber and a decreased performance in microbial fuel cells (MFCs), especially in the air-cathode MFC with a very small aqueous volume between the membrane and the cathode. Using an anion membrane in MFCs to substitute the proton membrane could reduce the pH increasing in the cathode by taking advantages of the ion transport characteristics of anion membranes, the feasibility of which has been demonstrated in two-chamber and air-cathode MFCs. However, how would the catalyst activity change upon the membrane types still needs to be further investigated. In this study the activities of different catalysts were investigated in air-cathode MFCs using the anion membrane and compared with those of MFCs using the cation membrane. Through pre-experiments, Nafion was proved to be a more effective adhesive for anion membrane compared with the colophony dissolved in chlorobenzene. Power densities of the air-cathode MFCs using the anion membrane were 446 mW/m^2, 68 mW/m^2 and 95 mW/m^2 respectively for Pt, Ag and MnO_2 as catalyst. These power densities were 16%, near 2 times and 7 times respectively higher than those of MFCs using the cation membrane with same catalysts. After one-month operation, the maximal power density of the air-cathode MFC using an anion membrane

with Pt catalyst remained at the same level, while that of the air-cathode MFC using a cation membrane decreased by 18%, indicating the higher catalyst activity and longer stable operation of air-cathode MFC using anion membrane.

Mathematical analysis of the mechanisms that control the transport of protons out of the biofilm anode

Andrew Kato Marcus, César I. Torres, and Bruce E. Rittmann

A major limitation for the biofilm anode of a microbial fuel cell is transporting protons out of the biofilm. Because anode-respiring bacteria (ARB) carry out half reactions at the anode, ARB reactions produce a significant amount of protons. Accumulating protons in the biofilm can lower pH and inhibit the activity of ARB; therefore, transporting protons out of the biofilm is necessary for efficient anode operation. Torres et al. (2008) showed that buffers in an acid-base equilibrium reaction can carry out protons from the biofilm to the bulk liquid and mitigate the pH problem. Understanding the coupling of acid-base equilibrium reactions and mass transport of buffer requires a systematic evaluation. In this study, we describe the Proton Condition in Biofilm (PCBIOFILM) model as a novel platform to analyze the mechanisms for out-transporting protons out of the biofilm anode. Processes in a biofilm-anode occur at different time scales and in different phases: acid-base equilibrium and mass transport occur much more rapidly with respect to many bacterial processes such as growth and decay; homogenous reactions include combining of buffers with protons, while heterogeneous reactions include synthesizing new cells from soluble substrates. PCBIOFILM uses the proton condition to efficiently link processes at different time scales and phases in the biofilm. Using PCBIOFILM, we identify dominant transport mechanisms in the biofilm anode and evaluate the impact of buffer concentration in the anode medium.

Thermophilic bacteria capable of electricity generation in microbial fuel cells

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Due to their high rates of metabolism, thermophilic bacteria are sometimes used in the production of biofuels, but thus far the study of their application in microbial fuel cells has been limited. No single organism has been shown to produce an electric current at thermophilic temperatures without the addition of an electron-carrying mediator of electrode reduction. The purpose of this study was to begin the process of discovering a thermophilic isolate capable of electrode reduction. Thermophiles from marine sediment in the Charleston, South Carolina harbor were shown to produce a current ten times higher in a sediment fuel cell than the mesophilic organisms taken from the same environmental source. This bacterial community was further enriched in sediment-free single-chamber fuel cells maintained at 60°C with acetate or cellulose as an energy source. Amplified 16S rRNA gene sequence analysis of the community in the biofilm on the anode revealed that the majority of clones were most closely related to Firmicutes, particularly the Gram-positive iron-reducing *Thermincola ferriacetica*. The clones present in the community analysis in lesser quantities were similar to Deferribacteres isolated from thermophilic environments. The organisms with similarity to these known iron-reducing bacteria are currently being investigated further for electricity generation.

Influence of operational conditions on microbial fuel cell performance

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In this work, the influence of organic load, pH and temperature on power generation was studied in a single chamber air-cathode membraneless microbial fuel cell (MFC) inoculated with mesophilic anaerobic sludge. The MFC was equipped with temperature and pH stabilization loops and was continuously fed with glucose as a source of carbon. Measurements of glucose and volatile fatty acid (VFA) concentrations in the effluent, power generation, as well as methane flow rate and composition were used to evaluate MFC performance in response to variations in organic load, anodic compartment pH, and temperature. Variation of the substrate concentration in the anodic compartment from 7 to 1100 mg-COD/L showed that exoelectrogenic microorganisms were inhibited at high substrate concentrations. Simultaneously, methane production rate followed the Monod kinetic equation. A comparison of electricity generation and methane production at different pH values showed a decrease in methanogenic activity at pH values below 6.6, as well as a decrease in power generation at pH values below 6.3. Finally, an increased anodic compartment temperature (from 25°C to 38°C) resulted in both increased power generation and methane production, i.e. increased competition between methanogenic and exoelectrogenic microorganisms for the common substrate. Overall, better power generation was observed at substrate concentrations between 100-300 mg-COD/L and a pH of 6.3-6.5.

The effects of buffer species on the hydrogen evolution reaction at circum-neutral pH

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Microbial electrolysis cells (MECs) can be used to convert organic matter in wastewaters to hydrogen fuel with the assistance of an applied voltage. The hydrogen evolution reaction (HER) is typically performed and studied in either extremely acidic or basic conditions whereas the HER may be performed in buffered, circum-neutral pH electrolytes in some MEC to facilitate microbial growth. The effects of buffer species on the HER at circum-neutral pH has not been previously well-defined and is the subject of this work. Buffering species such as phosphate, carbonate, and ammonium electrolytes were compared with sodium, potassium, and chloride electrolytes between pH 5 and 8 (30 °C). Protonated buffer species were typically present at greater concentrations than solvated, free protons in the studied conditions and were found to affect both the rates of proton donation to the HER and the efficiency of electron transfer. The HER kinetic effects were correlated with pKa's of the buffer electrolytes. The effects of buffer species on the HER were demonstrated on both Pt foil and Ni alloy.

