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Conductive iron oxide minerals accelerate syntrophic cooperation in methanogenic benzoate degradation

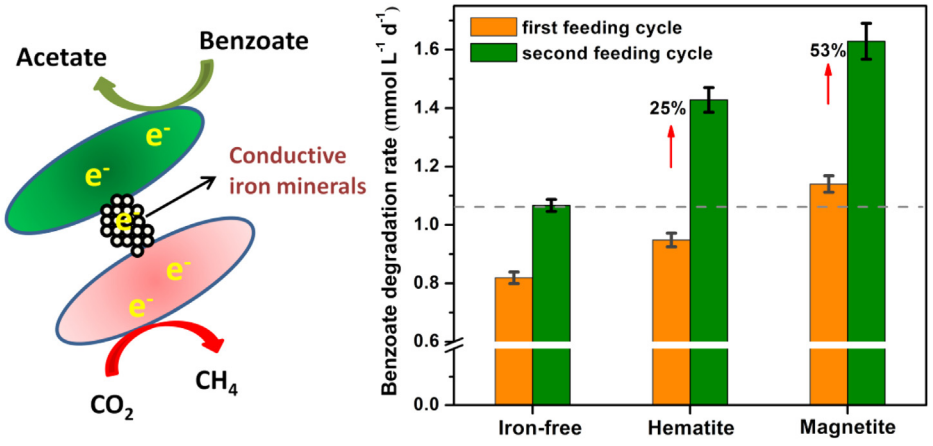
Li Zhuang [1], Jia Tang [1], Yueqiang Wang, Min Hu, Shungui Zhou *

Guangdong Key Laboratory of Agricultural Environment Pollution Integrated Control, Guangdong Institute of Eco-Environmental and Soil Sciences, Guangzhou 510650, China

HIGHLIGHTS

- Paddy soil contaminated with benzoate incubated with hematite and magnetite.
- Iron oxides addition enhanced methanogenic benzoate degradation by 25–53%.
- The facilitated syntrophy might involve direct interspecies electron transfer.
- *Bacillaceae*, *Peptococcaceae*, and *Methanobacterium* are potentially involved.

GRAPHICAL ABSTRACT



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ABSTRACT

Recent studies have suggested that conductive iron oxide minerals can facilitate syntrophic metabolism of the methanogenic degradation of organic matter, such as ethanol, propionate and butyrate, in natural and engineered microbial ecosystems. This enhanced syntrophy involves direct interspecies electron transfer (DIET) powered by microorganisms exchanging metabolic electrons through electrically conductive minerals. Here, we evaluated the possibility that conductive iron oxides (hematite and magnetite) can stimulate the methanogenic degradation of benzoate, which is a common intermediate in the anaerobic metabolism of aromatic compounds. The results showed that 89–94% of the electrons released from benzoate oxidation were recovered in CH_4 production, and acetate was identified as the only carbon-bearing intermediate during benzoate degradation. Compared with the iron-free controls, the rates of methanogenic benzoate degradation were enhanced by 25% and 53% in the presence of hematite and magnetite, respectively. This stimulatory effect probably resulted from DIET-mediated methanogenesis in which electrons transfer between syntrophic partners via conductive iron minerals. Phylogenetic

* Corresponding author at: Guangdong Institute of Eco-Environmental and Soil Sciences, No. 808 Tianyuan Road, Guangzhou 510650, China. Tel.: +86 20 37300951; fax: +86 20 37300951.
E-mail address: sgzhou@soil.gd.cn (S. Zhou).
[1] Both authors contributed equally to this work.

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analyses revealed that *Bacillaceae*, *Peptococcaceae*, and *Methanobacterium* are potentially involved in the functioning of syntrophic DIET. Considering the ubiquitous presence of iron minerals within soils and sediments, the findings of this study will increase the current understanding of the natural biological attenuation of aromatic hydrocarbons in anaerobic environments.

1. Introduction

Soil contamination with aromatic compounds is of particular environmental concern because of their toxic, mutagenic and carcinogenic properties and their recalcitrance to biodegradation [1]. Subsurface soils are mainly anaerobic or methanogenic due to the limited level and rapid depletion of oxygen; thus, anaerobic degradation of aromatic compounds is important for the understanding of their natural attenuation and the development of effective bioremediation strategies. The initial anaerobic transformation of aromatic compounds generally leads to the production of intermediates, such as benzoate and phenol, which is also transformed to benzoate before ring cleavage [2,3]. Thus, as a vital intermediate in the anaerobic metabolism of aromatic compounds, benzoate degradation usually plays an important role in removing aromatic compounds under anaerobic conditions.

In methanogenic environments, any electron acceptors other than CO_2 are limited or absent, the anaerobic oxidation of benzoate is thermodynamically unfavorable under standard conditions ($C_7H_5O_2^- + 7H_2O \rightarrow 3H_2 + HCO_3^- + 3CH_3COO^- + 3H^+$, $\Delta G^{0'} = +70.1$ KJ/reaction) but becomes favorable when methanogens consume intermediate products to maintain them at very low concentrations [4]. Thus, methanogenic benzoate degradation requires syntrophic metabolism between fermentative bacteria (syntrophs) and archaeal methanogens for complete mineralization to CO_2 and CH_4. The underlying feature of syntrophic processes is interspecies electron transfer via H_2 or formate exchange between syntrophic partners, which is termed interspecies H_2 (or formate) transfer and has long been assumed to be the exclusive mechanism of syntrophic methanogenesis [5,6].

