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Biochar as Electron Acceptor for Microbial Extracellular Respiration

Linpeng Yu[a,b,c], Yueqiang Wang[c], Yong Yuan[c], Jia Tang[c], and Shungui Zhou[c]

[a]Guangzhou Institute of Geochemistry, Chinese Academy of Sciences, Guangzhou, China; [b]Graduate University of Chinese Academy of Sciences, Beijing, China; [c]Guangdong Institute of Eco-environmental and Soil Sciences, Guangzhou, China

ABSTRACT

Biochar is a charred carbonaceous material that has recently been identified to provide many potential environmental and agricultural applications. Biochar amendments are shown to effectively improve the quality of soil and increase soil microbial biomass. However, the interactions between biochar and microorganisms and the mechanisms through which biochar influences soil microbial growth and activities remain unclear. In this study, we investigated the potential for biochar to function as an electron acceptor for microbial extracellular respiration and growth. Anaerobic incubation of *Geobacter sulfurreducens* revealed that biochar was used as a sole terminal electron acceptor, as evidenced by a 31-fold increase of biomass and gradual increase in reducing equivalents of biochar and the consumption of acetate after 15 d. An electron stoichiometry analysis showed that 58.7% of the electrons released from acetate oxidation could be recovered in biochar, which was comparable to that of humic substances (44.8%). The finding that biochar participates in microbial extracellular respiration may have important environmental implications considering the widespread existence of both extracellular-respiring microorganisms and black carbon in the environment.

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Biochar; electron-accepting capacity; extracellular respiration; *Geobacter sulfurreducens*

Introduction

Microbial extracellular respiration (MER) has been recognized as an extension of respiratory chains to the cell surface and a new pathway for microorganisms to gain energy in the process of reducing extracellular compounds (Richter et al. 2012). Recently, MER has been most thoroughly explored because of its underlying environmental implications such as its impacts on the biogeochemical cycling of organic carbon, nitrogen, sulfur, and iron (Wu et al. 2011). Importantly, and in most cases, MER is coupled with the biodegradation of organic contaminants, the detoxification of heavy metals, or the generation of bioenergy (Choppala et al. 2011; Hong and Gu 2009; Wang et al. 2013). Thus, much effort has focused on the isolation and identification of microorganisms that are capable of MER (termed electroactive bacteria). The most common extracellular electron acceptors used in the characterization of electroactive bacteria are Fe(III) oxides, quinones, and electrodes (Cervantes et al. 2004; Holmes et al. 2006; Luu et al. 2003).

Humic substance, a major fraction of organic carbon in soils and sediments, is a typical compound involved in quinone respiration and has been increasingly discussed for its roles as an electron shuttle in the reduction of iron oxides and biodegradation of contaminants (Martínez et al. 2013; Roden et al. 2010). Similar to humic substances, black carbon (e.g., activated carbon) was also demonstrated to be a good redox mediator in the biotic and abiotic transformation of organic pollutants (Tang et al. 2011; Van Der Zee et al. 2003). Biochar, a novel kind of black carbon produced by the thermal decomposition of biomass under limited oxygen conditions, has recently attracted more and more attention due to its potential environmental and agricultural applications (Kannann et al. 2013; Lou et al. 2011; Woolf et al. 2010).

It has been proposed as an effective means to sequester carbon and reduce the emission of greenhouse gases, improve the quality of soil, and increase the yield of crops (Ghani et al. 2013; Alburquerque et al. 2013; Sun and Lu 2014). In most previous studies, biochar was also shown to positively impact microbial activities and biomass (Lehmann et al. 2011; Liang et al. 2010; Rillig et al. 2010; Zhang et al. 2014). For instance, the presence of biochar resulted in tighter cycling and reduced loss of both nutrients and carbon and thereby an increased microbial biomass (Jin 2010). Another property of biochar is that it is electrically conductive and redox-active, and it may participate in microbial metabolic activities that involve electron transfer in MER.

For example, biochar seems to be an electron shuttle that facilitates the transfer of electrons to denitrifying microorganisms (Cayuela et al. 2013). Moreover, recent studies revealed that biochar amended in paddy soil was a growth stimulant for Fe(III)-reducing and dechlorinating bacteria (Tong et al. 2014). These results suggest that biochar might be involved in the MER. However, the functionality of biochar to support MER and microbial growth has not been evaluated yet. The ubiquity of both solid carbonaceous materials (black carbon) and a wide diversity of extracellular respiring microorganisms warrant a systematic evaluation of the interactions between them.