Optimization of process parameters of microbial fuel cell (MFC) with respect to bioelectricity generation and wastewater treatment

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Rapid industrialization has been resulting in the production of large quantities of wastewater and its treatment is highly imperative. Reducing the wastewater treatment cost and finding ways to produce useful products from wastewater has been gaining importance recently in view of environmental sustainability. Exploiting wastewater as substrate to generate electricity is considered as sustainable, green and promising alternative approach to meet the increasing energy needs and also as a substitute for fossil fuels. Electricity production especially using wastewaters as substrates employing microbial fuel cell (MFC) is in the early stages of research and development. Characteristics and composition of wastewater used as carbon source and design of MFC and configuration, nature and coating of electrodes, membrane electrode assembly, mediators, electrolytes used, nature of the inoculum (biocatalyst) used in anode chamber, operating conditions such as loading rate, feeding pH, temperature, retention time, etc. are some of the key process parameters which govern the overall efficiency of electricity generation. In this communication, we have made an attempt to evaluate the role process parameters such as type and nature of wastewater, nature of proton exchange membrane, nature of anodic inoculum used (aerobic and anaerobic), feeding pH of wastewater (acidophilic/neutral/basic), distance between electrode, nature of electrode materials (graphite, steel, nickel, copper and aluminum), fuel cell configuration (single and dual) and nature of anodic microenvironment (aerobic, anoxic and anaerobic) on the performance of the fuel cell based on power output parameters and substrate removal efficiency were presented and discussed based on experimental work carried out in our laboratory for past three years. All experiments were performed using mixed consortia as anodic biocatalyst.

Influence of operating conditions on the structure of electro-active bacterial communities in microbial fuel cells

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To improve electrical performances of microbial fuel cells (MFCs), a better understanding of the microbial interactions between the electrodes and bacteria, which are responsible for electricity production, is necessary. In order to assess the role of different operating conditions on community structure and electrical performances, we varied substrates and electrical conductivity of the system (open and close circuits). We inoculated different reactors with domestic wastewater and fed them with wastewater amended with starch, glucose, acetate or without amendment. DNA extraction of anode, suspension and cathode were performed at different times and the 16s rDNA was analysed by hybridization on 16S-based taxonomic microarrays and by cloning and sequencing. Two correlations were found: one between the bacterial communities and the substrate, where acetate is the best substrate for electricity generation and another one between electricity production and bacterial communities where electricity seems to drive communities in their development and stability.

A novel device that controls fuel cell voltage and steps it up to a level appropriate for powering sensors in aquatic environments

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Benthic microbial fuel cells are being developed as power supplies for sensors in aquatic environments. Through polarization experiments we have determined that the optimum voltage for power generation from marine sediments is usually between 0.4 and 0.5 V. Commonly, sensors are designed with a voltage requirement ranging from 3.5 to 12 V. We describe a combination potentiostat and DC to DC converter that controls the fuel cell voltage and converts it to a usable voltage in a single device. The passive potentiostat allows the user to set a discharge potential for the fuel cell. If the potential difference between the cathode and anode is below the setpoint, then the device stops drawing current so that the fuel cell can recover. When the cell potential is greater than the setpoint, the potentiostat passes enough current so that the setpoint voltage is maintained. The current charges a 10,000 uF capacitor which discharges at a higher voltage. The capacitor charges a Li-ion rechargeable battery, which, in turn, provides power output for a sensor. We will present 1) laboratory calibrations that show the efficiency of the DC to DC conversion is approximately 50% and 2) examples of field deployments in which the converter was used to power an acoustic receiver.

Quorum sensing in microbial fuel cell

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Recently it was found out that biofilm on the anode surface and nanowire production lead to increased power output in *Geobacter sulfurreducens* fuel cells, which imply that biofilm formation on the electrode surface may provide biological clue to the increase of power generation in MFC. That is to say, we can efficiently enhance the performance of MFC based on the biofilm control strategy. However, despite of its new possibility, there hasn't been any remarkable research on this subject in the MFCs as far as our knowledge. Therefore, in this study we investigated the potential of MFC performance enhancement based on the biofilm control techniques. Among various biofilm properties, we focused on biofilm's high cell density and quorum sensing (QS) that is the regulation mechanism of bacteria's group behavior in density dependent fashion. Agrobacterium tumefaciens A136 (Ti^-)(pCF218)(pCF372) bioassay proved the acyl-homoserine lactone type QS-activity of gram-negative bacteria in the mixed-culture inoculated to the MFC. Then, during the operation of two-chamber type MFC, the anode was taken out from MFC at each step of power generation development and the quorum sensing activity of each sample was tested with the same method. After confirming the evidence of QS-activity in MFC, we injected the acyl-homoserine lactone to the anode chamber to examine the effect on power generation of MFC.

Effects of applied voltages and oxygen concentrations at the anode on power of a microbial fuel cell

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Oxidation of organic matter at the anode of an MFC results in the production of electricity when anoxic conditions are maintained. Oxygen contamination of the anode chamber could therefore affect power generation in an MFC, and possibly the composition of the microbial community. Purging the anode chamber with air or pure oxygen for 16 hours, however, not affect power generation. After the oxygen in the anode chamber was scavenged by bacteria using aerobic oxidation of acetate, power sharply increased and returned to previous levels indicating no permanent damage in performance. When there was high concentrations of substrate in the anode chamber, the oxygen flux from the cathode chamber into the anode did not affect dissolved oxygen concentrations (DO). However, after 5 hrs of acetate starvation, the DO in the anode increased up to 0.31 mg/L, but it quickly reduced to below detectable levels when acetate (1 mM) was added. During operation of two MFCs in stack mode (in series), charge reversal occurred whereby one cell could charged the other cell. Applying a voltage of 2 V (the positive terminal of a power supply was connected to the anode) did not affect power generation. However, applying 3 V for 3 h resulted in a 15 h lag phase suggesting injury to the bacteria, but performance was restored when the applied voltage was removed with an external load of 1 kOhm.

