Biochar-Mediated Abiotic and Biotic Transformation of Halogenated Organic Contaminants

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Abstract

Prevailing global increases in population, urbanization, and agricultural production are resulting in increased pressures on water resources, especially as the use of chemicals in agriculture, industry, and medicine provide new challenges for water treatment and reuse. Organohalogen compounds are persistent contaminants whose accumulation in the environment presents a serious threat to ecosystem health and constrains water treatment technology. Recent advances in understanding pyrogenic carbons as electron shuttling and storing materials have exposed their capability of enhancing the dehalogenation and overall degradation of organohalide contaminants in soil, sediment, surface water, and wastewater systems. Biochar is a porous carbonaceous material produced during the thermochemical decomposition of biomass feedstock in the presence of little or no oxygen (pyrolysis). Interest in biochar for application towards environmental remediation are largely based on its three distinct benefits: (1) carbon sequestration to offset greenhouse gas emissions, (2) adsorption of (in-)organic contaminants and nutrients, and (3) a strong electron exchange capacity. Due to the innate complexity of biochar materials, several electron transfer mechanisms exist by which biochar may mediate contaminant degradation. These electron transfer pathways include electron-accepting and donating cycles through redox-active functional groups and direct electron transfer via conductive carbon matrices. These mechanisms are responsible for biochar's participation in multiple redox-driven biogeochemical transformations with proven consequences for effective organohalogen remediation. This literature review summarizes the current knowledge on the mechanisms and processes through which biochar can directly or indirectly mediate the transformation of organohalogen compounds under various environmental conditions. Perspectives and research directions for future application of biochars for targeted remediation strategies are also discussed.

Table of contents:

1. Introduction

Biochar, a subset of pyrogenic carbon, is produced through the thermochemical conversion of biomass by pyrolysis. This incomplete combustion of manures, agricultural wastes, and other organic debris at elevated temperatures (200 – 1000 °C) in an oxygen-deprived environment reduces the volume of biowastes and can concurrently be a method of bioenergy production, with biochar as an end product (Y. Yuan et al., 2017). Extensive studies report benefits of biochar amendment to soils, including carbon sequestration, improved retention of water and bioavailable nitrogen and phosphorous, reduced temperature sensitivity, reduced greenhouse gas emissions (CO_2, CH_4, and N_20), immobilization of contaminants, and overall improved crop yield and surface and groundwater quality (Cao et al., 2011; J. Chen et al., 2018; El-Naggar et al., 2018; Kumar et al., 2022; P. Liu et al., 2012; W. Yang et al., 2020; Y. Yuan et al., 2017). Due to its high efficiency in (ad)sorbing organic compounds, biochar has also been increasingly used for sorption-based remediation of contaminated soils, sediments, water, and wastewater (Häggblom et al., 2007; Krzmarzick & Novak, 2014a; Mohan et al., 2014; Ogura et al., 2021; Shakoor et al., 2020).

It has recently been demonstrated that pyrogenic carbon materials are capable of facilitating abiotic and biotic electron donating, accepting and conducting processes (S. Y. Oh & Chiu, 2009; Saquing et al., 2016a; Van Der Zee et al., 2003; W. Xu et al., 2010). The electron shuttling and conductive properties of biochar have been shown to stimulate mineral transformations by acting as an intermediate electron carrier between bacteria and iron (Kappler et al., 2014a; Wu et al., 2017a; Z. Yang et al., 2021). Biochar has also been shown to support natural biotic attenuation of organic contaminants through stimulation of redox processes (S. Y. Oh et al., 2013; Tan et al., 2019). This has led to the investigation of biochar as an amendment for supporting microbial degradation of halogenated contaminants (Tong et al., 2014; L. Yu et al., 2015).

Halogenated chemicals are integral components of many household products and industrial processes. They function as solvents, degreasing agents, biocides, pharmaceuticals, plasticizers, hydraulic and heat transfer fluids, intermediates for chemical synthesis, and flame retardants. The ubiquitous use of chlorinated compounds has resulted in their accidental or deliberate release into the environment. As such, organohalide contaminants, including polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs), polychlorinated biphenyls (PCBs), pesticides, and brominated flame retardants, among others, have been detected in groundwaters, surface waters, soils, sediments, and wastewater treatment systems (Häggblom et al., 2007; Krzmarzick & Novak, 2014b; R. Xu et al., 2021). This has raised major concern, due to the inherent toxicity, recalcitrance, and bioaccumulation potential of many halogenated organic compounds (Dann & Hontela, 2011; Du et al., 2017; Murati et al., 2020; Rusyn et al., 2014; She et al., 2013; Ulbrich & Stahlmann, 2004; Vorkamp & Rigét, 2014; G. Xu et al., 2021).

Electron shuttling compounds facilitate electron transfer and can serve as electron donors and/or acceptors. Depending on environmental conditions studies show that electron shuttling compounds have the capacity to promote oxidation or reduction of environmental contaminants, including many halogenated organics (Doong et al., 2014; Y. Wang et al., 2009). Thus, biochar's electron shuttling abilities make it a likely candidate for promoting the reductive dehalogenation of contaminants in environmental and engineered systems. Biochar has been shown to aid in electron shuttling for abiotic and biotic reactions (Kappler et al., 2014a; W. Xu et al., 2010). However, the advancement of biochar technology for industrial remediation and large-scale wastewater treatment applications hinges on developing a greater understanding of the mechanisms, conditions, and biochar characteristics that support redox mechanisms in environmental systems, as well as technical development for its scaling up. Thus, the purpose of this review article is to: I) Summarize known mechanisms by which biochar of varying properties facilitates abiotic and biotic transformations of halogenated organic pollutants, II) Provide an overview of published case studies that support the industrial application of biochars in environmental and engineered systems for promoting degradation of halogenated contaminants, and III) identify areas that require further research for the advancement of biochar materials towards organohalide remediation.

2. Natural vs engineered carbonaceous materials

While this review article will focus on research and applications of biochar, other related materials are often discussed for comparison. Thus, it is necessary to provide some distinctions between biochar and other pyrogenic carbons. Both biochar and activated carbon (AC) are pyrogenic carbonaceous materials produced by the thermochemical conversion of biomass feedstock. However, their production is defined by different criteria and purposes. Biochar is made from sustainably sourced biomass as a means of sequestering carbon as a recalcitrant form, and it is most used as a soil amendment. AC is produced from any carbon precursor and engineered to be used as a sorbent to remove contaminants from gases and liquids. Both materials have distinct history, bodies of literature, and separate scientific communities, but the research and applications of biochar and AC have increasingly overlapped in response to their shared sorptive and electrochemical properties. Both materials are now being used for soil remediation (Hagemann et al., 2018), and both have exhibited electron shuttling properties (Wu et al., 2017b; L. Yu et al., 2016). Because they are functionally similar, AC literature is oftentimes relevant to discussions of biochar, and vice versa. Importantly, the physicochemical properties of these materials exist on a spectrum and are dependent on the starting feedstock material and pyrolysis conditions. As such, biochar and AC should not be compared superficially, nor should any two biochars.

3. Electron flux through biochar: biochar conductivity and electron exchange capacity

Historically, the role of biochar and other black carbons in natural and engineered systems has been attributed solely to its function as an inert adsorbent (Cao et al., 2011), however biochar's newly emphasized ability to facilitate redox reactions that affect nutrient (N and P) and metal/metalloid (Fe, As, Hg, Se) speciation has profound implications for natural biogeochemical cycles and for the fate and transport of environmental contaminants. Biochar contains multiple redox-active moieties, including aromatic rings, oxygen and nitrogen containing functional groups, non-covalently bound redox-active metals, and persistent free radicals. Biochar is thought to mediate electron transfers via both conductor and battery-type mechanisms (Saquing et al., 2016a; Tan et al., 2019). The conductor mechanism involves electron transfer through conductive graphene domains of biochar, whereas the battery mechanism involves reversible electron flux through the redox-active functional groups on biochar surfaces (Sun et al., 2017). Electrical conductivity (EC) and electron exchange capacity (EEC) are two important parameters that can be used to characterize these electron shuttling and storing properties of different biochars. EC measures the electrochemical effect of biochar's graphene content (pi–pi electron network associated with conductive graphite regions; conductor mechanism), which allows for transfer of electrons between two (bio)chemical entities, and between differing redox micro-niches within biochar. EC can be quantified by measuring the electrical resistance across biochars of known dimensions and converting these measurements into conductivity (Gabhi et al., 2017). EC is a property that has stimulated interest in investigating the application of biochar as capacitor (Jiang et al., 2013; L. Zhang et al., 2014). On the other hand, EEC is an indirect measure of the abundance of redox active moieties (RAMs), or battery mechanism, including redox active metals and free radicals, present in biochar materials. EEC consists of both electron accepting capacity (EAC) and electron donating capacity (EDC). Quinone and poly-condensed aromatic moieties on the biochar surface are generally recognized as having both, electron-accepting and donating capabilities, while phenolic sites are more so recognized for their electron-donating capacity within biochars (Y. Yuan et al., 2017). Biochar has the ability to accept and donate several hundred micromoles of electrons per gram of biochar, with electron exchange capacities (EECs) reported as high as 2.28 mmol of electrons per gram biochar (Klüpfel, Keiluweit, et al., 2014a). The EC and EEC of a particular biochar may vary greatly depending on the chemical composition of the original biomass and the conditions of pyrolysis (temperature, residence time, gas ratios). A growing body of research on pyrogenic carbon materials supports a model where EC and EEC function synergistically to facilitate storage and shuttling of electrons to effectively moderate redox mechanisms within heterogenous biogeochemical systems (Sun et al., 2018; L. Yu et al., 2015). Because the significance of biochar-mediated redox activity has only been recognized recently, measurements of EEC and EC for biochar are still relatively scarce, and the relative importance of each parameter in supporting underlying electron exchange mechanisms in reductive biodegradations in

