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Effect of Inoculum Types on Bacterial Adhesion and Power Production in Microbial Fuel Cells

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Abstract Microbial fuel cell (MFC) is an emerging biotechnology to convert the organic substrates in wastewater to electricity by anaerobic electrogenic bacteria. The main challenge for MFC research is to elucidate the fundamental mechanisms of electron generation and transfer and to apply these mechanisms to improve the power production in the engineering operation. This study extensively investigated the effects of three inocula (*Geobacter sulfurreducens*, soil, and wastewater) on the power production and electrochemical characteristics (i.e., internal resistances, Coulombic Efficiency) of MFCs. The results showed that the extents of bacterial adhesion varied between mixed cultures (soil) and pure cultures (*G. sulfurreducens*). The voltage output increased 30% when bacterial adhesion was well-developed in the soil inocula. Meanwhile, the inoculum types clearly affected the internal resistance (R_{in}) and power production of MFCs. Pure culture inoculum (*G. sulfurreducens*) had the lowest R_{in} (165 Ω) and the highest Coulombic Efficiency (CE, 25.8%) and Energy Conversion Efficiency (ECE, 7.2%), while the mixed culture inocula (soil) with the high concentration of nonelectrogenic bacteria, exhibited the highest R_{in} (620 Ω), lowest CE (9.2%) and lowest ECE (2.4%). Additionally, a second-order correlation was established between the anode potential (P_A) and power output while an exponential correlation was established between the difference between anode and cathode potentials (ΔP_{C-A}) and power output.

Keywords Microbial fuel cells (MFCs) · Bacterial adhesion · Internal resistance · Redox potential (ORP) · Open circuit potential (OCP) · Coulombic efficiency (CE) · Energy conversion efficiency (ECE)

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Introduction

Microbial fuel cell (MFC) is an emerging biotechnology to harvest energy from wastewater. Utilizing anaerobic electrogenic bacteria to generate electrons during the degradation of chemical oxygen demand (COD) in wastewater, MFCs can simultaneously achieve the COD removal and the electricity generation [1–3]. Traditional MFCs consist of anaerobic anode and aerobic cathode compartments. In the anode compartment, anaerobic bacteria produce electrons from the degradation of organic substrates (e.g., acetate, glucose, and wastewater). Electric power is harvested as electrons flow through an external circuit to react with the electron acceptor (e.g., oxygen, ferricyanide) in the cathode compartment. Compared with the methane production in anaerobic sludge digestion processes, MFCs directly convert the chemical energy stored in wastewater into electricity, which is easier and safer to transport and store. In the past several years, a tremendous effort has been made to improve the power generation in the lab-scale MFC studies. Novel MFC configurations (e.g., single-chamber MFC, upflow MFC) [4, 5], electrode materials (e.g., graphite fiber, polymer coated electrode) [5–7], and engineering operational conditions (e.g., substrate concentration, temperature, electrode spacing) [8–10] have been extensively studied in order to increase the power generation in MFCs. The power density of MFCs has increased from 0.1 mW/m^2 to over 1,000 W/m^3 in microscale MFCs [32], although it remains in question whether a satisfactorily high power can be attained in large scale reactors.

As a biofilm-based process, the power generation in MFCs is affected by the interactions between bacterial cells and electrode surfaces [3, 11, 33]. Electrons generated from the COD removal in the bulk solution need to be transferred to electrode surfaces. Forming biofilms on electrode surfaces is an effective way to achieve electron transfer [11]. Biofilms as thick as 40–50 μm have been observed on the surface of carbon electrodes in MFCs [11]. Theoretically, higher extents of bacterial adhesion enable the transfer of more electrons from bacterial cells to electrodes. However, recent studies showed that several bacterial species such as *Geobacter sulfurreducens* PCA [12], *Shewanella oneidensis* MR-1 [13], and *Synechocystis* PCC6803 [13] were able to transfer electrons to a distant electron acceptor [12, 13]. This raises a question if whether the suspended bacteria in bulk solution contribute to electron transfer in MFCs. In order to enhance the power production of MFCs, it is critical to explicate the roles of bacterial adhesion in the electron transfer processes.

Due to the high cost, pure cultures are impractical for the real-world engineering operation. Mixed cultures (i.e., soil, wastewater) containing significant amounts of electrogenic bacteria can be used as the cost-effective inocula for MFCs. However, the nonelectrogenic bacteria (i.e., methanogenic bacteria, denitrifying bacteria) in mixed cultures consume organic substrates without generating electricity [14, 15]. Therefore, it is important to determine the difference between pure cultures and mixed cultures in terms of the effects on power generation and electronic characteristics of MFCs (i.e., internal resistance, coulombic efficiency, energy conversion efficiency).