Micro-Scale Dissection of Iron Reducing Bacterial Network

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Using cultures such as *G. sulfurreducens* and *S. oneidensis,* high Coulombic efficiencies have been achieved in microbial fuel cells. Potential losses, however, are still prevalent, and the power generated is low. In this research, we study the energy flow within the pili connected bacteria and bacterial network using micro-electro-mechanical systems (MEMS). Due to the planar nature of micro-fabrication techniques, single layer colonies and spatially controlled arrangements of bacterial networks can be isolated and characterized. Through these experiments, we strive to provide insight to questions such as the effect of having a single layer deep colony of bacteria on the electrode and measure energy conversion of the bacteria connected to the electrode by a single versus a volumetric array of pili, the effect of adding additional layers of bacteria, the potential of stacking cells at the micro-scale to increase potential, and the effect of pili length among other issues of interest.

Taking advantage of photosynthetic activity of *Rhodobacer sphaeroides* for electricity generation in a single-chamber configuration

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The amount of solar energy striking the Earth in a day is higher than society's energy consumption in a year; thus, solar-powered devices hold promise as relevant sources of alternative, non-fossil fuel derived energy. Among them, microbial devices for electricity generation are an interesting option to readily use natural photosynthetic systems for harnessing the power of sunlight. With this goal in mind, photosynthetic bacteria have been

used for hydrogen production [1,2] and current generation in microbial fuel cells (MFCs)[3,4]. Our research team is developing strategies to maximize sunlight-driven power generation using *on site* hydrogen utilization in single-chamber photobioelectrochemical fuel cells and with *Rhodobacter sphaeroides* as a model organism. Initial experiments with test-tube sized bioreactors resulted in sustainable light-driven electricity production with a maximum power point density of 790 mW/m^2, using platinum-coated Toray carbon paper electrodes[4]. Currently, we are developing catalytic transparent anodes that will allow surface area maximization for efficient hydrogen oxidation *on site*, without interfering with light penetration into the photosynthetic reactor. Several materials have been tested for the creation of transparent, conductive and catalytic electrodes, and several reactor configurations are being used to optimize overall process performance.

Waste Water Management for the Food Processing Industry using Continuous Flow Microbial Fuel

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The food processing industry typically generates waste water with high organic content that can be costly to dispose off. This study focuses on the use of a continuous Microbial Fuel Cells to significantly reduce the organic content in the waste water by harvesting energy. An innovative four-chamber Tank MFC was developed, consisting of 4 chambers of different sizes made of plexiglass elements and separate by panels of the same material. Each chamber contains an anode and cathode situated at few centimetres of distance. The total Volume of the Tank is 22.5 L which made this MFC exclusive as the first one reported in literature with this size. Furthermore the advantage of this novel MFC is that is PEM free; the continuous flow is used as a virtual membrane to force protons between anode and cathode. The COD removal of a food processing effluent was investigated using the Tank MFC. The system was operated under continuous flow, 5.7 L\h, HRT 3.19 h for 8 days. The starting COD level of the effluent was 14350 mg/L which decrease in 8 days down to 2500 mg/L, showing a COD removal of 82.58%. About 85 % of the reduction in COD was harvested in other energy forms, including electricity, hydrogen gas and microbial residues. The innovative tank MFC can provide a new strategy to offset the high cost for wastewater treatment and obtain additional energy from the by-products of the effluents.

Correlation of Biological Constraints and Electricity Production Using a Micro-Microbial Fuel Cell (MMFC)

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Major advancements in microbial fuel cell (MFC) reactor configuration have dramatically increased power production by reducing physical constraints. Recent work shows that the microbial kinetics of anode reduction can also limit power production in some systems. However, there is no information on how the density of cells (catalysts) on the anode influences system performance, and how system design and operation influences cell density. Previous data are compared simply on the basis of system geometry (e.g., anode area or MFC volume), with general disregard for the effect of catalyst density and the fact

that it varies during operation as microbes grow and decay. In this study, we designed a flow-through micro-microbial fuel cell using lab-on-chip micro-fabrication techniques to directly analyze biofilm density and system performance. Using an undefined mixed culture as inoculum and ferricyanide as catholyte, low levels of power were generated during acclimation stage. The biofilm could be directly observed under the microscope *in situ*. The system is being optimized to improve system performance while allowing directly visualization of biofilm thickness for the examination of biocatalyst density and the affect of flow rate or shear force on biofilm thickness and system performance.

Isolating cellulolytic bacteria for electricity generation in MFC fed with cellulose

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Electricity can be directly generated by bacteria in microbial fuel cells (MFC) from a variety of biodegradable substrates, including cellulose. However, maximum power densities produced in MFCs using cellulose have been lower than those achieved using soluble substrates likely as a result of slow hydrolysis rates of the particles. Both cellulolytic and exoelectrogenic microorganisms are needed in an MFC as no single strain has yet been shown to be able to generate electricity directly from cellulose. In this project we are isolating cellulolytic and exoelectrogenic microorganisms using specially-designed U-tube MFCs with cellulose as the sole electron donor. We enriched our microorganisms from a mixed culture MFC fed a paper industry wastewater and then used the dilution-to-extinction methods to transfer cell suspensions to the U-tube MFC. Successive enrichment and transfers in the U-tube MFCs was performed for 15 batches and still is being performed for more cycles. Dynamics and diversity of the microbial communities on the anode from the selected reactor after each cycle are monitored by PCR-DGGE based on the 16S rRNA gene. Preliminary results for the first 11 cycles suggest that the dominant populations are affiliated with uncultured *Enterobacteriales*, but further tests are ongoing to confirm this finding. This investigation will lead to a better understanding of the bacteria that are present in such cellulolytic systems and our ability to use these bacteria to obtain bioelectricity.

Long-Term Survival and Evolution of *S. oneidensis*: Applications for Microbial Fuel Cells

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Shewanella oneidensis is one of the model organisms used in microbial fuel cells. Here we characterize the long-term survival and evolution of *S. oneidensis* MR-1 and other strains, and select for traits that may lead to increased power output in the fuel cell. We show the ability of MR-1 to survive in long-term batch culture and demonstrate evolution in real-time by observing expression of the Growth Advantage in Stationary Phase (GASP) phenotype. In addition to observing strains with increased relative fitness, we have observed alterations in the outgrowth characteristics of the evolved strains. We plan to capitalize on this ability of MR-1 to adapt in directed evolution experiments designed to increase power output in the MFC. Two avenues of selection we are pursuing are (1) increased acid tolerance and (2) better electrode-associated biofilm formation. We have determined the long-term survival

patterns of several strains of *Shewanella* in different media and pH. We also have characterized biofilm formation for several acid-adapted MR-1 mutants.

