Stimulatory effect of magnetite on the syntrophic metabolism of *Geobacter* co-cultures: Influences of surface coating

Yunshen You\(^a,1\), Shiling Zheng\(^b,1\), Hongmei Zang\(^a\), Feng Liu\(^a\), Fanghua Liu\(^b,*\), Juan Liu\(^a,c,*\)

\(^a\) The Key Laboratory of Water and Sediment Sciences, Ministry of Education, College of Environmental Sciences and Engineering, Peking University, Beijing 100871, China
\(^b\) Key Laboratory of Coastal Biology and Biological Resources Utilization, Yantai Institute of Coastal Zone Research, Chinese Academy of Sciences, Yantai, China
\(^c\) Beijing Key Laboratory of Mineral Environmental Function, Peking University, Beijing 100871, China

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**Abstract**

Magnetite-mediated direct interspecies electron transfer (DIET) can facilitate syntrophic metabolism in natural microbial communities and also promote the performance of the engineered systems based on syntrophic interactions. In this study, the stimulatory effect of bare synthetic magnetite (Mt), humic acid coated magnetite, and SiO\(_2\) coated magnetite (Mt-SiO\(_2\)) on DIET in defined co-cultures of *Geobacter metallireducens*/*Geobacter sulfurreducens* were studied. Magnetite coated with Aldrich humic acid (HA) and Elliott Soil humic acid (HA\(_{ES}\)), respectively, were prepared, and the two kinds of humic acid influenced the ability of Mt to promote syntrophic metabolism of the co-cultures in a similar way. When weight concentration was the same, pure humic acid presented the stimulatory effect on DIET similar to bare magnetite. However, the presence of HA coating on magnetite surface caused 50% and 61%, respectively, decrease in the rates of ethanol consumption (\(R_e\)) and succinate production (\(R_s\)) in DIET processes. Pure HA in the same weight concentration as the HA coating in Mt-HA induced the similar metabolism rates as Mt-HA. In the Mt-HA mediated DIET, most electrons from ethanol metabolism were transferred to *G. sulfurreducens* selectively through the HA coating, and magnetite core hardly contributed to DIET processes. The SiO\(_2\) coating on magnetite resulted in 81% and 89%, respectively, decreases in \(R_e\) and \(R_s\), mainly because the non-conductive SiO\(_2\) layer hindered electron transfer between magnetite core and bacteria. After eight-day incubation with the co-cultures, bare magnetite nanoparticles formed relatively larger and more compact aggregates with cells than Mt-HA and Mt-SiO\(_2\), due to the different surface charge between bare and coated Mt. The generation of dissolved Fe(II) and HCl-extractable Fe(II) due to microbial reduction of magnetite by *G. metallireducens* and vivianite formation were observed along with DIET processes in all DIET experiments. Based on these results, different pathways of electron transfer in defined co-cultures of *Geobacters* with bare and coated magnetite nanoparticles were proposed. The findings in this study demonstrate the significant effects of surface properties on the ability of magnetite to stimulate DIET, which needs to be considered in order to comprehensively understand the role and mechanisms of mineral-mediated DIET in natural and engineered systems.

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* Corresponding authors at: College of Environmental Sciences and Engineering, Peking University, Beijing 100871, China (J. Liu); Yantai Institute of Coastal Zone Research, 17 Chunhui Road, Laishan District, Yantai, Shandong 264003, China (F. Liu).

E-mail addresses: fhliu@yic.ac.cn (F. Liu), juan.liu@pku.edu.cn (J. Liu).

1 Both authors contributed equally to this work.
1. INTRODUCTION

In the Earth’s critical zone where mineral-microbe interactions significantly impact many biogeochemical processes, a variety of microbes can utilize redox-active minerals as electron acceptors/donors for extracellular respiration (e.g. Lovley et al., 1987; Weber et al., 2006; Melton et al., 2014), naturally occurring batteries for electron storage (Byrne et al., 2015), or conduits for cell-to-cell electron transfer (Shi et al., 2016), etc. The phenomenon that electrical connections effectively accelerate electron transfer between microbial cells of different species is known as mineral-mediated direct interspecies electron transfer (DIET) (Shrestha and Rotaru, 2014). The electrical connections can be minerals, conductive pili, cytochromes, etc. (Lovley, 2017). Without the need for metabolite generation and exchange, DIET is expected to be more efficient than the conventional metabolite-mediated interspecies electron transfer (MIET) (Summers et al., 2010). Considering the ubiquity of redox-active minerals in the natural environment, mineral-mediated DIET may largely contribute to electrical connections between microbes (Shrestha and Rotaru, 2014), affect the physiology of the microorganisms in microbial consortia (Shi et al., 2016), and promote efficiencies of engineered systems based on syntrophic metabolism (Liu et al., 2012a; Cruz Viggi et al., 2014; Cheng and Call, 2016). Despite the importance of mineral-mediated DIET in engineered and natural systems, the research in this area is still in its infancy. Further studies are needed to reveal the correlation between the properties of minerals and their ability to stimulate metabolism in syntrophic co-cultures.