Recently, direct interspecies electron transfer (DIET), in which two species exchange electrons via electric currents through conductive solid conduits or microbial pili, has been proposed as a novel alternative to interspecies H_2/formate transfer for the methanogenic degradation of organic matter [[7] and references therein]. Methanogenesis based on DIET can take place in methanogenic wastewater digester aggregates via direct cell-to-cell contact [8], or in the co-culture of *Geobacter metallireducens* and *Methanosaeta harundinacea/Methanosarcina barkeri*, in which electrons released from ethanol oxidization are directly transferred to methanogens via conductive pili for CO_2 reduction [9,10]. Electrically conductive materials, including granulated activated carbon [11] or biochar [12], have been demonstrated to be capable of promoting DIET-mediated methanogenesis in the co-culture of *G. metallireducens* and *M. barkeri*, metabolizing ethanol to CH_4. Of more ecological relevance, naturally occurring iron minerals (hematite or magnetite) have been found to accelerate the methanogenic degradation of organic matter in paddy soils (acetate, ethanol and butyrate) [13–15] or anaerobic sludge digesters (propionate) [16]. In this pathway, electrically conductive materials function as electron conduits for direct electron transfer between syntrophic, organic matter-oxidizing bacteria and CO_2-reducing methanogens.

Because DIET is more efficient than interspecies H_2/formate transfer for microbial syntrophy [16–19], conductive mineral-mediated DIET might be crucial for natural methane emission in iron-rich soils and also has great potential for improving the performances of bioenergy processes and anaerobic digestion. The goal of this study was to evaluate the potential of the supplementation of electrically conductive iron oxides (hematite and magnetite) for facilitating the methanogenic degradation of benzoate, which would benefit the complete mineralization and removal of aromatic compounds under methanogenic conditions. Our experimental findings clearly demonstrate the stimulatory effect of conductive iron oxides on syntrophic benzoate degradation, and we identify the corresponding changes in the microbial community using high-throughput sequencing on an Illumina MiSeq platform.

Table 1
Description of the six experimental treatments.

Number	Treatment	Sodium benzoate added	Fe(III) oxide added
I	Benzoate-free control	None	None
II	Benzoate	8.6 mM (twice[a])	None
III	Hematite	None	25 mM hematite
IV	Hematite + benzoate	8.6 mM (twice)	25 mM hematite
V	Magnetite	None	25 mM magnetite
VI	Magnetite + benzoate	8.6 mM (twice)	25 mM magnetite

[a] The treatments were twice amended with 8.6 mM benzoate, and the second amendment was conducted after the first dose of benzoate was completely degraded.

2. Materials and methods

2.1. Anaerobic incubation

The soil used in this experiment was sampled from the 0- to 20-cm layers in a rice paddy field located at the South China Agricultural University (23°10.099′N, 113°21.696′E). The soil properties have been described previously [15,20]. Soil samples were air dried and sieved (<60 μm) before use. All batch experiments were conducted in 125-mL anaerobic serum bottles. Using 1 g of dry soil as the microbial inocula, methanogenic benzoate-degrading communities were enriched in 50 mL anaerobic medium supplemented with 8.6 mM benzoate. The anaerobic medium consisted of 10 mM NH_4Cl, 1 mM KH_2PO_4, 0.5 mM $MgCl_2$, 0.5 mM $CaCl_2$, 5 mM $NaHCO_3$, 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 0.05% Bacto yeast extract, and 1 mL L^{-1} each of a trace element solution and an Se/W solution [21]. Either hematite or magnetite synthesized in the laboratory was supplemented in the vials at a final concentration of 25 mM as Fe atoms. Hematite was synthesized by sintering lepidocrocite powder (prepared by mixing 0.08 M $FeCl_2$, 0.16 M $(CH_2)_6N_4$ and 0.8 M $NaNO_2$ in Milli-Q water) at 420 °C for 2 h with an incremental temperature increase of 2 °C min^{-1} [22]. Magnetite nanoparticles were synthesized by slowly adding a Fe(II)/Fe(III) acidic solution (0.8 M $FeCl_3$ and 0.4 M $FeCl_2$ in 0.4 M HCl) into a vigorously mixed NaOH solution (1.5 M). They were then purified by centrifugation and suspended in deoxygenated water [23]. The experiment consisted of six treatments that are described in detail in Table 1. All bottles were sealed with Teflon®-coated septa and aluminum crimp caps after being bubbled with N_2/CO_2 (80:20, v/v) gas for 30 min at a rate of 10 mL min^{-1}. All of the experiments were conducted in biological triplicate and incubated at a constant temperature of 30 °C in the dark. For the benzoate-amended cultures, an additional 8.6 mM benzoate was