One major effect of inoculum type on MFCs is the biofilm formation on electrodes. In the pure culture inocula, the biofilms are consisted of electrogenic bacteria, while in the mixed-culture inocula, a potion of bacteria in biofilms are nonelectrogenic bacteria [14]. The growth of nonelectrogenic bacteria assimilates substrates, increases the electron transfer resistances across the multilayered biofilms and, thus, reduce the overall power generation in MFCs. In order to enhance the power production of MFCs ultimately, it is critical to explicate the roles of inoculum type on the bacterial adhesion and electron transfer processes. Another effect of inoculum type on MFC is the internal resistance (R_{in}). An MFC with a low R_{in} has a low internal power loss and a high power output. Most studies have focused on the physical aspect of R_{in}, including MFC configuration, electrode material and operational conditions [4, 6, 9]. In fact, as a bioelectrochemical system, the R_{in} of MFCs may be well-related with the bacterial activities and inoculum types. It is critical to elucidate the physical and biochemical aspects of Rin in order to reduce the power loss inside MFCs and improve the overall power generation of MFCs.

There were three objectives in this study. First, the roles of bacterial adhesion in the power generation of MFCs was comprehensively investigated by comparing the power generation of bacteria attached on electrodes and bacteria growing in solutions. Second, three inocula (*G. sulfurreducens*, soil, and wastewater) with various populations of electrogenic bacteria were compared in order to elucidate the effects of inoculum types on R_{in} and power production. Third, the correlations between power production and electrochemical characteristics (e.g., electrode potentials, solution redox potentials) of MFCs were explored.

Materials and Methods

Microbial Fuel Cells

Two-compartment MFCs with a working volume of 100 mL were used in this study. MFCs consist of an anode compartment and a cathode compartment separated by a proton exchange membrane (PEM, Nafion 117, Ion power Inc.) and connected by an external circuit. The non-wet-proofing carbon cloth (E-TEK B1B, Somerset, NJ, USA) was used as anodes, and 30% wet-proofing carbon cloth (E-TEK B1A, Somerset, NJ, USA) was used as cathodes. The geometric area of the anode and cathodes was 6 cm^2. The area of PEM was 6 cm^2. The distance between the electrodes was 10 cm. The external resistance ($R_{ext.}$) of the MFCs was 1,000 Ω. The MFC tests were conducted in a 30 °C temperature control room. The voltage over R_{ext} was recorded by Keithley 2700 data logging system at 2-h time intervals.

The anode solution (1 L containing 0.31 g KCl, 0.13 g NaCl, 1 g sodium acetate (NaAc), 12.5 mL vitamin solution, 12.5 mL mineral supplement solution, 0.5 mL 0.1% resazurin, and 1 mM L-cysteine) was used to provide sufficient nutrients and organic carbon to the anaerobic bacteria in the anode chamber. The COD of the anode solution was initially set at 1,000 mg/L. The pH was maintained at 6.8–7.0 in the anode chamber by the phosphate buffer (5.84 g/L $NaH_2PO_4 \cdot H_2O$ and 15.47 g/L $Na_2HPO_4 \cdot 7H_2O$). The cathode solution contains 40 g/L potassium ferricyanide, which accepts the electrons and is reduced to ferrocyanide.

Inocula and Growth Medium

Three types of inocula—pure cell culture (*G. sulfurreducens*, *strain PCA* [16]) and mixed cultures (soil and domestic wastewater)—were examined in order to investigate the effects of electrogenic and nonelectrogenic bacteria on bacterial adhesion and power production of MFCs. *G. sulfurreducens* (strain PCA unless indicated otherwise) were obtained from American Type Culture Collection (ATCC) and cultured in the standard growth medium (ingredients of the growth medium: NH_4Cl 1.5 g/L, NaH_2PO_4 0.6 g/L, KCl 0.1 g/L, $NaHCO_3$ 2.5 g/L, NaAc 0.82 g/L, sodium fumarate 8.0 g/L, Wolfe's mineral solution

10 ml/L, and vitamin solution 10 ml/L) in anaerobic condition at 30 °C. The soil was collected at the University of Connecticut (UConn) Organic Farm (samples were taken at 10 cm below the ground surface), and the domestic wastewater was the influent at the UConn Wastewater Treatment Plant.

The three inocula had distinctly different features in terms of the populations of electricity-producing bacteria. *G. sulfurreducens* is a typical type of anaerobic electrochemically active bacteria broadly present in anaerobic sediment, soil, and activated sludge [1, 16, 17]. The soil taken at UCONN organic farm was previously proved to have a high concentration of methane- and hydrogen-producing bacteria. Wastewater inocula also contain a variety of electrogenic bacteria and nonelectrogenic bacteria [15, 31].

Internal Resistances and Electrode Potentials of MFCs

The internal resistance (R_{in}) is the resistance existing within the MFCs. It consumes the power generated by the MFCs and, thus, lowers the power generation efficiency. The R_{in} of the MFCs was measured by polarization curves. The external resistors (R_{ext}) were changed from 46 to 1,500 Ω during the measurement, and the voltage on each R_{ext} was recorded by a multimeter. The power output (P) generated was calculated according to $P=V^2/R$ (V is the voltage, R is the R_{ext}) and plotted with respect to R_{ext}. When R_{ext} equaled R_{in}, the power output reached the maximum value (P_{max}) [18].