Although a variety of engineered materials, such as carbon cloth and activated carbon (e.g. Liu et al., 2012a; Chen et al., 2014b), have been studied for catalyzing syntrophic metabolism via DIET, but the interspecies electron transfer mediated by common minerals in nature is more environmentally relevant. Among all iron oxide minerals that have been investigated in DIET studies so far, magnetite (Fe₃O₄) is the most efficient iron oxide in promoting syntrophic metabolism most efficiently (Kato et al., 2012b). In the medium with ethanol as the electron donor and fumarate as the electron acceptor, only G. metallireducens can oxidize ethanol, and fumarate can only accept electrons from G. sulfurreducens. The presence of magnetite can stimulate syntrophic growth via DIET in these defined co-cultures (Liu et al., 2012a, 2015). Similarly, in co-cultures of G. sulfurreducens and Thiobacillus denitrificans (Kato et al., 2012b), or Geobacter spp. and methanogens, magnetite nanoparticles (NPs) were also able to promote syntrophic metabolism via DIET (Kato et al., 2012a; Cruz Viggi et al., 2014; Li et al., 2015). The underlying mechanisms for magnetite-mediated DIET are still under debate. Some researchers proposed that the chains of magnetite NPs serve as electron conduits for DIET by bridging electron-donating and electron-accepting cells (Kato et al., 2012a, b; Cruz Viggi et al., 2014). However, it was also suggested that magnetite NPs can work as an equivalent of OmcS, a multi-heme c-type cytochrome associated with the pili of G. sulfurreducens, to facilitate DIET between G. metallireducens and OmcS-deficient G. sulfurreducens (Liu et al., 2015). No definitive conclusions have been made regarding how magnetite NPs transfer electrons within microbial consortia and what properties of magnetite dominate its ability to stimulate syntrophic metabolism via DIET.

In addition to complex pathways for electron exchange between microorganisms participating in magnetite-mediated DIET (Wang et al., 2016), the variable properties of magnetite particles in the natural environment also make it difficult to determine the detailed mechanism of magnetite-mediated DIET. In previous studies, the stimulatory effect of magnetite on DIET has been mostly attributed to its relatively high conductivity (Kato et al., 2012b; Cruz Viggi et al., 2014; Chen et al., 2014b; Li et al., 2015; Cheng and Call, 2016). Magnetite has mixed-valence states of Fe in an inverse spinel structure. Fe(II) and Fe(III) cations in the edge-sharing chains of FeO₆ octahedra facilitate rapid hopping of electrons along the octahedral sublattice, accordingly resulting in high electrical conductivity (10⁻²–10⁻¹ Ω⁻¹ cm⁻¹) (Cornell and Schwertmann, 2003). The conductivity of magnetite is sensitive to many factors including environmental variables (temperature, redox potential, etc.) and the mineral properties of magnetite (crystallinity, stoichiometry, surface properties, etc.) (e.g. Rosencwaig, 1969; Latta et al., 2011). Changes in these factors may influence the ability of magnetite to stimulate DIET. In addition to conductivity, other mineral properties, such as aggregation state, particle size, etc., may also affect electron transfer at the microbe-mineral interface (Liu et al., 2013, 2016), and accordingly impact the efficiency of magnetite-mediated DIET. In most previous DIET studies, synthetic magnetite particles were used under precisely controlled experimental conditions. The effects of environmental variables or mineral properties on the abilities of magnetite to stimulate DIET were not sufficiently considered, which might result in the different efficiencies of magnetite-mediated DIET observed in laboratory and natural systems. Therefore, studying the relationship between mineral properties and the ability of magnetite to stimulate syntrophic metabolism is important for understanding the role of magnetite-mediated DIET in natural microbial communities and also helpful for improving the performance of engineered systems based on syntrophic interactions.

