Promoting mechanism of electronic shuttle for bioavailability of Fe(III) oxide and its environmental significance

Yuning Yang, Lei Chen, Yu Jiang, Zheng Xu, Shaobo Gao, Mingxiao Li, Rui Li and Beidou Xi **IWA**

ABSTRACT

The biological reduction process of Fe(III) not only strongly affects the circulation of C, N, O, P and other elements in the environment, but also plays an important role in the transformation and degradation of organic and inorganic pollutants. Most Fe(III) oxides existing in nature have low bioavailability due to their poor solubility or strongly crystalline form with stable chemical properties. Addition of a substance having redox activity can form an electron shuttle cycle between Fe(III) oxide and the microorganism, which can not only enhance the bioavailability of Fe(III) and accelerate electron transfer, but also improve the removal efficiency of contaminants. This paper compares and analyzes several common redox active substances, for their promoting effect and limiting factors of Fe(III) bioavailability. Moreover, the mechanism by which the electron shuttle promotes the bioavailability of Fe(III) oxide is discussed. This review demonstrates that the electron shuttle promotes the Fe(III) bioreaction process for the degradation and removal of heavy metals, polycyclic aromatic hydrocarbons, azo dyes and other pollutants, which is of great environmental significance.

Key words | bioavailability, electronic shuttle, Fe(III) oxide, organic matter, promotion mechanism

Yuning Yang
Yu Jiang
Zheng Xu
Shaobo Gao
Mingxiao Li
Rui Li (corresponding author)
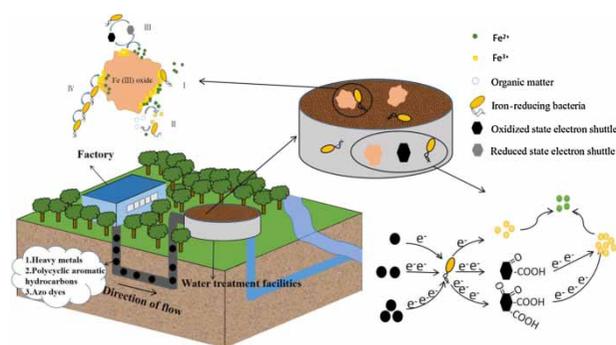**Beidou Xi IWA**
State Key Laboratory of Environmental Criteria and
   Risk Assessment,
Chinese Research Academy of Environmental
   Sciences,
Beijing 100012,
China
E-mail: *518lirui@163.com*

Yuning Yang
Lei Chen
Zheng Xu
Municipal and Environmental Engineering College,
Jilin Jianzhu University,
Changchun, Jilin 130118,
China

Yu Jiang
Beidou Xi
Environmental and Chemical Engineering College,
Shanghai University,
Shanghai 200444,
China

Shaobo Gao
Beidou Xi
Water Recourse and Environment,
China University of Geosciences (Beijing),
Beijing 100083,
China

HIGHLIGHTS

- The electron shuttle can promote the electron transfer between Fe(III) oxide and microorganisms, and enhance the bioavailability of Fe(III).
- The electron shuttle can promote Fe(III) bio-reduction coupled with the degradation of environmental pollutants.
- Initial concentration, pH, preparation conditions and structural complexity could affect the effect of the electron shuttle.
- Concentration is the main limiting factor of the electron shuttle promoting the bioavailability of Fe(III).

GRAPHICAL ABSTRACT

INTRODUCTION

Iron is the fourth most abundant element in the earth's crust. From the perspective of geochemistry, iron is mostly present in sediments in the form of iron oxide, especially insoluble hydrated iron oxide (Liu *et al.* 2007). Bioreduction reactions with Fe(III) oxide as the electron acceptor can degrade macromolecular organic matter in groundwater (Botton & Parsons 2007; Liang *et al.* 2009) and landfill leachate (Li *et al.* 2018), oxidize ammonia nitrogen in soil and water (Park *et al.* 2009; Yang *et al.* 2012), and decompose organic matter in deep-water lakes and deep seas (contribution rates of 44% and 75%) (Roden & Wetzel 2002; Thomsen *et al.* 2004). The Fe(III) bioreduction process not only strongly affects the cyclical transformation of key elements such as C, N, O and P in nature, but also plays an important role in the transformation and degradation of organic and inorganic pollutants (Lovley *et al.* 1991). The main Fe(III) oxides include goethite (α-FeOOH), hematite (α-Fe$_2$O$_3$), lepidocrocite (γ-FeOOH), maghemite (γ-Fe$_2$O$_3$) and Fe(OH)$_3$ (ferrihydrite, also known as amorphous iron). These forms of iron oxide are basically insoluble, and their biological activity in decreasing order is: Fe(OH)$_3$, γ-Fe$_2$O$_3$, γ-FeOOH, α-Fe$_2$O$_3$, and α-FeOOH. The higher thermodynamic stability and stronger crystal structure of Fe(III) oxide result in lower bioavailability and lower pollutant removal efficiency. In recent years, the promotion of the bioavailability of Fe(III) oxide by electronic shuttle has gradually become a research hotspot.