The potentials of anodes and cathodes in MFCs were measured using a potentiostat (Gamry Reference 600), with the target electrode (anode or cathode) as the working pole, and an Ag/AgCl reference electrode as the counter pole and the reference pole. The open circuit potential (OCP), the maximum potential that a MFC can generate [14], was measured as the potential difference between anodes and cathodes in the open circuit using a potentiostat.

The Coulombic Efficiency and Energy Conversion Efficiency of MFCs

The coulombic efficiency (CE) and energy conversion efficiency (ECE) are the critical criteria to evaluate the MFC performance. The electricity or energy produced in MFCs must be high enough in order to justify the full-scale application in wastewater treatment. The CE is defined as the ratio of the actual charge generated to the theoretical charge generated if the substrate is completely converted to electricity. The calculation was based on Eq. 1 [3], where I is the current, ΔCOD is the COD change in the anode solution, v is the volume of the anode chamber, 4 is the moles of electrons transferred when 1 mol of oxygen is oxidized, 32 g/mol is the molecular weight of oxygen, and F is the Faraday constant (96485).

$$\varepsilon = \frac{\int_0^t I dt}{\Delta COD \frac{4}{32} vF} \tag{1}$$

The ECE is defined as the following two ratios. The first is the ratio of the power produced to the theoretical heat (termed as η_1), where the theoretical heat was calculated based on the heat obtained through the combustion of the defined organic substance (e.g. acetate, glucose) [3]. The second is the ratio of the power produced to the mass of organic substances consumed (termed as η_2), where the mass of organic substances consumed was calculated on basis of the COD change during the MFC operation. These two ratios were calculated based on Eqs. 2 and 3, where V is the voltage in Volts, I is the current in Amps, t

is the time in seconds, *m* is the mass of acetic acid that participates in the reaction in grams, *M* is the molecular weight of the substrate (acetic acid in this study, 60 g/mol), and ΔH is the enthalpy change when the organic substance is completely combusted to CO_2 and H_2O. In the acetate-fed MFCs, the theoretical heat is calculated based on the enthalpy change of acetic acid in combustion (ΔH is 874.5 KJ/mol).

$$\eta_1 = \frac{\int_0^t VIdt}{\Delta H \frac{m}{M}} \tag{2}$$

$$\eta_2 = \frac{\int_0^t VIdt}{\Delta COD} \tag{3}$$

Effect of Temperature on the Power Generation in MFCs

Anaerobic bacterial activities are critically affected by temperatures [19, 20], especially electrogenic bacteria [21]. Therefore, temperature was expected to have an impact on the power generation in MFCs. In this study, a set of MFCs inoculated with 1 g/L soil was started and operated at 15 °C for 2 cycles and then operated at 30 °C to determine the effects of temperature on the performance of MFCs.

Bacterial Adhesion in MFCs

The effects of bacterial adhesion on power production were examined in parallel tests by comparing the power production of bacteria attached on anodes and the bacteria suspended in anode solutions. When testing the power production of bacteria attached on anodes, the anodes with the attached bacteria were removed from a well-working MFC and then placed into a new MFC with fresh anode solution. The power production in the new MFC was considered as the contribution of the attached bacteria. When testing the power production of bacteria suspended in anode solution, the well-grown bacterial anode solution in a well-working MFC was filled into a new MFC with a new anode. The power production in the new MFC was considered as the contribution of the suspended bacterial in anode solution.

Two types of inocula—10 g soil/L growth media and 100 ml pure *G. sulfurreducens* solution/L grow media—were compared for the extents of bacterial adhesion. MFCs were operated for multiple cycles to obtain stable conditions. One cycle was termed as the time from inoculation to the peak voltage and later to the depletion of substrates. In this study, the duration of 1 cycle was about 1 week.

Quantification of Bacterial Adhesion by Quartz Crystal Microbalance

The Quartz Crystal Microbalance (QCM, Maxtek Inc), a high-resolution mass sensing technique, was used to measure the biomass change on electrode surfaces. QCM has been used in the quantitative studies of biofilm formation on solid surfaces [22, 30]. In the QCM tests, the bacterial solution (*G. sulfurreducens*) was continuously pumped into the flow chamber (volume 100 μL) of the QCM. The bottom of the flow chamber was the electrode. The frequency change of the electrode surface was measured and correlated with the biomass change by the software (EC-Lab) in a real time mode.

Scanning Electron Microscopy Observation of Bacterial Adhesion

Bacteria adhesion on the anode surfaces was observed using a scanning electron microscopy (SEM; Model: Joel 6335F). A small portion of anodes with attached bacteria (5×5 mm, small enough not to affect the electricity generation) was cut off and fixed for 12 h at 4 °C in a mixed solution containing 2.5% paraformaldehyde,1.5% glutaraldehyde, and 0.1 M cocadylate buffer (pH 7.4). The sample was then washed three times with cocadylate buffer before dehydrated in a series of ethanol/water solution (the volume ratios of ethanol are 25%, 50%, 70%, 85%, 95%, 100%, each step took 15 min) [4]. The sample was completely dried in an anaerobic environment at 30 °C and sputtered with gold at 2.2 kV and 10 mA for 2 min.

