Letter

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# Wood-Derived Black Carbon (Biochar) as a Microbial Electron Donor and Acceptor

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## Abstract

Research on the environmental impacts of black carbon has focused largely on sorption. Besides being a strong geosorbent, black carbon is redox-active and may facilitate abiotic and microbial transformation. Using a wood-derived black carbon (biochar) and the bacterium *Geobacter metallireducens* (GS-15), we showed that air-oxidized biochar served as an electron acceptor to enable acetate oxidation, and that chemically or biotically reduced biochar served as an electron donor for nitrate reduction. The bioavailable (to GS-15) electron storage capacity (ESC) of the biochar, estimated based on acetate oxidation and nitrate reduction, was 0.85 and 0.87 mmol $e^-$/g, respectively, comparable to the ESCs of humic substances and other biochars measured electrochemically. We propose that black carbon should be regarded as a rechargeable reservoir of bioavailable electrons in anaerobic environments. The redox cycling of biochar in natural and engineered systems and its impact on microbial processes and contaminant fate merit further investigations.

## Introduction

Black carbon and natural organic matter are the two major types of carbonaceous geosorbents that control the fate and transport of hydrophobic contaminants in the environment.[1-3] Until recently, research on the impact of black carbon on contaminant fate has focused on adsorption, where black carbon was considered to be chemically inert toward adsorbates. This assumption is common in sediment remediation and risk assessment.[4-6] However, studies in recent years have shown that black carbon, such as soot, activated carbon, graphite, char, carbon nanotubes, and graphene oxide, is not merely a passive adsorbent but a catalytically active material that can mediate the abiotic reduction of nitrogenous compounds.[7-10] While the mechanisms are not fully understood, the catalytic ability of black carbon appears to stem from its graphitic structure and/or redox-active functions such as (hydro)quinones. First, through adsorption of reactants and conduction of electrons and hydrogen atoms, high-purity graphite can mediate the reduction of nitro and azo aromatic compounds, heterocyclic nitramines, and nitrate esters, even when these compounds are physically separated from the reductant.[9,11,12] Second, similar to natural organic matter, black carbon that contains quinone groups may undergo reversible redox reactions, which explains the catalytic ability of less graphitic (more functionalized) black carbon such as soot and biochar.[7,8,10] Indeed, the capacity of biochar to reversibly donate and accept electrons has been quantified by Klüpfel et al. in an electrochemical cell[13] and was attributed to the redox-facile quinone groups in biochar. Depending on the source biomass and the pyrolysis temperature, the electron storage capacity (ESC) of plant-based biochar was up to 2 mmol $e^-$/g,[13] on a par with those of dissolved and particulate organic matter.[14,15] Note that, in contrast to the first mechanism, which involves *transfer* of electrons through conductive (i.e., graphene) domains of black carbon,

the second mechanism involves *storage* of electrons in biochar through charging and discharging (i.e., reduction and oxidation) of the quinone functions.

In addition to being a geosorbent and mediator of abiotic redox reactions,[14,16] natural organic matter such as humic acid is also an important electron acceptor[17,18] and donor[19] for anaerobic microbes. This suggests black carbon that has a significant quinone content (and thus ESC) may similarly support microbial transformation by serving as *both* an electron donor and acceptor. In this study, we tested this hypothesis using a wood-based biochar and the organism *Geobacter metallireducens*. We showed the biochar can be an electron acceptor to enable acetate oxidation, and an electron donor to support nitrate reduction to ammonium, by *G. metallireducens*. We also estimated the microbially accessible ESC of this biochar. The implications of our results for the potential roles of black carbon in biogeochemistry, pollutant fate, and engineering applications are discussed.

## Materials and Methods

*Biochar.* Soil Reef biochar (The Biochar Company, PA) produced through pyrolysis of Southern Yellow hardwood chips at 550 $^{o}$C was used. This commercial biochar has been used in pilot[20] and full-scale bioretention cells and infiltrations strips for stormwater treatment in Delaware and Virginia. The properties of the biochar are given in Table S1 in the Supporting Information (SI). Biochar was sieved and the 250-500 μm fraction was oxidized in continuously aerated deionized water[13] (50 g/L) over 60 h. The oxidized biochar was filtered, vacuum-dried, autoclaved (121 $^{o}$C, 15 min), and purged with $N_2/CO_2$ (80:20) before use. Total organic carbon (TOC) analysis of the

rinsates/filtrates suggests the washed/oxidized biochar contained little dissolved organic carbon (Table S2, SI).

