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雄黄矿区沉积物中砷形态转化的微生物作用
机制

Microbial mediated arsenic transformation in realgar mine
sediments

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摘要

砷污染及其危害已逐渐成为一个全球性的环境问题。砷的污染来源于地质方面,但在中国砷污染主要来源于采矿及冶金业,导致土壤、地下水及河流砷污染。砷在生态系统中有非常显著的毒性作用,而且随着环境条件的改变,固体物质中砷很容易转变为移动的砷,从而引起更大的环境风险。因此,需要充分了解固体物质中砷的迁移转化特征。目前,砷的生物地球化学调查表明自然环境中微生物在砷的化学形态及循环方面起到至关重要的作用,已有几个微环境实验研究表明有机物在原位被氧化导致砷的迁移。由于有机废水的排放和纳米材料在环境修复中的应用,这些有机物和纳米颗粒不可避免会进入到环境中。然而这些有机物和纳米颗粒在生态系统中的作用还不清楚。所以研究碳源及纳米颗粒对沉积物中微生物群落结构及功能的影响,特别是对微生物异化还原金属矿物进而影响到沉积物中砷的迁移转化显得尤为重要,为今后砷污染环境生物修复提供理论依据。

本文采用实验室恒温培育的方式,利用PCR-DGGE和real-time PCR等分子生物技术,在厌氧和有氧条件下,研究了微生物对雄黄矿区沉积物中砷的释放。另外还分别考察了碳源和纳米颗粒对沉积物中微生物群落结构和功能微生物丰度的影响,并分析了纳米颗粒对微生物还原的As(III)的竞争吸附作用,获得雄黄矿区沉积物中砷的迁移转化规律。本论文的主要结果包括以下方面:

1、雄黄矿区沉积物中砷的释放主要是微生物的作用,厌氧条件下(以As(III)为主)释放的砷是有氧条件(以As(V)为主)的4倍多,并伴随铁的释放;环境条件的改变对沉积物中微生物群落结构有影响,主要功能微生物(如金属还原微生物)的丰度有所增加;微生物直接还原As(V)在控制砷迁移转化的过程中比Fe(III)氧化物还原贡献更大,沉积物中主要优势种群是由 α 和 γ 变形杆菌和酸杆菌门所控制。

2、碳源对沉积物中砷的释放影响显著,其中乙醇释放砷的量最多($3531.2 \mu\text{g}\cdot\text{L}^{-1}$,占可交换态及碳酸盐结合态的66.1%),而添加腐殖质(HA)的沉积物中砷释放量最少($1925.6 \mu\text{g}\cdot\text{L}^{-1}$,占可交换态及碳酸盐结合态的36.1%),迁移到水体砷的最终去向可能还受到形成As-DOM(可溶有机碳)和As(III)-Fe-HA络合物的影响;碳源对沉积物中微生物群落结构有影响,其富集的微生物中优势种群主要是由 β 、 γ 和 δ 变形杆菌和厚壁菌门所控制,其中*Anaeromyxobacter*、*Clostridium*

和 *Azotobacter* 可能对砷的释放起关键作用。

3、纳米颗粒对沉积物中砷的释放及微生物群落结构有显著影响，其中 SiO_2 纳米颗粒对砷的释放有促进作用， Fe_2O_3 和 Fe_3O_4 纳米颗粒对砷的释放有抑止作用；富集的主要优势种群是由厚壁菌门及 α 和 γ 变形杆菌所控制，主要功能微生物(如金属还原微生物)的丰度有所增加；三种纳米颗粒在以乙酸钠作为电子供体的环境下明显促进微生物的异化铁还原，这些导体或半导体纳米颗粒在微生物与金属氧化物(如铁、锰)之间可能形成一种电子传导网络。

4、沉积物中砷释放的可能机制：(1)沉积物中砷主要被 Fe(III) 氧化物等吸附；(2)当存在 DOM、硅和磷酸根后，吸附于铁氧化物表面(或粘土)的砷部分进行解吸；(3)DOM 被铁氧化物吸附；(4)在微生物作用下释放出铁；(5)由于铁的架桥作用形成 As-Fe-HA 络合物；(6)吸附在铁氧化物表面的 As(V) 被微生物直接异化还原为 As(III) 随后释放到水体中；(7)微生物随后以有机物作为电子供体， Fe(III) 和 As(V) 作为电子受体，进行异化还原反应，随后释放出 Fe(II) 和 As(III) ；(8)当有 Fe(III) 的纳米颗粒时， As(III) 再次被其吸附而沉淀到固液界面；(9)当有 SiO_2 纳米颗粒时，由于纳米硅与 As(III) 存在竞争吸附，减少了 As(III) 被再次吸附的机会。