By adding a redox active material to form an electron shuttle between Fe(III) oxide and microorganisms, the rate of electron transfer between the microorganism and the extracellular electron acceptor can be accelerated. In addition to the common humic substances (HS) (Lovley & Woodward 1996), a variety of redox active substances have been discovered that accelerate the reduction of Fe(III), such as quinonoids (O'Loughlin 2008), biochar (Zhou *et al.* 2017), activated carbon (Wu *et al.* 2017), sulfur species, and microbial autocrine substances. By virtue of a unique phenolic hydroxyl group and a quinone group structure, these redox active substances can form an electron shuttle cycle of mutual conversion of oxidized and reduced states between microorganisms and Fe(III) oxides. The electron shuttle cycle acts as a bridge for electron transfer, improves electron transport efficiency (Dunnivant *et al.* 1992; Scott *et al.* 1998), and promotes the transition of crystalline Fe(III) to an amorphous state, thereby improving the bioavailability of Fe(III) (Li *et al.* 2018). Although domestic and foreign scholars have carried out a large amount of research on the promotion mechanism of Fe(III) bioavailability, the effects, restrictive factors and environmental applications of this process have not yet been systematically summarized and analyzed. In this paper, the research progress of related frontiers in recent years is reviewed, and the promotion mechanism of Fe(III) bioavailability, the Fe(III) reduction effect and restrictive factors of different redox active substances are discussed. From promoting the removal of contaminants such as heavy metals, polycyclic aromatic hydrocarbons, and nitrogen dyes, the environmental significance of the electron shuttle to promote the bioavailability of Fe(III) is evaluated, which provides

reference and consideration for the selection and application of redox active substances.

MECHANISM OF ACTION OF ELECTRON SHUTTLE FOR IMPROVING FE(III) BIOAVAILABILITY

There are many possible mechanisms for the bioreduction of Fe(III) oxide: (1) direct contact: this involves the direct transfer of electrons by contact between insoluble Fe(III) oxide and the extracellular membrane protein, (I); (2) chelation and dissolution: the small molecule organic matter interacts with the insoluble Fe(III) oxide to form a complex, which promotes the contact between the iron-reducing bacteria and Fe(III) oxide after dissolution (II); (3) mediated reduction: the microorganism first transmits the electrons to the redox active substance and reduces it; then the reduced substance transfers electrons to Fe(III) oxide, and also returns to an oxidized state itself to continue to participate in a new round of electron transfer (III); (4) Electronic transition: electrons are transferred multiple times between microorganisms and finally transferred to Fe(III) oxide (IV) (Chi *et al.* 2007) (Figure 1). Among these mechanisms, the mediated reduction has the best promoting effect on Fe(III) bioavailability because it does not require direct contact of microorganisms with Fe(III) oxide.

In an anaerobic microenvironment, iron-reducing bacteria with strong metabolism can significantly affect the degradation of various organic pollutants (saccharides, organic acids, aromatic hydrocarbons, etc.) during the bioreduction of Fe(III) oxide. Therefore, this reaction is critical in the repair and treatment of contaminated environments. The addition of a redox active material capable of forming an electron shuttle cycle can effectively promote this bioreduction reaction. Iron-reducing bacteria can transfer electrons from saccharide compounds to substances with redox activity, thereby promoting the formation of electron shuttles, which can oxidize carbohydrates (glucose) to CO_2 (Manfred *et al.* 2009). Under anaerobic conditions, iron-reducing bacteria can also use Fe(III) oxide as an electron acceptor and organic acid as an electron donor (Champine & Goodwin 1991). The quinone and hydroquinone functional groups in specific organic compounds can continuously lose electrons through the redox reaction to form an electron shuttle cycle, and oxidize formic acid, lactic acid and pyruvate to CH_3COO^- and CO_2. In addition, the metal reduction bacterium is the first microorganism that can oxidize aromatic compounds with Fe(III) as an electron acceptor. This electron shuttle can catalyze the degradation of various aromatic compounds (such as benzene, toluene, phenol, cresol, p-cresol, phenol, aniline, benzoic acid, p-hydroxybenzoic acid, benzaldehyde, p-hydroxybenzaldehyde, phenylethyl alcohol, p-hydroxyphenylethanol, etc.)
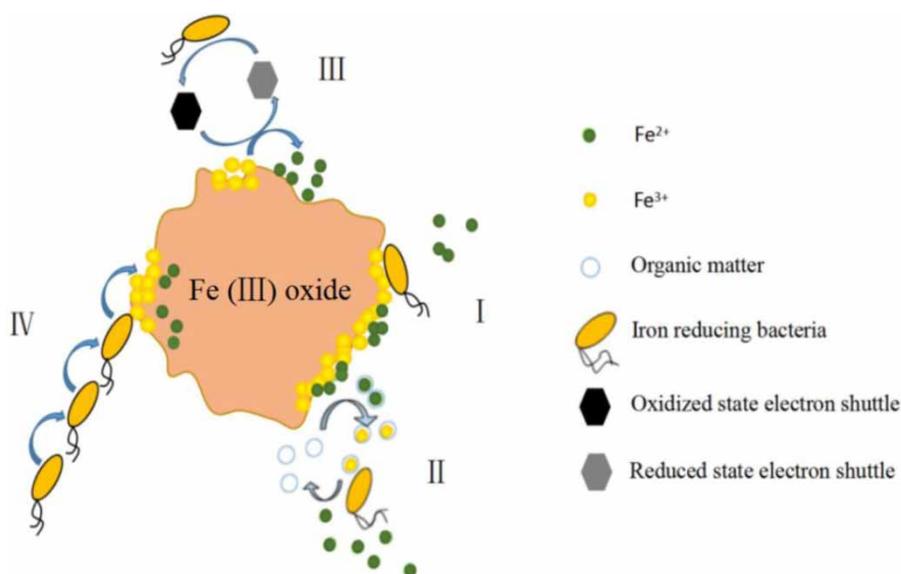
Figure 1 | Mechanism of microbial reduction of iron oxides (Liu *et al.* 2018). (I) direct contact; (II) chelating; (III) mediated reduction; (IV) electronic hopping.