*Microorganism. Geobacter metallireducens* (GS-15) was chosen for this study because it can use humic acid as both an electron acceptor[17] and donor[19] but cannot use $H_2$ as an electron donor,[21] which was present (5%) in the glove box and thus might exist in reactors. GS-15 (ATCC 53774) was grown on 5 mM each of acetate and nitrate in a modified ATCC 1768 medium. GS-15 oxidizes acetate to $CO_2$ and reduces nitrate dissimilatorily to ammonium through nitrite.[22] After an 18-h incubation at 30 $^oC$, culture was centrifuged at 1100g for 15 min, washed 4 times with an anoxic medium ($N_2/CO_2$-purged ATCC 1768 without electron donor, electron acceptor, or $NH_4^+$) and re-suspended to a density of $7.0(\pm1.6)\times10^9$ cells/mL, as measured by optical density at 600 nm. Further details about cell density estimation are given in SI.

*Acetate Utilization Experiment.* Serum bottles (125 mL) were set up in a glove box ($N_2/CO_2/H_2$, 75:20:5) in quintuplicates, each containing 104 mL of the anoxic medium (above) with known quantities of oxidized biochar (2 or 4 g) and cells (~$2\times10^8$/mL). Cysteine (158 μmol, <5% of the electrons from acetate oxidation) was added to each bottle to scavenge oxygen. Additional bottles were prepared in triplicates as controls: oxidized biochar (no cells), cells only, cells plus cystine (no biochar), and blank (medium only). The measured pH was 6.9±0.1 throughout each experiment. All reactors were sealed with butyl rubber stoppers and aluminum crimps, foil-wrapped, spiked with 0.4 mmol of sodium acetate (~4.0 mM), and incubated at 30 $^oC$.

*Nitrate Reduction Experiments.* Upon completion of the acetate utilization experiment, reactors containing 2 g of biochar were placed in a glove box and the (biologically reduced) biochar was retrieved and washed 5 times with 30 mM deaerated bicarbonate buffer and twice with anoxic medium to remove residual acetate and cells. Reactors and controls were set up in triplicates as described above, except either oxidized or biological reduced biochar was used, and ~0.45 mmol of nitrate was spiked instead of acetate. To further test our hypothesis, a second nitrate reduction experiment was conducted using chemically reduced biochar. Air-oxidized biochar was reduced in 100 mL of 75 mM sodium dithionite (Fisher, Pittsburgh, PA) solution overnight in a glove box and washed thoroughly with 30 mM bicarbonate buffer and anoxic medium. The reduced biochar was used to prepare nitrate reduction experiments as outlined above, except cysteine was omitted.

*Sampling and Analyses.* Liquid samples were collected from reactors at pre-determined times, diluted, filtered (0.22-μm), and split for cation and anion analyses by ion chromatography (IC). The sampling and analytical methods are described in SI.

## Results and Discussion

*Acetate Utilization*. As shown in Figure 1, aqueous acetate concentrations were constant in both controls and the blank over 6 days, indicating that physical losses (e.g., sorption to biochar) were minimal and that GS-15 grown on acetate could not utilize acetate without an electron acceptor. In the presence of 2 g of oxidized biochar, acetate was consumed immediately and continuously. The rate of acetate utilization was 0.66±0.10 mM/d for the first 2 d but slowed and approached zero at later times. This suggests air-oxidized biochar could support acetate oxidation by GS-15, presumably by acting as an electron sink, though its capacity to do so appeared to be finite. With

4 g of oxidized biochar, the initial rate of acetate utilization doubled to 1.3±0.1 mM/d, and acetate was completely degraded by day 6. Thus, both the rates and extents of acetate utilization support the hypothesis that air-oxidized biochar could serve as a microbial electron acceptor.