various environments requires further attention. Recently, Sun et al. (2018) developed a dual-interface electron transfer detection system using scanning electrochemical microscopy techniques in order to simultaneously measure and distinguish between electron transfer via surface functional groups and via carbon matrices (graphene domains) in biochar. While the results of this study did underestimate the contribution of surface functional groups compared to previous reports, the new method helps to distinguish between the relative contributions of different electron transfer mechanisms to the total electron transfer ability of any pyrogenic carbon. The review by Yuan *et al.*(2017) provides a thorough overview of general biochar characteristics in their relation to redox capacity of the material.

3.1. How electron mobilizing properties of biochar mediate redox reactions

The redox properties of biochar are proposed to be responsible for the facilitated transformation of contaminants through mechanisms including, but not exclusive to, reduction, nucleophilic substitution, and beta-elimination, depending on the chemical structure of the contaminants and mechanisms of biochar mediation (Ai et al., 2019; Pignatello et al., 2017). This redox shuttling capacity of biochar has been harnessed for the enhancement of biotic/abiotic reductions of iron and other metalloids, as well as biological-mediated reductions of toxic contaminants (Huggins et al., 2014; S. Y. Oh et al., 2013; Tan et al., 2019; L. Yu et al., 2015). Studies differentially report that biochar enhancement of contaminant degradation depends on EEC or EC properties. For example, Zhang et al.(2019a) found that stimulation of microbial (*Geobacter sulfurreducens*) PCP dechlorination by biochars prepared from different feedstocks depended mainly on the EC of the biochar and not on EEC. However, kinetic modeling by Yu et al. (2015) revealed that EEC-promoting surface redox-active moieties and the EC of biochar produced at 900°C contributed to 56% and 41%, respectively, of the biodegradation rate of PCP by a mixed dechlorinating microbial consortia. Many other studies lack direct measurement of biochar EC and EEC but correlate dehalogenation reactions with properties of biochar that are known to contribute to EEC, such as content of redox-active functional groups and biochar-associated metals. The effects of biochar electrochemical properties on contaminant dehalogenations are likely to vary depending on the physicochemical properties of the biochar in relation to the surrounding environment and the structure of the contaminant of interest. A comprehensive understanding of the varied effects of biochar in the systems to which it is applied is requisite for the future large-scale application of biochar as a catalytic remediation additive.

3.2. The role of oxygen-containing functional groups

Oxygenated functional groups, including phenolic, carbonyl, and other quinones, are widely recognized as important surface-bound functional groups on biochar that contribute to its EEC (Chacón et al., 2020; Y. Yuan et al., 2017). Compositionally, low H/C along with high O/C

ratios of biochar have also been correlated with enhanced conductor mechanism of electron transfer through biochar, where higher O/C ratio is considered indicative of greater presence of oxygen-containing functional groups. This effect is likely due to the rapid electron exchange (charging and discharging) occurring at redox-ready oxygenated functional groups on the char surface (Sun et al., 2017). It should be noted that surface functional groups also contribute to the sorption characteristics of biochar, which can mediate biochar-contaminant interactions (R. Zhao et al., 2021).

Quinoid chemical moieties have been shown to be the important active sites on pyrogenic carbon materials used in the reductive degradation of nitro and azo compounds (Kemper et al., 2008; Van Der Zee et al., 2003; X. Yu et al., 2011). Quinone functional groups are likewise implicated in biochar's stimulation of reductive dehalogenations. For example, Li et al. (2019) found positive correlations between the quinoid content of biochar, its measured EEC, and the enhancement of electron transfer from the dissimilatory iron reducing bacteria *Shewanella putrefaciens* CN32 to aged zero valent iron (NZVI), thus accelerating reductive dechlorination of pentachlorophenol (PCP).

3.3. The role of oxygenated redox-active metals

Redox- active metals like Fe and Mn are commonly found in the feedstocks from which biochar is produced, and their contribution to EEC depends on their speciation and distribution as either mineral deposits, metal oxides, or organo-mineral complexes on the biochar surface, which is dependent on their feedstock content and the conditions of pyrolysis. Metal deposits in biochar can occupy a wide range of oxidation states and thus participate in various redox reactions with exogenous electron donors and acceptors (Chacon et al., 2017; Chacón et al., 2020; Dieguez-Alonso et al., 2019; Klüpfel, Keiluweit, et al., 2014b; Risch et al., 2017; Sander et al., 2015). It is important that metal/mineral deposits on the biochar surface also impact sorption characteristics of biochar, as discussed by Zhao et al. (2021).

In a recent study, Tan *et al.* (2018) observed an increase in the rate and extent of ferrihydrite reduction in the presence of *S. oneidensis* MR-1 cells and biochar correlated with increased Ni content of the biochar feedstock. Interestingly, the incorporation of Ni into aromatic graphene domains was sterically similar to the structure of electron-carrying hemes that are present in the outer-membrane cytochromes of microorganisms (Okamoto et al., 2013, 2014). If transition metal (like Ni) incorporation into aromatic domains of biochar can simulate heme-like catalytic function, the conductor mechanism of electrons through these graphene regions may be enhanced by formation of electron transfer conduits through biochar, promoting direct transfer of electrons for Fe(III) reduction, in this case. Heme-like moieties may also aid in microbial electron transfer to and from biochar by simulating interspecies electron transfer

mechanisms. This study shows great promise for enhancing the conductor mechanism of biochar through selection of Ni enriched biomass (Tan et al., 2018). Although evidence for the effects of Ni in biochar on remediation of organic contaminants remains to be seen, the results of this study have implications for turning plants grown for phytoremediation of ferronickel mine land into biochar enriched for the potential remediation of other pollutants elsewhere.

Recent efforts have been made to improve the redox properties of biochar by preloading the biomass material either before or after pyrolysis (B. Chen et al., 2011a; M. C. Wang et al., 2015). Biochars that were prepared using a metal preloading method (Mn or Fe) exhibited increased electron donating and accepting capacities (EDC and EAC), with a disproportionately enhanced EDC (Chacón et al., 2020). It is possible that this preparation method increased the biochar redox properties either by exposing additional functional groups through increased surface and internal pore areas or by affecting the formation ratios of active phenolic vs inactive aliphatic C-O groups. However, x-ray diffraction (XRD) analysis showed that the dominant Fe and Mn minerals on the preloaded biochar surfaces were most likely calcium and/or manganese containing metal oxides (Chacón et al., 2020), which exhibit the ability to mediate redox reactions (Tipsawat et al., 2018; Woo et al., 2003). Therefore, it is possible that the presence of metals in redox-active forms on biochar surfaces are responsible for contributing to EEC and overall redox functions.