CONTACT Yong Yuan yuanyong@soil.gd.cn Guangzhou Guangdong Institute of Eco-environmental and Soil Sciences, Guangzhou, 510640, China; Shungui Zhou sgzhou@soil.gd.cn Guangdong Institute of Eco-environmental and Soil Sciences Guangzhou, 510640, China.

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Therefore, in this study, we evaluated the potential of biochar to function as an electron acceptor for MER and microbial growth. *Geobacter sulfurreducens*, a model microorganism of extracellular respiration, was used for the respiration of biochar. The functionality of biochar as an electron acceptor in MER was also tested in comparison with those of humic substance and fumarate, and the environmental implications of this work are discussed.

Materials and methods

Biochar preparation and characterization

Biochar was produced from coconut shell or mature rice straw obtained from a local paddy field in Guangdong Province, China. After being dried at 80°C for 48 h to remove moisture, the coconut shell and rice straw samples were cut into 2–3-cm pieces. The pieces were placed into a cylindrical quartz tube in an electric furnace and pyrolyzed under a N_2 flow of 1.2 L/min. The temperature of the furnace was programmed to increase at a rate of approximately 20°C/min and was held at different values (500 or 900°C) for 1 h. After cooling to room temperature, the charred materials were milled to approximately 0.15 mm and sieved through a 100-mesh sifter. The obtained biochars were designated as R500, R900 (rice straw biochars), C500 and C900 (coconut shell biochars). The elemental composition and surface area of the biochars were analyzed using an elemental analyzer (Vario EL Cube, Elementar Co., Germany) and Quantachrome QuadraWin (ASIQMO002-2, Contador Instrument Co., USA), respectively (Table 1).

Measurements of electron-accepting capacity

Electrochemical experiments were performed using an electrochemical workstation (CHI660D, Chenhua Co., Ltd., Shanghai, China) with a conventional three-electrode cell at ambient temperature (Yuan et al. 2011). A graphite plate (2 cm × 2 cm), a platinum sheet and a saturated calomel electrode (SCE) were used as the working electrode, counter electrode and reference electrode, respectively. Chronoamperometry (CA) measurements were performed in a phosphate buffer solution (PBS, 0.10 M, pH = 7.0) with 0.1 M KCl electrolyte at an applied potential of − 0.49 V (vs. SHE) under constant stirring and N_2 flow. To quantify the electron-accepting capacity (EAC) of biochar and humic acid (HA), mediated electrochemical reduction was conducted using a synthesized electron shuttle (zwitterionic viologen 4,4′-bipyridinium-1,1′-bis(2-ethylsulfonate)) as previously described (Gorski et al. 2013). All of the solutions were deoxygenated by purging with oxygen-free N_2 for 2 h before the electrochemical measurement.

Microorganism and culture conditions

G. sulfurreducens strain PCA was routinely anaerobically cultured in nutrient broth with acetate and fumarate (NBAF) as previously described (Coppi et al. 2001). Cells at the exponential phase were harvested by centrifugation (8000 rpm, 10 min), and the cell pellet was washed three times with a sterile bicarbonate buffer (pH = 7.0). For the growth experiments, the fumarate-free NBAF medium with 3 mM acetate as an electron donor and 20 g/L R900 (or 2 g/L humic acid, purchased from Sigma-Aldrich) as electron acceptors were sonicated by a bath sonicator for 10 min to disperse the biochar (or humic acid) suspensions and autoclaved at 121°C for 30 min, followed by purging with N_2/CO_2 (80%: 20%) for 2 h to thoroughly remove the oxygen.

All of the serum bottles contained 50 ml of the culture media and were sealed tightly with a butyl rubber stopper, and aluminum cap. *G. sulfurreducens* was inoculated into each of the culture medias to provide an initial cell density of approximately 0.8×10^6 cells/ml. The growth of *G. sulfurreducens* with each type of biochar or humic acid was run in triplicate and maintained statically in an incubator for 15 d at 30 ± 1°C. The pH values of all of the media were controlled at 7.0 ± 0.1 by a bicarbonate buffer during the entire incubation period. The water soluble extract of R900 was prepared by filtering (at 0.22 um) the sterilized R900 suspension (20 g/L) of the fumarate-free NBAF medium after a 48-h equilibration. The obtained filtrate was used as a control for the growth experiments using the same experimental procedure as mentioned above. Additionally, the growth experiment of *G. sulfurreducens* in the complete NBAF medium with fumarate (40 mM) as a sole electron acceptor was also performed under the same conditions for a comparison with the biochars.

