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Electron shuttles enhance the degradation of sulfamethoxazole coupled with Fe(III) reduction by *Shewanella oneidensis* MR-1

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ABSTRACT

The ability of anthraquinone-2,6-disulfonate (AQDS) and riboflavin to enhance the sulfamethoxazole (SMX) degradation coupled with the Fe(III) reduction by *Shewanella oneidensis* MR-1 was investigated. The results indicated that the SMX degradation rate was 38.5% with an initial SMX concentration at 0.04 mM. For the overall performance of AQDS and riboflavin mediated SMX degradation and iron reduction, the SMX degradation rate was gradually increased with the enhancement of iron reduction. Riboflavin had a stronger enhancement on SMX degradation and iron reduction than AQDS, but the enhancement was not positively correlated with electron shuttles concentration. A quantitative characterization of the electron transfer capacity (ETC) of the electron shuttles showed that the ETC was higher for riboflavin than AQDS. The *S. oneidensis* MR-1 16S rRNA gene copies results indicated that electron shuttles had a positive effect on the microbial activity of *S. oneidensis* MR-1. The LC—MS result indicated that the products of the SMX biodegradation were 3-amino-5-methylisoxazole and 4-aminobenzenesulfonic acid, which suggested that the SMX biodegradation was caused by S—N bond cleavage. This study indicates that the biochemical mechanisms play a vital role in SMX transformation and Fe(II) generation in this system.

1. Introduction

Antibiotics are used globally to treat common diseases and for the preventative care of animals. Sulfonamides are artificially synthesized antibiotics with broad-spectrum antibacterial effects and widely used in animal husbandry, aquaculture, and human medicine. Most of these antibiotics are not completely metabolized and enter the soil and aquatic environment in the form of manure (Phillips et al., 2004; Cui et al., 2017).These drugs accumulate in the environment and may lead to bacterial drug resistance and endanger human health and environmental safety through the food chain (Allen et al., 2010).

Studies have shown that the biodegradation process of sulfonamide antibiotics in the environment is slow, which causes long-term residues (Barnes et al., 2008). Sulfamethoxazole (SMX) exists in a variety of forms in the environment and it has a complex degradation mechanism; this makes its biodegradation and biotransformation more difficult in soils/sediments (Muller et al., 2013). The concentration of SMX in the surface- and wastewater can be detected up to 0.008 and 0.032 μM (Kolpin et al., 2002; Peng et al., 2006). A number of studies have indicated that the biodegradation rate of SMX in a soil environment/sewage treatment system is about 50% (Lin and Gan, 2011; Joss et al., 2006). The biodegradation of SMX in the environment is influenced by many factors, including microbial species, drug level, pH and carbon source (Eibes et al., 2011; García-Galán et al., 2012). Domestic and foreign scholars have identified some stable biodegradable products of SMX, such as 3-amino-5-methylisoxazole, 4-amino-N-hydrobenzesulfonamide, 4-aminobenzesulfonamide, and aniline (Jiang et al., 2014; Ricken et al., 2013). These results show that the initial degradation of SMX is caused by breaking the N—C and S—N bonds.

Iron-reducing microorganisms are widespread on the earth. *Shewanella oneidensis* can obtain energy to support cell growth by reducing iron (Myers and Nealson, 1988). Iron-reducing microorganisms play an important role in mineral dissolution and deposition; they reduce Fe(III) to Fe(II) also can utilize other electron acceptors, such as S (0), Mn(IV), and U(VI) (Lovley et al., 2004). The process of iron reduction affects the transmission of organic and inorganic pollutants in the environment, which has important environmental significance. Previous studies have shown that iron-reducing microorganisms are resistant to sulfonamide antibiotics (Kang et al., 2013; Groh et al., 2007) and that iron reduction can be used to enhance the degradation of sulfonamide antibiotics in the environment (Mohatt et al., 2011).

Electron shuttles accelerate the electron transfer rate between

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Glossary

S. oneidensis MR-1	*Shewanella oneidensis* MR-1
SMX	sulfamethoxazole
AQDS	anthraquinone-2,6-disulfonate
ETC	electron transfer capacity
EAC	electron accepting capacity
EDC	electron donating capacity
ABTS	2,2′-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt
DQ	diquat dibromide monohydrate
HPLC	high performance liquid chromatography
RT-qPCR	real-time quantitative polymerase chain reaction
MEO	mediated electrochemical oxidation
MER	mediated electrochemical reduction

