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Biochar-Facilitated Microbial Reduction of Hematite

Shengnan Xu,[†,#] Dinesh Adhikari,[†] Rixiang Huang,[§] Hua Zhang,[†,‡] Yuanzhi Tang,[§] Eric Roden,[∥] and Yu Yang*[,†]

[†]Department of Civil and Environmental Engineering, University of Nevada, Reno, Nevada 89557, United States

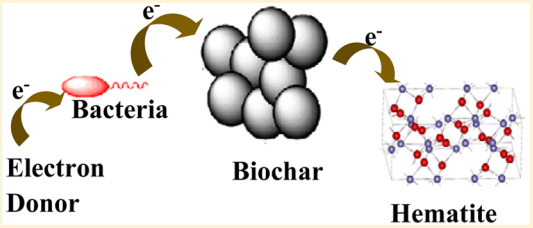
[‡]College of Environmental Science and Engineering, Guilin University of Technology, Guilin, Guangxi 541004, China

[§]School of Earth and Atmospheric Sciences, Georgia Institute of Technology, 311 Ferst Drive, Atlanta, Georgia 30332-0340, United States

[∥]Department of Geoscience, University of Wisconsin—Madison, 1215 W. Dayton Street, Madison, Wisconsin 53706, United States

Supporting Information

ABSTRACT: As an important component of soil organic matter (SOM), the transformation of pyrogenic carbon plays a critical role in the biogeochemical cycles of carbon and other redox-active elements such as iron (Fe). Herein, we studied the influences of wheat straw-derived biochars on the microbial reduction of 100 mM of hematite by the dissimilatory metal reducing bacteria *Shewanella oneidensis* MR-1 under anoxic conditions. The long-term microbial reduction extent and initial reduction rate of hematite were accelerated by more than 2-fold in the presence of 10 mg L^{-1} biochar. Soluble leachate from 10 mg L^{-1} biochar enhanced Fe(III) reduction to a similar degree. Microbially prereduced biochar leachate abiotically reduced hematite, consistent with the apparent electron shuttling capacity of biochar leachate. Electron paramagnetic resonance (EPR) analysis suggested that biochar leachate-associated semiquinone functional groups were likely involved in the redox reactions. In addition to electron shuttling effects, biochar particles sorbed 0.5−1.5 mM biogenic Fe(II) and thereby increased the long-term extent of hematite reduction by 1.4−1.7 fold. Our results suggest that Fe redox cycling may be strongly impacted by pyrogenic carbon in soils with relatively high content of indigenous pyrogenic carbon or substantial application of biochar.



INTRODUCTION

Natural organic matter (NOM)-mediated microbial redox reactions are important for the biogeochemical cycles of carbon and redox-active elements, such as iron (Fe).[1−3] Under anoxic conditions, dissimilatory Fe(III)-reducing microorganisms are able to utilize humic substances as electron acceptors to oxidize organic compounds and gain energy for metabolic activities.[4] As an electron shuttle, reduced humic substances can further transport electrons to poorly soluble Fe(III) (oxyhydr)oxides to facilitate their reduction.[5] Quinone moieties in NOM have been shown to be the main functional group in mediating the electron transport between bacteria and Fe(III) oxides.[6−8]

Existing studies on the NOM-facilitated redox reactions have focused on aqueous phase and to a lesser extent solid-phase humic substances.[9,10] In contrast, the impact of pyrogenic carbon on the reduction of Fe has received limited attention, despite the fact that pyrogenic carbon contains much more aromatic carbon and also likely a higher quinone content compared to humic substances.[11−13] Pyrogenic carbon, mainly generated from incomplete combustion of biomass, contributes to 5−36% of total organic carbon in soils.[14,15] Aromatic functional groups account for up to 90% of the carbon in pyrogenic organic matter, and substantial quinone contents have been detected in various pyrogenic carbons.[16] This unique structural feature suggests that pyrogenic carbon may have the capability to facilitate electron transport during redox reactions. Previous studies have shown that biochar can facilitate the reductive degradation of toxic organic compounds.[17−19] However, limited knowledge is available for the role of pyrogenic carbon in the redox reactions for Fe.[20]