Reduction of biochar in the microbial fuel cell

A two-chamber (total volume of 150 ml for each glass chamber) microbial fuel cell (MFC) was constructed using carbon cloth (4 cm × 5 cm) and carbon felt (5 cm × 7 cm) as the anode and cathode electrode, respectively. The electrodes were connected by a titanium wire (~0.5 mm in diameter) with an external resistance of 1000 Ω. The fumarate-free NBAF

Table 1. Elemental composition, surface area and ash content of four different biochars used in the present study.

	Elemental analysis							
	Percentage by weight (%)				Atomic ratio			
Samples	C	H	O	N	O/C	H/C	BET surface area (m^2/g)	Ash (wt %)[a]
C500	74.14	2.53	17.47	0.48	0.18	0.41	5.87	7.4
C900	71.43	1.38	21.39	0.45	0.22	0.23	9.62	8.9
R500	53.06	1.74	23.57	0.68	0.33	0.39	5.03	28.6
R900	46.90	1.18	24.96	0.78	0.39	0.30	10.85	31.7

[a] Represent the mass percentage of total ash (produced at 550°C for 6 h in a muffle furnace) to the biochar.

medium in the anode chamber and phosphate buffer solution (0.1 M, pH 7.0, with 0.1 M KCl as an electrolyte) in the cathode chamber were separated by a proton exchange membrane.

G. sulfurreducens was inoculated into the anode to form a biofilm on the electrode, and the cathode was purged with air continuously until a steady cell voltage of approximately 0.39 V was achieved. Before the electrochemical experiments, the cathode was flushed with N_2 for 2 h and sealed to maintain an anaerobic environment. When the cell voltage of MFC declined to the baseline and remained constant, the deoxygenated biochar suspension (200 mg/L) or the humic acid solution (120 mg/L) was spiked into the cathode under a continuous N_2 flow. The cell voltage was collected by a 32-channel voltage collection instrument (AD8223, China).

Real-time quantitative PCR

The genomic DNA was extracted from the cells in triplicate from 2 ml of biochar or HA culture media using a PowerSoil™ DNA isolation kit (Mo Bio Laboratories, Carlsbad, CA, USA) according to the manufacturer's instructions (Tong et al. 2014). The extracted DNA samples were quantified by real-time fluorescent quantitative PCR (qPCR) based on the 16S rRNA gene numbers of *G. sulfurreducens* as previously described (Tong et al. 2014; Xu et al. 2013). Briefly, the PCR reaction mixture (total volume of 25 μl for each reaction) contained 10.1 μl of RNase-free water, 12.5 μl of SYBR green master mix (SYBR® Premix Ex Taq™ II, TAKARA), 0.2 μl of each primer (10 μM), and 2 μl of template DNA. The qPCR was performed on a Bio-rad MyiQ™2 Two-color Real-Time PCR detection system with triplicate reactions for each DNA sample. The specific primers of qPCR were Geo16S_494F (5′-AGGAAGCACCGGCTAACTCC-3′) and Geo16S_825R (5′-TACCCGCRACACCTAGT-3′). The qPCR of the plasmid containing the targeted gene fragment and prepared at serial dilutions was conducted simultaneously and in the same plate to generate a calibration curve. Other detailed conditions and programs for the qPCR were as described in our previous study (Xu et al. 2013). DNA concentrations in the samples were expressed as the average copy number of 16S rRNA genes per ml of culture.

Scanning electron microscopy

To evaluate the attachment of *G. sulfurreducens* to the biochar particles, the morphology and distribution of cells on the biochar fractions were analyzed using a scanning electron microscope (SEM, S-4800, Hitachi Co., Japan). The protocol of preparing SEM samples was as follows. At the end of the incubation time (15 d), 20 ml of the medium was filtered with a 0.45-μm membrane, and the cells on the filtration residue were fixed with 2.5% glutaraldehyde for 6 h, followed by a gradual dehydration using 25%, 50%, 75%, and 100% ethanol solutions for 10 min, respectively. Then, the samples were freeze-dried and spray-coated with a thin film of platinum prior to SEM observation (at 20 kV).

