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# Conductive Magnetite Nanoparticles Accelerate the Microbial Reductive Dechlorination of Trichloroethene by Promoting Interspecies Electron Transfer Processes

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Microbes thrive on the energy they gain and conserve by "moving" electrons from low-potential electron donors to higher-potential electron acceptors occurring in natural environments. Many types of natural donors and acceptors are freely diffusible gases or soluble species that are easily transported and metabolized within living cells. On the other hand, certain microorganisms (often referred to as "electroactive" or "electrochemically-active") are capable of respiring insoluble electron donors or acceptors, including solid-state electrodes in microbial electrochemical systems (MESs).[1–3] To do this, these electroactive bacteria employ various strategies, ranging from the use of electron-transfer proteins located on the outer membrane (e.g., cytochromes), conductive microbial appendages (e.g., nanowires), to soluble redox shuttles (e.g., pyocyanin).[4]

Although electroactive microorganisms have found applications as electrocatalysts in a number of different bioelectrochemical processes (e.g., from bio-energy generation to groundwater bioremediation), the ecological and evolutionary bases of extracellular electron transfer (EET) remain poorly elucidated, and practical strategies for boosting EET only marginally explored. Recent studies have suggested that, in sedimentary environments, microorganisms with EET capabilities may take advantage of the electric currents running through conductive minerals, which connect spatially segregated bio-geochemical redox processes.[5]

Here, we evaluate the possibility that electrically conductive magnetite ($Fe_3O_4$) nanoparticles can enhance the reductive dechlorination (RD) of trichloroethene (TCE), an ubiquitous groundwater pollutant, by allowing electrons to be transferred—extracellularly—from acetate-oxidizing microorganisms to TCE-dechlorinating microorganisms. More specifically, we explore if such an interspecies electron transfer (IET) can occur between an anaerobic mixed culture (hereafter named "MES-culture") previously shown to be capable of anaerobically oxidizing acetate using a polarized graphite electrode (anode) as direct extracellular electron acceptor,[6] and an anaerobic mixed culture (hereafter named "RD-culture") previously shown to be capable of dechlorinating TCE [to cis-dichloroethene (cis-DCE), vinyl chloride (VC), and ethene] with a polarized graphite electrode (cathode) serving as direct extracellular electron donor.[7,8]

To this aim, an initial set of batch experiments was conducted in which small samples of the two cultures (5 mL of RD-culture and 1 mL of MES-culture) were transferred into 120 mL anaerobic serum bottles containing anaerobic mineral medium,[9] amended with acetate (1.5 mmol) and TCE (0.03 mmol), and supplemented with either 15 mL of a filtered (0.2 μm) suspension of magnetite nanoparticles or 15 mL of filtered (0.2 μm) deionized water. The final liquid volume in the bottles was 75 mL. Magnetite nanoparticles were prepared as described by Kang and colleagues.[10] The filtered suspension used in the batch experiments contained approximately $1.5 \times 10^7$ particles per mL (Supporting Information, Figure S1), with a total Fe concentration of 0.16 mmol $L^{-1}$ (as determined by ICP–MS). Flow cytometry revealed the presence of two dominant morphotypes of individual particles in the filtered suspension, with average diameters of $95 \pm 7$ nm and $119 \pm 8$ nm. Scanning electron microscopy (SEM; Figure S2) confirmed that the diameter of particles was in the range 80–150 nm. Finally, energy dispersive X-ray (EDX) spectra confirmed that the nanoparticles were abundant in iron and oxygen (Figure S2). Upon setup, all of the serum bottles were incubated statically, in the dark, at room temperature (21–25 °C).