In this study, we investigated the role of biochars in the reduction of hematite by the dissimilatory Fe(III)-reducing bacterium (DIRB) *Shewanella oneidensis* MR-1. Biochar was used as a representative pyrogenic carbon, because it is widely present in soils and there are increasing interests in the application of biochar as agricultural soil amendments.[21] The central goal of this work is to compare hematite reduction by *Shewanella oneidensis* MR-1 in the presence and absence of biochars, and to elucidate the influences of physicochemical properties of biochar, especially the content of semiquinone radicals. Our focus on the reduction of crystalline Fe(III) oxide differs from a recent study about the biochar-promoted reduction of synthetic ferrihydrite.[22] Unlike poorly crystalline Fe(III) (oxyhydr)oxide ferrihydrite, crystalline Fe(III) oxide phases such as hematite do not typically undergo major recrystallization during microbial reduction,[22,23] which often plays a critical role in governing the long-term extent of

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amorphous Fe(III) oxide reduction.[24] Rather, accumulation of aqueous Fe(II) and their sorption on the Fe(III) oxide and DIRB surfaces limit the long-term extent of reduction;[25,26] therefore, both aqueous- and solid-phase ligands that complex with Fe(II) can increase the extent of Fe reduction by delaying/retarding Fe(II) sorption on the mineral and cell surfaces.[27] The fact that biochar can bind a significant amount of Fe(II) and other divalent cations[28] raises the possibility that biochar could influence the rates of crystalline Fe(III) oxide reduction through both electron shuttling and Fe(II) binding effects. Such possibility is tested in this study.

MATERIALS AND METHODS

Materials. Biochars were produced by pyrolyzing wheat straws at two different heat treatment temperatures (HTTs) (250 and 500 °C), and each of the two biochars were further fractionated into two size fractions (0.25−0.5 mm and <0.25 mm). The biochars prepared at 250 °C with the size of 0.25−0.5 mm and <0.25 mm were termed 250A and 250B, respectively; biochars prepared at 500 °C: 500A (0.25−0.5 mm) and 500B (<0.25 mm). Details of the treatment process and extensive characterizations of these four biochars can be found in Supporting Information (SI). Elemental composition, surface area, and solid-state ^{13}C nuclear magnetic resonance (NMR) spectroscopy analysis of functional groups are listed in Table 1 and Figure S1. Hematite was used as received (≥99%, Fisher Scientific, Pittsburgh, PA), and the mineral phase was confirmed by X-ray diffraction (XRD) analysis.[29] All other chemicals used are above analytical grade.

Table 1. Characteristics of the Biochars Synthesized at Different Heat Treatment Temperatures (HTTs) with the Size Range of 0.25−0.5 mm and <0.25 mm[a]

	Biochar 500A	Biochar 500B	Biochar 250A	Biochar 250B
HTT (°C)	500	500	250	250
particle size (mm)	0.25−0.5	<0.25	0.25−0.5	<0.25
carbon (%)	60.2 ± 4.3		54.0 ± 2.2	
nitrogen (%)	2.1 ± 0.8		1.8 ± 0.2	
BET-N_2 (m^2 g^{-1})	3.3		2	
	fractions of functional groups (%)			
functional group	Biochar 500A	Biochar 500B	Biochar 250A	Biochar 250B
alkyl	0.72		20.3	
n-alkyl, methoxyl	0.19		7.4	
O-alkyl	1.9		16.7	
aromatic	90.8		33.3	
O-substituted aromatic	5.2		12.1	
carbonyl and carboxylic	1.1		10.1	

[a]Quantitative assessment of the different carbon types was determined using ^{13}C nuclear magnetic resonance (NMR) spectroscopy.

Shewanella oneidensis MR-1 was cultured following published methods.[30] Briefly, cells were grown aerobically in Luria−Bertani (LB) broth in an incubator at 30 °C. After the 14-h incubation, cells were collected at mid log phase and washed three times using 20 mM PIPES buffer (pH 7). Finally the bacteria pellets were obtained by centrifugation at 10 000*g* for 5 min.