(Lovley *et al.* 1989; Lovley & Lonergan 1990; Kazumi *et al.* 1995). The specific reaction equations are shown in Table 1.

PROMOTING EFFECT OF DIFFERENT TYPES OF ELECTRONIC SHUTTLES ON FE(III) BIOAVAILABILITY

Humus (HS)

HS is a complex polymer compound formed by long-term physical, chemical and biological reactions of animals, plants and microbial residues. It is generally black or dark brown and is a major component (50%–65%) of natural organic matter. HS is widely found in soil, sediments and aquatic environments, and it can be divided into humic acid (HA), fulvic acid (FA) and humin (Pettit 2004). HA contains many functional groups, such as carboxyl, hydroxyl, and sulfhydryl groups.

HA promotes the reduction efficiency of hematite higher than glucose, and can also synergize with glucose to improve the bioavailability of Fe(III). Unlike glucose, HA can form an electron shuttle to promote the biological reaction process (Stern *et al.* 2018). Lovley (Lovley & Woodward 1996) proposed that HA promotes the bioavailability of Fe(III) oxides in two stages: first, iron-reducing bacteria oxidize acetate, and HA is reduced as an electron acceptor; second, the reduced HA transfers electrons to Fe(III).

Table 1 | Degradation reactions of different contaminants

Electron acceptors	Degradation reaction equation
Glucose	$C_6H_{12}O_6 + 24C=O + 12H_2O \rightarrow 6HCO_3^- + 24C\text{-}OH + 6H^+$ $24C\text{-}OH + 24Fe(III) \rightarrow 24Fe(II) + 24C=O + 24H^+$
Formic acid	$HCOO^- + 2C=O + H_2O \rightarrow HCO_3^- + 2C\text{-}OH$ $2C\text{-}OH + 2Fe(III) \rightarrow 2Fe(II) + 2C=O + 2H^+$
Pyruvic acid	$CH_3COCOO^- + 2C=O + 2H_2O \rightarrow CH_3COO^- + HCO_3^- + 2C\text{-}OH + H^+$ $2C\text{-}OH + 2Fe(III) \rightarrow 2Fe(II) + 2C=O + 2H^+$
Benzoic acid	$C_6H_5COOH + 30C=O + 19H_2O \rightarrow 7HCO_3^- + 30C\text{-}OH + 6H^+$ $30C\text{-}OH + 30Fe(III) \rightarrow 30Fe(II) + 30C=O + 30H^+$
Phenol	$C_6H_5OH + 28C=O + 17H_2O \rightarrow 6HCO_3^- + 28C\text{-}OH + 6H^+$ $28C\text{-}OH + 28Fe(III) \rightarrow 28Fe(II) + 28C=O + 28H^+$

Jiang *et al.* (Jiang *et al.* 2018) firstly confirmed that HA can promote the bioavailability of Fe(III) oxide. Their results showed that the first reaction rate of Fe(III) oxide bioreduction was increased at least 27 times compared with no HA addition, and the second reaction rate was increased 7 times.

HS concentration is a key factor in promoting Fe(III) bioavailability. Jiang *et al.* (Jiang *et al.* 2018) investigated the effects of various HS standards on reducing ferrihydrite by *Geobacter sulfureducens*, and concluded that the minimum concentration of HS was 5–10 mg C/L to promote the electron shuttle process. In another study, Wolf *et al.* (Wolf *et al.* 2009) reported that HA promoted the reduction of ferrihydrite by *G. metallireducens*. When the HA concentration was as low as 0.59 mg C/L, it still promoted the reduction of ferrihydrite. This may be due to the different abilities of various HS and microbes to transfer electrons. The study of HS promoting the bioavailability of Fe(III) has been carried out around soluble iron oxides, but the promotion of solid humus is still unclear. It is also important to thoroughly explore the optimal initial concentration of HS.

Carbonaceous material

Carbonaceous materials include biochar, soot, activated carbon (AC), graphite, carbon nanotubes, and graphene oxide (Langley & Fairbrother 2007; Fu *et al.* 2015; Saquing *et al.* 2016). They have a highly porous structure, high alkalinity and abundant surface functional groups, which have great potential for carbon sequestration, soil fertility and quality (Woolf *et al.* 2010; Mohan *et al.* 2014; Inyang *et al.* 2016). The carbonaceous material contains many redox functional groups with high conductivity, and it has the same electron shuttle capability as HS. However, unlike the mechanism of HS in promoting the bioavailability of Fe(III) oxide, the carbonaceous material can transmit electrons through multiple oxidation reduction reactions (Kappler *et al.* 2014).