As shown in Figure 1, regardless of the presence of magnetite nanoparticles, TCE was dechlorinated to cis-DCE and, though to a lower extent, VC. This dechlorination pathway is consistent with *Desulfitobacterium* spp. (a bacterial group capable of dechlorinating TCE to cis-DCE[11]) and *Dehalococcoides* spp. (a bacterial group capable of dechlorinating TCE to VC and ethene[12]) being the dominating members of the RD-culture. Even though at the end of incubation (day 38) the extent of dechlorination was nearly the same (Figure 2a), bottles containing magnetite nanoparticles exhibited a 2.3-fold higher rate of TCE dechlorination (Figure 2b). Although a greater stimulatory effect was observed on cis-DCE formation, VC formation was positively influenced by the presence of conductive nanoparticles (Figure 2b, insert), also, clearly indicating the occurrence of specific interaction(s) between *Dehalococcoides* spp. and magnetite. Importantly, abiotic control experiments carried out in the presence of magnetite nanoparticles allowed

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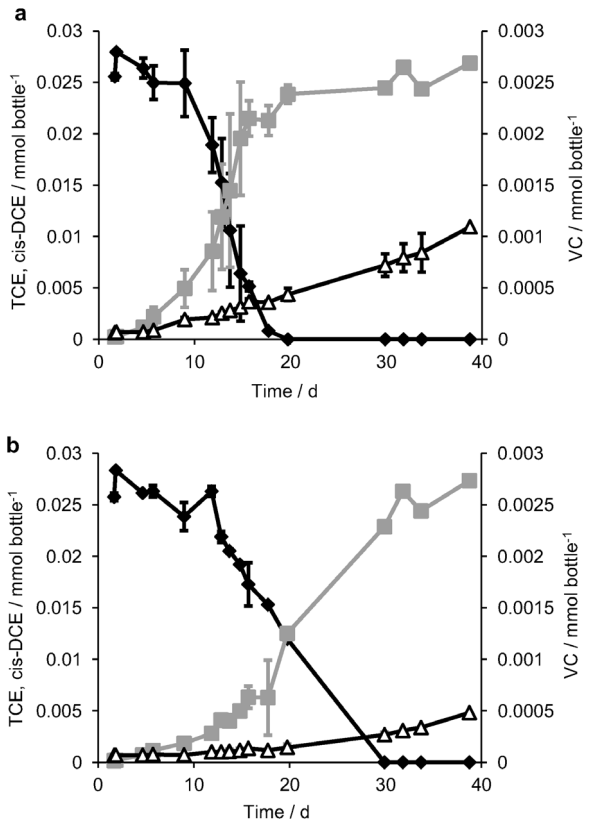


**Figure 1.** Time course of TCE dechlorination with acetate (20 mmol $L^{-1}$) as electron donor in anaerobic serum bottles a) supplemented, or b) not supplemented with conductive magnetite nanoparticles. Error bars (when larger than symbols) represent ±1 standard deviation of replicated experiments. ◆: TCE, ■ : cis-DCE, △: VC.