Evaluation of methane production in microbial fuel cells generating electricity from cellulose

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Methanogenesis is not a desirable reaction in microbial fuel cells (MFCs) as it shifts the flow of electrons available from the substrate away from electricity generation. The objectives of this research were to evaluate the effect of methane production on performance of cellulose-fed MFCs, and study the diversity of methanogens involved in the process. Two-compartment MFCs were inoculated with a ruminal microbial consortium and fed colloidal cellulose (0.5 g l^{-1} d^{-1}) as the sole substrate. MFCs were operated at 39ºC for 3 months with a 20-Ω circuit resistance. Electrical current production increased from 0.3 mA during the start-up period to 2.3 mA after three months of operation, resulting in 19% coulombic efficiency and a maximum volumetric power density of 3.5 W m^{-3}. During the start-up period, 7.6 ± 0.1 ml of methane was produced, however the methane production decreased to below the detection limit (< 0.012 ml) after 3 months of operation. Denaturing gradient gel electrophoresis of PCR-amplified 16S rRNA genes using archaea-specific primers revealed that the reduction in methanogenesis was accompanied by a decrease in the diversity of methanogens. Sequencing analysis of partial 16S rRNA genes indicated that the most predominant methanogens were related to the family *Methanobacteriaceae*. HPLC analysis revealed that electricity generation from cellulose was associated with production of acetic, propionic, butyric, isobutyric, valeric, isovaleric, and lactic acid, with acetic acid being predominant. This study demonstrates that methanogenesis competes with electricity generation at the early stages of MFC operation but operating conditions suppress methanogenic activity over time.

Synergetic effects of microbial binary cultures on microbial fuel cell performance

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A binary culture of *Lactococcus lactis* and *Shewanella oneidensis* was studied for an efficient conversion of glucose into electricity in a continuously-operated chemostatic electrochemical reactor. The homolactic fermentation bacterium *L. lactis* fermented glucose almost exclusively to lactate – the favourite electron donor for the electricigen *S. oneidensis*. The latter bacterium cannot utilize sugars directly as electron donor in microbial fuel cells. *L. lactis* alone showed no electrochemical activity, while the maximum obtained current density for *S. oneidensis* in a pure culture in lactate based medium was about 12 µA/cm², which confirms literature reports about this microorganism. However, in a binary culture with glucose as primary fuel, the current density increased by 140% to about 27 µA/cm². The study was followed using electrochemical, HPLC-analytical, and electron microscopic techniques. The examination of the metabolic interactions between two defined species of microorganisms eventually will help to understand the complex food web among mixed bacterial communities in microbial fuel cells treating complex organic compounds.

Microbial Fuel cells for Electricity Generation and Waste Water Treatment

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Waste water treatment has begun to be focused on waste reuse and energy recovery due to increasing environmental concern and finite fossil fuels. It is known that bacteria can be used to generate electricity that can be harvested in Microbial Fuel Cells (MFCs). MFCs are a relatively new technique in waste water treatment which converts organic matter into electricity using bacteria as Biocatalyst. With this objective our project is aimed to demonstrate electricity generation from microbes using cation Exchange membrane (CEM) and potassium hexacyano ferrate electrolyte (PE) mediated Dual chamber MFC. Although electricity was generated continuously for around 12 hours till the microbes used were biochemical active, the use of CEM and PE made the system costly and unsafe for any further upgradation. To overcome the use of CEM and PE, Sediment based MFC was constructed using Waste water sediment of uppal lake, Hyderabad. The battery of four cells in series is performing flawlessly since twelve weeks generating significant output voltage and current, sufficient enough to run small electronic devices (Viz. LED, Calculator etc.). To further widen the scope of the project it was aimed to use MFCs for Waste Water treatment simultaneously generating Electricity. In this set up a salt bridge mediated dual chamber MFC was designed which substituted the use of CEM and PE. This anaerobic Waste Water treatment based MFC showed significant reduction in COD levels by 70% and simultaneously generating Electricity.

Hydrogen production from glycerol via fermentation and microbial electrolysis cells (MECs)

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Glycerol is a major byproduct of biodiesel production: for every 100 gallons of biodiesel, 10 gallons of glycerol are produced. It is projected that over 100 million gallons of glycerol will be produced by 2010 as a result of increasing biodiesel production, thus creating a glut in the market for glycerol. An alternative use for glycerol is conversion to hydrogen, which is a clean and highly efficient energy carrier. Traditional fermentation of glycerol using mixed cultures produced gas yields of 160 ml/g COD/L, compared to glucose fermention produced gas yields of 300 ml/g COD/L. Analysis of the final products showed that the reason for this was that glycerol fermentation preferentially formed 1,3-propanediol, a chemical not produced by glucose fermentation. Different pretreatment methods and inocula sources did not increase gas production in glycerol. Microbial electrolysis cells (MEC) are being investigated as an alternative to fermentation for hydrogen production from glycerol. It is expected that MEC's will bridge the difference between glucose and glycerol gas production, making hydrogen production from glycerol more efficient and practical.

Correlation of biofilm growth on the anode with electricity generation in Single Chamber Microbial Fuel Cells (SCMFC) treating different substrates

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Single-chamber microbial fuel cells (SCMFC) are advantageous than two-chamber MFCs due to low internal resistance, rapid mass transfer from anode to cathode, and small operating volume. The adhesion of electrogenic bacteria at the anode is important for electricity generation. The electricity generation with different stages of bacterial adhesion on anode surface was investigated in this project. Domestic wastewater was used to colonize the biofilm growth on carbon cloth anodes. The Cyclic Voltammogram (CV) measurement was conducted daily on the anode surface to evaluate power generation along with biofilm growth. CV curve was flat on the 1st day of inoculation, indicating there was no apparent electrochemical reaction at the anode. From the 2nd day, slight peaks appeared on the CV curve, signifying the oxidation of the substrates at the anode. The CV peaks got sharper with time and stabilized after the 4th day of inoculation, with the voltage reaching at 0.3 V, indicating bacterial adhesion completed on the anode and biofilm started growing. The characterization of biofilm at the anode by electrochemical and microbiological methods is underway. This knowledge is important for selecting anode materials to shorten the startup time for MFCs and increase power generation. Two pure substrates (ethanol and acetate) and one mixed substrate (liquid fermentation products from an acidogenic biofermentor) were tested. Columbic efficiencies of 25% and 16% were obtained by using ethanol and acetate respectively. The liquid fermentation products from the biofermentor contained acetic acid, butyric acid and ethanol, and reached at a columbic efficiency of 20%. The utilization of different substrates in SCMFC is being studied to identify the type of substrate with high columbic efficiencies and power generation.