5、在含砷沉积物和铬污染土壤中分别筛选获得了能够还原 As(V) 和 Cr(VI) 的菌株，在雄黄矿区沉积物中筛选获得 8 株能够还原 As(V) 的菌株，其中菌株#3 和菌株#4 对 As(V) 还原能力最强，经测序鉴定，菌株#3 是 *Firmicutes* 的 *Bacillus* sp.，菌株#4 是 γ -*Proteobacteria* 的 *Pseudomonas* sp.，证实沉积物中存在直接还原 As(V) 的微生物；在铬污染的土壤中筛选获得 6 株有 Cr(VI) 还原能力的菌株，其中菌株 XMCr-6 还原能力最强，48 h 内能完全还原 $100 \text{ mg}\cdot\text{L}^{-1}$ 的 Cr(VI) ，测序鉴定属于 *Firmicutes* 的 *Bacillus cereus*；菌株 XMCr-6 还原 Cr(VI) 的机制是细胞碎片上的酶促反应，先还原 Cr(VI) 后吸附固定到细胞上；还原后的 Cr(III) 在有机配体充分的条件下，主要是形成可溶的 organo- Cr(III) 络合物，在有机配体不足条件下，主要是形成 Cr_2O_3 的纳米颗粒。

通过本文的研究，不同环境条件的改变对雄黄矿区沉积物中微生物群落结构及功能的变化与砷的释放关系有了更为深入的认识，探讨了碳源和纳米颗粒对沉积物中砷释放的影响，揭示在不同条件下砷的迁移转化与沉积物微生物种群分布及功能组成的关系，并对沉积物中微生物释放砷的机理提出了初步的构想，为进

一步明确沉积物对周边环境影响以及今后生物修复提供理论依据。

关键词：砷；沉积物；*Geobacter*；PCR-DGGE；纳米颗粒

厦门大学博硕士学位论文摘要库

Abstract

Arsenic contamination has become a serious environmental problem in many countries. It is mobilized through a combination of natural processes such as weathering reactions, biological activity and volcanic emissions as well as through a range of anthropogenic activities. Most environmental problems are the result of arsenic mobilization under natural conditions. However, arsenic could be discharged into environment through mining, combustion of fossil fuels and the use of arsenical pesticides and herbicides.

The potentially high adverse effect of arsenic on the ecosystem is well known. Moreover, arsenic toxicity and mobility is highly related to its oxidation state. The tailing sediments from abandoned mine areas and in particular arsenic contamination of agricultural soils and crops pose serious management issues, because immobile arsenic can be easily transformed into a mobile phase as the result of changes in environmental conditions. The contamination of soils, ground and surface water by arsenic from tailing sediments represent significant threats to human health. Consequently, it is essential to fully understand the transport characteristics of arsenic.

To date, biogeochemical investigations on the fate of arsenic have revealed that microorganisms play a crucial role in the chemical speciation and cycling of arsenic in nature. Numerous studies offer direct evidence that anaerobic metal-reducing bacteria play an important role in the formation of toxic, mobile As(III) in sediments. However, little is known about the relationship between dynamic distribution of microbial community and arsenic release from sediments under flooded anaerobic condition. Furthermore, relatively little has been done on the effect of organic carbon and foreign nanoparticles on microbial activities in tailing sediments at Shimen County. With increased applications of engineered nanostructures, releases of such materials to environment such as sediments are inevitable. Increasing concerns have been raised on how this release would affect microbial community in sediments. However, there are limited and inconsistent data regarding the effect of nanoparticles on the sediments microbial community. Therefore, a better understanding of how

microorganisms respond to nanomaterials can help to address environmental and health concerns brought about by the use of nanomaterials.

In this study, we used anaerobic incubation with a constant temperature, the effect of nanoparticles and carbon sources on the sediments bacterial community has been studied with molecular biology approaches. We analyzed the dynamic characteristics of microbial As(V) and Fe(III) reduction, in order to clarify the internal relationship between arsenic release and microbial metal dissimilatory reduction and provide the necessary theoretical basis for revealing the mechanism of microbial arsenic release in tailing sediments. The main results obtained are as following:

1. Although the biotic and abiotic processes may all play a role in arsenic mobilization, it has been concluded that microorganisms play the defining role in catalysing the redox transformations that ultimately control the mobility of the arsenic. Sediments incubated under anaerobic conditions showed Fe(III) reduction concomitant with arsenic mobilization with time, principally as As(III). Incubation under aerobic conditions, however, resulted in release of arsenic from the sediments was predominantly dissolved As(V). The amount of arsenic released from sediments under anaerobic conditions was 4 times of that under aerobic conditions. The sediments bacterial community structure changed and some key bacterial abundance significantly increased with the changes in environmental conditions. These results suggested that the directly reduction of As(V) made greater contribution to arsenic release from sediments than reductive dissolution of arsenic-rich Fe(III) oxyhydroxides. *Alpha* and *gamma* *Proteobacteria* and *acidobacteria* were the dominant population in tailing sediment.

2. The arsenic release from sediments had a significant impact on amended with organic carbons, wherein the concentrations of arsenic in sediments amended with ethanol was the highest ($3531.2 \mu\text{g}\cdot\text{L}^{-1}$) and amended with humic acid was the least ($1925.6 \mu\text{g}\cdot\text{L}^{-1}$), while there was a significantly negative correlation between arsenic mobilization and the formation of As(III)-Fe-HA complexes. Amended with organic carbons had a significant effect on the microbial community structure in the sediments, *Beta*, *gamma* and *delta* *Proteobacteria* and *Firmicutes* were the dominant population

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