It has been suggested that the cysteine/cystine couple can mediate electron transfer in syntrophic acetate oxidation.[23] Although neither cysteine nor cystine is known to be a substrate for GS-15,[21] it might be possible that the added cysteine was abiotically oxidized by biochar to cystine, which was then used by GS-15 to oxidize acetate. However, no acetate was lost in controls containing cells and stoichiometric amount (1.6 mmole) of cystine (Figure 1), indicating cystine was not an electron acceptor or mediator for acetate oxidation, and that GS-15 transferred electrons directly to biochar. Assuming acetate oxidation stopped due to the finite electron accepting capacity of 2 g of biochar, and given that 8 electrons would be exported per acetate ion oxidized, the ESC of the oxidized biochar accessible to GS-15 was calculated to be 0.77 mmol/g. The calculations are detailed in SI. As discussed below, the actual ESC of the biochar was probably greater than 0.77 mmol/g due to the presence of other electron sources.

*Nitrate Reduction*. Result of a nitrate reduction experiment with biologically reduced biochar is shown in Figure 2(a). Without cells, nitrate was stable in the anoxic medium containing oxidized biochar. In all controls receiving cells, nitrate was removed instantly but only to a limited extent, with or without oxidized biochar. Ammonium (and traces of nitrite) was detected, indicating that nitrate was indeed reduced. The possible electron sources in these controls were the cysteine and cells added. *Geobacter* species are known to store electrons in the periplasmic and outer-surface cytochromes,[21] and rest cells of GS-15 could reduce Pu(VI) and U(VI) without external electron

donors.[24] As shown in SI, the amount of electrons carried by cysteine and cells, estimated based on ammonium yields, was 0.173 mmol in each reactor. Accounting for the additional electrons, the ESC of the air-oxidized biochar in acetate utilization experiments would be ~0.85 mmol/g. In contrast to the controls, reactors containing cells and microbially reduced biochar harvested from acetate utilization experiments showed sustained nitrate removal (Figure 2(a)) and concomitant formation of ammonium (Figure S1(b), SI). This indicates the electrons stored in biochar from acetate oxidation were subsequently retrieved by GS-15 for nitrate reduction.

Attempt to include an abiotic control containing microbially reduced biochar without added cells was unsuccessful, because the washing procedure could not eliminate all GS-15 cells associated with biochar (Figure S2, SI) and nitrate reduction would commence after an initial lag. To verify that reduced biochar cannot reduce nitrate abiotically, and to confirm that biochar reduced either biologically or chemically can be an electron donor for microbial nitrate reduction, an additional experiment was conducted using dithionite-treated biochar. As shown in Figure 2(b), nitrate was not removed by dithionite-reduced biochar without cells, and was removed to a limited extent in the biotic control, as discussed earlier. The $NH_4^+$ yields in all controls were 25–30%, suggesting the nitrate removed from solution was only partially reduced. In reactors with dithionite-reduced biochar and cells, nitrate was removed faster and more extensively and the removal stopped at 72 h. Interestingly, $NH_4^+$ continued to form and reached a plateau at 192 h. Based on the ammonium yield of 78.0% (Figure 2(b) and SI), the bioaccessible ESC of dithionite-reduced biochar was calculated to be 0.87 mmol/g.

The *bioavailable* ESC of Soil Reef biochar is comparable to the ESCs of wood and grass biochar prepared at 500 $^{o}$C, 0.59 and 1.04 mmol/g,[13] respectively, measured electrochemically using the electron transfer mediators 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS, $E_H^{o'}$ = 0.70 V) and 4,4′-bipyridinium-1,1′-bis(2-ethylsulfonate) (ZiV, $E_H^{o'}$ = –0.41 V). This is somewhat surprising. We expected the bioavailable ESC to be smaller because 1) the mediator molecules would access virtually all quinone groups including those that were sterically hindered, whereas GS-15 presumably could access only exposed quinone groups at/near biochar's exterior, and 2) the electrochemical method can measure ESC (i.e., reduce/oxidize quinone groups) over a wider range of redox potentials than the microbes could achieve thermodynamically.[25] Further studies are necessary to obtain the ESC and redox potential distribution of Soil Reef biochar in order to determine what portion of its total ESC is bioavailable.

*Mechanism*. Our results show that, similar to acetate, humic acid, and anthrahydro-quinone-2,6-disulfonate (a humic acid surrogate),[19,26] chemically or biologically reduced biochar supported nitrate reduction by GS-15 to ammonium (Figures 2(b) and S1). In contrast, GS-15 reduced nitrate only to nitrite with a graphite cathode as electron donor.[27] This suggests that, like humic acid (which *stores* electrons) and unlike graphite (which *conducts* electrons), biochar supports microbial transformation through reversible redox reactions of its quinone groups rather than by electron conduction through its graphene domains. While conductivity may be involved in black carbon-mediated abiotic electron transfer reactions,[11,28] it does not explain the ability of biochar to store electrons from acetate and subsequently release them to nitrate in a separate experiment.