The added concentration of carbonaceous material is a key factor affecting the bioavailability of Fe(III) oxide. Kappler *et al.* (Kappler *et al.* 2014) studied the effects of different concentrations of biochar prepared with wood chips on the bioreduction process of Fe(III) oxide, and found that higher concentrations (5 g/L, 10 g/L) of biochar can improve the

bioavailability of Fe(III) oxide. The reduction efficiency of ferrihydrite, anthraquinone-2,6-disulfonic acid (AQDS) and biochar synergistically promoted the bioavailability of Fe(III). When the biochar concentration was lower than (0.5 g/L, 1 g/L), the Fe(III) biological reaction was inhibited. Moreover, Wu *et al.* studied the maximum reduction rate of ferrihydrite promoted by activated carbon, and found that it was as high as 0.127–0.434 mM/h, which was similar to the Fe(III) reduction rate promoted by biochar (0.12–1.49 mM/h). Also, they found that activated carbon (AC-N2, AC-N4) was more potent after pretreatment with nitric acid (Wu *et al.* 2017). However, the mechanism for the improvement in bioavailability of Fe(III) oxides by solid-phase carbon materials and the influencing factors need to be further explored.

Quinonoids

Quinonoids are a class of substances with a quinoid structure, which are simple, diverse and widely found in the natural environment. They can be mainly classified into four types: benzoquinone, naphthoquinone, phenanthrene-quinone and anthracene. Many researchers have used quinonoids as analogs or substitutes for humus to promote the bioavailability of Fe(III) oxides (Lovley *et al.* 1998; Wolf *et al.* 2009). Unlike HS, quinonoids do not exhibit properties such as complexation and adsorption of Fe(III)

oxides (Royer *et al.* 2002; Piepenbrock *et al.* 2011; Masayuki *et al.* 2013; Eusterhues *et al.* 2014).

Under the action of sodium 2-sulfonate (AQS), the bio-reduction efficiencies of goethite and hematite were significantly improved, and the content of Fe(II) in the reaction product was increased by 0.118 and 0.149 mg/L, respectively (Yu *et al.* 2016). In addition, AQS extended the service life of ferrihydrite, and the Fe(III) reduction rate was increased by 43.9% (Zang 2011). Low concentrations (10 μM) of quinonoids also promoted the efficiency of Fe(III) bioreduction. Due to the structural diversity of quinonoids, the current research is limited to several common quinonoids, whose results do not represent all quinonoids. Therefore, further studies on the reaction mechanisms are still needed.

Oxidized sulfur compounds

Sulfur compounds are widely present in the environment and usually exist in the form of sulfides, sulfates or simple substances, which promote the bioavailability of Fe(III) oxides. At present, there are two explanations for the mechanism of action of sulfur compounds: (1) Iron-reducing bacteria reduce S_0 to sulfide. Then, sulfide reduces Fe(III) oxide and is simultaneously reoxidized to S_0 (Figure 2(I)) (Nevin & Lovley 2000); (2) Acidophilic bacteria can simultaneously produce S_0 oxidation and Fe(III) bioreduction
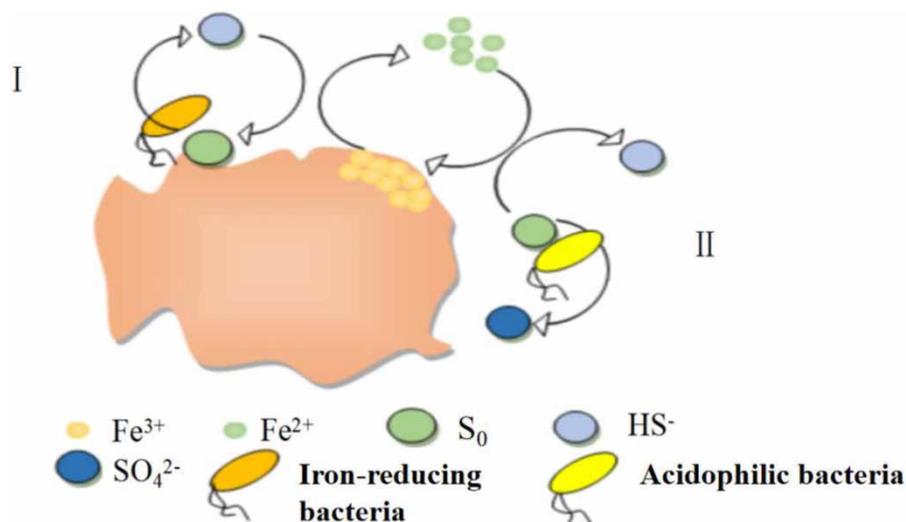
Figure 2 | Sulfur species mediated iron oxide reduction mechanism.

under anaerobic conditions. Then, S_0 forms H_2S and SO_4^{2-} by disproportionation reaction, and one part of Fe(III) is reduced by acidophilic bacteria, while another part is indirectly reduced by H_2S (Figure 2(II)) (Osorio *et al.* 2013). In both mechanisms, Fe(III) oxide is not required to be in direct contact with the microorganism. Thereby, the rate of redox reaction can be increased.

Flynn *et al.* (Flynn *et al.* 2014) found that S_0 enhanced the bioavailability of Fe(III) oxide by simulating alkaline groundwater, and the Fe(III) reduction rate was increased by three times. Wang *et al.* (Wang *et al.* 2015) added S_0 to the cultures of *S. oneidensis* and *G. sulfurreducens* under alkaline conditions and found that the reduction rate of ferrihydrite was increased by 1.7 and 1.8 times, respectively. In addition, Wang *et al.* (Wang *et al.* 2015) found that the ability of AQDS to promote Fe(III) bioavailability was much higher than that of S_0. Therefore, in most natural environments, except for those with high sulfur content, HS and quinonoids are more capable of promoting Fe(III) reduction than sulfur species.