Magnetite, widely found in soils, sediments, surface waters, atmospheric aerosols, and highly corrosive environments (King et al., 1982; Baer et al., 2010; Pearce et al., 2014; Vikesland et al., 2016), commonly possesses surface properties that are distinctly different from those of its synthetic counterpart (Baer et al., 2010; Salazar-Camacho et al., 2013; Swindle et al., 2014). One common feature of magnetite grains in natural sediments or soils is the presence of a thin Si-containing coating on surface that might be related to the complex water-mineral interactions in Earth’s near-surface environment (Baer et al., 2010; Salazar-Camacho et al., 2013). Another feature is that natural magnetite particles are often coated by humic substances, such as humic acids (HAs), to varying extents (Swindle et al., 2014), which is probably due to the high affinity of ubiquitous HA to magnetite surface (Jiang et al., 2014). Moreover, magnetite NPs coated by silica or
HA have been widely studied in the fields of wastewater treatment, catalysis, bioengineering, etc. (Levy et al., 2002; Ge et al., 2008; Jiang et al., 2014; Zhang et al., 2014). Due to the aforementioned reasons, the effects of silica or HA coating on the ability of magnetite NPs to stimulate DIET in defined G. metallireducens/G. sulfurreducens co-cultures was investigated in this study. The rates of ethanol metabolism and succinate production, as well as electron recovery, in the co-cultures amended with bare magnetite, silica-coated magnetite, and HA-coated magnetite, respectively, were compared. The influences of surface modification on electron transport pathway in magnetite-mediated DIET, bio-reduction of magnetite NPs, and aggregation state of magnetite NPs with bacteria have been systematically investigated. The findings herein revealed that the surface coating had significant impacts on the ability of magnetite to stimulate DIET by alternating the pathway or efficiency of electron transport through magnetite, which provided fundamental insights into the mechanisms of magnetite-mediated DIET in natural microbial communities.

2. MATERIALS AND METHODS

2.1. Particle synthesis

The bare magnetite (Mt) NPs were synthesized by adding 1.5 M NaOH solution to a stoichiometric mixture of FeCl₃ (0.8 M) and FeCl₂ (0.4 M) in 0.4 M HCl under vigorous stirring 1.5 M NaOH solution to a stoichiometric mixture of et al., 2010). The synthetic Mt NPs (0.3 g) described above were added to 100 mL of 0.11 M sodium silicate at 80°C were synthesized according to literature procedures (Hu et al., 2002; Ge et al., 2008; Jiang et al., 2014; Zhang et al., 2014). Due to the aforementioned reasons, the effects of silica or HA coating on the ability of magnetite NPs to stimulate DIET in defined G. metallireducens/G. sulfurreducens co-cultures was investigated in this study. The rates of ethanol metabolism and succinate production, as well as electron recovery, in the co-cultures amended with bare magnetite, silica-coated magnetite, and HA-coated magnetite, respectively, were compared. The influences of surface modification on electron transport pathway in magnetite-mediated DIET, bio-reduction of magnetite NPs, and aggregation state of magnetite NPs with bacteria have been systematically investigated. The findings herein revealed that the surface coating had significant impacts on the ability of magnetite to stimulate DIET by alternating the pathway or efficiency of electron transport through magnetite, which provided fundamental insights into the mechanisms of magnetite-mediated DIET in natural microbial communities.

2.2. Characterization of synthetic particles

The weight concentration of particles in all stock suspensions was determined by measuring the weight difference of particle suspensions before and after drying in the anoxic glovebox (Section S1). In addition, the weight percentage of magnetite in coated Mt NPs and the solid-state Fe(II)/Fe(III) ratio in the synthetic bare and coated Mt NPs were measured by acid digestion (Section S1). The results indicate that Fe₂O₄ in the synthetic Mt-SiO₂, Mt-HA, and Mt-HAES NPs were about 81.5%, 89.9%, and 87.6%, respectively. The crystal phase, particle size, and morphology, as well as magnetic properties of synthetic NPs, were studied by X-ray diffraction (XRD), transmission electron microscopy (TEM), and vibrating sample magnetometer (VSM), respectively. The binding of HA or SiO₂ coating to magnetite surface in the coated Mt NPs was investigated by Fourier transform infrared spectroscopy (FTIR). Surface charge and aggregation state of synthetic NPs were studied by zeta potential analysis and dynamic light scattering (DLS), respectively. The details of the measurements and sample preparation are described in the Supporting information (Section S1).