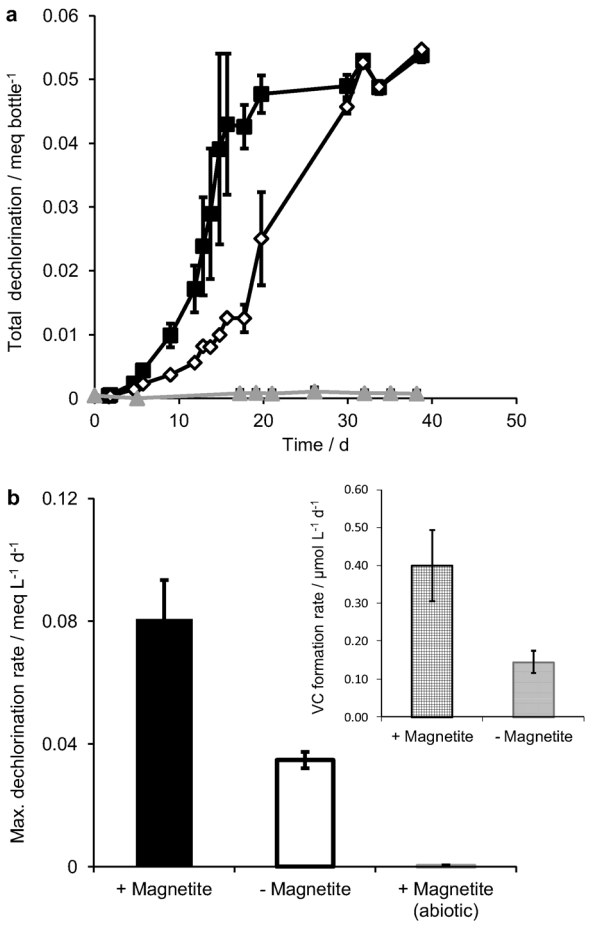


**Figure 2.** a) Cumulative respiratory electrons channeled to TCE dechlorination, and b) maximum rates of TCE dechlorination for the batch experiments depicted in Figure 1. The effect of magnetite nanoparticles on the maximum rate of VC formation is highlighted as an insert in (b). Error bars (when larger than symbols) represent ±1 standard deviation of replicated experiments. ■: + magnetite, ◇: − magnetite, ▲: + magnetite (abiotic).

to rule out that the stimulatory effect was due to the particles directly catalyzing the reductive dechlorination of TCE (Figure 2a and b).

Even though the magnetite nanoparticles were added to the bottles in relatively small amounts (< 10 mg $L^{-1}$ as total iron), the redox cycling of soluble iron species [$Fe^{II}/Fe^{III}$] possibly produced from their dissolution could have contributed to IET between the MES-culture and the RD-culture. To verify this possibility a batch experiment was conducted in which an inoculum consisting of the MES-culture and the RD-culture was supplemented with either a filtered suspension of magnetite nanoparticles (0.16 mmol $L^{-1}$ as total iron), soluble $Fe^{III}$ (0.16 mmol $L^{-1}$ ferric chloride), or no iron-based amendments (control test) in the presence of TCE (0.03 mmol) and acetate (1.5 mmol) (Figure S3). The rate of TCE dechlorination in the presence of soluble $Fe^{III}$ was very similar to that observed in the control tests, and substantially lower (by a factor of ca. 2) than that observed in the presence of magnetite nanoparticles. These results are a clear indication that soluble iron species did not serve as electron shuttles in IET between members of the two cultures.

Alternatively, to verify the hypothesis that magnetite nanoparticles served primarily as "conduits" of electrons between dechlorinating (i.e., the RD-culture) and non-dechlorinating (i.e., the MES-culture) microorganisms, a new set of batch experiments was conducted. These new experiments included four different treatments (A–D), as depicted in Figure 3. When anaerobic serum bottles (total volume 75 mL) were incubated with the RD-culture (2 mL to a final liquid volume of 37 mL) and magnetite nanoparticles (2 mL), but in the absence of the MES-culture (treatment B), the rate of TCE dechlorination (in the presence of 20 mmol $L^{-1}$ of acetate) was nearly indistinguishable from that observed in bottles containing only the RD-culture (treatment A; Figure 3). This trend did not change after the bottles were subjected to a second feeding cycle (i.e., a new spike of TCE after the first one had been completely consumed; Figure 3). More specifically, even though the rates of TCE dechlorination during the second feeding cycle were all substantially higher than those observed during the first feeding cycle, likely due to the growth of dechlorinating (and non-dechlorinating) microorganisms, the increase was nearly identical for treatments A and B. This result confirmed that the suspension of nanoparticles was not capable to directly stimulate the metabolism of dechlorinating microorganisms, for example by providing some key (trace) elements.

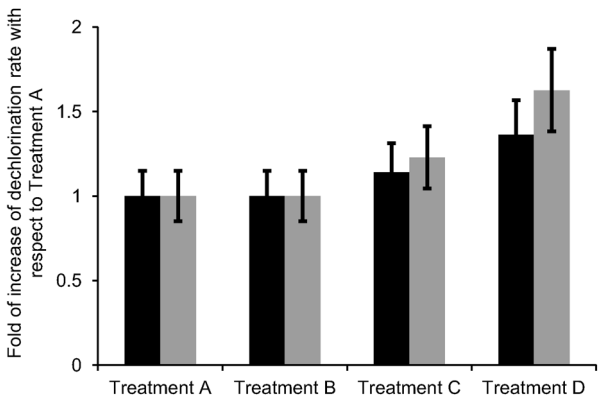


**Figure 3.** Effect of magnetite nanoparticles and the MES-culture on the maximum rate of TCE dechlorination by the RD-culture, in the presence of acetate (20 mmol $L^{-1}$). Error bars (when larger than symbols) represent $\pm 1$ standard deviation of replicated experiments. Treatment A: RD-culture, treatment B: RD-culture + magnetite, treatment C: RD-culture + MES-culture, treatment D: RD-culture + MES-culture + magnetite. ■: 1st feeding cycle, ■: 2nd feeding cycle.