The Optical Density of Anode Solution in MFCs

Bacterial growth is affected by substrate concentrations [4, 23]. Sufficient substrates are able to increase the power production of MFCs while excessive substrates may, however, trigger the growth of nonelectrogenic bacteria and lower the power production efficiency. Wastewater-seeded MFCs were operated at three COD concentrations (100, 200, and 500 mg/L) to compare the effect of substrate concentration on bacterial growth and power generation of MFCs. One milliliter of anode solution adjacent to anode surfaces was taken during the operational period of MFCs to measure the optical density using a UV-1700 spectrophotometer (Shimadzu Inc., Japan) following the standard procedure.

Water Quality Measurements in MFCs

The COD of the anode solution was measured with HACH high range (0–1,500 mg/L) COD vials and DR 220 spectroscopy (HACH, Loverland, CO, USA). The redox potential (ORP) and pH of the anode solution was measured with Accumet AP72 ORP meter and Accumet pH meter biweekly.

Results and Discussion

Electricity Production of Attached Bacteria and Suspended Bacteria in MFCs

Bacterial adhesion to anode surfaces affected the power production in soil-seeded MFCs and *G. sulfurreducens*-seeded MFCs at different extents. Bacteria adhesion played the major role for power production in *G. sulfurreducens*-seeded MFCs, while the suspended bacteria in anode solution were responsible for power production in soil-seeded MFCs (Fig. 1). In the *G. sulfurreducens*-seeded MFCs, the attached bacteria on anode generated nearly 600 mV after being inoculated into new MFCs, while the bacteria suspended in anode solutions generated less than 50 mV. In contrast, in soil-seeded MFCs, the attached bacteria on anode only generated less than 100 mV, while the bacteria suspended in solution generated 400 mV (Fig. 1). The power production of these two inocula was agreeable with the SEM images. Anodes taken from *G. sulfurreducens*-seeded MFCs exhibited a high extent of bacterial adhesion on the surfaces (Fig. 2a), while anodes taken from soil-seeded MFCs had a low extent of bacterial adhesion on the surfaces (Fig. 2c).

These results demonstrated that the attached bacteria on anode surfaces are efficient at power production in the pure-culture inocula (i.e., *G. sulfurreducens* in the study), while the

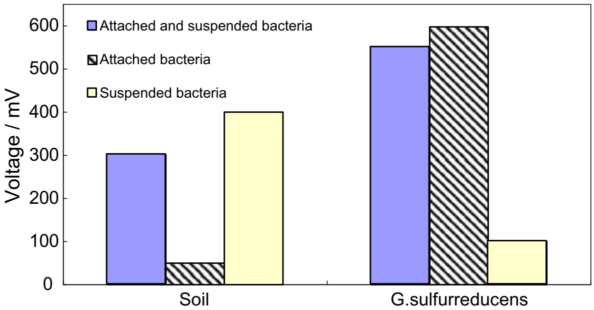
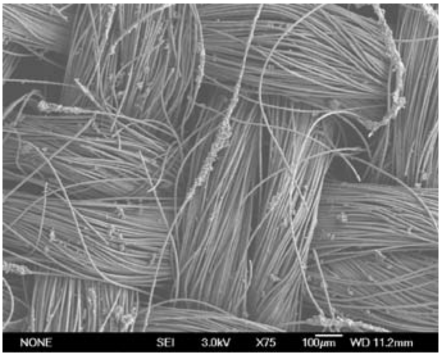
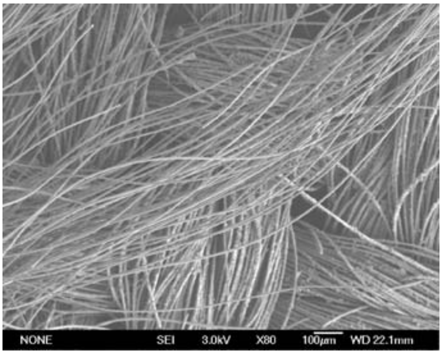


Fig. 1 The voltage production of MFCs with both bacteria-attached anode and bacterial suspension solution, MFCs with the bacteria attached on anodes, and MFCs with the bacteria suspended in solution (voltage measured on the seventh day of the operation)

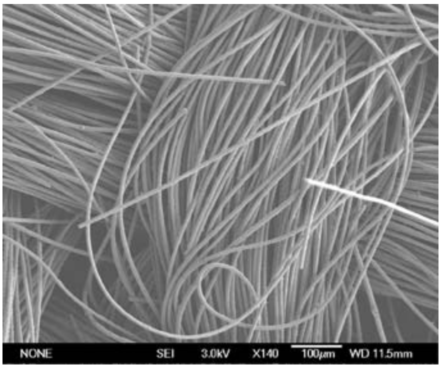
suspended bacteria in solution are efficient at power production in mixed-culture inocula (i.e., soil in this study). The possible reason is that the mixed-culture inocula contain bacterial species capable of generating electrons without attaching on the electrode surfaces. Several findings verified the existence of the bacterial species such as *G. sulfurreducens* PCA [12], *S. oneidensis* MR-1 [13], and *Synechocystis* PCC6803 [13] in