Hematite Reduction with Biochar Particles. Microbial reduction experiments were conducted in 20 mM PIPES buffer (pH 7) containing 100 mM of hematite (200 mM Fe) and 20 mM sodium L-lactate (Alfa Aesar, 98+%). Biochar was preautoclaved and added to the solution to achieve a concentration of 10 mg/L. To establish anoxic conditions, the solutions were purged with N_2 gas for at least 1 h at room temperature and transferred to amber serum vials. The headspace was flushed with N_2 for 2 min, and the vials were immediately crimped with rubber stoppers. Washed MR-1 cells were added to achieve a final concentration of $10^{8.5}$ cells mL^{-1}. The cell-mineral suspensions were shaken at 100 rpm at room temperature. At different intervals within 240 h, triplicate samples were sacrificed for analysis of Fe(II). Using the same procedure, biotic controls without biochar (MR-1 cells + hematite + lactate) were conducted in parallel to study the microbial reduction of hematite in the absence of biochars. A parallel experiment with pasteurized cells (heated at 80 °C for 1 h) was conducted to study the possible reduction of hematite in the presence of dead cells. Systems without lactate (MR-1 cells + hematite + biochar) were used to demonstrate whether biochar can act as electron donor to facilitate the reduction of hematite. Biochar-mediated abiotic reduction of hematite in the presence and absence of lactate was also analyzed to demonstrate the contribution of abiotic processes to the reduction of hematite with biochar. In addition, the background release of Fe(II) from biochars was analyzed by mixing the biochars with MR-1 cells in PIPES buffer and measuring the time-dependent accumulation of Fe(II) in solution phase (SI, Figure S2).

At different intervals, triplicate samples were sacrificed for analysis of Fe(II) production using the ferrozine assay.[31,32] Briefly, at the predetermined time intervals, HCl was pipetted into the serum bottles to achieve a final concentration of 0.5 N in the suspensions, and the acidified suspensions were reacted overnight in order to remove Fe(II) adsorbed on solid surfaces (hematite, cells, and/or biochar). Then, suspensions were passed through a 0.2-μm polycarbonate filter to remove solids. Ferrozine reagent (Acros Organics, 98+%), 1 g/L buffered in 50 mM PIPES (pH 7), was added into the filtrate with a volume ratio of ferrozine reagent buffer: filtrate of 10:1. The absorbance of the yielded pink color was detected at 562 nm using an UV−vis spectrometer (Evolution 260 BIO, Thermo Scientific).

Both dissolved and acid-extractable Fe(II) were measured on day 4 and day 9 for systems with and without 10 mg/L biochar. Acid-extractable Fe(II) was measured using the methods described above, and dissolved Fe(II) was determined by measuring syringe-filtered (0.2 μm) Fe(II) fraction before the acid extraction process. Adsorbed Fe(II) was calculated as the difference between acid-extractable and dissolved Fe(II).

Hematite Reduction with Biochar Leachates. Biochar (500A, 500B, 250A, 250B) leachates were obtained by mixing the biochar particles (10 mg/L) with 20 mM PIPES buffer (pH 7) and shaken at 100 rpm for 24 h under anoxic conditions. The biochar particles were then removed using 0.45-μm filters, and the filtrate was hereafter referred to as biochar leachate. Concentrations of dissolved organic carbon (DOC) in the biochar leachates were measured by analysis of dissolved organic carbon using Shimadzu TOC-VCSH (Kyoto, KYT, Japan). Hematite with a concentration of 100 mM was added to the solution of biochar leachate, and the suspensions were bubbled with N_2 to produce anoxic environment. Washed MR-

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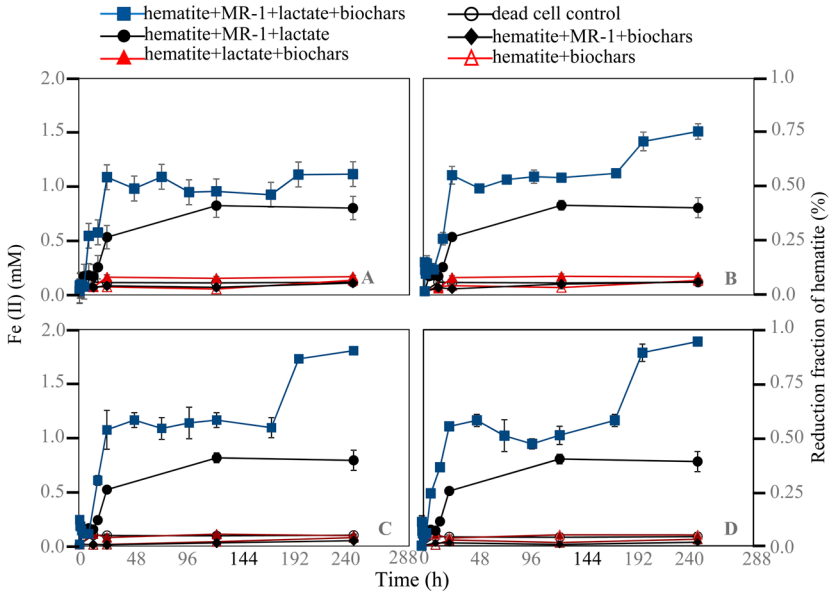