Wang *et al.* (Wang *et al.* 2017) used underground sediments as research objects and concluded that low-concentration sulfates have limited promotion effect, while high-concentration sulfates can significantly improve Fe(III) reduction efficiency. However, Hansel (Hansel *et al.* 2015) found that low concentrations of sulfate (200 μM) can also promote the bioavailability of goethite and hematite. The difference between the two results may be due to the different experimental conditions in the two studies. Eitel (Eitel & Taillefert 2017) used electrochemical methods and found that low molecular weight organic sulfur compounds such as cysteamine, cysteine, homocysteine and glutathione can improve the bioavailability of ferrihydrite. The degree of reduction was found to be proportional to the complexity of the sulfur species. In summary, both pH and concentration can affect the bioavailability of Fe(III) by sulfur compounds, and the promotion effect of low concentration sulfur compounds is limited, which is not conducive to their applications. At present, the mechanism of action is still unclear.

Microbial autocrine

In addition to artificially added HS and quinonoids, microorganisms in the natural environment can secrete a variety of redox active substances in their own metabolic processes, which can form electron shuttles involved in extracellular electron transport to enhance Fe(III) oxide bioavailability. Wang and Newman (Wang & Newman 2008) considered that microbial-derived flavin mononucleotides (FMN) may play multiple roles in the reduction of Fe(III) oxides: (1) As a cofactor for cytochrome C (MtrC), FMN promotes the bioavailability of lepidocrocite, hematite and goethite; (2) FMN forms an electron shuttle system to promote Fe(III) bioreduction when reducing lepidocrocite; (3) FMN acts as an unreactive electron in the reduction of hematite and goethite.

The phenazine compound secreted by microorganisms has a lower redox potential than the weakly crystalline Fe(III) oxide and can reduce Fe(III) oxide to promote its bioavailability. Hernandez *et al.* (Hernandez *et al.* 2004) found that *Pseudomonas chlororaphis* PCL1391 isolated from the rhizosphere of plants can produce phenazine-1-carboxamide, which can form an electron shuttle to promote the bioavailability of weakly crystalline iron oxides. *S. oneidensis* secretes sulfhydryl compounds such as cysteine, homocysteine, glutathione and cysteamine, participates in the electron shuttle cycle, and promotes the bioavailability of Fe(III) oxide (Cooper 2016). Until now, there is no consensus on the mechanism by which microbial autocrine promotes the bioavailability of Fe(III) oxide and this area needs further study.

ENVIRONMENTAL SIGNIFICANCE OF ELECTRON SHUTTLE TO PROMOTE FE(III) BIOAVAILABILITY

The promotion of Fe(III) bioavailability by electronic shuttle can accelerate the redox removal of environmental pollutants. At present, this strategy has been widely used in the treatment of trace metals, polycyclic aromatic hydrocarbons, azo dyes and other pollutants.

Heavy metals

The promotion of Fe(III) bioavailability by electronic shuttles can affect the geochemical cycle of heavy metal elements. Han Rui (Han 2016) studied six different electron transport proteins as research objects, and found that

cytochrome C enhanced the bioavailability of Fe(III). Cytochrome C improved the reduction rate of Cr(VI) by 66.7%, and played a significant role in controlling Cr(VI) in the environment.

Polycyclic aromatic hydrocarbons

The electron shuttle promotes the Fe(III) bioreaction process, which can degrade pollutants such as polycyclic aromatic hydrocarbons (Xu 2012; Zhou 2018). It was found that HS- and AQS-enhanced the Fe(III) bioreaction process increased the degradation rate of nitrobenzene by 10.3% and 1.2% (Xu 2012). Moreover, the AQS-enhanced Fe(III) bioreduction process promoted the degradation of 4-nitroacetophenone, and the removal rate of 4-nitroacetophenone was increased by 34% when AQS was added (Zang 2011). Another study found that the biochar prepared at 600 °C had the best effect on the degradation of pentachlorophenol (PCP) and could degrade 97.6% of PCP (Zhou 2018).

Azo dyes

Azo dyes are widely used in the printing and dyeing industry. These dyes are difficult to biodegrade and cause significant pollution to the environment. The electron shuttle can promote the Fe(III) bioreaction to accelerate the decolorization of azo dye wastewater. The newly formed Fe(II) also has a flocculation effect to reduce the color of wastewater. The Fe(III) bioreduction process enhanced by

exogenous riboflavin can promote the decolorization of cationic red X-GRL dye (Cationic Red x-GRL, X-GRL). When the concentration of riboflavin reached 5 mM, the reaction efficiency was increased by two times (Xiao et al. 2017). The Fe(III) bioreduction process enhanced by riboflavin also decolorized methyl orange (MO) and naphthol green B (NGB) dyes.

Table 2 details the impact of several electronic shuttles on the removal of environmental pollutants. It can be concluded that the effect of different electronic shuttles is quite different through comparison. When the pollutant is a heavy metal, the activated carbon will play a certain role in inhibition; when the pollutant is organic, the promotion effect of HA is better.