Adapting a denitrifying biocathode for perchlorate reduction

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Perchlorate is an emerging water contaminant, and cost-effective treatment methods are needed. Previous research, conducted in a half cell with exogenous electron shuttles, suggests that perchlorate-reducing bacteria (PCRB) may use a cathode as an electron donor. We investigated a full MFC with a denitrifying biocathode for perchlorate reduction, as a means to explore the diversity of biocathode-utlilizing microorganisms and the possibility of cost-effective treatment of high-strength perchlorate wastes. A granular-carbon electrode MFC was seeded with activated sludge, and operated with acetate in the anode and nitrate in the cathode. The initial nitrate concentration was 20 mgN/L, and over six months nitrate was decreased stepwise to 5 mgN/L, concurrent with stepwise increases in perchlorate concentrations from 0 to 10mg/L. With the initial 20 mgN/L nitrate, full removal was achieved and the maximum current density, relative to the cathode surface area, was 18.4 mA/m^2. With 5 mgN/L nitrate and 10 mg/L perchlorate, the effluent perchlorate was less than 1 mg/L and the maximum current density was 9.1 mA/m^2. The 5 mgN/L nitrate in the cell could only have contributed 5.1 mA/m^2. Given the lack of soluble electron donor in the medium, the extent of perchlorate reduction, and the steady improvement in perchlorate reduction after each perchlorate increase, these tests strongly suggest perchlorate-reducing bacteria can utilize the cathode as an electron donor. Ongoing tests are exploring the

performance with no nitrate, and exploring the microbial community structure of the biocathode.

A model of power production by *Geobacter* spp. at fuel cell anodes

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A model was developed to simulate electrical power production by *Geobacter* spp. using parameters derived primarily from electrochemical measurements of *G. sulfurreducens* and *G. metallireducens* colonizing carbon electrodes. The model considers a thin layer of bacteria that oxidize electron donor and transfer electrons to redox proteins at their outer membrane. The formal midpoint potential of this redox protein pool (-0.2 to 0 V) was based on cyclic voltammetry and differential pulse voltammetry observations. The actual state of this pool (oxidized vs. reduced) depended upon the rate of electron donor oxidation and the rate of electron transfer to the anode. Electrons were transferred to the anode via a direct pathway with a characteristic resistance (10^8 to 10^{11} Ω) that was estimated from electrochemical impedance spectroscopy measurements and from published data for conductive pili produced by *Geobacter* spp. The rate of mass transfer of electron donor to the microbial biofilm and Monod kinetics were also included. The model was used to simulate current, potential of bacterial and anode surfaces, and total power output over a range of applied external resistance values for a membrane electrode assembly fuel cell. Model results highlighted the importance of the outer membrane redox state and the concept of cell-to-electrode resistance. A plateau in current at low external resistance values demonstrated that the rate of electron donor oxidation by bacteria can become limiting in MFCs built to minimize internal resistances. The model will be useful for investigating factors affecting electricity production, designing novel MFCs, and interpreting experimental results.

Novel bioprocessing technologies based on microbial consortia

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Current technologies for the conversion of biomass to ethanol incorporate a biomass pretreatment step and use genetically-engineered microbes to simultaneously hydrolyze the plant biomass and ferment the soluble sugars to ethanol and other organic acids. This is a complex process whose optimization relies on the integration of process engineering, fermentation technology, enzyme engineering and metabolic engineering. In Nature, plant biomass is efficiently degraded by the concerted action of microbes with different metabolic capabilities. Such microbial consortia cooperate to degrade the plant biomass, ferment the soluble products and completely oxidize the fermentation products to return the light-driven fixed CO_2 back to the atmosphere. Fe(III)-reducing organisms such as *Geobacter* bacteria are important components of these consortia and completely oxidize fermentation products

such as H_2 and acetate to metal oxides, a process that could be harnessed to design *Geobacter*-powered fuel cells growing in consortia with biomass-degrading organisms. Our laboratory is interested in developing technologies for the conversion of biomass to ethanol using microbial fuel cells (MFCs). Here we describe the development of bioprocessing schemes using MFCs that integrate pretreated agricultural waste and biomass-degrading microbial consortia composed of a biomass-degrading and fermentative bacterium and the model electricigen *Geobacter sulfurreducens*. We show that the nature of the metabolic interaction, e.g., interspecies H_2- or organic acid- transfer, between the consortia partners as well as genetic manipulation of the metabolic capabilities of the microbial members can be used to customize the bioprocessing scheme while increasing ethanol conversion rates and the overall energetic output of the system.

Mitigation of the effect of cathode contamination in microbial fuel cells using simple designs and low cost materials

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Cathode design greatly affects microbial fuel cell (MFC) performance. Cathode contamination is inevitable in a single chamber MFC yet it is impossible to study the magnitude of this effect in that format. Therefore to study the effect of contamination at the cathode on current generation two chamber MFCs must be used. The study was performed with the cathode in two positions: completely submerged in the catholyte and raised while maintaining electrical contact, allowing wicking of the catholyte and increased exposure to air. The advantages of the two chamber MFC design used in this study include: the assembled and filled fuel cell is autoclavable and the cathode can easily be moved from the submerged to air exposed position while maintaining sterility. When the cathode was submerged and the catholyte was inoculated with *Bacillus megaterium*, *Shewanella oneidensis* or *Escherichia coli* current generation was greatly decreased as compared to sterile. As the cathodes were raised, allowing contact with the catholyte by wicking, the current rose to levels comparable with sterile cathode MFCs. The reduced performance of submerged cathodes is most likely due to the microbial culture in the cathode greatly reducing the available oxygen for completion of the cathode reaction. This shows simple designs with low cost materials can be used to mitigate effects of cathode contamination.