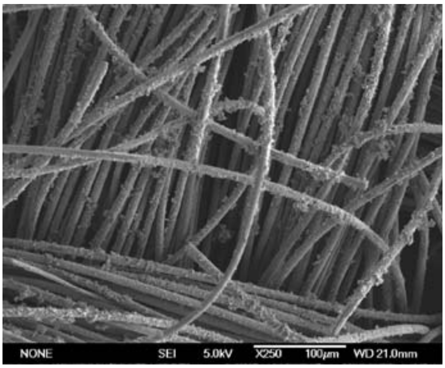
a. Bacteria-attached anode in *G.sulfurreducens* -seeded MFCs in the 1st week



b. Anode in bacterial suspension in *G..sulfurreducens*-seeded MFCs in the 2nd week



c. Bacteria-attached anode in soil-seeded MFCs in the 1st week



d. Anode in bacterial suspension in soil-seeded MFCs in the 2nd week

Fig. 2 The anodes with attached bacteria and the anodes in bacterial suspension in *Geobacter sulfurreducens*-seeded and soil-seeded MFCs mixed-culture inocula. These bacteria possess conductive surface appendages that can transfer electrons to electron acceptors from a distance (40–50 μm) [11].

Another finding was that bacterial adhesion in soil-seeded MFCs was slower than in *G. sulfurreducens*-seeded MFCs. After operating for 1 week, *G. sulfurreducens* developed good biofilms on anodes (Fig. 2a), while soil exhibited poor adhesion on anodes (Fig. 2c). However, soil also developed good adhesion on anodes (Fig. 2d). There are two possible reasons for the slow bacterial adhesion in the soil-seeded MFCs. First, compared with the pure cultures in which bacterial cells freely float in solutions, a portion of bacteria cells were adsorbed on soil particles, which delayed the release of bacterial cells into the solutions in the soil-seeded MFCs and hindered the bacterial adhesion to electrode surfaces. Second, soil contains a variety of nonelectrogenic bacteria that compete with electrogenic bacteria for the space on the electrode surfaces, which slowed down the bacterial adhesion process and power production. The microbial community analysis verified the existence of nonelectrogenic bacterial species in the anode biofilms [14].

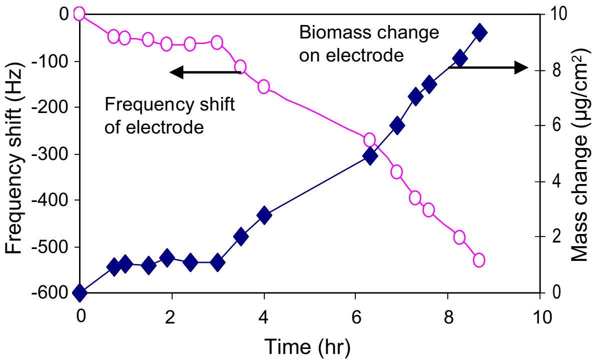
To demonstrate the fast adhesion rate of *G. sulfurreducens*, the QCM technique was used to measure the frequency (Hz) shift of an electrode and the biomass change (μg/cm^2) on the electrode in a real-time mode during the adhesion period. In less than 10 h after inoculated with bacterial cells, the frequency of the electrode decreased to −550 Hz (Fig. 3). The biomass change calculated based on the frequency shift showed that the biomass rapidly increased to 9.3 μg/cm^2 during the process, indicating the fast adhesion of *G. sulfurreducens* to the electrode surface.

Although soil-seeded MFCs produced power without sufficient amounts of attached bacteria on anodes, a good extent of bacterial adhesion was necessary for higher power generation. In soil MFCs, the voltage was 300 mV in the first week when the bacterial adhesion was not well developed, and the voltage increased to 401 mV after two more weeks when the bacterial adhesion was well developed on anodes (Fig. 1). With higher extents of bacterial adhesion, more electrogenic bacteria were in contact with the anodes. Thereby, the electrons generated by these bacteria could be directly transported to the anodes, which had a much lower activation resistance than the electron transfer from solution [3].

Effects of Internal Resistances on Power Production

The existence of nonelectrogenic bacteria (i.e., methanogens) in mixed-culture inocula consumed substrates without generating electricity, which may increase the internal resistance (R_{in}) and lower the power generation. This study verified that the R_{in} of MFCs

Fig. 3 The frequency shift or mass change of the QCM electrode in *G. sulfurreducens* solution during bacterial adhesion period