CONCLUSIONS AND PROSPECTS

The introduction of redox active substances to construct an electronic shuttle between Fe(III) oxide and microorganisms not only enhances Fe(III) bioavailability and accelerates electron transfer, but also improves pollutant removal efficiency. However, the promoting effect and mechanism of action of redox active substances for enhancing the bioavailability of Fe(III) need to be studied further in terms of the following aspects: (1) analysis of the diversity of microbial species that can utilize electron shuttles; (2) exploration of the decisive factors for bioavailability of different forms of Fe(III) oxide under different

Table 2 | Typical electronic shuttles promoting the degradation reaction of organic pollutants

Pollutants	Electron shuttle	Degradation rate (rate of change)/%	References
Cr(VI)	Cytochrome C	98.4 (+66.7)	Han (2016)
PCP	Biochar (600 °C)	97.6 (+50.6)	Zhou (2018)
	Biochar (800 °C)	72.1 (+25.1)	
4-Nitroacetophenone	AQS (10 μmmol/L)	0.6 (+0.0)	Zhang (2011)
	AQS (1,000 μmmol/L)	0.8 (+0.2)	
Nitrobenzene	HA	88.8 (+10.3)	Xu (2012)
	AQS	80.7 (+1.2)	
	Riboflavin	60.3 (−18.2)	
2,4,6-Trinitrotoluene	AQDS	69.2 (+17.3)	Borch et al. (2005)
hexahydro-1,3,5-trinitro-1,3,5-triazine	AQDS	49.6 (+37.1)	Kwon & Finneran (2006)
	HA	54.5 (+42.0)	

environmental conditions, and studying each redox active material-mediated electronic shuttle cycle; (3) analyze the electron shuttle from the perspectives of thermodynamics, kinetics, chemistry, materials science, etc., and study the mechanism of action of the shuttle on improving the bioavailability of Fe(III) oxide; (4) Application of this strategy to actual contaminated water and soil samples.

ACKNOWLEDGEMENTS

REFERENCES

Borch, T., Inskeep, W. P., Harwood, J. A. & Gerlach, R. 2005 Impact of ferrihydrite and anthraquinone-2,6-disulfonate on the reductive transformation of 2,4,6-trinitrotoluene by a Gram positive fermenting bacterium. *Environmental Science & Technology* **39** (18), 7126–7133.

Botton, S. & Parsons, J. R. 2007 Degradation of BTX by dissimilatory iron-reducing cultures. *Biodegradation* **18** (3), 371–381.

Champine, J. E. & Goodwin, S. 1991 Acetate catabolism in the dissimilatory iron-reducing isolate GS-15. *Journal of Bacteriology* **173** (8), 2704.

Chi, G. Y., Zhang, Z. W., Chen, X. & Shi, Y. 2007 Research advances in the mechanisms and affecting factors of soil Fe(III) dissimilatory reduction. *Chinese Journal of Ecology* **26** (12), 2075–2080.

Cooper, R. E. 2016 Bacterial Iron and Manganese Reduction Driven by Organic Sulfur Electron Shuttles. Georgia Institute of Technology, Atlanta, USA.

Dunnivant, F. M., Schwarzenbach, R. P. & Macalady, D. L. 1992 Reduction of substituted nitrobenzenes in aqueous solutions containing natural organic matter. *Environmental Science & Technology* **26** (11), 2133–2141.

Eitel, E. M. & Taillefert, M. 2017 Mechanistic investigation of Fe(III) oxide reduction by low molecular weight organic sulfur species. *Geochimica Et Cosmochimica Acta* **215**, 173–188.

Eusterhues, K., Hädrich, A., Neidhardt, J., Küsel, K., Keller, T. F., Jandt, K. D. & Totsche, K. U. 2014 Reduction of ferrihydrite with adsorbed and coprecipitated organic matter: microbial reduction by *Geobacter bremensis* vs. abiotic reduction by Na-dithionite. *Biogeosciences* **11** (18), 4953–4966.

Flynn, T. M., O'Loughlin, E. J., Bhoopesh, M., Dichristina, T. J. & Kemner, K. M. 2014 Sulfur-mediated electron shuttling during bacterial iron reduction. *Science* **344** (6187), 1039–1042.

Fu, H., Liu, H., Mao, J., Chu, W., Li, Q., Alvarez, P. J., Qu, X. & Zhu, D. 2015 Photochemistry of dissolved black carbon released from biochar: reactive oxygen species generation and phototransformation. *Environmental Science & Technology* **50** (3), 1218.

Han, R. 2016 A Study on the Extracellular Electron Transfer Processes Mediated by Outer Membrane C-Type Cytochromes of Shewanella oneidensis MR-1. South China University of Technology, Guangzhou, Guangdong Province, China.

Hansel, C. M., Lentini, C. J., Tang, Y. Z., Johnston, D. T., Wankel, S. D. & Jardine, P. M. 2015 Dominance of sulfur-fueled iron oxide reduction in low-sulfate freshwater sediments. *Isme Journal* **9** (11), 2400.

Hernandez, M. E., Andreas, K. & Newman, D. K. 2004 Phenazines and other redox-active antibiotics promote microbial mineral reduction. *Applied and Environmental Microbiology* **70** (2), 921.

Inyang, M. I., Gao, B., Ying, Y., Xue, Y., Zimmerman, A., Mosa, A., Pullammanappallil, P., Yong, S. O. & Cao, X. 2016 A review of biochar as a low-cost adsorbent for aqueous heavy metal removal. *Critical Reviews in Environmental Science & Technology* **46** (4), 406–433.

Jiang, R., Qu, Z., You, P. & Qu, D. 2018 Effect of biochar on photosynthetic microorganism growth and iron cycling in paddy soil under different phosphate levels. *Science of the Total Environment* **612**, 223–230.