Analytical techniques

The concentrations of acetate in the culture media were determined by ion chromatography (ICS-90, DIONEX, USA) coupled with an IonPac™ AG14A - 7 μm guard column (4 mm, 50 mm), AMMS 300 micromembrane suppressor, RFIC™ IonPac™ AS14A −7 μm analytical column, and a DS5 Detection Stabilizer conductivity detector. An eluent solution, containing 8.0 mM Na_2CO_3 and 1.0 mM $NaHCO_3$, was pumped at a flow rate of 1.0 ml/min. A regenerant (0.05 M methanesulfonic acid) was used to regenerate the suppressor's ability for suppressing eluent conductivity. The reducing equivalents of biochar and humic acid were analyzed using the ferrozine technique as previously described (Ratasuk and Nanny 2007; Van Der Zee et al. 2003). The concentration of Fe(II) produced from the redox reaction between reduced biochars (or humic acid) and Fe(III) was used to calculate the molar numbers of electrons transferred from the samples to Fe(III).

Results

Growth of Geobacter sulfurreducens *with biochar as an electron acceptor*

In the presence of 20 g/L biochar (R900), *Geobacter sulfurreducens* grew rapidly and the 16S rRNA gene copies of *G. sulfurreducens* increased significantly after a 15-d static culture (Figure 1a), indicating that *G. sulfurreducens* could use biochar as a sole terminal electron acceptor. The quantity of *G. sulfurreducens* at 15 d was approximately 24.3% of the biomass grown with furmarate (40 mM) as an electron acceptor. In contrast, a control culture of *G. sulfurreducens* with a water-soluble extract of R900 showed little change in the number of 16S rRNA gene copies. This demonstrated that the particulate fraction of biochar was mainly responsible for the growth of *G. sulfurreducens*. Under the same condition, humic acid (2 g/L) also supported the growth of *G. sulfurreducens*, but the maximum number of 16S rRNA gene copies was approximately 54.5% of that for biochar, which may be attributed to the difference in the effective concentrations of the electron acceptors.

Three other types of biochar (C500, C900 and R500) were also tested for their electron-accepting capacities to support the extracellular respiration and growth of *G. sulfurreducens*. As shown in Figure 1b, the quantities of *G. sulfurreducens* increased profoundly in the presence of C500, C900 and R500 (20 g/L) after a 7-d incubation, and the 16S rRNA gene copies in these treatments were comparable to each other. However, no further growth of *G. sulfurreducens* was observed in the treatments of both C500 and R500 at 15 d compared with those at 7 d, indicating that the electron-accepting functional groups had been completely consumed.

In contrast, C900 maintained continuous growth of *G. sulfurreducens*, and there was a significant increase in the 16S rRNA gene copies after 15 d. This phenomena was consistent with their different EACs (101.2, 142.6, 236.8 μmol e^-/g biochar for R500, C500 and C900, respectively). SEM images revealed that *G. sulfurreducens* was in a short-rod shape and colonized biochar particles (Figure 2). Interestingly, the cells