The effects of settling on cellulose degrading microbial fuel cells

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Microbial fuel cells (MFCs) use bacteria to oxidize organics (including waste materials) and concurrently generate electricity through the capability of some microorganisms to reduce extracellular electron acceptors. Research on MFCs has usually involved dissolved substrates, but many liquid wastes have a significant insoluble fraction, including cellulose. This feedstock is of great interest for bioenergy production, and it creates a unique situation as an electron donor in an MFC because it requires a microbial community that couples the use of an insoluble electron donor and acceptor. Single-bottle air-cathode reactors were evaluated in both settled (carrying over substrate from the previous batch cycle) and unsettled conditions. Both soluble and insoluble forms of cellulose were used in this

evaluation, as well as either a binary culture comprised of cellulolytic and anode-reducing phenotypes or a mixed culture obtained from a wastewater treatment facility. Cellulose degradation, pH, power generation, and production of byproducts were all measured to determine whether settling affected the efficiency of the fuel cell in terms of both cellulose degradation and power generation.

Comparison of acetate, glucose and xylose in microbial fuel cells with humic acid as mediator

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Power production with acetate, xylose and glucose was investigated in two-chamber microbial fuel cells (MFC) since these are the main compounds formed by hydrothermal treatment of lignocellulosic biomass. Humic acid (HA) was tested as a model compound for the hydrolysed lignin and was expected to have a mediating effect due to the phenol structure. Na-acetate resulted in the highest voltage (550-600 mV) and power density (240 mW/m^2, 24 cm^2 anode surface). Usage of glucose and xylose resulted in maximum power densities of 76 mW/m^2 and 63 mW/m^2 at a lower voltage level of 380 mV and 420 mV, respectively. With glucose and xylose as substrate the maximum power density increased versus HA-concentration to 110 mW/m^2 and 84mW/m^2, respectively at 2 g HA/l. pH decreased with glucose and xylose as substrate from 6.7 to 5 due to formation of acetic acid and propionic acid by fermentation reactions.

Effect of increasing anode surface area-to- reactor volume ratio in Microbial Fuel Cells

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The study of anode materials in Microbial fuel cells (MFC) is essential to decrease MFC cost and improve operational effectiveness. The anode side of a Microbial Fuel Cell contains bacteria under anaerobic conditions. Bacteria shuttle electrons to the anode and although it is not completely determined if the biofilm is fully responsible for the electron transport, a high anode surface area will benefit the current production since there is more space to receive electrons. Carbon graphite is the preferred anode material since it is highly conductive; fully available in different forms and textures; and cheap. In this work we studied the effect of increasing the anode surface area-to-volume ratio using carbon graphite. Experiments were performed in constructed single chamber microbial fuel cells (SCMFC) using air cathodes. MFCs had the following conditions: graphite plate, one layer of graphite pellets and several layers of graphite pellets. The MFCs were inoculated in batch mode for approximately two weeks with artificial wastewater (AW) containing anaerobic sludge as bacteria source. Measurements were taken after these two weeks of bacteria

enrichment. It resulted that the higher the surface area with pellets, the higher the current generated and therefore the columbic efficiency. The worst performances were in fact obtained for the MFCs using a graphite plate anode which has a very smooth surface and therefore the smallest surface for the biofilm. Results obtained confirmed that by improving the anode configuration, in particular the anode surface area, the MFC performance can be improved.

Hydrogen production from swine wastewater

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Hydrogen can theoretically be generated from wastewater in two ways: 1) fermentation in which methanogenic hydrogen consumption is uncoupled from hydrogen production; and 2) electrochemically assisted organic matter oxidation in a modified microbial fuel cell. Both methods were tested using wastewater from a swine facility. Uncoupling hydrogen consumption by methanogens in fermentation was attempted using several strategies including pH control, controlled hydraulic retention time, and autoclaving of wastewater with heat pretreatment of the inoculum. These measures all failed to produce practical hydrogen recoveries. In a microbial electrolysis cell (MEC), the difference in the potential created by the bacteria at the anode and the potential at the cathode is increased by applying a small voltage to allow hydrogen production at the cathode. Hydrogen produced by swine wastewater in this capacity is more efficient than the fermentation manipulation. Preliminary data indicates practical hydrogen recoveries in an MEC. In addition, considerable COD removal suggests that this system can also make significant contributions to the treatment of the wastewater.

An industrial perspective of microbial fuel cells: economic requirements and barriers to application

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Sustainable solutions in the areas of energy, water, and human health are crucial to continuing human progress and, at the same time, represent areas of significant economic growth. Microbial fuel cells (MFCs) have the potential to impact each of these areas, but in order to be implemented, they must offer a net advantage over alternatives. With the intent of stimulating discussion and future work, we will examine the economics of MFCs and their sensitivity to material costs and design parameters and present the hurdles that face commercializing this new technology.

Electrochemical dynamics of hydrogenation from treating organic wastewater in biofuel cell

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Bio-fuel cell is useful tool to convert organic matter into biohydrogenation in treatment of organic waste and wastewater. A bio-fuel cell was composed of two chambers with inserted anode and cathode containing proton permeable ionic exchange membrane. The anodic chamber as the bio-reactor was seeded with mesophilic clostridium sp., and was able to couple with cathodic chamber with a buffer solution as cathodic electrolyte. The electrochemical process was measured by using a monitoring system. The electrochemical dynamic process was investigated by the testing platform of common geometry. The critically overlooked parameters of bio-fuel cell were identified to increase hydrogenation. The amperage and voltage in the biofuel cell were recorded under variable conditions when treated the wastewater. The structure of electrode material was investigated by electronic microscopy and X-ray. The bio-fuel cell operated under normal atmospheric pressure. The mathematic model was setup by electrochemical dynamics and the differential matrix of reacted process was calculated by the finite element analysis method. It is important that the dynamics can help to understand how the system was measured and evaluated the performance.