Redox active metals associated with biochar may also play a more targeted role in remediation of halogenated contaminants. Jin et al. (2015) reported that biologically reduced iron(III) oxyhydroxides can form reactive secondary Fe(II) minerals that may sequentially and abiotically reductively dechlorinate DDT under dissimilatory iron reducing conditions. As such, the reductive transformation of DDT by Fe(II) minerals has been previously demonstrated (F. B. Li et al., 2010). Reduced iron oxide species have also been reported to enhance the degradation of carbon tetrachloride (Erbs et al., 1999; S. Kim & Picardal, 1999). Interestingly, reduction of other trace metals (Ag^{I}, Au^{III}, and Cu^{II}) to their zero valent forms by iron(II)/iron(III) hydroxides (green rust) has also been proposed to enhance the reductive dechlorination of carbon tetrachloride (O'Loughlin et al., 2003). Thus, the reduction of iron (oxide and oxyhydroxide) species by dissimilatory iron reducing bacteria, along with the redox interplay of various other biochar-associated metals with reduced iron species may be important factors in biochar-mediated dehalogenation reactions under iron reducing conditions. In studies by Li et al. (2019) where biochar was used to activate zero valent iron for PCP dechlorination, wheat straw biochar produced at 600 °C exhibited the greatest maximum PCP degradation rate (k_{max}), compared to wheat straw biochars produced at other temperatures (k_{max} values of 1.56mg, 2.45, 1.78, and 1.39 $L^{-1}d^{-1}$, for biochars produced at 500°C, 600°C, 700°C, and 800°C respectively). The greater PCP reduction efficiency by biochar produced at 600°C was attributed to the higher content of iron(II) sorbed to this char. Thus, engineering of biochars towards their mediation of

effective dehalogenation reactions may involve selection of feedstocks high in iron and other metals, metal preloading of feedstocks, and/or post pyrolytic metal precipitation treatments.

3.4. The role of persistent free radicals (PFR)

The presence of transition metals in biochar feedstock also favors the formation of free radicals during the thermal decomposition process (pyrolysis) (Fang, Liu, et al., 2015). Even though free radicals are typically short-lived, the aromatic structure of biochar can stabilize the unpaired electrons of free radicals through resonance, increasing their half-life from a few hours to months and even years (Fang, Liu, et al., 2015; Liao et al., 2014). These persistent free radicals (PFRs) appear as either aryl radicals (carbon-centered) or as semi-quinoid radicals (intermediate of the phenolic C-OH and quinoid C=O groups), and phenoxy radical and have been detected in biochar at relatively high concentrations (Y. Qin et al., 2018; J. Yang et al., 2016), thereby influencing the electron exchange capacity of biochar, as discussed in the recent review by Yuan et al. (J. Yuan et al., 2022). Importantly, metal and phenolic compound pretreatments were found to increase the concentrations of PFRs and to affect the resulting ratios of PFR types in biochar, indicating that manipulation of metals and phenolics in biomass before pyrolysis could be used to control PFR production in biochar products (Fang, Liu, et al., 2015). PFRs react with molecular oxygen to produce reactive oxygen species (ROS) that catalyze many different reactions, including the degradation of contaminants (Fang et al., 2014; Fang, Zhu, et al., 2015). For a summary of biochar treatments, resulting PFR contents, and results of 2,4,4′-trichlorobiphenyl degradation experiments performed by Fang, et al. (2015), See Table 1.

Fang et al. 2014 determined that biochars typically contain PFRs at concentrations around 10^{18} unpaired spins per gram of biochar. Experiments with biochar and hydrogen peroxide (H_2O_2) correlated decreasing biochar PFRs with increasing trapped hydroxyl radicals (·OH). This showed that PFRs within biochar were main contributors to the reactions with H_2O_2 that resulted in formation of trapped ·OH, which can facilitate the efficient degradation of polychlorinated 2-chlorobiphenyls (see Table 1.) (Fang et al., 2014). In free radical quenching studies performed by Fang et al. (2015), it was confirmed that PFRs within biochar could react with molecular oxygen to form hydrogen peroxide, which could then react with PFRs further to generate ·OH, indicating that these mechanisms could very possibly occur in biochar systems where oxygen is present. This study confirmed that ·OH generated from biochar PFRs could effectively degrade diethyl phthalate (Fang, Zhu, et al., 2015), thus PFR generation is a likely mechanism by which biochar may accelerate degradation of organohalides and other contaminants in environmental and engineered systems.

The degradation of *p*-nitrophenol (PNP) in the presence of biochars prepared from a variety of starting materials (pine wood, corn stalks, peanut shells, rice, and wheat straw) was positively correlated

with the intensity of biochar-PFR and with the detection of ·OH in the aqueous phase. Although, quenching of ·OH did not affect the extent of PNP degradation, whereas displacement of biochar adsorbed PNP did significantly decrease PNP degradation. These results support a mechanism involving the surface-bound degradation of PNP with direct contact to PFRs (J. Yang et al., 2016). Interaction of biochar PFRs with molecular oxygen can also result in the formation of superoxide radicals ($O_2^{\cdot-}$), which were shown to account for 20−30% of sulfate radical ($SO_4^{\cdot-}$) generation in suspensions with biochar and persulfate, and sulfate radicals are also known to degrade PCBs (Fang et al. 2012).

Biochar-associated semiquinone radicals may also be involved in electron transfer from *Shewanella putrefaciens* CN32 for the activation of aged nanoscale zero valent iron (NZVI) in accelerating reductive dechlorination of PCP (H. Li et al., 2019). NZVI is a highly reactive material used for remediation purposes/technology. Li et al. 2019 observed a strong linear relationship between PCP degradation rate (k_{max}) and the area under the curve of the electron paramagnetic resonance (EPR) signal (T_{area}) for semiquinone radical detection (R^2=0.98, p=0.006), supporting the importance of semiquinone radicals in enhancing the degradation of PCP in this mechanism. Further research should investigate the roles of PFRs in other biochar-mediated dehalogenation reactions.

4. The role of sorption

Biochars are carbon-rich, chemically heterogenous, and characterized by negative surface charge, high aromaticity, high ion-exchange capacity, high porosity, and high surface area (S. Y. Oh et al., 2013; Y. Yuan et al., 2017). These characteristics facilitate strong sorption and retention of metal(loid) cations like Cr, Cu, Pb, Cd, Hg, Fe, Zn, and As, as well as strong sorption and retention of many organic compounds (Dang et al., 2020; Luo et al., 2022; Z. Wang et al., 2020). Organic molecules sorbed to biochar were previously commonly assumed to be chemically and biologically inert. This paradigm has shifted recently, due to the realization of biochar's sorbent and electrochemical properties. As opposed to the solvent-like sorption behavior of amorphous organic matter, biochar sorbs molecules in a competitive and nonlinear fashion to its surfaces and within its pore structures, where they remain immobilized, yet physically exposed and chemically active (Nguyen et al., 2004, 2007; S.-Y. Oh et al., 2012). Due to its prominent sorption mechanisms, molecules that have or can adopt a planar configuration, like polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) sorb particularly strongly to biochar (Cornelissen et al., 2004, 2005). As such, studies have demonstrated the sorption of various organic contaminants of concern to biochar, including but not limited to triazine and organo-phosphorous pesticides, steroid hormones, dyes, phenolics, polynuclear aromatics, and antibiotics (Mahmoud et al., 2012; Mohan et al., 2014; Uchimiya et al., 2012), with typical adsorption capacities within the range of 100-200 mg/g (Luo et al., 2022). Biochar also sorbs a wide variety of organohalide molecules. Biochar

addition significantly increased the pentachlorophenol (PCP) sorption capacity of paddy soil (Tong et al., 2014), almond shell activated biochar was able to adsorb the nematode fumigant dibromo-chloropropane from deionized water and well water (Klasson et al., 2013), and Mohan et al. used biochar to remove chlorophenols from water (Mohan et al., 2014).

4.1. Abiotic catalysis at the char surface

Sorption of both organic and inorganic compounds to biochar surface influences their mobility, bioavailability, and reactivity in various natural and engineered systems where biochar is present or applied (R. kou Xu et al., 2011) Traditionally, sorption of organic contaminants to black carbons has been assumed to hinder their degradation by removing them from reactions occurring in the aqueous phase. However, it is becoming increasingly clear that sorption of a molecule to the surface of pyrogenic carbon instead transports that molecule to a different, yet still highly dynamic/reactive microenvironment at the char surface (S.-Y. Oh et al., 2012). Soot and graphite have been shown to enhance the abiotic reduction of nitroaromatic compounds by the thioreductant dithiothreitol (Oh and Chiu 2009). Biochar, activated carbon, and graphite have been shown to enhance the abiotic reductive degradation of explosive compound RDX 1,3,5-Trinitro-1,3,5-triazinane by hydrogen sulfide (Kemper et al. 2008), and Xu et al. (2010) have shown that biochar can accelerate the abiotic reduction of nitroglycerine by hydrogen sulfide.