Figure 1. Production of Fe(II) and reduction fraction of hematite during the microbial reduction of hematite in the presence of biochars 500A (A), 500B (B), 250A (C), and 250B (D). 500A/B denotes biochars produced at 500 °C, and 250A/B denotes biochars produced at 250 °C. Size of biochar 500/250 A ranged 0.25−0.5 mm, and 500/250B was smaller than 0.25 mm. MR-1 represents *Shewanella oneidensis* MR-1. The hematite + MR-1 + lactate treatment was conducted to analyze the microbial reduction of hematite by MR-1 in the absence of biochars. The dead cell control was used to determine the reduction of hematite by biochar in the absence of biotic Fe(III) reduction. Error bars represent the standard deviations obtained from triplicate experiments (the same for all the following figures).

1 cells ($10^{8.5}$ cells mL^{-1}) and lactate (20 mM) were added, and Fe(II) production was monitored as described above.

Electron Shuttling Experiment. The potential for electron shuttle-mediated hematite reduction by biochar leachate was determined following the method from a previous study.[5] Briefly, 20 mM sodium L-lactate, 10 mg L^{-1} of biochar particles, and $10^{8.5}$ cells mL^{-1} MR-1 were mixed in 20 mM PIPES buffer (pH 7). After 24-h anoxic incubation, cells and biochar particles were removed by 0.2-μm filters in an anaerobic chamber (COY, Grass Lake, MI), and preautoclaved hematite particles were added to the filtrate to achieve a final concentration of 100 mM. At different intervals, samples were sacrificed for Fe(II) analysis. A set of control experiments were set up using the same procedure, which contained MR-1 cells but no biochar leachates.

Influences of Shaking Speed. The influences of shaking speed on the reduction of hematite and the adsorbed fraction of Fe(II) were studied using biochar particle 250B (hematite + MR-1 + lactate +10 mg L^{-1} biochar 250B) at different shaking speeds (0, 100, and 200 rpm). Both dissolved and acid-extractable Fe(II) were measured on day 0, 1, 5, and 10.

Electron Paramagnetic Resonance (EPR) Analysis. EPR spectroscopic analysis was applied to detect semiquinone radicals associated with biochars. Fixed amounts (0.0015 g) of biochar particles were added to the EPR test tubes. The samples were measured with a Bruker EMXPlus EPR spectrometer (Billerica, MA). Major parameters for EPR measurement were as follows: microwave frequency 9.38 GHz, microwave power 20 dB (or 2.0 mW), sweep width 200 G, and sweep time 30 ms. Blank EPR tubes were also placed in EPR spectrometer to measure the baseline for adjustment. The area under the curve of the EPR signal (X_{area}), as an indicator for the amount of semiquinone radicals, was integrated using the software of Xenon 1.1b.44 version (Bruker, Billerica, MA).

To study the impact of microbial activities on the content of semiquinone radicals, EPR analysis was applied to biochar samples before and after microbial reduction. Biochar particles (500A, 500B, 250A, and 250B) were mixed with 20 mM PIPES buffer (pH 7) to a final concentration of 500 mg/L, and the leachates were collected by syringe filtration with a 0.45 μm membrane filter and measured for EPR signal. MR-1 cells ($10^{8.5}$ cells mL^{-1}) were added into the biochar leachates. After 24 h microbial reduction, the suspension was further filtered, and the filtrate was used for EPR measurements.

Statistical Analysis. Statistical analyses were performed using IBM SPSS Statistics.

RESULTS AND DISCUSSION

Enhancement of Hematite Reduction by Biochars. Compared to biochar-free controls, the 240-h reduction extent of hematite was substantially enhanced (ca. 50−100% compared to biochar-free controls) by all four types of biochars (Figure 1). Only negligible Fe(III) reduction took place in the presence of biochar and absence of lactate, suggesting that MR-1 could not utilize the biochars as an electron donor. Similarly negligible amounts of reduction were observed in cell-free controls both with and without lactate. In addition, the presence of biochar also increased the initial reduction rate, obtained from the linear regression for the Fe(II) concentration versus time within the first 24 h, from 0.019 mM h^{-1} for the control to 0.039−0.047 mM h^{-1} (SI, Table S1, Figure S3). These results demonstrate that biochar enhanced not only the long-term (240 h) reduction extent for hematite but also its initial reduction rate.