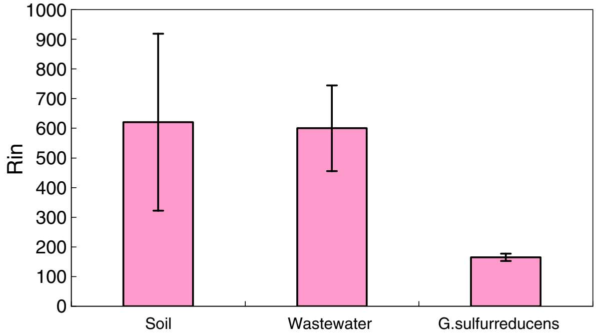


Fig. 4 The internal resistances (R_{in}) of MFCs with different inocula

was reversely correlated with the population of nonelectrogenic bacteria (Fig. 4). Pure *Geobacter*-seeded MFCs had the lowest R_{in} (165±12 Ω). Mixed-culture-seeded MFCs had nonelectrogenic bacteria, which led to higher R_{in}. The R_{in} of wastewater-seeded MFCs was 600±298 Ω, and R_{in} of soil-seeded MFCs was 620±144 Ω. The R_{in} of MFCs consists of two components: the electrolyte ohmic loss caused by the movement of ions through the electrolyte and the electrode ohmic loss caused by the movement of electrons through the electrode and wires [3, 33]. The electrolyte ohmic loss is mainly affected by the electron travel distance and the resistance of PEM. Because the MFCs tested in this study had the same electrode distance, dimensions, PEM, and electrode materials, the electrolyte ohmic loss should be the same for all the MFCs tested. Thereby, the difference in R_{in} of MFCs in this study was caused by the electrode ohmic loss related with bacterial adhesion and bacterial activity. The diverse microbial communities in soil inocula led to the adhesion of non-electronic bacterial species on the anode surfaces [3] and increased the resistances of electron transfer through the biofilms.

Unlike the previous results that R_{in} was reversely correlated with the power production [4, 5], there was no clear correlation between R_{in} and power density in this study. High power (535 mW/m^2) was generated at high R_{in} (1,000 Ohm), and low power (2.7 mW/m^2) was generated at low R_{in} (180 Ohm; Fig. 5). The power density at the same R_{in} also varied greatly. For instance, the average power density at the R_{in} of 200 Ω ranged from 3 to 1,300 mW/m^2. Moreover, the majority of MFCs (16 of 22 tested) had R_{in} of 180–600 Ω, which was much lower than the reported R_{in} values (>1,000 Ω) of two-chamber MFCs [10, 24, 25]. There were two reasons for the lower R_{in} in this study. First, the area ratio of the PEM to the anode was 1.0 in this study, which was much higher than those (1/8) in the

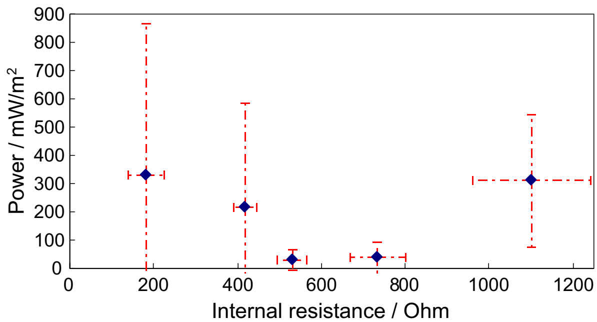


Fig. 5 The variation of internal resistance (R_{in}) and power density in MFCs

study of [10]. This higher ratio enabled a fast transfer of protons and electrons between anode and cathode chambers and lowered the R_{in}. Second, ferricyanide used in this study as the electron acceptor has a higher redox potential than oxygen used in the studies of [24] and [25], which accelerated the electron transfer from anode to cathode (Table 1).

In addition, the R_{in} of MFCs decreased with the increase of temperature, the R_{in} of MFCs operated at 15 °C was 100 Ω higher than at 30 °C, and the voltage was only 1/8 of that at 30 °C (Table 2). This demonstrates that R_{in} is affected by bacterial activities. It has been found that a high metabolic rate leads to a high electron-generating rate and a high power production [14, 33]. Temperature is well known to affect the metabolic activity of anaerobic bacteria [19, 20], especially electrogenic bacteria [21]. The tests verified that higher temperatures increased the bacterial activity, which in return enhanced the power output and lowered R_{in}.

Effects of Electrode Potentials, Solution Redox Potentials on Power Production

The anode potential of MFCs was usually below −300 mV [14]. It has been known that a lower anode potential and a higher cathode potential are necessary for higher power production. However, there is no quantitative correlation between the electrode potential and the power production of MFCs. In this study, the anode potential, cathode potential, and anode ORP were measured and correlated with the power production. The results showed that there was a second-order correlation between the anode potential (termed as P_A) and the power production (Fig. 6). MFCs with negative anode potentials (<−400 mV) corresponded with high power outputs (>0.4 W/m^2), while MFCs with positive anode potentials (>−300 mV) corresponded with low power outputs (<0.2 W/m^2). This reverse correlation can be explained by the characteristics of the electron producing processes occurring in anode chambers. The biochemical reactions taking place at the anode are affected by the bacterial metabolic rates [33]. A higher bacterial metabolic rate results in a higher electron production rate and a lower anode potential.

The difference between the cathode potential and the anode potential (termed as $\Delta P_{C\text{-}A}$) exhibited an exponential correlation with the power production (Fig. 7). Since the voltage of MFCs is determined by the difference between anode and cathode potentials, a high value of $\Delta P_{C\text{-}A}$ corresponded with a high power output. When $\Delta P_{C\text{-}A}$ was over 700 mV, the power density of MFC was 0.5 W/m^2. When $\Delta P_{C\text{-}A}$ was below 100 mV, the power density attained was less than 0.02 W/m^2.