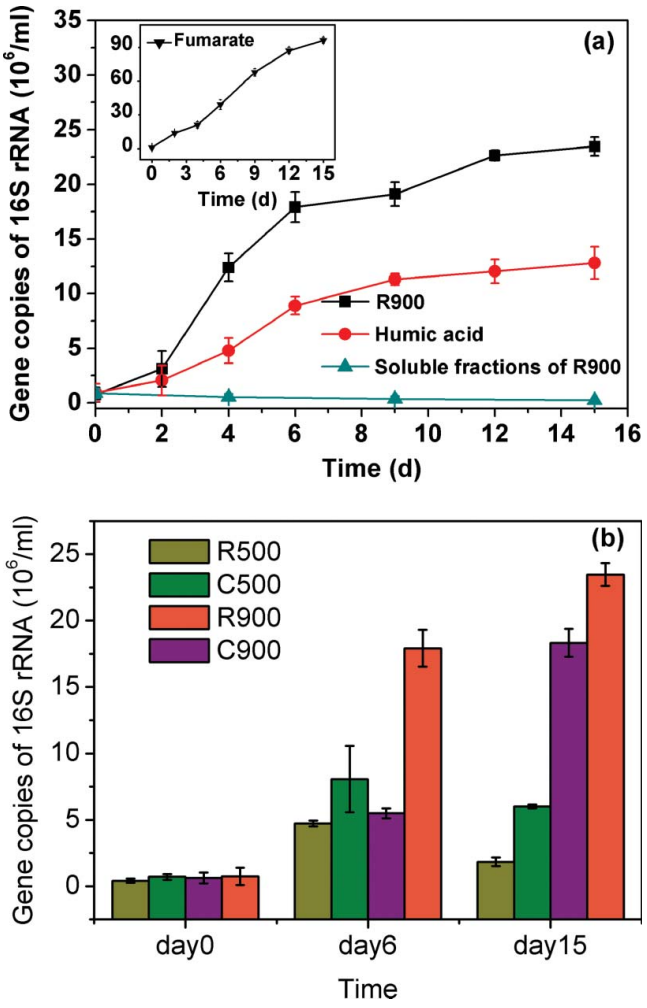

Figure 1. (a) Growth of *G. sulfurreducens* with R900, humic acid, fumarate (inset) or water soluble fractions of R900 as electron acceptor. (b) Growth of *G. sulfurreducens* with three other types of biochars as electron acceptor. 1%-inocula (v/v) of *G. sulfurreducens* was incubated anaerobically in the presence of biochars (20 g/L) or humic acid (2 g/L) at 30°C and pH 7.0 ± 0.1. The water soluble fractions of R900 were prepared by filtration (0.22 μm) of the R900 suspension (20 g/L) and the filtrate was used for the preparation of fumarate-free NBAF medium. The results are expressed as mean ± SD of three independent replicates (n = 3).

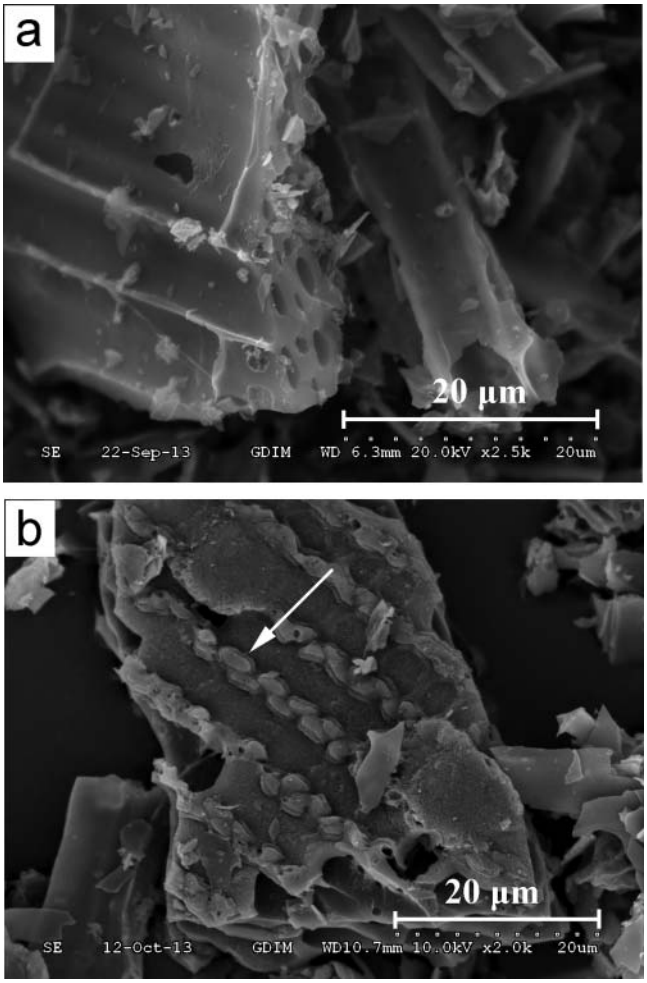

Figure 2. SEM images of cell-free biochar control (a) and *G. sulfurreducens* growing on the surface of biochar particles (b). Biochar (R900, 20 g/L) was incubated with 1%-inocula of *G. sulfurreducens* from the exponential phase for 15 d, and then the biochar samples were used for SEM observation. Arrow indicates the cells on the surface of biochar particles.

were in orderly rows on the surface of the biochar and formed a close contact with each other.