Kappler, A., Wuestner, M. L., Ruecker, A., Harter, J., Halama, M. & Behrens, S. 2014 Biochar as an electron shuttle between bacteria and Fe (III) minerals. *Environmental Science & Technology Letters* **1** (8), 339–344.

Kazumi, J., Haggblom, M. M. & Young, L. Y. 1995 Degradation of monochlorinated and nonchlorinated aromatic compounds under iron-reducing conditions. *Applied and Environmental Microbiology* **61** (11), 4069–4073.

Kwon, M. J. & Finneran, K. T. 2006 Microbially mediated biodegradation of hexahydro-1,3,5-trinitro-1,3,5-triazine by extracellular electron shuttling compounds. *Applied and Environmental Microbiology* **72** (9), 5933–5941.

Langley, L. A. & Fairbrother, D. H. 2007 Effect of wet chemical treatments on the distribution of surface oxides on carbonaceous materials. *Carbon* **45** (1), 47–54.

Li, R., Jiang, Y., Xi, B., Li, M., Meng, X., Feng, C., Mao, X., Liu, H. & Jiang, Y. 2018 Raw hematite based Fe (III) bio-reduction process for humified landfill leachate treatment. *Journal of Hazardous Materials* **355**, 10–16.

Liang, T., Zhou, S. & Li, F. 2009 Interactive reaction of polychlorinated compound dechlorinating transformation at the soil interface. *Progress in Chemistry* **21** (4), 1262–1263.

Liu, C., Zachara, J. M., Foster, N. S. & Strickland, J. 2007 Kinetics of reductive dissolution of hematite by bioreduced

anthraquinone-2,6-disulfonate. *Environmental Science & Technology* **41** (22), 7730–7735.

Liu, G. F., Zhu, J. Q., Yu, H. L., Jin, R. F., Wang, J. & Zhou, J. T. 2018 Progress in the reduction of iron oxides by microorganisms mediated by electron shuttle. *Earth Science* **43** (S1), 157–170.

Lovley, D. R. & Lonergan, D. J. 1990 Anaerobic oxidation of toluene, phenol, and p-cresol by the dissimilatory iron-reducing organism, GS-15. *Applied and Environmental Microbiology* **56** (6), 1858–1864.

Lovley, D. R. & Woodward, J. C. 1996 Mechanisms for chelator stimulation of microbial Fe (III)-oxide reduction. *Chemical Geology* **132** (1–4), 19–24.

Lovley, D. R., Baedecker, M. J., Lonergan, D. J., Cozzarelli, I. M., Phillips, E. J. P. & Siegel, D. I. 1989 Oxidation of aromatic contaminants coupled to microbial iron reduction. *Nature* **339** (6222), 297.

Lovley, D. R., Phillips, E. J. & Lonergan, D. J. 1991 Enzymic versus nonenzymic mechanisms for iron (III) reduction in aquatic sediments. *Environmental Science & Technology* **25** (6), 1062–1067.

Lovley, D., Fraga, J., Blunt-Harris, E., Hayes, L., Phillips, E. & Coates, J. 1998 Humic substances as a mediator for microbially catalyzed metal reduction. *Acta Hydrochimica Et Hydrobiologica* **26** (3), 152–157.

Manfred, W., Andreas, K., Jie, J. & Meckenstock, R. U. 2009 Effects of humic substances and quinones at low concentrations on ferrihydrite reduction by *Geobacter metallireducens*. *Environmental Science & Technology* **43** (15), 5679–5685.

Masayuki, S., Jihai, Z., Christian, S. D., Martin, O., Andreas, K. & Thomas, B. 2013 Dissimilatory reduction and transformation of ferrihydrite-humic acid coprecipitates. *Environmental Science & Technology* **47** (23), 13375–13384.

Mohan, D., Sarswat, A., Ok, Y. S. & Pittman Jr., C. U. 2014 Organic and inorganic contaminants removal from water with biochar, a renewable, low cost and sustainable adsorbent–a critical review. *Bioresource Technology* **160**, 191–202.

Nevin, K. P. & Lovley, D. R. 2000 Potential for nonenzymatic reduction of Fe (III) via electron shuttling in subsurface sediments. *Environmental Science & Technology* **34** (12), 2472–2478.

O'Loughlin, E. J. 2008 Effects of electron transfer mediators on the bioreduction of lepidocrocite (γ-FeOOH) by *Shewanella putrefaciens* CN32. *Environmental Science & Technology* **42** (18), 6876–6882.

Osorio, H., Mangold, S., Denis, Y., Ñancucheo, I., Esparza, M., Johnson, D. B., Bonnefoy, V., Dopson, M. & Holmes, D. S. 2013 Anaerobic sulfur metabolism coupled to dissimilatory iron reduction in the extremophile *acidithiobacillus ferrooxidans*. *Applied and Environmental Microbiology* **79** (7), 2172–2181.

Park, W., Nam, Y. K., Lee, M. J. & Kim, T. H. 2009 Anaerobic ammonia-oxidation coupled with Fe^{3+} reduction by an

anaerobic culture from a piggery wastewater acclimated to NH_4^+/Fe^{3+} medium. *Biotechnology and Bioprocess Engineering* **14** (5), 680–685.

Pettit, R. E. 2004 Organic matter, humus, humate, humic acid, fulvic acid and humin: their importance in soil fertility and plant health. *CTI Research* 1–17.