Xu et al. (2015) studied the abiotic degradation of a variety of halogenated organics sorbed to black carbon (biochar) via reactive sulfur species (sulfide, thiosulfate, sulfite), including the halogenated heterocyclic amines 2,6-dichloropyridine and 2,6-dibromopyridine, the nitrated and halogenated aromatics 3-bromonitrobenzene, 1,3,5-tribromobenzene, and the chlorinated aliphatic insecticide γ-hexachlorocyclohexane. Of these compounds studied, only 3-bromonitrobenzene was transformed significantly over a period of 3 days in the presence of sulfide and graphite. Over 95% of 3-bromonitrobenzene was transformed to 3-bromonitrobenzene, indicating that 3-bromonitrobenzene degradation proceeded by reduction of the nitro group, and not by reductive dehalogenation (W. Xu et al., 2015). While these results shed light on the importance of sorption as a mechanistic mediator of redox transformations, the role of sorption to pyrogenic carbon in mediating the dehalogenation of organohalogens require more systematic investigation.

On the other hand, Oh et al. (2012) demonstrated in batch experiments that graphite, granular activated carbon (GAC), and diesel soot could enhance the abiotic dehalogenation of 2-4 dibromophenol (DBP) via sulfide. Complete removal of DBP in the presence of graphite or GAC and 97% removal in the presence of soot was observed within 12 hours. In the absence of graphite, soot, or GAC, total transformation of DBP required 72 hours reaction time. While this study included sorption isotherm experiments that indicated minimal sorption of DBP to the pyrogenic carbons, mass balances of measured

transformation products do not account for the majority of removed DBP. While other reduction products and adducts or complexes between DBP and hydrogen sulfide are possible, their production is not confirmed in this system. Yet, the detection of 4-bromophenol(4BP) and 2-bromophenol(2BP) (29% and <13% of DBP conversion to 4BP and 2BP, respectively), in the presence of graphite confirms the enhancement of abiotic debromination by pyrogenic carbons, and indicates greater favorability for debromination of DBP at the ortho position (S.-Y. Oh et al., 2012). Further research should focus on understanding the mechanisms by which pyrogenic carbon enhances abiotic reductive dehalogenations of compounds like DPB. Interestingly, sorption appeared to play a minimal role in this study, indicating that abiotic dehalogenations mediated by biochar may not require sorption to the char surface. These types of reactions could be enhanced by aqueous components of biochar in the bulk media, or by transient contact with the char surface.

Green rusts (layered iron(II) and iron(III) hydroxides) are known reductants of methanes , however they typically react very little or not at all with chlorinated ethylenes (CEs). Ai et al. (2019) demonstrated that addition of biochar could eliminate the kinetic hindrance of the reaction between green rust (GR) and CEs (perchloroethylene (PCE), trichloroethylene (TCE), cis-dichloroethylene, and trans- dichloroethylene), resulting in 85% of CE removal within 24 hours and 100% removal by 48 hours, with acetylene as the main product (Ai et al., 2019). Importantly, the observed pseudo-first-order rate constant for TCE dechlorination, k_{app} (h^{-1}), increased linearly with BC concentration (slope of 1.76 $Lg^{-1}h^{-1}$), with minimal negative correlation to GR concentration (slope of −0.030 $Lg^{-1}h^{-1}$), pointing to the BC phase as rate limiting and as the likely site of dehalogenation. Compared to studies of CE dechlorination done in the absence of BC, Ai et al. (2019) observed shifts in product distributions and in the trends of CE reactivity related to degree and position of chlorination. This divergence may reflect differences in dechlorination reactive sites mediated by biochar sorption, as supported by the positive relationships between BC concentration, CE sorption to BC, and CE transformation rates. A summary of these studies by Ai et al. (2019) can be found in Table 1. (2019) used x-ray photoelectron spectroscopy to detect quinoid-type groups, condensed polyaromatic structures, and graphitic domains present in their char, which may indicate a role of electron-mediated reactivity of biochar in CE dehalogenation. Other studies also report changes in reductive pathways upon amendment with pyrogenic carbons and other conductive materials (Borch et al., 2005; S. Y. Oh et al., 2002; S. Y. Oh & Chiu, 2009), although the differentiation between sorption-based and electrical mechanisms of compound transformation is difficult to make. Additionally, studies on bone chars as electron conductors and redox mediators are scarce, and further validation of the roles of sorption and electrical mediation of dehalogenation by bone chars is required. Likewise, the catalytic synergy between GR and BC should be validated using more commonly used biochar types, such as those derived from hardwood, green plant, and manure feedstocks.

5. Electrochemical properties of the aqueous fractions of biochar

Biochar leachate has been shown to retain redox-mediation properties akin to solid biochar. Xu et al. (2016) observed that biochar leachate stimulated microbial reduction of hematite (Fe(III)) to a similar degree as solid biochar particles. Biochar leachate enhanced Fe(III) reduction by 172−468%, compared to 111-229% for solid biochar particles. Additionally, microbially pre-reduced biochar leachate abiotically reduced hematite, demonstrating that, like solid biochar, biochar leachate can act as electron shuttle between microbes and minerals. The rates and extents of abiotic hematite reduction by microbially pre-reduced biochar leachate showed a strong correlation with the semiquinone radical content of the original biochar. The same correlations were much weaker for biochar particles. While this effect could be confounded by the effects of other redox attributes of solid biochars, like surface functional groups and conductivity, this provides indirect support that the electrochemical properties of biochar leachates are due to their free radical content (S. Xu et al., 2016a). While direct measurement of radical species in leachate would bolster this hypothesis, the low concentration of aqueous components in biochar leachate makes direct measurements difficult.

Further validation of biochar leachate electrochemical properties would add further complexity towards understanding the redox mediating properties of biochar systems while also providing new mechanisms towards applying biochars for remediating aqueous-phase contaminants. Yet, in a similar set of experiments as described above , Kappler et al. (2014) did not observe any stimulating effects of biochar leachate on the reduction of ferrihydrite in the presence of the *Shewanella oneidensis* MR-1. Additionally, Wu et al. (2017a) incubated activated carbon leachate with *Shewanella oneidensis* MR-1 for reduction of ferrihydrite and found no substantial difference in the extent of Fe(III) reduction compared to biotic or abiotic controls. In both studies, biochar leachates were prepared by filtering anoxic biochar suspensions in deionized water through 0.22 μm filters. However, in the study by Xu et al., leachates were prepared by filtering biochar suspensions through 0.45um filters. Thus, the difference in biochar particle size exclusion between these studies could account for some differences in the composition and function of the leachates.

Understanding the roles of the different phases of biochar (aqueous and solid) may be critical for successful application of biochar amendments for contaminant degradation. Although biochar sorption may benefit abiotic and biotic (see discussion below) contaminant degradations, there is concern that the strong sorption of some organic contaminants to biochar may reduce their bioavailability and inhibit their degradation by bacteria. Strong contaminant sorption of biochar along with reduced contaminant degradation can cause long-term concerns due to increased persistence. Therefore Lokesh et al. (2020) recently proposed the use of aqueous phase biochar leachate for accelerating the degradation of organohalogens without affecting their bioavailability. Lokesh et al. (2020) applied aqueous biochar fractions to substantially enhance the reductive biodegradation of the halogenated antimicrobial triclosan

(TCS) by *Shewanella putrefaciens* CN32 (Table 2). Aqueous biochar that was pre-reduced by strain CN32 was reported to reductively dehalogenate TCS by 26.9 -93.0%. Anaerobic control incubations with unreduced aqueous biochar resulted in only 1.65-27.0% TCS dehalogenation. This indicates that electron shuttling by aqueous biochar compounds may play a role in biochar's ability to support reductive dehalogenations.

6. Biochar and microbial dehalogenations: microenvironments, communities, and metabolisms

Under specific environmental or engineered conditions (redox state, pH, organic matter content, nutrient availability, etc., bacteria can metabolize organohalides either via oxidative and hydrolytic substitution, isomerization, or reductive dehalogenation (Lu et al., 2010). While aerobic metabolism of many organohalides may be thermodynamically and biochemically feasible, the oxidative stress caused by aerobic metabolism of halogenated compounds greatly inhibits this mechanism (Krzmarzick & Novak, 2014a; Nikel et al., 2013). Nevertheless, several aerobic bacteria have been found to degrade halogenated organic compounds, including polychlorinated biphenyls (Field & Sierra-Alvarez, 2008), haloalkanes (Belkin, n.d.; Hardman, 1991; Janssen et al., 1987; Vollmer et al., n.d.), chlorobenzenes (Goulding et al., 1988; Guerin, 2008), chlorophenols (Olaniran & Igbinosa, 2011; Solyanikova & Golovleva, 2004), and chlorohexanes (Miyauchi et al., 1998).