Oxidation of acetate and concomitant reduction of biochar by G. sulfurreducens

The consumption of acetate by *G. sulfurreducens* in the presence of the biochar is shown in Figure 3. The acetate concentration in the biochar culture medium with *G. sulfurreducens* decreased gradually over time, whereas acetate in the cell-free control medium remained nearly constant, which suggests that acetate was utilized by *G. sulfurreducens*. In the presence of humic acid, acetate was also oxidized but to a lesser extent compared with that of biochar. This is consistent with the relatively low cell density of *G. sulfurreducens* (Figure 1a).

Electron transfer from the oxidation of acetate to biochar (R900) by *G. sulfurreducens* was demonstrated by the increased reducing equivalents of biochar. As shown in Figure 3, the reducing equivalents of R900 in the presence of *G. sulfurreducens* was negligible at the initial time of incubation, but then

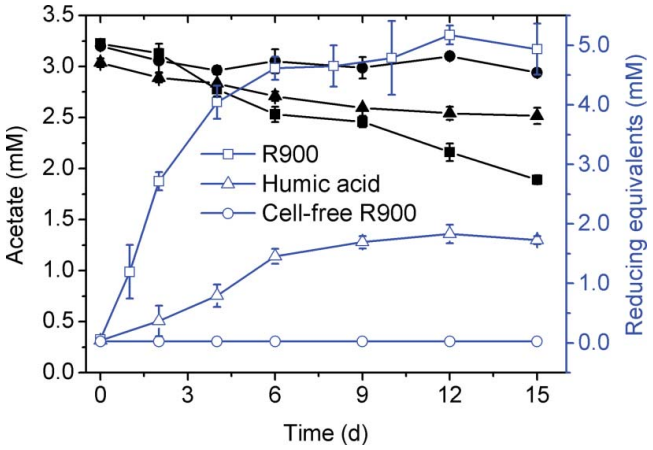

Figure 3. Changes of acetate concentrations (full symbols) in the presence of R900 (■) or humic acid (▲) with *G. sulfurreducens*, or cell-free R900 (●), and the reducing equivalents of electron acceptors (the corresponding open symbols). Biochar (R900, 20 g/L) and humic acid (2 g/L) were incubated with 1%-inocula of *G. sulfurreducens* statically at 30°C for 15 d, while the cell-free R900 was run simultaneously under the same conditions and used as a control. Err bars represent the standard deviation of three independent replicates.

increased to 4.93 mM after 15 d. In contrast, R900 in the abiotic control medium kept approximately the same reducing equivalents during the entire culture period. The amount of microbial-transferred electrons agrees well with the electron-accepting capacity (EAC) of R900 as determined by electrochemical experiments (Table 2). By comparison, the reducing equivalent of R900 was much higher than that of humic acid (1.83 mM) under the same conditions, which suggests their different contents of the surface redox functional groups. Based on these data, the observed electron recovery from the oxidation of acetate to the reduction of biochar was 58.7%, compared to 44.8% for humic acid (Table 2).

Biochar as an electron acceptor in a microbial fuel cell

To further demonstrate the potential of biochar to function as an electron acceptor, a MFC was constructed where the biofilm anode of *G. sulfurreducens* was separated from the biochar suspension (R900, 200 mg/L) in the cathode by a proton exchange membrane (Figure 4a). The biochar particles in the MFC were reduced (Figure 4b), as evidenced by the increased current when the deoxygenated biochar suspension was spiked anaerobically into the MFC. Then, the current leveled off gradually and was maintained for 5.5 h, suggesting that most of the oxygen functional groups in the biochar had been reduced. In the presence of biochar that was previously reduced by $NaBH_4$, there was a small peak in the current, but then it decreased to the background level rapidly within 1 h, which suggests that the reduced biochar could no longer accept the microbial electron. In comparison, *G. sulfurreducens* reduced humic acid (120 mg/L) in a similar way as biochar but with a higher current response than that of biochar (Figure 4b). Integration of the current curve revealed that biochar and HA accepted electrons at a capacity of 262.3 μmol e^-/g biochar and 931.2 μmol e^-/g HA, respectively.

Discussion

In this study, we demonstrated that biochar functions as an electron acceptor for the anaerobic growth of *G. sulfurreducens*. This functionality is similar to those of activated carbon and solid humic substances reported in previous studies (Roden et al. 2010; Van Der Zee et al. 2003). Electrochemical measurements showed that all of the tested biochars had modest EACs, and the EAC of R900 was comparable with that of biochars and activated carbon (AC) in previous studies (Kluepfel et al. 2014; Van Der Zee et al. 2003), where AC was verified to accept microbial extracellular electron at a capacity of approximately 315 μmol e^-/g.