183 It is important to distinguish these two mechanisms. In recent years, direct interspecies electron
184 transfer (IET) has been illustrated.[29,30] It was proposed black carbon such as activated carbon[31]
185 and biochar[32] can promote IET by conducting electrons in between microorganisms. Chen et al.[32]
186 attributed the stimulated "IET" observed in co-cultures of GS-15 with *Geobacter sulfurreducens*
187 or *Methanosarcina barkeri* to electron *conduction* through biochar, even though all the biochars
188 used were poorly conductive. Electron balance calculations show that a portion of the electrons
189 released during ethanol oxidation by GS-15 was missing, suggesting biochar acted as an electron
190 sink (0.5 mmol/g).[32] That is, Chen et al.'s results are actually consistent with ours and support the
191 electron *storage* (i.e., quinone) mechanism. Our study thus provides an alternative mechanism to
192 black carbon-promoted IET. Depending on its properties (e.g., abundance of redox-active groups
193 and aromaticity), black carbon may support microbial activities through different mechanisms.
194
195 *Implications and Applications*. The important role of dissolved and solid-phase organic matter in
196 geomicrobiology as electron acceptor[18,33] and donor[19,34] is well-known. Organic matter possesses
197 an ESC between 0.5 and 7 mmol/g OC[14,33,35] and represents a significant reservoir of electrons in
198 anaerobic environments. Kappler et al.[36] recently showed that, analogous to humic acid, biochar
199 facilitated electron transfer from *Shewanella oneidensis* to ferric minerals; i.e., biochar served as
200 a microbial electron acceptor. Our study further shows that biochar can be *both* an electron donor
201 and acceptor and may possess a bioavailable ESC comparable to that of dissolved and particulate
202 organic carbon. Therefore, biochar and other black carbon should be regarded as *rechargeable*
203 reservoirs of bioavailable electrons. Given the high annual global emission rate of black carbon
204 (8.4 MT/y,[37,38] two thirds of which is derived from burning of wood and other biomass) and its
205 ubiquity in soil and sediment,[1] the redox cycling of black carbon in anaerobic environments and

its potential impacts on microbial processes (e.g., iron/sulfate reduction and methanogenesis[18]) and contaminant fate (e.g., nitrate[20]) merit further investigations.

In recent years biochar has been promoted as a beneficial soil amendment,[39-43] and many studies have claimed that its addition promotes denitrification in soil.[40,43,44] While different mechanisms have been suggested,[43-45] few were substantiated by direct evidence. Our study offers a plausible mechanism for biochar-supported microbial denitrification, and provides experimental evidence to support that claim.

It should be noted that GS-15 was selected for its suitability to test our hypothesis, not for nitrate treatment. While GS-15 reduces nitrate to ammonium with biochar, microbes that use humic acid as electron donor to reduce nitrate to $N_2$ are widespread in soil[34,46] and presumably can also use biochar to do the same. This is supported by a recent field study.[20] Based on the hypothesis that biochar can be microbially charged (e.g., via organic carbon oxidation) and discharged (e.g., via nitrate reduction), field tests were conducted where nitrate was injected into a pilot bioretention cell amended with Soil Reef biochar (18% v/v) and a control cell without biochar. More than one third of the added nitrate was removed in the biochar-amended cell relative to the control (which showed no nitrate removal), while the effluent ammonium levels from both cells were similarly low, suggesting $N_2$ was the predominant product of denitrification in the biochar-amended cell.

We propose that, because of its ability to support both microbial oxidation and reduction, its rechargeable nature[13], its considerable ESC, and its high stability and low mobility relative to humic substances, biochar would be a useful electron storage medium to support and promote

biodegradation of contaminants in bioretention and other engineered treatment/remediation systems.

## Notes

The authors declare no competing financial interest.

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## ASSOCIATED CONTENT

### Supporting Information Available

Results of biochar and rinsate analyses, sampling and analytical methods, ESC calculations, ammonium and nitrite concentration profiles, and SEM image of washed, microbially reduced biochar. This material is available free of charge via the Internet at http://pubs.acs.org/.