Table 1 The electrochemical characteristics of MFCs seeded with *Geobacter sulfurreducens*, wastewater, and soil.

	G..sulfurreducens-seeded MFCs	Wastewater-seeded MFCs	Soil-seeded MFCs
R_{in} (Ω)	150	400	1,200
Open circuit potential (OCP, mV)	625	785	684
COD removal (%)	79	85	82
Coulombic efficiency (CE, %)	25.8	20.5	9.2
Energy conversion efficiency (η_1, %)	7.2	5.2	2.4
Energy conversion efficiency (η_2, J/g COD)	1045	759	343
Maximum power on R_{ext} (P_{max}, mW)	0.60	0.47	0.10

Only the MFC with the highest power output in each inoculum is shown

Table 2 The voltage output and internal resistance (R_{in}) of MFCs at 15 and 30 °C.

	15 °C	30 °C
Voltage (mV)	30	250
R_{in} (Ω)	800	700
Power (mW)	0.9	62.5

In contrast to P_A and $\Delta P_{C\text{-}A,}$ the cathode potentials (P_C) were fairly constant and were not correlated with power production (data not shown). The values of P_C ranged at 200–300 mV in all MFCs tested, which was probably due to the cathode reaction of ferricyanide. Unlike the anaerobic biochemical reactions in anode chambers, the cathode reactions are pure electrochemical reactions, and the cathode potential was consistent at a fixed ferricyanide concentration (40 g/L in this study).

The ORPs of anode solution varied widely from −210 to 250 mV (Fig. 8). The power production was low at positive ORPs. The power density was less than 0.02 W/m^2 when the ORP was higher than −30 mV. This could be explained by the presence of electron acceptors (i.e., oxygen, nitrate) at high ORPs, which inhibited the activity of anaerobic electrogenic bacteria [26–29]. On the other hand, a negative ORP did not necessarily lead to a high power output. At ORPs lower than −100 mV, the power density ranged from 0.06 to 0.52 W/m^2. The possible reason was that low ORPs (<−100 mV) were suitable for both electrogenic and nonelectrogenic bacteria. In some cases, nonelectrogenic anaerobic bacteria (i.e. methanogenic, acidogenic bacteria) outcompeted the electrogenic bacteria in the MFCs with mixed cultures inoculums [15, 31], which resulted in a low power production.

Effects of the Inoculum Types on the MFC Performance

MFCs with three types of inocula (*G. sulfurreducens*, soil, and wastewater) were compared in order to elucidate the effects of the inoculum types on the electrochemical characteristics including OCP, R_{in}, CE, ECE, and maximum power density (P_{max}). The MFCs with the highest power production in each inoculum were compared in Table 1.

A significant difference in OCP was observed among MFCs with three inocula, with wastewater having the highest OCP (785 mV), soil having the second highest OCP (684 mV), and *G. sulfurreducens* having the lowest OCP (625 mV; Table 1). The possible

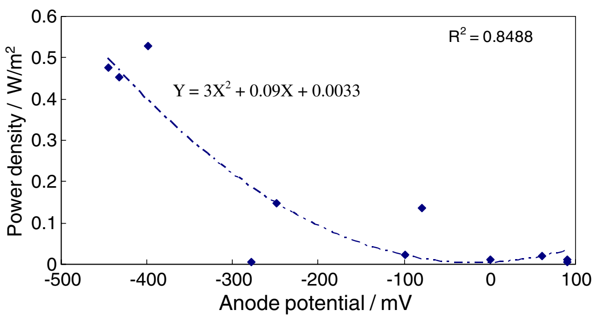


Fig. 6 The variation of power density with anode potential (P_A) in MFCs

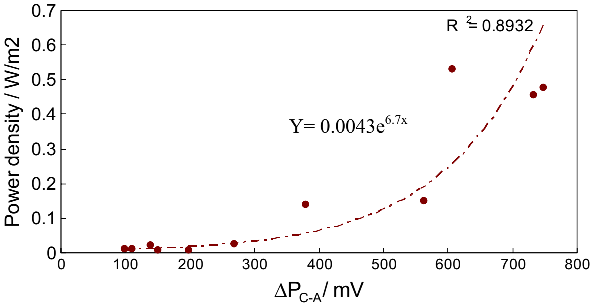


Fig. 7 The variation of power density with the difference between cathode and anode potential ($\Delta P_{C\text{-}A}$) in MFCs

reason for the difference in OCP was the different electrochemical or biochemical reactions carried out by various microbial communities in the inocula [3]. Compared to pure culture (*G. sulfurreducens*), the mixed cultures (wastewater and soil) had diverse electrogenic bacterial species capable of degrading intermediate substrates in anode chambers, which enhanced the oxidation reactions and increased the OCP of anode electrodes. Moreover, there were two reasons for the higher OCP of wastewater-seeded MFC than soil-seeded MFC. First, anaerobic bacteria in wastewater were more accustomed to the aquatic environment in MFCs than bacteria in soil, which allowed a fast degradation of organic substrates and led to a higher OCP. Second, in soil, certain portions of bacterial cells were absorbed on soil particles, which inhibited the adhesion of electrogenic bacteria to the electrode surfaces and slowed the degradation of organic substrates.