Anaerobic bacteria seem to be more efficient than their aerobic counterparts in removing halogen atoms from polyhalogenated compounds (Fantroussi et al. 2000), and they are thus much more widely studied. Anaerobic microbial dehalogenation of organic compounds can occur co-metabolically or metabolically, coupled to energy generation (Häggblom et al., 2007). Microbial reductive dehalogenation relies primarily on the action of specific reductive dehalogenase enzymes (de Jong & Dijkstra, 2003). The most commonly reported and studied dehalogenating bacteria include bacteria from the genera *Dehalococcoides*, *Dehalobacte*r, *Dehalogenimonas*, *Desulfitobacterium*, *Desulfuromonas*, and *Clostridium* (Ebrahimbabaie & Pichtel, 2021; Nijenhuis & Kuntze, 2016). Because microbial degradation is a key factor determining the fate of organohalides in the environment, much work has been done to develop safe and effective strategies to accelerate the biotic attenuation of organohalide contaminants in environmental and in wastewater treatment systems (Ahn et al., 2008; Benabdallah El-Hadj et al., 2007; Ebrahimbabaie & Pichtel, 2021; Häggblom et al., 2006; Kastanek et al., 2007; Nijenhuis & Kuntze, 2016).

The addition of biochar to environmental systems has been shown to stimulate the biotic attenuation of halogenated contaminants, stimulating interest in understanding the role of biochar in supporting these unique microbial metabolisms. Biochar imparts several distinct physicochemical characteristics to environments where it is introduced. The high surface area of biochar can increase microbial habitat space and heterogeneity, and it has been suggested that biochar with high porosity provides refuge for

microorganisms when added to soil (Warnock et al., 2007; X. Zhu et al., 2017). However, the effect of micro-porosity on microbial community structure and activity is unclear, as increased surface area due to porosity is mostly associated with small nanopores, which are too small for microorganisms to access. Regardless, it has been observed (using X-ray μ-CT) that wood-derived biochar with micro- and nano pores was colonized by a higher number of bacteria than grass (*Miscanthu*)-derived biochar with an overall larger pore size distribution (Schnee et al., 2016). While pores that are too small for microorganisms do not provide additional surface area for attachment, they may help retain and slowly release nutrients necessary for microbial growth.

The porous structure of biochar is known to contribute to decreased soil bulk density, increased water holding capacity, and retention of various nutrients (N, P, K), trace metals (Cu, Zn), and substrates through sorption (X. Chen et al., 2011; Mia et al., 2017; Yue et al., 2017). Biochar also sorbs significant amounts of heavy metals (As, Cd, Pb and Ni), and numerous organic contaminants (Beesley et al., 2011; Cao et al., 2011; B. Chen et al., 2011b) The often alkaline pH range of biochars can lead to an increase in overall system pH (Cui, Yin, Chen, Quan, Ippolito, Liu, Yan, Ding, et al., 2019a; Ippolito et al., 2014; S. Liu et al., 2017). Furthermore, biochar has been shown to increase the cation exchange capacity (CEC) of soils (Cui, Yin, Chen, Quan, Ippolito, Liu, Yan, Ding, et al., 2019a). CEC describes the ability of soil to retain inorganic and organic cations and can likewise predict a soil's ability to retain certain soil organic carbons, nutrients, and contaminants, all of which have vast implications for microbial metabolism and contaminant degradation. Biochar's impact on CEC is likely derived from its large number of functional groups and negative surface charge. In fact, Cui et al. (2019a) found that biochar amendment to soils resulted in an increase in the number of functional groups in soil (-OH, CH2, aromatic C=O, and C-O-C), as determined by Fourier transform infrared spectroscopy (FTIR), an increase in CEC, and a 9-94% decrease in the concentration of adsorbable organohalides in soil, compared to unamended soil. The magnitude of these effects depended on the concentration of biochar added (Cui, Yin, Chen, Quan, Ippolito, Liu, Yan, Ding, et al., 2019a).

Biochar also increases organic carbon content of the systems to which it is applied. For example, upon biochar addition to wetland soils, Cui et al. (2019b) observed a 48–261% increase in soil organic carbon compared to controls. Yet, much of biochar organic carbon is recalcitrant and not available as food source for microbes (X. Yang et al., 2018). However, the less complete carbonization of biochar produced at lower pyrolysis temperatures results in more biodegradable carbon sources provided by these chars (Gregory et al., 2015). Lokesh et al. (2020a) found positive correlations between the total organic carbon (TOC) contents of corn biochar (400°C), swiss biochar, and activated carbon and the fitted pseudo first-order reaction rate constants for triclosan degradation in batch experiments with aqueous extracts of the pyrogenic carbons and *Shewanella putrefaciens* CN32. Considering the confounding differences between

these materials (specific surface area, EC, EEC), it is not entirely surprising that when all chars were grouped together for analysis, no significant correlation between TOC and triclosan degradation rate was found (Pearson or Spearman test, $p > 0.20$) (Lokesh et al., 2020a). However, it is plausible that higher concentrations of bioavailable carbon supplied by biochar may stimulate co-metabolic degradation of contaminants, as has recently been shown for the co-metabolic degradation of tetrabromobisphenol A (Lefevre et al., 2016). Thus, future work should continue to investigate the role of biochar-associated organic carbon as a microbial substrate in bioremediation mechanisms.

Differences in colonizing bacterial community composition between different biochars were found to be strongly influenced by the pyrolysis temperatures of the biochar (Dai et al., 2017), which could be in response to the degree of carbonization of the biochar, differences in surface functional groups, conductivity, or a combination of various factors. Schnee et al. (2016) found that bacterial community composition on biochar surface is biochar-specific in soil environments, such that distinct bacterial communities were found on the surfaces of biochars prepared from different feedstocks. It is notable that Schnee et al (2016) recorded changes in some physical habitat features of biochar such as porosity, surface area, and pore size distribution after long-term colonization by fungi and bacteria, indicating that microorganisms may alter the physical environment of biochar surfaces over time (Schnee et al., 2016). Oxidative aging can also change the surface chemistry of biochar, altering its sorption efficiency and potentially affecting it's redox properties over time (Mia et al., 2017). The possibility that long-term microbial colonization and chemical weathering could alter the physicochemical properties (EC and EEC, surface functional groups) of biochar should likewise be investigated when considering the application of biochar for stimulating long-term attenuation of contaminants.

There are thus a variety of ways in which biochar may induce shifts in microbial environments and communities towards affecting organohalide degradation. Interestingly, Kjellerup et al. (2014) found that the addition of granular activated carbon (GAC) to sediments contaminated with PCBs increased the population of dehalorespiring bacterial phylotypes. Other researchers have reported that biochar stimulates increases in the abundances of bacteria playing key roles in contaminant degradation. For example, Ni et al (2017) reported increases in *Arthrobacter* and *Flavobacterium*, both of which are involved in the degradation of persistent organic pollutants such as polycyclic aromatic hydrocarbons (Ni et al., 2017). Quantitative real-time PCR analysis of batch experiments with rice paddy soil revealed that biochar amendments stimulated the proliferation of known iron(III)-reducing and dechlorinating bacteria and enhanced the degradation of pentachlorophenol (Tong et al., 2014). However, the effect of biochar in supporting these functional groups of microorganisms and specifically their metabolic activities towards organohalogen degradation is multivarious and requires further investigation.

6.1. How biochar amendment alters the soil microbial respiration

Several studies describe increases in microbial abundance and increased soil respiration following soil biochar amendment (J. Chen et al., 2018), and multiple studies have reported increases in soil enzymatic activity upon biochar addition (Cui et al., 2013; Huang et al., 2017; S. Liu et al., 2017; X. Zhu et al., 2017). In a study by Cui et al. (2019b), the amendment of wheat straw (*Triticum aestivum L.*) biochar to organohalide contaminated saline-alkali soil increased soil enzyme activities of urease, alkaline phosphatase, and sucrase (by 14-57%, 2-147%, and 1-75%, respectively) and decreased the activity of soil dehydrogenases (by 13-60%). The magnitude of these enzymatic activity changes was dependent on the amount of biochar added. Interestingly, dehydrogenases are used by microorganisms to oxidize organic compounds, and their activity has been used as a marker for the overall microbial activities in contaminated soils (Kaczyńska et al., 2015). Yet, the increased activities of other enzymes (urease, alkaline phosphatase, and sucrase) point toward general stimulation of microbial metabolic activities in response to biochar addition. An increase in general microbial activity might also enhance co-metabolic organohalide degradation. Cui et al. (2019b) did observe significant reductions in adsorbable organic halogen contaminants after biochar addition in their study, indicating that dehydrogenase activity has limitations as a predictable measure of biological attenuation in contaminated soils.