Bottles which contained both the RD-culture (2 mL) and the MES-culture (2 mL) but not magnetite (treatment C), displayed slightly higher, though not statistically different, dechlorination rates (by a factor of 1.1 to 1.2) compared to bottles containing the RD-culture only (Figure 3). In principle, this finding could indicate the existence of a cooperative metabolism between microorganisms in the RD-culture and those in the MES-culture, resulting in a (although limited) stimulatory effect on the reductive dechlorination of TCE. Considering that the MES-culture had not been previously exposed to TCE and did not exhibit any dechlorinating activity (data not shown), it is possible that the stimulatory effect resulted from a direct electron exchange between microorganisms in the MES-culture and the RD-culture, taking place via conductive biological appendages (e.g., extracellular cytochromes and nanowires).[13,14]

Such a direct (i.e., microbe-to-microbe) IET mechanism has been recently documented in co-cultures of *Geobacter* species with other "electroactive" microorganisms. As an example, ethanol oxidation coupled to fumarate reduction, by an adaptively evolved co-culture of *G. metallireducens* and *G. sulfurreducens* was found to proceed via direct IET, rather than via diffusion of reduced carriers such as $H_2$ or formate.[15] Interestingly, the co-culture formed microbial aggregates exhibiting a remarkable electrical conductivity, further supporting the occurrence of direct IET. Direct IET also appeared as an important process in multi-species aggregates from a methanogenic digester in which *Geobacter* and *Methanosaeta* species predominated.[16]

The occurrence of *Geobacter* spp. in the MES-culture used in this study was probable. Indeed, the predominance of *Geobacter* spp. in acetate-fed bioelectrochemical systems has been documented in a large and ever-increasing number of publications.[17–19] Furthermore, a molecular characterization of the MES-inoculum by fluorescent in situ hybridization (FISH) confirmed that δ-proteobacteria (the phylum of *Geobacter*) were the dominating members of the culture, accounting for about 50% of total bacteria.

As shown in Figure 3, the most striking increase in the rate of TCE dechlorination (a factor of 1.4 in the first feeding cycle and 1.6 in the second feeding cycle) was noticed when the RD-culture, the MES-culture, and the magnetite nanoparticles were simultaneously present in the serum bottle. This finding is consistent with the hypothesis that conductive nanoparticles wire up acetate-oxidizing bacteria to TCE-dechlorinating bacteria, resulting in more efficient IET and, in turn, cooperative metabolism (Figure 4). TCE dechlorination was negligible in control experiments in the presence of the RD-culture, the MES-inoculum, and the magnetite nanoparticles, but in the absence of acetate (data not shown), demonstrating that IET was ultimately driven by catabolic acetate utilization. Similar results were recently reported by Kato and colleagues, who observed that supplementation of soil microbes with conductive nanoparticles (in the presence of an excess of carbon source) resulted in the acceleration of methanogenesis in terms of lag time and production rate, while supplementation with an insulative iron oxide did not.[20] Since the presence of conductive nanoparticles stimulated the growth of *Geobacter* species, the authors suggested that this microorganism grew under syntrophic association with methanogens, and IET could occur via electric currents flowing through the conductive minerals. In a very elegant co-culture investigation, the same research group reported that magnetite nanoparticles facilitated IET from *Geobacter sulfurreducens* to *Thiobacillus denitrificans*, accomplishing acetate oxidation coupled to nitrate reduction.[21]