Biofuel cell of producing power and bio-hydrogen by shifting operation from treating organic wastewater

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The technologies of bio-fuel cell are rapidly developing. In waste and wastewater treatment, bio-fuel cell can be a practical technique for the conversion of organic matter into biomass energy while biofuel cell as bio-reactors can treat domestic and organic waste and wastewater. A bio-fuel cell was composed of two chambers with inserted anode and cathode containing proton permeable ionic exchange membrane. The anodic chamber as the bio-reactor was seeded with photo-synthetic or hydrogenation bacteria, and was able to couple with cathodic chamber with a buffer solution as cathodic electrolyte. The bio-fuel cell process was controlled by using a monitoring system. The objective of the present experiment was to test the amperage and voltage production capability of an anodic chamber as bioreactor to treat wastewater coupled with a cathodic chamber, and operated under variable conditions. The mesophilic clostridium sp. was isolated from cow manure and then used as inoculum to seed the anode chamber for hydrogen production which was measured by Micro GC. The hydrogen concentration exceeded over 65 %, when steady state was reached. The structure of electrode material was investigated by electronic microscopy and X-ray. The bio-fuel cell operated under normal atmospheric pressure and generated a steady state current and voltage of 65 microampere and 175 millivolt. The system was able to function using highly concentrated organic wastewater and may have commercial utility for producing low amounts of electrical power for remote locations.

Power production by *Shewanella oneidensis* MR-1 compared to a mixed culture in four different batch microbial fuel cell configurations

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There is some debate as to whether or not pure cultures can produce more power than mixed cultures in microbial fuel cells. Power production from the facultative anaerobe *Shewanella oneidensis* MR-1 was compared to an undefined mixed culture obtained from a wastewater treatment facility in four different types of batch-fed MFC reactor configurations. Two were single chamber reactors each with a brush anode sized to the reactor volume (28ml and 300ml). One reactor was a traditional two chamber H-type reactor with a carbon paper anode and air cathode separated by a Nafion membrane. The fourth reactor configuration was similar to the two chamber reactor with the addition of a third nitrogen sparged chamber located between the anode and cathode chambers for the purpose of reducing oxygen diffusion into the anode chamber. In all cases, the mixed culture was able to produce 17 to 75% more power than *S. oneidensis* MR-1.

Microbial fuel cells: electricity from waste?

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The use of energy in waste water treatment is becoming a significant issue, with municipal water and waste treatment in the USA accounting for 1.4% of the total national electricity usage and estimated to cost in excess of $4 billion annually. Lowering electricity consumption in this area will help to reduce CO_2 emissions and offset the economic cost of waste water treatment. Research into renewable fuel technology has begun to address the issue of utilising the metabolic diversity of certain prokaryotes housed within "microbial fuel cells" as a means of electricity generation from waste. This project aims to focus upon the design, construction and optimization of microbial fuel cells initially using pure bacterial cultures and simple growth substrates. The use of bacteria such as *Shewanella oneidensis* strain MR-1 within single species microbial fuel cells has eliminated the use of expensive soluble electron "shuttle vectors" as *S. oneidensis* MR-1 can directly transfer electrons to an insoluble terminal acceptor or electrode. *Shewanella oneidensis* MR-1 is ideal as a model organism for use in this investigation due to a range of electron transfer mechanisms, a fully sequenced genome and established genetic manipulation systems. The project goals are to investigate the construction, optimisation of microbial fuel cells using a variety of model and actual waste streams. The use of environmentally derived bacterial populations within microbial fuel cells will give the opportunity to identify, evaluate and isolate key species capable of electricity generation; and subsequently optimise the consortial activities upon a range of waste substrates.

Maximizing Power Production in a Stack of Microbial Fuel Cells by Multi-Unit Optimization

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Power generation in a Microbial Fuel Cell (MFC) from renewable carbon sources is one of potential alternatives to fossil fuel utilization. Although power density in MFCs increased by

several orders of magnitude during the last decade, attainable power density of a single MFC is relatively low, in a range of 20-200 mW L_R^{-1} and the working voltage of 0.3-0.5 V. Therefore, a stack of MFCs might be required to achieve significant voltages and power outputs. In this study a recently proposed optimization method, multi-unit optimization, was used to maximize power production in a stack of two continuous-flow microbial fuel cells (MFCs). Multi-unit optimization uses multiple units to optimize process performance. The main advantage of the multi-unit optimization algorithm is its fast convergence toward the optimum in comparison with other types of optimization algorithms, such as maximum power point tracking (MPPT), which are based on temporal perturbations. Two single chamber air-cathode MFCs equipped with carbon felt anodes and gas diffusion cathodes were used in the experiment. External loads of these MFCs were controlled on-line using a data acquisition and control system based on the multi-unit optimization algorithm. Optimal external resistance of 35-40 Ω with a corresponding power density of 69-84 mW L_A^{-1} (A=anode volume) was achieved within the first hour of the test. Also, stable MFC performance during external perturbations (i.e. temperature variations) has been demonstrated.

Clone library analysis of anodic microflora in MFCs enriched from methanogenic sludge and paddy soil at various temperature

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The recovery of bio-energy from waste biomass has attracted much attention in promoting utilization of biomass. Mediator-less MFC (microbial fuel cell) has been expected as a next-generation technology to efficiently generate "electricity" from waste biomass as one of most convenient energy. This study analyzed microbial population of electricity-generating microflora that was enriched from thermophilic methanogenic sludge and paddy soil under mesophilic and thermophilic conditions. The electricity-generating microflora was enriched in the air-cathode type MFC, in which 400 ohm of loading resistance was connected between anode and cathode. Unwoven carbon fiber cloth was used as an anode material. Artificial wastewater used contained starch as a carbon and electron source. The electricity-generation and the microbial diversity were investigated within the temperature range of 25 – 65 degree of centigrade. During 40 days of batch experiment, a certain amount of electricity generation was observed in all enrichment cultures accumulated from both methanogenic sludge and paddy soil. There was no significant difference of electricity output between mesophilic and thermophilic conditions. In the PCR-DGGE analysis targeting on 16SrDNA, it was observed that wide variety of microorganisms were enriched on anode surface regardless of the temperature. Clone library analysis showed that these microorganisms were classified into several phylogenetically different microbial groups. It is suggested that various kinds of microorganisms universally existing in natural habitat could be involved in the electricity generation. This work was supported by New Energy and Industrial Technology Development Organization (NEDO), Japan.