Gregory et al. (2015) applied willow (*salix* sp) biochar to organochlorine and arsenic co-contaminated soils and observed a significant increase in soil dehydrogenase activity, after 60 days of treatment. In this study, three of four soils amended with biochar underwent reductions in levels of dichlorodiphenyltrichloroethane (DDT) and its breakdown products (350°C biochar at 1% and 2% application and 550°C biochar at 2%). Biochar-treated soils also underwent 10-fold and 4-fold reductions, respectively, in concentrations of the isomers hexachlorocyclohexane (HCH), alpha-HCH, and gamma-HCH (lindane) (Gregory et al., 2015). Observed magnitudes of contaminant degradation also depended on the amount of biochar applied in some cases. It should be noted that not all treatments were successful in decreasing the levels of these contaminants significantly, and other contaminants of interest in this study were not substantially degraded with biochar amendment (aldrin, dieldrin, delta-HCH, and endrin ketone). See Table 4 for details. Yet, 16S rRNA gene amplicon sequencing did reveal shifts in the microbial community structure upon biochar addition, with increases in the proportions of some known hydrocarbon degraders *(Chryseobacterium, Flavobacterium, Dyadobacter and Pseudomonadaceae*) and organochlorine degraders (*Chloroflexi*, genus *Luteibacter* and *Burkholderia*) capable of breaking down HCH isomers. However, these microorganisms were already present at low numbers as part of the native microbial community in control samples, so their increase in abundance after biochar addition may have resulted from an overall and non-specific increase in microbial activity. While biochar may support microbial proliferation and thereby enhance non-targeted co-metabolic dehalogenation of contaminants, the

complexity of environmental systems and the variety of different contaminant structures makes natural attenuation by this method inherently difficult to predict without considering other concurrent, and possibly more dominant, processes supported by biochar.

6.2. Biochar sorption can alter chemical interactions with microbes

The sorption properties of biochar can alter the distribution of contaminants between solid and aqueous phases, altering their ability to interact with the microbial community. Gregory et al. (2015) noted a temporary but substantial decrease in the concentration of water extractable arsenic after biochar addition to organochlorine contaminated soil. The authors of this study concluded that biochar sorption of the heavy metal reduced the concentration of soluble arsenic, thus relieving microbial communities of arsenic toxicity-related stress and resulting in an overall increase in microbial growth and concurrent organochlorine degradation (Gregory et al., 2015). Thus, an additional benefit of biochar might be the sequestration and neutralization of toxic compounds that could otherwise inhibit bacterial growth.

Toxic chemicals in wastewater influents can likewise negatively affect nutrient removal processes, especially nitrification, the important first step in biological nitrogen removal. As such, addition of the antibiotic ciprofloxacin (CIP, 5mg/L) to sequencing batch reactors reduced the ammonium uptake efficiency (AOE) and nitrate generation efficiency (NGE) by 75–76%. However, CIP-dosed reactors with biochar experienced an 80% reduction in available CIP, along with only 15–16% reductions of AOE and NGE. Additionally, while CIP substantially decreased the relative abundances of *Nitrospira* (nitrite-oxidizing bacteria ubiquitous in WWTPs*)* in reactors by 45-50% compared to unamended control reactors, the abundances of *Nitrospira* bacteria in CIP-dosed reactors with biochar increased compared to control reactors. Moreover, biochar addition into CIP-dosed reactors stimulated increased removal of CIP from the reactors, beyond what would be expected by adsorptive removal alone. Thus, it appears that the sorptive sequestration of CIP by biochar mitigated toxicity and restored microbial function in these reactors to an extent which supported the co-metabolic degradation of the antibiotic (Y. Kim & Oh, 2021). And, while pyrogenic carbons (traditionally activated carbon, and more recently biochar) are commonly used as adsorbents to remove contaminants during wastewater and drinking water treatment, very few studies have investigated biochar amendments as stimulants of bioattenuation in these systems, and further work is required in this area.

There is concern that strong sorption by bulk biochar particles may reduce the bioavailability of contaminants and consequently inhibit their susceptibility to biodegradation. Strongly sorbed organohalogens can cause long-term concerns due to their persistence. Alternatively, increased concentrations of contaminants at the biochar surface may create microenvironments enriched with organohalides to support halorespiration by obligate organohalide-respiring microorganisms. In a series of

soil column studies, Cui et al. (2019) showed that biochar addition reduced the downward transport of the chlorinated anti-microbial 2,4,6-trichlorophenol (2,4,6-TCP) in soil. Immobilization of 2,4,6-TCP via sorption to organic functional groups at the biochar surface facilitated degradation of 2,4,6-TCP over several months (Cui, Yin, Chen, Quan, Ippolito, Liu, Yan, & Hussain, 2019). Additionally, Qin et al. (2020) demonstrated the benefit of biochar adsorption with improved microbial consumption of the sorbed contaminants acetone, chlorobenzene, and toluene as carbon sources. Thus, biochar sorption could be beneficial towards attenuation of some contaminants of interest. However, the transformation of organic contaminants with higher degrees of recalcitrance may not benefit from biochar addition. For the application of biochar towards remediation of any contaminant of interest, it will thus be necessary to consider the interaction of sorption mechanisms with susceptibility towards abiotic and biotic degradations within a given system. In systems where biochar sorption is a concern, application of aqueous biochar leachates may be an alternative remediation strategy as suggested by Lokesh et al. (2020a). These results place emphasis on the adsorption properties of pyrogenic carbons and their effects on contaminant biodegradation, specifically with respect to bioavailability and toxicity of various forms of intermediate degradation products in different phases of a system. Since it has been shown that the distribution of microorganisms in solid and aqueous phases of heterogenous environments is highly microorganism specific (Y. Wang et al., 2014), the effect of biochars on direct microbial contaminant degradation will depend on the distribution and bioavailability of the contaminant with respect to the distribution of degrading microorganisms in the solid and aqueous phases of a particular environment. These mechanisms should be considered when investigating and utilizing pyrogenic carbons for the degradation of contaminants.

6.3. Biochar as electron shuttle and terminal electron acceptor for microbes

The degradation of halogenated organic compounds relies primarily on anaerobic reductive processes, which may be accelerated by increasing the energetic favorability of electron transfer to these compounds as terminal electron acceptors (in absence of oxygen) for microbial processes. The function of pyrogenic carbons as auxiliary electron transfer materials provides an additional mechanism for supporting contaminant degradation. Known electron shuttling compounds have been shown to enhance anaerobic reductive dehalogenation of organohalide contaminants. For example, Doong et al. (2014) demonstrated that the naturally occurring quinones 2-hydroxy-1,4-naphthoquinone (lawsone) and 1,4-naphthoquinone and the model compound AQDS can enhance degradation rate and efficiency of carbon tetrachloride in the presence of *Geobacter sulfurreducens* and ferrihydrite under iron reducing conditions. The pseudo-first-order rate constants (k_{obsCT}) for dechlorination of carbon tetrachloride in the presence of these compounds were reported to be 4.6–7.4, 2.4–5.8, and 5.4–5.8 times higher than in the absence of a quinone compound,

respectively. Wang et al. (2009) demonstrated the ability of AQDS to increase the dechlorination rate of 2,4-dichlorophenoxyacetic acid by up to four times in the presence of the humic-reducing facultative anaerobe *Comamonas koreensis*. Dechlorination of 2,4-dichlorophenoxyacetic acid by microbially pre-reduced AQDS implicates the electron shuttling mechanism of AQDS for mediating these results.

The quinone content of humic substances has long been implicated as an important electron acceptor (Klüpfel, Piepenbrock, et al., 2014; Lovley et al., 1996) and donor (Lovley et al., 1999) for anaerobic microorganisms. Given the significant quinone content of pyrogenic carbons, it is not surprising that they too can act as an electron shuttle for microbial respiration under anaerobic conditions. Saquing et al. (2016b) demonstrated that biochar can simultaneously be an electron acceptor to enable acetate oxidation and an electron donor to support the reduction of nitrate to ammonium by *Geobacter metallireducens*. The function of pyrogenic carbons as intermediate electron carriers between *Shewanella* spp. and Fe(III) minerals has been demonstrated to increase iron reduction (Kappler et al., 2014b; Wu et al., 2017c; S. Xu et al., 2016b). Oxidation by treatment with HNO_3 increased the quinone and hydroquinone functional group (-OH, C=O-C, C=O, and phenolic -OH) content of activated carbon. HNO_3 treated activated carbon enhanced Fe(III) reduction in incubations with the bacterium *Shewanella oneidensis* MR-1, compared to controls and incubations with untreated activated carbon (Wu et al., 2017c). These results support the role of oxygenated functional groups as critical components of pyrogenic carbons for enhancing microbial metabolisms and provoke the modification of these materials towards increased quinone content to enhance electron shuttling functions.