There was also a significant difference in R_{in} among three inocula. The soil-seeded MFCs had much higher R_{in} (1,200 Ω) than wastewater-seeded (400 Ω) and *G. sulfurreducens*-seeded MFCs (150 Ω; Table 1). As discussed before, the R_{in} of MFCs was dependent upon bacterial adhesion and bacterial species (Fig. 4). With a high amount of inorganic particles and nonelectrogenic bacteria present in soil inocula, the adhesion of electrogenic bacteria to the anode surfaces [3] was low in soil-seeded MFCs, and thus the MFCs had the highest R_{in} among three inocula.

Different inocula had similar COD removal rates but different power production. The COD removal rates for the three inocula were between 79–85%, while the wastewater-seeded MFCs and the *G. sulfurreducens*–seeded MFCs achieved higher CEs (20.5% and 15.8%) than the soil-seeded MFCs (9.2%; Table 1). Correspondingly, the ECEs (in terms of theoretic heat (η_1) and COD consumption (η_2)) of *G. sulfurreducens*-seeded MFCs were

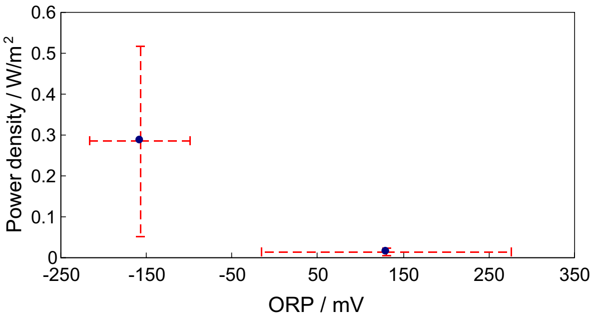


Fig. 8 The variation of power densities with the anode solution redox potentials (ORPs) in MFCs

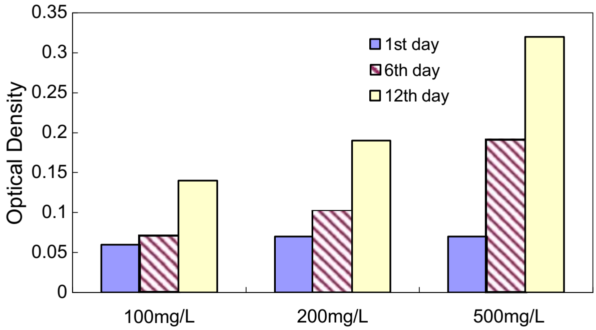


Fig. 9 The optical density of the anode solutions at different CODs in wastewater-seeded MFCs

higher than soil-seeded MFCs. Moreover, *G. sulfurreducens*-seeded MFCs had a much higher P_{max} (when R_{ext} equaled R_{in}) than wastewater- and soil-seeded MFCs. There were two reasons for the higher power production of pure cultures than mixed cultures. First, the nonelectrogenic bacteria in mixed cultures consumed organic substrates for bacterial growth instead of electricity production. The optical density of the anode solution in wastewater-seeded MFCs at different CODs showed a steady increase over the 12-day operational period (Fig. 9). The optical density increased twice at COD 100 mg/L, three times at COD 200 mg/L and almost five times at COD 500 mg/L. The results clearly indicated bacterial growth in the solution, which consumed the substrate without producing electricity. Second, bacterial adhesion of pure cultures was better than that of mixed cultures (Figs. 2a and c), which reduced the electron transfer resistances (R_{in}), and increased the power production (CE, ECE, and P_{max}).

Conclusions

This study extensively investigated the effect of inoculum type on bacterial adhesion, R_{in} and power production. The correlation between the power production and electrochemical characteristics of MFCs was established. Four major findings were obtained in this study. First, *G. sulfurreducens* had a faster adhesion rate than soil. Adhesion of electrogenic bacteria to the anodes increased the power production, although the suspended bacteria in the soil-seeded MFCs were capable of generating electrons. Second, bacterial activity and bacterial adhesion are important factors that affect R_{in} in MFCs. Higher populations of nonelectrogenic bacteria in mixed cultures increased R_{in}. However, there was no clear correlation between R_{in} and power production. Third, two good quantitative correlations (anode potential (P_A) vs. power production, the difference between anode and cathode potentials ($\Delta P_{C\text{-}A}$) vs. power production) were established. Fourth, the inoculum types had significant effects on the MFC performance. The difference in the populations of electrogenic bacteria in the inocula tested (*G. sulfurreducens*, soil and wastewater) resulted in different CE, ECE, R_{in}, and power production.

The results of this study verified the disturbance caused by nonelectrogenic bacteria on R_{in} and power production of MFCs and suggested that a careful selection of the inocula could enhance the performance of MFCs. Further study on R_{in} is needed to completely understand the correlation between R_{in} and power production. Moreover, a molecular study on the bacterial communities in the biofilms might reveal more details regarding the interference of nonelectrogenic bacteria in the MFCs.

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