Although we used a comparatively low concentration (10 mg L^{-1}) of biochars, the relative enhancement of hematite reduction in the presence of biochars overlapped with that observed for the reduction of ferrihydrite reported by Kappler et al.[22] In the latter study, 58% of ferrihydrite was reduced by

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Shewanella oneidensis MR-1 without biochar, whereas 77−103% of ferrihydrite was reduced in the presence of 5−10 g L^{-1} biochar. Biochar-promoted increase in hematite reduction was also comparable to the impact of dissolved humic substances. For instance, Royer et al.[33] showed that 500 mg C L^{-1} humic substances increased the 5-day reduction of hematite by a factor of 1.1−3.5. To the best of our knowledge, this is the first report on biochar-mediated reduction of a crystalline Fe(III) oxide. Although crystalline Fe(III) oxides are reduced more slowly than ferrihydrite because of their different redox potentials, larger particle size, and smaller surface area,[34−36] they often constitute the dominant form of Fe(III) oxides in soils and sediments.[37]

Importance of Biochar Leachate. Reduction of hematite was also promoted by biochar leachate (Figure 2). The

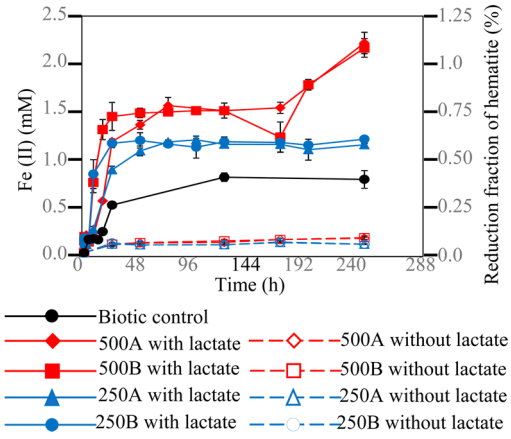

Figure 2. Production of total Fe(II) during microbial reduction of hematite in the presence of biochar leachate. Filled and open symbols show results for MR-1 cultures with and without lactate, respectively.

enhancement factors (%), i.e., the difference between 240-h Fe(II) concentration in samples and control divided by that in the control, were 186%, 180%, 48%, and 56% for biochar leachates from 500A, 500B, 250A, and 250B, respectively. Without lactate, the reduction of hematite was much slower, indicating only minor contribution from biochar leachate as an electron donor (Figure 2). With biochar leachate, the initial reduction rate ranged 0.019−0.047 mM h^{-1}, 1−3.6 times of the control value (SI, Table S1, Figure S4).

We compared the enhancement of extents of hematite reduction by the presence of biochar particles and leachate (Figure S5). With biochar particles, the greatest enhancement was observed within 24 h for most of the biochars except biochar 250A, which showed the highest enhancement at 240 h. The highest enhancement factors were 229%, 111%, 128%, and 207% for biochar 500A, 500B, 250A, and 250B, respectively, whereas the values were 172−468% for biochar leachate (Figure S5). Only for rare cases were the enhancement factors for biochar particles substantially higher than that for biochar leachate, such as at 8 h for biochar 500A. In most cases, the enhancement due to biochar leachate was similar or even higher than biochar particles. For examples, at 240 h, for biochar 500A and 500B, the biochar leachate had enhancement factors higher than that of the biochar particles (Figure S5).

The importance of biochar leachate was also supported by the results for the reduction of hematite with different doses of biochars (Figure S6). In response to an increased concentration of biochar particles, the concentration of biochar leachate (measured as DOC) increased disproportionally. For example, when the biochar concentration increased from 10 mg L^{-1} to 2.5 g L^{-1}, the DOC increased from 1.5 to 2.4 mg C L^{-1} to 4.5−26.5 mg C L^{-1} (Figure S7). This result can be tentatively explained by the limited solubility of biochar, although the nature and physicochemical properties of these biochar leachates warrant further study. The impact of biochar dose on hematite reduction was studied using biochar 250B (Figure S6). When the concentration of biochar 250B was increased to 500, 1000, 2500 mg L^{-1}, the hematite reduction did not increase proportionately. Multiple-variant linear regression was used to describe the relative change in the amount of hematite reduced at 24, 120, and 240 h:

$$R_r = aR_b + bR_L + c \quad (1)$$

where R_r is the relative change in the amount of hematite reduced, R_b is the relative change in total biochar concentration, R_L is the relative change in the biochar leachate concentration, and a, b, and c are constants. In all cases, the relative change was calculated based on the value for samples with 10 mg/L biochar. The multivariant regressions fit the results fairly well, with a always negative and b always positive (Figure S8). These results demonstrate the importance of biochar leachate in facilitating the reduction of hematite and differ from the results of Kappler et al.,[22] which did not find significant contribution from biochar leachate, probably because the wood-derived biochar did not release a substantial amount of leachate.