Because both humic substances and biochar contain quinone functional moieties, it is possible that microorganisms capable of humic substance reduction can reduce biochar as well. Zhang et al. (2013) reported on a *Dehalobacter* species that relied on solid humin to dehalogenate the brominated flame retardant tetrabromobisphenol-A (C. Zhang et al., 2013), and other *Dehalobacter* species have been found that use biochar as an electron shuttle for co-metabolic growth (S. Chen et al., 2015). Quinone functional groups of humic substances have also been implicated as electron shuttling components that were requisite for pentachlorophenol dechlorination by a mixed culture (C. Zhang & Katayama, 2012), and for accelerating degradation of PCBs in marine sediments (D. Zhang et al., 2019), Thus, many more microorganisms may exist in nature that profit from the presence of solid electron shuttles for growth and contaminant transformations. Given the ubiquity of pyrogenic carbons in natural soil and sediment systems (Gustafsson & Gschwend, n.d.; Middelburg et al., 1999), it seems possible that bacteria capable of reducing humic substances could also reduce pyrogenic carbons. Of the huge variety of known terminal reductases, some of them could be capable of specifically or nonspecifically reducing biochar surface functional groups. However, further is needed to understand the nuances of microbial propensity for use of biochars as electron acceptors and the specific properties of the varied terminal reductases that allow them to do this.

Nonetheless, the discovery of biochar's electron shuttling and conductive properties has led to investigations into its use for stimulating biotic attenuation of organic contaminants. Biochar's electron shuttling capacity has been suggested to effectively increase biodegradation of azo-dyes (Tan et al., 2019; Van der Zee & Cervantes, 2009) nitro-herbicides and explosives (S. Y. Oh et al., 2013), aromatic hydrocarbons (phenanthrene) (Leglize et al., 2008), antimicrobial compounds (Lokesh et al., 2020a), and various organohalides (Lefèvre et al., 2018; Tong et al., 2014; L. Yu et al., 2015). Lokesh et al. (2020b) recently demonstrated the electron shuttling ability of aqueous biochar (<2nm) towards organohalide degradation. Aqueous biochar, prepared by filtering biochar suspensions through 0.45 μm filters, was reduced in incubations with the dissimilatory metal-reducing bacteria *S. putrefaciens* CN32. The microbially pre-reduced aqueous biochar was then filter-sterilized through 0.2 μm filters and applied to successfully dechlorinate triclosan (TCS) in batch reactions. The recovery of chlorine based on residual triclosan and generated Cl^- ranged from 73.6 to 85.2%, implying that a major fraction of TCS was fully dechlorinated. This indicates that the biochar colloids or other leachate compounds may play a major role in biochar's ability to stimulate biotic reductive dehalogenations. While other studies have applied biochar to stimulate biodegradation of organohalides, this study stands out because it experimentally confirms an electron shuttling mechanism for microbes in biochar-mediated dehalogenation.

6.4. Biochar promotes interspecies electron transfer

Another mechanism by which biochar may support microbial metabolism is through its stimulation of direct interspecies electron transfer (DIET), a form of syntrophy that involves the exchange of electrons directly between bacterial cells without exogenous electron shuttles (Kouzuma et al., 2015; Lovley, 2012; Stams & Plugge, 2009). DIET involves electron transfer through conductive microbial pili and nanowires that transfer electrons with organic metallic-like conductivity. For example, the bacterium *G. metallireducens* can utilize ethanol as an electron donor but cannot use fumarate as an electron acceptor, and *G. sulfurreducens* cannot use ethanol as an electron donor but can reduce fumarate. Growth of the two organisms in medium containing ethanol as the electron donor and fumarate as the electron acceptor requires DIET. When Chen et al. (2015) added biochar to co-cultures of these two organisms under conditions requiring DIET, ethanol metabolism significantly increased. Liu et al. (2012) observed similar results when they amended syntrophic cultures of G. *metallireducens* and *G. sulfurreducens* with activated carbon. Typically, these co-cultures require an adaption period to transition to ethanol metabolism, however the addition of activated carbon substantially reduced the lag phase in this study from around 30 days to just 2 days.

To investigate the mechanism of syntrophy being facilitated or enhanced by pyrogenic carbon, both studies separated biochar from the cell growth medium at the end of the incubations to quantify differential

protein mass. Chen et al. (2015) reported that 79% of the co-culture protein was associated with the biochar fraction after 10 days if incubation. Chen et al. noted that, even though biochars in their study were 1000 times less conductive than GAC, biochars accelerated DIET-dependent ethanol metabolism similarly to GAC. Additionally, biochar leachate only minimally promoted ethanol consumption in this study compared to controls and reactions amended with biochar solids. Ethanol consumption in batch studies by Chen et al. (2015) ranged from about ~12% in the abiotic controls, ~25% in the biochar leachate with co-cultures, and 53-80% in the co-cultures amended with biochar solids. Thus, one explanation for the ability of these materials to stimulate DIET was that they provided a conductive surface for cells to attach and exchange electrons. While proximity of cells to each other on the char surface could promote electron exchange via DIET, scanning electron microscopy images of colonized biochar showed a lack of cell aggregation that is typical of co-cultures participating in DIET. Liu et al. (2012) carried out control incubations with glass beads instead of activated carbon as an inert attachment surface. The glass beads did not stimulate syntrophic metabolism, indicating that the effect of biochar in promoting co-metabolisms between *G. metallireducens* and *G. sulfurreducens* involves more than simple surface attachment. Additionally, gene deletion mutants of *G. sulfurreducens* incapable of producing pili or pili-associated cytochromes (OmcS), were incapable of DIET in the absence of activated carbon. When grown with activated carbon, these strains performed similarly to wild-type co-cultures (Liu et al., 2012). Thus, it appears likely that *Geobacter* species can directly transfer electrons to each other through biochar conductive regions. In this mechanism, the flow of electrons through biochar could be promoted by simple charge differences via reduction of the char surface by electron donating microorganisms and simultaneous oxidation at distal locations on the char surface by electron accepting microorganisms.

In another study, biochar was reported to promote the DIET that occurred in a complex up-flow anaerobic sludge blanket (Z. Zhao et al., 2015). Here, biochar significantly stimulated the enrichment of 16S rRNA gene sequences closely related to *Geobacter* and *Methanosaeta* species known to participate in DIET, resulting in acceleration of organic waste conversion to methane. Thus, it appears that biochar's support for DIET mechanisms may have implications for a variety of anaerobic systems. DIET and other syntrophic electron exchange mechanisms have not currently been studied for biochar's role in biological reductive dehalogenations, however this mechanism should be explored.

Other mechanisms of microbial syntrophy, besides DIET, include exogenous molecule to molecule interspecies electron exchange. These include production of exogenous molecules such as cysteine, formate, H_2, sulfide, and various enzymes as electron shuttles (Stams et al., 2006). It is possible that biochar may provide a nutrient-rich and protected environment where syntrophic organisms can aggregate in close association to exchange electrons exogenously. Importantly, biochar may sorb exogenous electron transfer molecules, thereby affecting their bioavailability. Biochar sorption may concentrate these compounds at

the char surface for more efficient electron transfer by biochar-associated microorganisms. Alternatively, biochar sorption may sequester microbial electron shuttling molecules into nanopores thereby greatly limiting their bioavailability. The effect of sorption on this mechanism of syntropy would likely vary based on the sorptive behaviors of different molecules, as well as the pore size distribution of the biochar and composition of surrounding system to which the biochar is applied. While these mechanisms have not been investigated, they should be considered when applying biochar towards bio-attenuation of organohalides by syntrophic dehalogenating bacteria.