The present results for the growth of *G. sulfurreducens* provide a potential explanation for the observations that biochar amendments in the paddy soil increased the relative abundance of Fe(III)-reducing bacteria or quinone-respiring biomass (Tong et al. 2014). Previously, Chen et al. (2014) suggested that *Geobacter metallireducens* used pine biochar as an electron acceptor with an approximate EAC of 400 μmol e^-/g biochar. This was consistent with the present study, which indicated that biochar or black carbon acting as electron acceptors for a diversity of electroactive microorganisms may be a general feature.

It should be noted that quinone moieties, phenolic moieties and condensed aromatics in biochar are the possible redox-active moieties responsible for its EAC (Kluepfel et al. 2014). These functional groups vary with pyrolysis conditions, heat-treatment temperatures, residence time, and feedstock; thus, EAC values can vary significantly among different biochars, leading to their varied functionalities in microbial respiration and cell yield. For example, biochars prepared at a high temperature (900°C) possessed better electrochemical properties and thus were more favorable in MER than those prepared at a low temperature (500°C).

A possible reason for this phenomenon may be attributed to the formation or exposure of new redox-active moieties at higher charring temperatures because the surface areas of R900 and C900 increased significantly compared to those of R500 and C500. In addition, the redox pairs of microbial outer membrane cytochromes (OMCs) or proteins that are essential for electron transfer from cells to extracellular solid acceptors may vary among different microorganisms (Busalmen et al. 2010; Shi et al. 2012). Thus, the specific electron transport pathways and the potential difference between microbial terminal components of electron transfer chains and biochar surface redox moieties might finally determine the effective EACs of biochars in terms of different electroactive microorganisms (Bond 2010).

SEM images showed that cells of *G. sulfurreducens* were attached to the surface of biochar particles. Although this phenomenon was not evidence of biochar reduction, it provided the possibility of direct electron transfer between them as *G. sulfurreducens* was not able to excrete electron shuttles (MacDonald et al. 2011).

The hole in the electron equivalent balance (Table 2 and Figure 3) in the incubation experiments of *G. sulfurreducens* was similar to a previous study (Van Der Zee et al. 2003) and may be explained in part by the cell yield because part of the acetate consumed might be assimilated for the production of the increased biomass rather than the oxidation reaction in the tricarboxylic acid (TCA) cycle (Esteve-Núñez et al. 2005). The electron transfer from the electron donor (acetate) to biochar was also demonstrated by the MFC. In the MFC, microorganisms in the anode chamber donate electrons to the anode by

Table 2. Microbial oxidation of acetate, the EACs and the reduced milliequivalents of the biochar and humic acid, and the electron recoveries for these reactions.

Chemicals (concn, g L^{-1})	EAC (μmol e^-/g)	Acetate consumed (mM)	Microbially reduced milliequivalents (mmol e^-/L)	Electron recovery (%)
R900 (20)	258.1 ± 29.5	1.04 ± 0.05	4.93 ± 0.62	58.7
Humic acid (2)	971.2 ± 63.3	0.51 ± 0.08	1.83 ± 0.15	44.8