Extracellular Electron Transport. Biologically reduced biochar leachate abiotically reduced hematite at extents of 20−80% higher than those observed with filtrate from MR-1 cultures grown in the absence of biochar leachate (Figure 3).

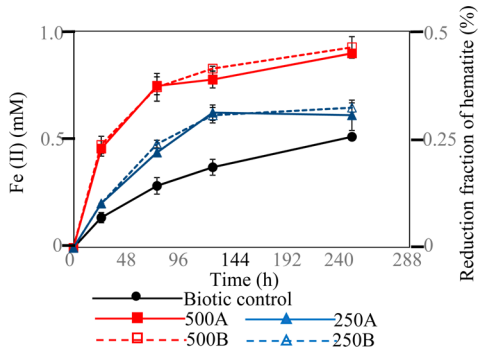

Figure 3. Production of Fe(II) during the abiotic reduction of hematite by the microbially prereduced biochar leachate. For biotic controls, the bacteria was incubated without biochars, and then the filtered solution was used to react with hematite abiotically.

The ability of biochar leachate-free MR-1 culture filtrate to reduce hematite was consistent with the results from Marsili et al.[38] and von Canstein et al.,[39] which demonstrated that MR-1 released flavin-like substances, with the capacity to shuttle electrons at a rate of 0.62 μmol Fe(III) min^{-1} g $protein^{-1}$. The flavin-like substances enabled the extracellular electron shuttling process of MR-1 for the respiration of insoluble oxide minerals (hematite in our study).

Our results clearly demonstrate that biochar leachate can serve as an extracellular electron shuttle that drives abiotic hematite reduction. This extracellular electron transport will contribute to the enhancement of hematite reduction, especially for its initial reduction rate. Such extracellular electron transport is thought to be mediated by the semiquinone

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radicals.[40] As the biochar leachate concentration is relatively low, direct analysis for biochar leachate-associated semiquinone radical is challenging. Hence, EPR analysis was first performed to analyze the biochar particle-bound semiquinone radicals, of which the physicochemical nature is presumably similar to those bound with biochar leachate (Figure S9). Significant EPR signals were detected for all four biochars, with a g value (a parameter indicating the type of free organic radicals) of 2.0029, 2.0029, 2.0036, and 2.0035 for biochar 500A, 500B, 250A, and 250B, respectively (Figure S9). These values were relatively low compared to those observed for humic substances (2.0037−2.0048).[8] Liao et al.[41] reported g value of 2.0036−2.0053 for radicals in biochars produced from corn stalk, rice straw, and wheat straw. Previous studies indicate that the g value is a good index for distinguishing different types of radicals, with $g < 2.003$ for carbon-centered and $g > 2.004$ for oxygen-centered radicals.[41−43] The g values are 2.0028 and 2.0026 for graphitic carbon and polycyclic aromatic carbon radical, respectively, and it is normally above 2.004 for semiquinone radicals.[44,45] Our results suggest that biochar 500A and 500B mainly contained carbon-centered radicals (mainly aromatic carbon), whereas there were mixed oxygen-centered (including semiquinone) and carbon-centered radicals for biochar 250A and 250B. Carbon-centered radicals can also be contributed by semiquinone groups, because of the resonance effects and lower activation energy for carbon-centered semiquinone radicals compared to those oxygen-centered radicals.[42] The exact nature of radicals in biochar warrants further investigation, especially considering that the complex matrix of biochar can also shift the g value for radicals.