It is well documented that many dehalogenating bacteria rely on hydrogen-producing acetogens such as *Syntrophomonas* and *Syntrophobacter* for growth (Stams et al., 2006). In these syntrophic interactions, anaerobic microorganisms oxidize organic compounds to produce H_2, which serves as electron donor for reductive dehalogenation (Fennell et al., 1997). For example, the complete mineralization of 3-chlorobenzoate has been shown to rely on a consortium of four different microorganisms; one dechlorinating organism which converts 3-chlorobenzoate to benzoate, another microorganism which degrades benzoate, one H_2 consuming methanogen, and an acetate consuming methanogen (Shelton & Tiedje, 1984). A similar microbial consortium appears to be important for anaerobic PCB degradation by *Dehalococcoides mccartyi* (S. Wang et al., 2019), as well as for fermentative degradation of chlorinated alkenes and alkanes (such as vinyl chloride and 1,2 dichloroethane) (Bradley & Chapelle, 1999). Summaries of recent experiments investigating biochar as amendment to dehalogenating enrichment cultures for biodegradation of organohalides can be found in Table 3. Further experiments are necessary that explore the effect of biochar on exogenous electron shuttling by dehalorespiring bacteria, considering its potential impact on the future development of biochar technology for remediating halogenated pollutants.

7. Biochar competes with indigenous redox processes for electrons

The effects of biochar amendment will also rely on the indigenous abiotic and biotic redox processes occurring in a given system. Biochar electron shuttling can compete with other electron transfer processes, which can affect the primary redox functions of a system and mediate the efficacy of biochar mediated reactions. Some studies have recently investigated the use of biochar to stimulate degradation of pentachlorophenol (PCP). Addition of biochar to PCP contaminated soils was shown to improve soil sorption properties, promote microbial growth, and accelerate electron transfer from microbial cells to PCP, all of which have served to accelerate reductive dechlorination of PCP, up to 76% in some cases (Tong et al., 2014; C. Zhang et al., 2019b). Biochar addition has also accelerated electron transfer and degradation of PCP in the lab in the absence of soil (L. Yu et al., 2015) (Table 2) demonstrating that biochar-mediated electron transfer can occur independently from soil components like humic substances (L. Yu et al., 2015). Biochar has also been shown to stimulate reductive dechlorination of lesser-chlorinated phenols in soil,

indicating that biochar stimulation of the total and sequential mineralization of PCP degradation products is possible (Cui, Yin, Chen, Quan, Ippolito, Liu, Yan, Ding, et al., 2019a; Tong et al., 2014; W. Wang et al., 2020).

However, biochar amendment does not universally improve remediation of organochlorides. In a study by Zhu et al. (2018), biochar addition inhibited PCP degradation in PCP-contaminated soil under anaerobic conditions. See Table 4 for details. While biochar addition enhanced dissimilatory iron and sulfate reduction, it simultaneously inhibited PCP degradation in this study. Compared to the control, the addition of biochar significantly increased the relative abundances of an unknown taxa SB-1 (which increased from 4.5 to 26.5% relative sequence abundance), *Dehalobacteriaceae*, *Pelobacteraceae*, *Desulfobulbacea*e, and *Desulfobacteraceae*, and significantly decreased the relative abundances of *Clostridiaceae* and *Peptococcaceae*. Significant shifts in the archaeal community were also described (M. Zhu et al., 2018). These results suggest that the biochar effect on microbial communities and metabolisms is strongly impacted by the strength and mechanisms of redox processes that are indigenous to a particular system and the metabolic capabilities of the microorganisms present.

In another recent study, Zhu et al. (2020) found that the addition of biochar to soil in anaerobic batch experiments resulted in the inhibition of both soil redox processes and the reductive dechlorination of PCP. Additionally, the extent of PCP degradation was linearly and negatively correlated with the amount of biochar added, where the addition of 1 % and 5 % biochar decreased the reduction of PCP by 40.6 % and 67.2 % compared to controls, respectively. Additionally, biochar amended treatments saw promotion of iron and sulfate reduction with accelerated methanogenesis, indicating that biochar may not necessarily redirect electrons towards organohalide degradation if alternative electron accepting processes outcompete those organohalide reducing processes that are accelerated by biochar, or if biochar promotes competing redox processes under selective conditions. Thus, the success of biochar amendments for enhancement of biodegradation of organohalides will rely on a comprehensive understanding of the multiple nuanced electron transfer processes and competing redox processes in relation to the reduction potential of a given system.

8. Conclusions and Future Perspectives

The future application of biochars for targeted remediation strategies will rely on a better understanding of the diversity of physicochemical properties of biochar based on feedstock, pyrolysis conditions, and pre- and post-pyrolysis treatments. Currently, the observed material heterogeneity of different biochars poses challenges for the comparability among studies, especially when compounded with the variability inherent to the systems in which biochars are being applied and investigated. Numerous recent studies support the idea that biochar has the potential to advance remediation of organohalide

pollutants in soils, sediments, and waters. However, comparing and interpreting results across studies continues to be a challenging task. While much work has been done to understand the impacts of pyrolysis temperature, feedstock, and metal content on biochar redox properties (Chacón et al., 2020), these findings need to be systematically and experimentally correlated with their impact on the degradation of various halogenated organic contaminants in a variety of different aquatic and terrestrial ecosystems.

Biochar organohalide surface interactions play a critical role in the transformation and degradation of some halogenated contaminants either by sorption of the contaminant to the highly reactive biochar surface for chemical reduction, by sequestering the contaminant from the bulk media and reducing microbial toxicity, or by diffusion of the contaminant into biochar pores for enhanced microbial organohalide respiration in reduced microenvironments. Sorption to biochar surfaces may alter contaminant stereochemistry and conformation, thus affecting reaction mechanisms and product speciation. Sorption can also differentially affect the bioavailability of parent and daughter compounds to microorganisms in proximity to the biochar surface, which can result in concentration gradients and shifts in product speciation in response to biodegradation. These complex and multifaceted interactions between biochar, contaminants, and microbes are difficult to study independently in natural systems. However, these diverse biotic and abiotic interactions need to be considered when attempting to apply biochar to stimulate or enhance the degradation of a halogenated organic contaminants.

Biochar electron donating and accepting properties and role as electron shuttle, electron donor, or acceptor for microbial respirations is correlated to shifts in ecosystem redox potentials, e.g. as consequence of water table fluctuation or organic carbon availability. As shown by Zhu et al. (M. Zhu et al., 2018), under certain conditions biochar addition might inhibited PCP degradation in anaerobic environments while simultaneously enhancing dissimilatory iron and sulfate reduction. This emphasizes that it is of critical importance for successful biochar-based remediation strategies that we develop a better understanding of the mechanisms that determine biochars redox properties under fluctuating redox conditions in natural systems. While much is known about the various redox active sites and components of biochar, there is a lack of understanding of how biochar's electrochemical properties enhance, compete, or coexist alongside other redox active components in the environment. Considering that microbial reductive dehalogenation is often associated with other prominent redox conditions that allow for methanogenesis, sulfate reduction, and iron(III) reduction, it seem important to understand how the presence of biochar interferes with these reactions if we hope to reliably apply biochars to stimulate microbial biodegradation of organohalides in the future.

The variety of mechanisms for electron flux through and by biochar adds an additional layer of complexity to understanding biochar's behavior on heterogenous systems. Electrons can be transferred to and from biochar via surface functional groups, especially quinoid type moieties, supporting a geobattery

mechanism. Graphene regions of biochar support a conductor mechanism whereby electrons can travel between different reactive sites by shuttling through biochar. These mechanisms have been shown to support contaminant degradation, and the formation of supplementary reactive chemical species, such as persistent free radicals or nucleophilic sulfur species at the char surface and as components of aqueous biochar fractions. Additionally, metal and mineral deposits on biochar can participate in redox mechanisms and support free radical formation. It has also been demonstrated that biochar leachate can retain redox properties, including PFRs, which may be important for biotic and abiotic redox mechanisms that do not rely on sorption or other catalytic biochar surface interactions. However, these specific biochar-mediated electron transfer mechanisms will require further research to better understand their contribution and overall relevance for contaminant degradation.

In summary, making use of biochar's nuanced sorptive, geo-battery, and electron conductor mechanisms for in situ remediation of halogenated organic contaminants depends on a comprehensive understanding of the numerous abiotic and biotic mechanisms that affect the degradation of a given organohalide. The design of biochar catalysts for organohalide remediation relies on a systematic understanding of how biochar feedstock and pyrolysis conditions relate to its sorptive and electrochemical properties, biochar's effects on environmental redox conditions and microbial metabolism, and ultimately empirical evidence of enhance degradation for contaminant groups of interest. The work by Chacon et al. (2020) and others on designing biochars with specific electron exchange properties (e.g. by mixing feedstock with redox active metals prior to pyrolysis) set the stage for the advent of engineered biochars with specific redox properties. These kinds of studies will continue to shed light on how we can actively manipulate the many nuanced properties of biochars for the development of effective contaminant remediation strategies in natural and engineered ecosystems.

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