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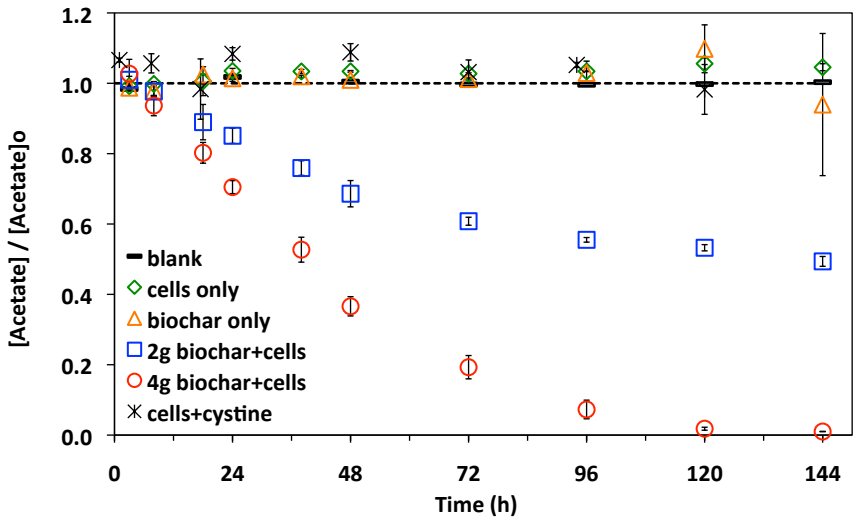


Figure 1. Acetate utilization in batch reactors containing anoxic medium only (blank), cells only (without biochar), cells with 1.6 mmol of cystine (no biochar), 2 g of oxidized biochar without cells (biochar only), and cells plus 2 g or 4 g of oxidized biochar. For biotic biochar reactors and controls, error bars represent one standard deviation from quintuplicate and triplicate reactors, respectively. The initial acetate concentration was approximately 4 mM for all reactors.