Piepenbrock, A., Dippon, U., Porsch, K., Appel, E. & Kappler, A. 2011 Dependence of microbial magnetite formation on humic substance and ferrihydrite concentrations. *Geochimica Et Cosmochimica Acta* **75** (22), 6844–6858.

Roden, E. E. & Wetzel, R. G. 2002 Kinetics of microbial Fe (III) oxide reduction in freshwater wetland sediments. *Limnology and Oceanography* **47** (1), 198–211.

Royer, R. A., Burgos, W. D., Fisher, A. S., Unz, R. F. & Dempsey, B. A. 2002 Enhancement of biological reduction of hematite by electron shuttling and Fe(II) complexation. *Environmental Science & Technology* **36** (9), 1939–1946.

Saquing, J. M., Yuhan, Y. U. & Pei, C. C. 2016 Wood-derived black carbon (Biochar) as a microbial electron donor and acceptor. *Environmental Science & Technology Letters* **3** (2), 62–66.

Scott, D. T., McKnight, D. M., Blunt-Harris, E. L., Kolesar, S. E. & Lovley, D. R. 1998 Quinone moieties act as electron acceptors in the reduction of humic substances by humics-reducing microorganisms. *Environmental Science & Technology* **32** (19), 2984–2989.

Stern, N., Mejia, J., He, S., Yang, Y., Ginder-Vogel, M. & Roden, E. E. 2018 Dual role of humic substances as electron donor and shuttle for dissimilatory iron reduction. *Environmental Science & Technology* **52** (10), 5691–5699.

Thomsen, U., Thamdrup, B., Stahl, D. A. & Canfield, D. E. 2004 Pathways of organic carbon oxidation in a deep lacustrine sediment, Lake Michigan. *Limnology and Oceanography* **49** (6), 2046–2057.

Wang, Y. & Newman, D. K. 2008 Redox reactions of phenazine antibiotics with ferric (hydr)oxides and molecular oxygen. *Environmental Science & Technology* **42** (7), 2380–2386.

Wang, Z., Zhi, S., Liang, S., White, G. F., Richardson, D. J., Clarke, T. A., Fredrickson, J. K. & Zachara, J. M. 2015 Effects of soluble flavin on heterogeneous electron transfer between surface-exposed bacterial cytochromes and iron oxides. *Geochimica Et Cosmochimica Acta* **163**, 299–310.

Wang, X. N., Sun, G. X., Li, X. M., Clarke, T. A. & Zhu, Y. G. 2017 Electron shuttle-mediated microbial Fe(III) reduction under alkaline conditions. *Journal of Soils & Sediments* **18** (1), 1–10.

Wolf, M., Kappler, A., Jiang, J. & Meckenstock, R. U. 2009 Effects of humic substances and quinones at low concentrations on ferrihydrite reduction by *Geobacter metallireducens*. *Environmental Science & Technology* **43** (15), 5679–5685.

Woolf, D., Amonette, J. E., Streetperrott, F. A., Lehmann, J. & Joseph, S. 2010 Sustainable biochar to mitigate global climate change. *Nature Communications* **1** (5), 56.

Wu, S., Fang, G., Wang, Y., Zheng, Y., Wang, C., Zhao, F., Jaisi, D. P. & Zhou, D. 2017 Redox-active oxygen-containing functional groups in activated carbon facilitate microbial

reduction of ferrihydrite. *Environmental Science & Technology* **51** (17), 9709–9717.

Xiao, X., Liu, Q. Y., Li, T. T., Zhang, F., Li, W. W., Zhou, X. T. & Yu, H. Q. 2017 A high-throughput dye-reducing photometric assay for evaluating microbial exoelectrogenic ability. *Bioresource Technology* **241**, 743–749.

Xu, C. 2012 *Coupled Attenuation of Nitrobenzene by Bacterial Dissimilatory Reduction of Iron Oxides*. Jilin University, Changchun, Jilin Province, China.

Yang, W. H., Weber, K. A. & Silver, W. L. 2012 Nitrogen loss from soil through anaerobic ammonium oxidation coupled to iron reduction. *Nature Geoscience* **5** (8), 538–541.

Yu, L., Wang, S., Tang, Q. W., Cao, M. Y., Li, J., Yuan, K., Wang, P. & Li, W. W. 2016 Enhanced reduction of Fe(III) oxides and methyl orange by *Klebsiella oxytoca* in presence of

anthraquinone-2-disulfonate. *Applied Microbiology and Biotechnology* **100** (10), 4617–4625.

Zang, H. 2011 *The Study of Kinetic Characteristics of the Biotic/ Abiotic Reductions of Iron Oxides and the 4- Nitroacetophenone Biodegradation Mediated by Goethite*. Xi'an University of Architecture and Technology, Xi'an, Shaanxi Province, China.

Zhou, L. Y. 2018 *Enhanced Reduction of Aged ZVI by Shewanella putrefaciens to Degradate Organic Pollutants in Groundwater*. South China University of Technology, Guangzhou, Guangdong Province, China.

Zhou, G. W., Yang, X. R., Marshall, C. W., Li, H., Zheng, B. X., Yan, Y., Su, J. Q. & Zhu, Y. G. 2017 Biochar addition increases the rates of dissimilatory iron reduction and methanogenesis in ferrihydrite enrichments. *Frontiers in Microbiology* **8**, 1–14.