Use of high area carbon particles and conducting polymers for the direct electron transfer and power enhancement in microbial fuel cells

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In recent years, increasing efforts have been made to develop mediator-less microbial fuel cells (MFCs), which electrochemically active bacteria are used as catalyst in anodic compartment. Mediator-less MFCs have longer lifetime and are more practical than mediator-type MFCs. Only few bacteria such as *Shewanella putrefaciens* and *Geobacter sulfurreducens* have been used for this purpose. Electrons can be directly transferred to the electrode through cytochromes localized in the outer membrane. In this presentation, however, we show a direct electron transfer could be possible although non-electroactive microorganism is used. We used constructed mediator-less MFCs using *Proteous vulgaris*, which is not classified as electrochemically active bacteria family. Under ordinary conditions, electrons cannot be transferred from the inside of bacteria to the electrode. To make direct electrons transfer possible, we mixed *P. vulgaris* with high area carbon (Vulcan X-72) to form bacteria-containing carbon paste and spread it onto carbon cloth, an anode. This way we could generate ca. 268 mW m^{-2} while virtually no electricity was produced when *P. vulgaris* was suspended in solution or made as a biofilm. This result indicates carbon black can function as electron transfer relay that makes direct electron transfer possible. In the case of mediator-type MFC, a dramatic power enhancement has been achieved when electropolymerized polypyrrole(Ppy) was deposited onto the electrode. With Ppy-coated the reticulated vitreous carbon (RVC), maximum power density of 1.2 mW cm^{-3} was obtained. Effects of other variables such as ionic strength, kinds of mediators, and amount of bacteria have also been examined.

Comparison between Lead Dioxide and Platinum Based Carbon Cathodic Electrodes in a Microbial Fuel Cell for Sulfide Removal

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Sulfide is ubiquitously present in organic wastewater and difficult to treat due its toxicity. Microbial fuel cell (MFC) technologies were demonstrated to be a feasible approach for sulfide removal in this study. Further more, Lead dioxide (PbO_2) and platinum based carbon (Pt/C) cathodic electrodes were compared in an H-type microbial fuel cell (MFC) utilizing sulfide and glucose as electron donors in the anode compartment. Contrast was drawn from several aspects such as power generation and contaminant removal under variously initial sulfide concentrations (from 0 mg/L to 100 mg/L with 20 mg/L as a step). PbO_2 cathodic electrode showed a relative advantage to Pt/C one. The maximum open circuit voltage was 1.254±0.060 V for PbO_2 and 0.797±0.054 V for Pt/C. sulfide oxidation in the anode resulted in the maximum power density of 1051.67±320.20 mW/m^2 cathodic electrode for the former and 127.53±40.65 mW/m^2 cathodic electrode for the latter. Sulfide removal rate of 88.15±4.37% and 80.32±10.57% were achieved respectively for the two cathodic electrodes during the 48 h operation, correspondingly COD removal rate of 15.20±7.45% and 13.91±7.54%, singly. Long-term operation narrowed the discrepancy between the two electrodes. It appeared that MFCs with PbO_2 cathodic electrode could be scaled up to treat sulfide containing wastewater successfully with electricity recovery when its stability was further improved.

Organism selection in MFC application using A CASCADE system (computer-assisted strain construction and development engineering)

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CASCADE is an *in silico* screening platform for discovering predictive relations between genomic fingerprints of organisms and their metabolic capabilities using a machine learning system. We obtained genomic information, along with experimental or logic data and link them with the metabolic efficiencies of diversified organisms. The relations were used to select smart metabolic-efficient microorganisms for the applications that leverage microorganisms' metabolic capabilities to metabolize biomass to produce renewable energy in the Microbial Fuel Cells (MFC). The distribution of gene usages in 137 metabolic function categories of 503 organisms was compiled, and used to learn across multiple organisms to extract the correlation patterns from the genetic make-ups and electrogenic properties of the organisms. A predictive model based on clusters generated from the metabolic function categories was built. Electrogenic organisms selected from the model were tested and the experiment results are consistent with the predictions where two out of three, two out of two and none out of one organisms tested in clusters containing the highest number, the second highest number and the least number of electrogenic organisms predicted respectively are experimentally shown to be electrogenic. We found seven metabolic pathways that might contribute to either 'Peak High' or 'Last Long' for power output in our experimental setting where "Fructose and Mannose Metabolism High" category appears much higher (34.33%) in the total 67 electrogenic organisms than in the population as a whole (13.87%) and the category "One Carbon Pool by Folate High" appears 26.87% in the electrogenic organisms compared to 10.97% in the population as a whole. Therefore they may be important characteristics for being electrogenic.

Electricity generation using a non-membrane upflow microbial fuel cell without catalysts on the cathode

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A non-membrane Upflow Microbial Fuel Cell (NM-UMFC) system with smashed sponge replacing the conventional PEM was developed and investigated in this paper. The glucose solution with COD concentration of 100~200 mg/l was continuously fed as the substrate, and without any metal catalyst on the cathode. The results showed that: 1) When the carbon-felt was used as the anode, no obvious plorization was occurred on the anode, and the anodic operational potential was -280 ± 20 mV, the anodic resistance was 19 ± 4 ohm. 2) Several kinds of carbon materials included carbon-paper contained platinum as catalyst, carbon-paper, carbon-felt, activated carbon fiber were used as the cathode. The cathodic potential in open-circuit state were 476 ± 2 mV, 129 ± 2 mV, 309 ± 2 mV, 397 ± 8 mV, respectively. The cathodic resistance were 154 ± 2 ohm, 204 ± 2 ohm, 164 ± 2 ohm, 48 ± 2 ohm, respectively. 3) When using activated carbon fiber as the cathode, the digested sludge from a municipal wastewater treatment plant was used as the inocula in the anode chamber, after 5 days' starting-up, the NM-UMFC could reach to a stable operational state, and during the next one week's operation, the anode surface power density was 103 ± 3 mw/m^2 and the volume power density was 822 ± 20 mw/m^3.