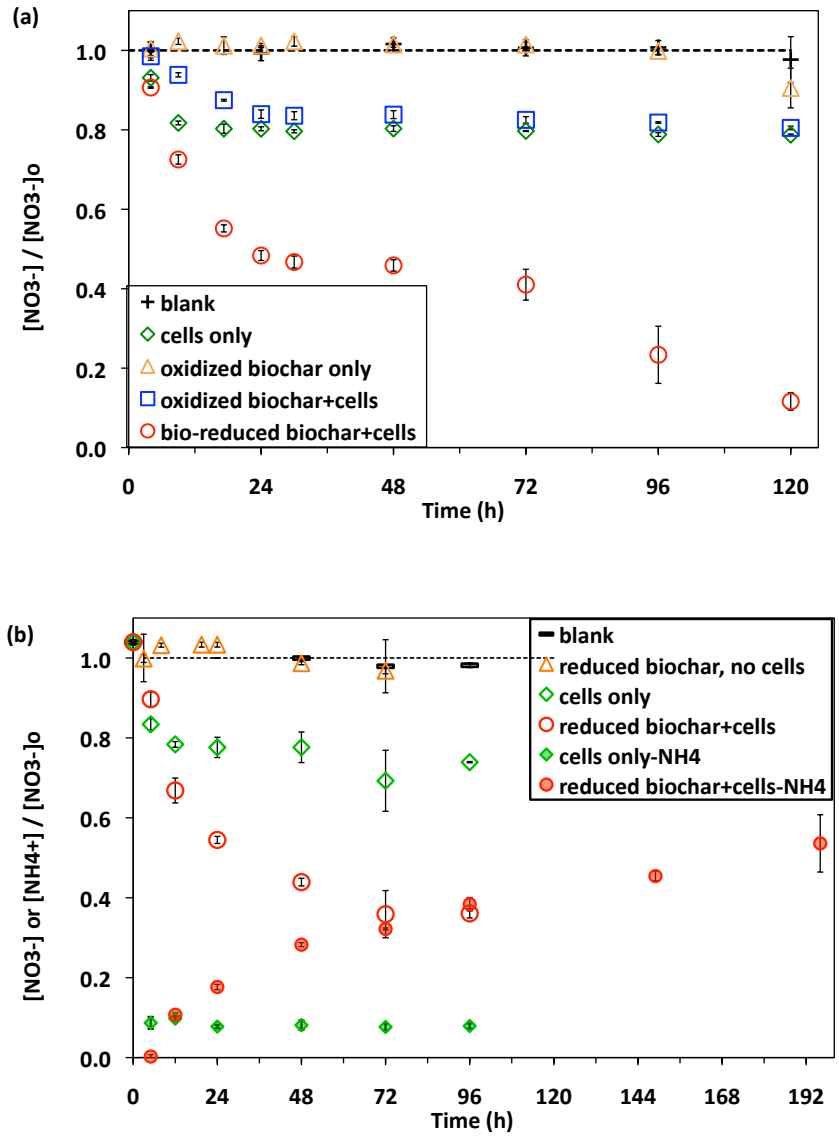


Figure 2. (a) Nitrate reduction in batch reactors containing anoxic medium (blank), cells only, 2 g of oxidized biochar with and without cells, and 2 g of biologically reduced biochar plus cells. (b) Nitrate reduction in reactors containing anoxic medium (blank), cells only, 2 g of dithionite-reduced biochar (no cells), and 2 g of dithionite-reduced biochar plus cells. No cysteine was used in this experiment. $NH_4^+$ concentrations are also shown for cells-only control and biotic biochar reactors. Initial nitrate concentration was approximately 4.4 mM for all reactors/controls. Error bars represent one standard deviation from triplicate reactors.

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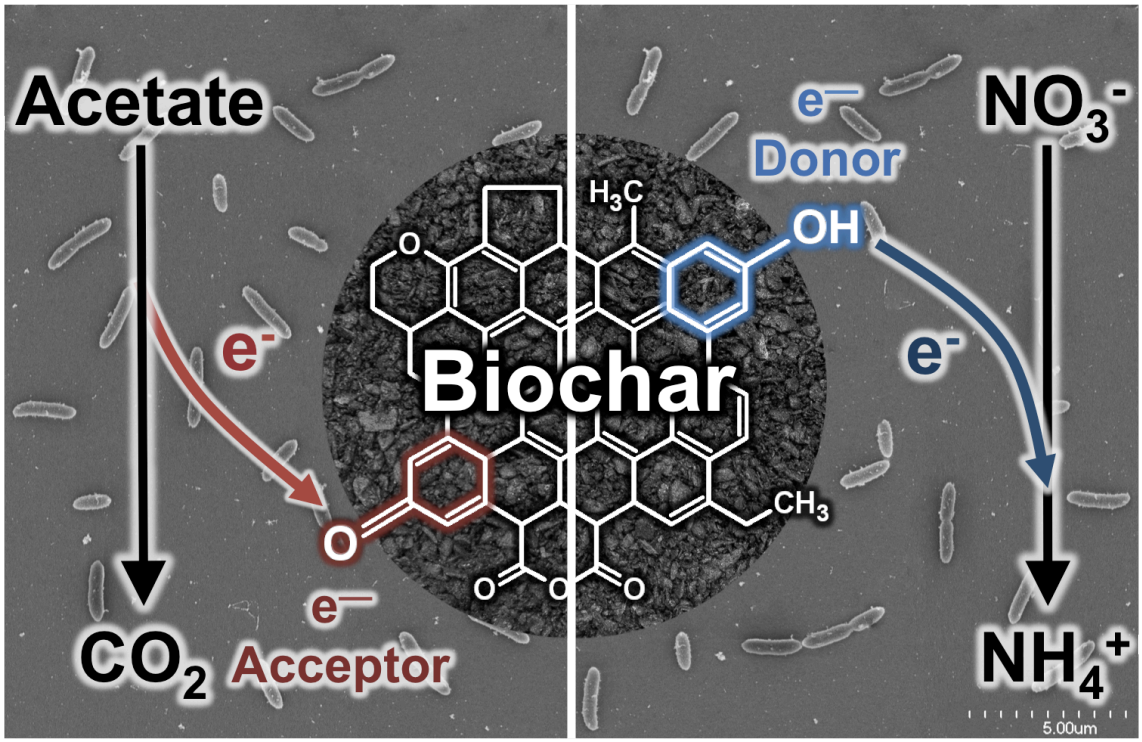


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