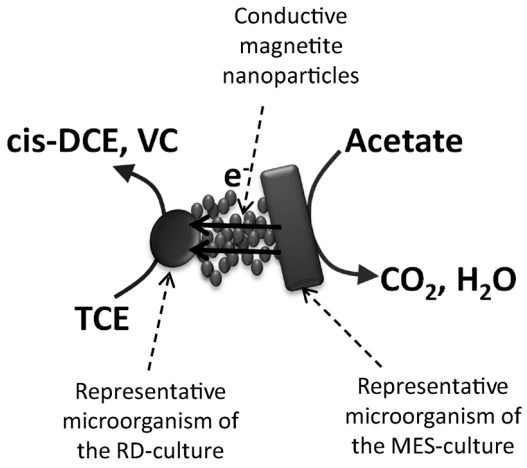


**Figure 4.** Conceptual illustration of the proposed interspecies electron transfer between dechlorinating and non-dechlorinating microorganisms, through conductive magnetite nanoparticles.

In conclusion, this study confirms the hypothesis that magnetite nanoparticles accelerate the reductive transformation of TCE by promoting cooperative metabolisms among dechlorinating and non-dechlorinating microorganisms, based on IET processes. Considering that conductive minerals are ubiquitous in subsurface environments, these processes are likely to occur naturally at many contaminated sites and are expected to contribute greatly to a wide number of geochemical cycles, also involving the degradation of contaminants. Moreover, this

study raises the intriguing possibility that conductive magnetite nanoparticles can be employed in engineered groundwater remediation systems such as chemical[22] or bio-electrochemical[23] permeable reactive barriers to improve the kinetics of contaminants transformation. Finally, the application of magnetite nanoparticles is envisaged as a promising strategy to boost microbe–electrode interactions and extracellular electron transfer processes in the emerging bioelectrochemical applications.

## Experimental Section

**Synthesis and characterization of magnetite nanoparticles**: Magnetite nanoparticles were synthesized by chemical precipitation of $Fe^{2+}$ and $Fe^{3+}$ ions in an alkaline (1.5 M NaOH) aqueous solution, according to the protocol developed by Kang and colleagues.[10] Prior to being used, the aqueous suspension was filtered across a hydrophilic membrane (pore size 0.2 μm), to eliminate particles with a diameter larger than 200 nm. For scanning electron microscopy (SEM) analysis, 500 μL of the prepared suspension of magnetite nanoparticles was dropped onto a glass slide, incubated at 30 °C for 1 h to evaporate the liquid phase, and finally coated with gold powder. Magnetite nanoparticles were also characterized by flow cytometry (Apogee A50, Apogee Flow System, UK). Histogram software (version 2.6) was used to plot the density diagrams (color plot and histograms) of log-transformed light signals, scattered off of each particle in line with the 375 nm laser light beam (Forward Scatter, or FSC) and perpendicular to it (Side Scatter, or SSC). Photomultipliers were set at a voltage of 210 and 360, respectively.

**Source cultures**: The anaerobic dechlorinating culture ("RD-culture") used as inoculum for batch experiments contained approximately $2\times10^7$ bacteria per mL, as determined by fluorescent in situ hybridization (FISH) with specific oligonucleotide probes. The culture was thoroughly characterized by FISH in a previous study,[8] with *Desulfitobacterium* and *Dehalococcoides* species accounting for nearly the totality of bacterial cells. The "MES-culture" was obtained from the effluent of the anode chamber of a microbial electrochemical system (MES) continuously fed with acetate. Under sustained operation, over 90% (on a molar basis) of the influent acetate was oxidized at the anode of the MES with concomitant electric current generation. The MES-culture contained approximately $6\times10^7$ bacteria per mL, 50% of which were δ-proteobacteria.

**Analytical methods**: Volatile components (TCE, cis-DCE, VC, ethene, methane) were quantified by injecting 100 μL of serum bottle headspace (taken with a gas-tight syringe) into a Perkin–Elmer GC 8500 gas chromatograph (2 m × 2 mm glass column packed with 60/80 mesh Carbopak B/1% SP-1000 Supelco; $N_2$ carrier gas 18 mL min$^{-1}$; oven temperature 190 °C; flame ionization detector temperature 260 °C).

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**Keywords:** bioremediation · bacteria · dechlorination · electron transfer · nanoparticles

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