The intensity of the EPR signal (for 0.0015 g samples) was much smaller for biochar 250A and 250B compared to 500A and 500B, with the X_{area} of 1809, 1621, 23.7, and 7.8 for biochar 500A, 500B, 250A, and 250B, respectively. This is consistent with the higher aromatic carbon content in biochar 500A and 500B, as a result of higher HTT (Figure S1, Table 1). A recent study showed that increased pyrolysis temperatures can greatly increase the amount of radicals in biochars.[41] The X_{area} for biochar EPR signals, as an index for the relative amount of semiquinone radicals, correlated strongly with the 240-h abiotic reduction of hematite by microbially prereduced biochars (Pearson correlation coefficient $r = 0.99$, $p = 0.015$), supporting the importance of semiquinone radicals in the electron shuttling process. The direct correlation between EPR signal and the reduction of hematite by biochar leachate ($r = 0.99$, $p = 0.002$) further suggests the critical role of electron shuttling in the reduction of hematite by biochar leachate. However, no strong correlation was found between EPR signal and the 240-h reduction extent or initial reduction rate of hematite in the presence of biochar particles, possibly due to the contribution of other processes mediated by particle-phase biochars.

The potential critical role of semiquinone radicals in the extracellular electron transport process was further supported by the changes in EPR signals of biochar leachate following exposure to MR-1 (Figure 4). Weak EPR signals were detected for all the four biochar samples (500 mg L^{-1} biochar in 20 mM PIPES buffer) (Figure 4). However, after reacting with cell suspension for 1 h, the EPR signals of biochar 500A and 500B were significantly enhanced with g value of 2.0028 and 2.0028, and X_{area} of 204.1 and 772.2. After 24-h anaerobic incubation with cell suspension, the EPR signals were even stronger with X_{area} of 221.4 and 935.5 for biochar 500A and 500B, respectively (Figure 4A and 4B). In contrast, there was no

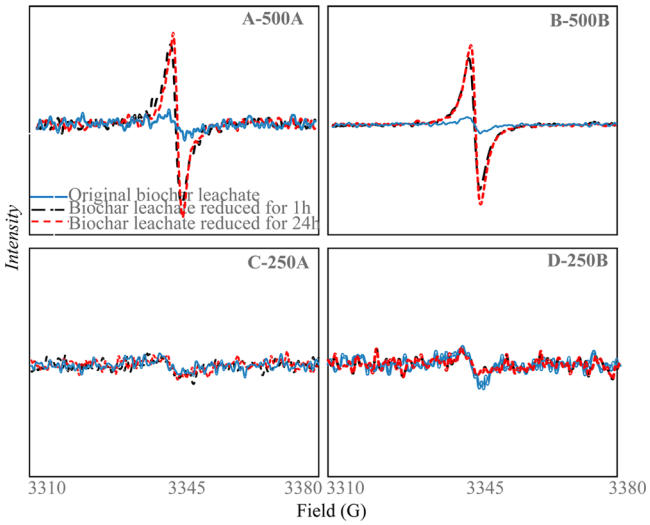

Figure 4. Electron paramagnetic resonance (EPR) signals detected for biochar leachate before and after the microbial reduction for 1 and 24 h. Panel A, B, C, and D represents results for biochar 500A, 500B, 250A, and 250B, respectively.

substantial enhancement in EPR signals of biochar 250A and 250B after reacting with MR-1, consistent with their relatively low fraction of aromatic and semiquinone functional groups (Figure S9, Table 1). This result supports that biochar leachate-bound semiquinone functional groups can be involved in the extracellular electron transport. Together with the reduction of hematite in the presence of biochar leachate and the response of hematite reduction to biochar dose, our evidence suggests the importance of biochar leachate in enhancing hematite reduction, mainly through the electron shuttling processes potentially mediated by the semiquinone functional groups.

Complexation of Fe(II) with Solid Biochar. In addition to the electron shuttling process, biochar particles also have the capacity to bind divalent cations,[28] thus possibly decrease or delay the accumulation of both aqueous and oxide/DIRB cell surface-bound Fe(II), consequently facilitating the long-term reduction of hematite.[24,27] In the biochar-free systems, adsorbed Fe(II) accounted for 30.9 ± 4.4% of total acid-extractable Fe(II) after 96 h. In the presence of biochars, adsorbed Fe(II) was higher, accounting for 38.1 ± 0.7%, 42.9 ± 2.1%, 41.7 ± 1.1%, and 60.2 ± 0.9% of total Fe(II) for samples in the presence of biochar 500A, 500B, 250A, and 250B, respectively (Figure 5). The presence of biochar thus increased the fraction of sorbed Fe(II) by 1.23−1.95 times after 96 h, and 1.41−1.73 times for samples after 216 h. On the basis of a previous study,[46] the maximum monolayer sorption of Fe(II) on hematite can reach 125 μmol g^{-1}. In our control system, we found around 28 μmol g^{-1} Fe(II) was sorbed on hematite, occupying 22% of its surface. The sorption on biochar can potentially release a substantial fraction of hematite surface and enhance its long-term reduction extent. The increased sorption of Fe(II) to biochar appears to facilitate long-term hematite reduction by serving as an additional sink for biogenic Fe(II), analogous to results obtained previously with mineral phases (aluminum oxide and clays).[27]