microorganisms and extracellular electron acceptors by acting as electron acceptors for microorganisms and electron donors of oxides (Hong et al., 2007). Methylene blue and neutral red are good electron shuttles with high redox activity. Methylene blue can serve as the electron shuttle of *S. oneidensis* MR-1 to enhance the anaerobic decolorization and detoxication of azo dye (Liu et al., 2016), but the current studies provided few evidence about the electron transfer mechanism of methylene blue. Neutral red has good biocompatibility, and it is mostly used in the research of the electrical properties of microbial fuel cells. However, Kastury et al. (2015) found that neutral red had certain toxic effects on microorganisms and plants. Many studies have reported that humics are the ideal electron shuttle because humics not only accelerate the electron transfer but are inherently stable. Due to the complex structure of humics in the environment, they are difficult for microorganisms to degrade; therefore, anthraquinone-2,6-disulfonate (AQDS) has been regarded as a type of humus quinone model for experimental studies (Hong et al., 2007). The concept of humus respiration was first proposed in the journal *Nature*, where it was reported that *Geobacter metallireducens* oxidized many organic compounds and hydrogen using the humus model substance AQDS, which supported bacteria growth (Lovley et al., 1996). Later studies showed that the microbes that respired in humus used humic acid or AQDS as an electron acceptor and they all belonged to the Fe (III)-reducing bacterial group *Geobacteraceae*. In addition, this group used oxides of organic substrates as the electron acceptor to support their own growth (Coates et al., 1998). Riboflavin is the synthetic precursor to the flavoproteins flavin mononucleotide and flavin adenine dinucleotide (Von Canstein et al., 2008). A large proportion of the *Shewanella* genus, including *Shewanella oneidensis* MR-1, strengthens the electron transport process by using riboflavin as an extracellular electron shuttle (Von Canstein et al., 2008). Riboflavin can enhance the reduction of iron oxides by iron-reducing microorganisms (Xia et al., 2016).

In this study, the iron-reducing bacteria *Shewanella oneidensis* MR-1 was selected as a model strain to determine the promoting effect of AQDS and riboflavin on the degradation of SMX and the reduction of iron under laboratory conditions. The electron transfer capacity (ETC) values of the electron shuttles and the 16S rRNA gene copies of *S. oneidensis* MR-1 were quantified to explore the intensification mechanisms of the SMX biodegradation. The products and degradation pathway of the SMX were also investigated. This study provides the scientific basis for the prevention and control of sulfonamides pollution in the environment and the use of electron shuttle materials.

2. Materials and methods

2.1. Chemicals

SMX (98%), AQDS (98%), riboflavin (98%), 2,2′-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt (ABTS) (99%) and diquat dibromide monohydrate (DQ) (99%) were supplied by Aladdin Industrial Corp (City of Industry, CA, USA). Methanol, acetic acid, and acetonitrile were of high-performance liquid chromatography (HPLC) grade and the other reagents were analytical grade. The basic properties of the two kinds of electron shuttles are shown in Table 1.

2.2. Microorganisms and culture conditions

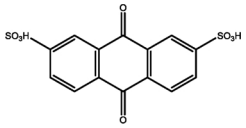
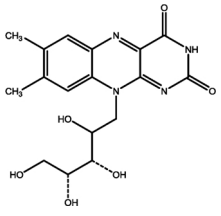
Source of bacterium: The iron-reducing bacteria *S. oneidensis* MR-1 (ATCC 700550) was provided by the soil microbiology laboratory of the Northwest Agriculture and Forestry University (Shanxi, China).

Luria Bertani (LB) medium (g/L): beef extract 5.0, peptone 10.0, NaCl 5.0, pH = 7.0; the agar powder was added to the solid medium at 1.5–2% and sterilized at 121 °C for 30 min.

Medium for *S. oneidensis* MR-1 growth (g/L): $KH_2PO_4{\cdot}7H_2O$ 3.0, $Na_2HPO_4{\cdot}7H_2O$ 12.8, NH_4Cl 1.0, NaAc 2.0, yeast extract 2.0 (Baron et al., 2009).

Preparation of bacterial suspension: The *S. oneidensis* MR-1 strain was inoculated onto the LB solid medium and the bacteria were transferred to the LB liquid medium after 24-h incubation at 30 °C, followed by incubation in a constant-temperature shaker at 30 °C for 24 h. The fermented liquid was centrifuged at 4000 r/min for 10 min, rinsed three times in sterilized 0.9% physiological saline after pouring

Table 1
The list of the basic properties of two kinds of electron shuttles.

Electron shuttles	abbreviation	chemical structure	structural formula	MW	E_0[a]/mV
anthraquinone-2,6-disulfonate	AQDS		$C_{14}H_6O_8S_2$	366.32	−184
Riboflavin	VB_2		$C_{17}H_{20}N_4O_6$	376.37	−208

[a] E_0: standard redox potential (pH = 7, standard hydrogen electrode) (Wolf et al., 2009; Van der Zee et al., 2003).