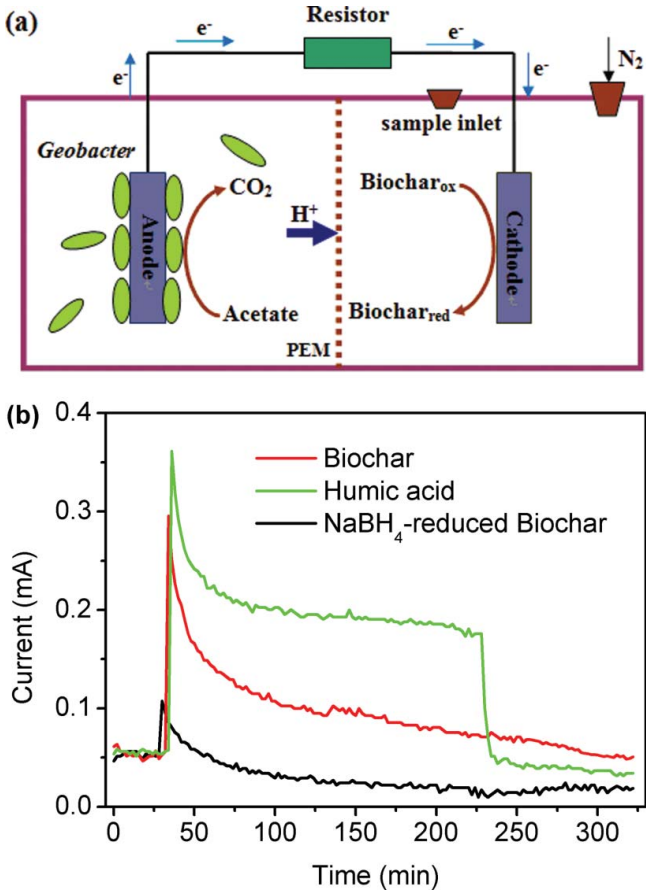

Figure 4. (a) A structural diagram of the MFC and (b) the reduction currents of biochars and humic acid by *G. sulfurreducens* in the MFC. The anode biofilm of *G. sulfurreducens* and the deoxygenated biochar suspension (R900, 200 mg/L) or the humic acid solution (120 mg/L) in PBS buffer (0.1 M, pH 7.0, 0.1 M KCl) were separated by a proton exchange membrane (PEM), and the MFC was run at an ambient temperature in the dark.

oxidizing organics. The released electrons transfer from the anode to the cathode via an electric wire and reduce the electron acceptors in the cathode chamber.

Therefore, the quantities of transferred electrons in the MFC depend on the electron-accepting capacities of the electron acceptors. In general, the electrochemical activity of R900 in the MFC was lower than that of HA (Figure 4b). The difference in the currents may be due to the higher abundance and the easier access of redox-active groups in dissolved HA compared to solid biochar particles. It is worth noting that oxygen could not contribute to the currents in the MFC because it was thoroughly removed from the cathode chamber, which was demonstrated by the resazurin assay (an oxygen indicator). Furthermore, the quantities of electrons transferred from acetate to R900 or HA in the MFC were close to their EACs, indicating the complete exclusion of oxygen from the cathode and an alternative method for determining the EACs of the solid materials. Therefore, it can be concluded that biochar can be reduced by *G. sulfurreducens* not only by direct contact but also when separated via an electrical wire.

The biochar in the reductive forms may further serve as an electron donor for other microorganisms. For example, it was demonstrated that biochar functioned as an electron shuttle to facilitate electron transfer to ferrihydrite (Kappler et al. 2014) and denitrifying microorganisms (Cayuela et al. 2013), thus decreasing the N_2O emission and impacting the biogeochemical cycling of nitrogen. In addition, biochar and granular activated carbon (GAC) were observed to stimulate direct interspecies electron transfer (DIET) in co-cultures of *Geobacter metallireducens* and *M. barkeri* and thereby the production of methane (Chen et al. 2014; Liu et al. 2012).

The underlying mechanisms were assumed to occur due to the mediation of electron transfer through the electrical conductor. However, the present study on biochar respiration suggests that it may be possible that their surface oxygen functional groups (e.g., quinones) shuttle electrons between donor microorganisms and acceptor microorganisms because biochars in the reductive forms might simultaneously function as an electron donor for acceptor microorganisms that have higher surface redox potential than the reduced biochar. Essentially, biochar respiration may be the same as that of humic acid for the reason that they are just different forms of quinone respiration. Therefore, biochar respiration can also provide an alternative explanation for decreasing the emission of methane in certain soil environments by biochar amendments due to the competition between quinone respiration and methanogenesis (Cervantes et al. 2000; Feng et al. 2012).

The observation that biochars can participate in microbial respiration may have important implications. Because most soil organic carbon materials are in the solid form and ubiquitous in the environment, they may constitute a large pool of electron acceptors or shuttles to accelerate the bioreduction of metal minerals, organic and inorganic contaminants such as nitrate, arsenate and chromate. In addition, the large surface area, porous structure of biochar, and its ability of retaining nutritional substances make it an excellent habitat for microbes. As a result, biochar amended in the environment may be not only a superior sorbent of contaminants but also a catalyst in enhancing the redox reaction rates of some important biogeochemical processes (e.g., reduction of iron oxide). This role of biochar may impact both certain biological activities and abiotic redox reactions in the environment.

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