The impact of Fe(II) complexation with biochar particles was further supported by the influence of shaking speeds on long-term hematite reduction by MR-1. Results showed that when the shaking speed increased from 0 to 200 rpm, the 240-h reduction extent of hematite in the presence of biochar 250B

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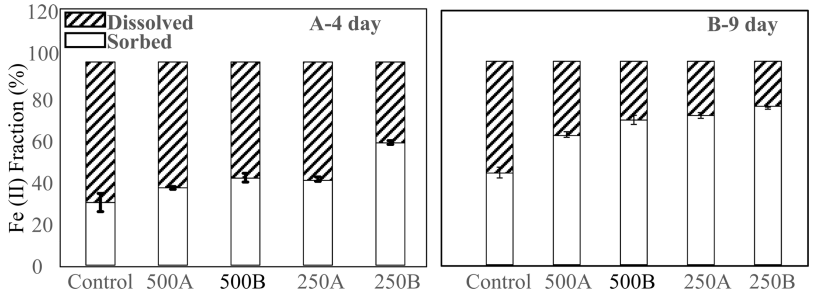

Figure 5. Fraction of dissolved and sorbed Fe(II) after 4 and 9 days of microbial reduction of hematite in the presence of biochars (hematite + MR-1 + lactate + biochars). Dissolved Fe(II) was measured by analyzing Fe(II) in the filtered solution, and sorbed Fe(II) was determined as the difference between acid-extractable Fe(II) and dissolved Fe(II). Control represents results from the controls without biochars.

increased from 0.42 ± 0.02% to 1.0 ± 0.01% of the initial hematite concentration (Figure S10). Correspondingly, the fraction of sorbed Fe(II) increased from 40% to 86%, when the shaking speed increased from 0 to 200 rpm. The simplest explanation for these findings is that increased shaking speed facilitated sorption of Fe(II) by biochar particles and therefore the Fe(II) reduction extent. The presence of biochar 250B led to the highest fraction of sorbed Fe(II) compared to other biochars, which was consistent with the highest reduction of hematite in the presence of biochar particles (Figure 1), despite the fact that the semiquinone radical amount and electron shuttling capacity of biochar 250B were not the highest (Figure 3, SI, Figure S9). These results pointed to a significant role of Fe(II) complexation with biochars in the facilitated reduction of hematite.

Environmental Implication. Our results demonstrated that both biochar particles and leachate can facilitate the microbial reduction of hematite, likely through electron shuttling as well as the removal of aqueous Fe(II) through adsorption. Such processes have broad implications for the reduction of crystalline Fe(III) oxides in the presence of pyrogenic carbon, which occurs ubiquitously in natural environments.[47] Aqueous biochar leachate had similar or even greater effects as compared to biochar particles in enhancing the reduction of hematite, mainly through the electron shuttling process. In addition, Fe(II) binding by biochar particles can enhance the long-term hematite reduction. These combined reaction pathways provided new perspectives for evaluating the linkages between the biogeochemical cycles of Fe and carbon in permanently or transiently anoxic soils and sediments.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.5b05517.

Table S1, initial reduction rate for hematite; Figure S1, NMR for biochar; Figure S2, release of Fe (II) from biochar; Figure S3, regression for determining initial reduction rate for samples with biochar; Figure S4, regression for determining initial reduction rate for samples with biochar leachate; Figure S5, enhancement factor for the reduction of hematite; Figure S6, response of hematite reduction to the dose of biochar; Figure S7, release of DOC from different doses of biochar; Figure S8, multivariance regression for the enhancement factors; Figure S9, EPR for biochars; Figure S10, response of reduction to shaking speed (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*Phone: 775-682-6609. E-mail: yuy@unr.edu.

Present Address

[#]Department of Civil and Environmental Engineering, University of Alberta, Edmonton, AB T6G 2R3, Canada.

Notes

The authors declare no competing financial interest.

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