2.3. DIET assay

The growth conditions of Geobacter sulfurreducens DL1 (ATCC 51573) or Geobacter metallireducens GS-15 (ATCC 53774) single strains were described in details in Electronic Annex Section A2. Anaerobic co-cultures of G. metallireducens and G. sulfurreducens were established with the equal amount (0.5 mL) of each Geobacter in the strictly anoxic NBF medium (10 mL) with 10 mM ethanol and 40 mM fumarate (Liu et al., 2012a, 2015; Chen et al., 2014a, 2014b). To compare the stimulatory effect of different materials on DIET, bare and coated Mt NPs with the same Fe concentration (15 mM) were added to the co-cultures and incubated at 30°C. Because dynamic aggregation-disaggregation processes of NPs in suspensions may change their available surface area, the weight concentration, not surface area, of NPs was chosen as the normalizing factor. According to the measured percentages of magnetite in coated Mt NPs, 1.16 g L⁻¹ bare Mt, 1.42 g L⁻¹ Mt-SiO₂, 1.29 g L⁻¹ Mt-HA, and 1.32 g L⁻¹ Mt-HAES were, respectively, added in order to keep the same concentration of magnetite. Before NPs were added into the co-cultures, they were suspended in DDW and then stored in serum bottles capped with thick rubber stoppers inside the glovebox.
were suspended in a small amount of milli-Q water and autoclaved at 120 °C. No changes were observed in XRD patterns of the synthetic NPs due to autoclaving. In addition, control experiments of the co-cultures with pure SiO$_2$, Aldrich HA, and Elliott Soil HA, respectively, were also conducted. In all DIET experiments, at desired time intervals, 0.7 mL aliquots of suspension were taken using sterile syringes under anoxic condition. The concentrations of ethanol, acetate, and succinate in the filtrates were measured simultaneously with high performance liquid chromatography (HPLC) as previously described (Nevin et al., 2008). All experiments were performed in triplicate.

Control experiments of single culture with synthetic NPs were conducted under the similar conditions for DIET experiments. Briefly, a 5% inoculum of *G. metallireducens* or *G. sulfurreducens* was incubated in 10 mL anoxic NBF medium with 10 mM ethanol and 40 mM fumarate. Then, the control experiment was initiated by adding 1.16 g L$^{-1}$ bare Mt, 1.42 g L$^{-1}$ Mt-SiO$_2$, 1.29 g L$^{-1}$ Mt-HA, or 1.32 g L$^{-1}$ Mt-HAES, respectively. The incubation temperature for the single culture studies was 30 °C. The concentrations of ethanol, acetate, and succinate as a function of time were monitored as described above.

2.4. Changes of bare or coated Mt NPs in DIET processes

The concentration change of bioavailable Fe(II) in the system due to microbial reduction of NPs was studied by measuring the concentrations of 0.5 M HCl-extractable Fe(II) as a function of time during DIET experiments. 0.5 M HCl has been widely used as an extraction agent to separate bioavailable Fe(II), the portion of Fe(II) that is produced by or available for bacterial respiration, from crystalline Fe(II) in iron oxide minerals or sediments (Fredrickson et al., 2004; Emmerich et al., 2012). No measurable Fe$^{2+}$ ions are released from magnetite in 0.5 M HCl within 12 h (Sidhu et al., 1981), so 0.5 M HCl cannot increase Fe(II) concentration due to the abiotic dissolution of magnetite. In this study, 0.5 M HCl-extractable Fe(II) includes dissolved Fe(II) and a portion of solid-phase associated Fe(II) in bare or coated Mt. The concentrations of HCl-extractable Fe(II) was measured by adding 0.2 mL sample to 5 mL of 0.5 M HCl. Then, the mixture was continuously shaken in the dark under anoxic condition for 12 h. After that, the product was filtered by 0.2 mm polyether-sulfone (PES) syringe filters, and the Fe(II) concentration in the filtrate was determined by the ferrozine assay (Liu et al., 2012b). The ferrozine solution was 1 g L$^{-1}$ in 50 mM HEPES buffered at pH 7. Moreover, concentrations of dissolved Fe(II) in samples were also quantified by filtering 0.5 mL of sample using a 0.45 μm sterile PES syringe filters. Fe(II) concentration in the filtrates was determined by adding 0.2 mL of the filtrate to 0.8 mL ferrozine solution as described above and measuring the absorbance at 562 nm. For comparison, the concentration change of HCl-extractable and dissolved Fe(II) in single culture experiments amended with different NPs was also studied using the similar method as described above.

In order to study aggregation state of cells with NPs, the end-products after the 15-day incubation of the co-cultures with different materials (when ethanol and fumarate were mostly consumed) were gently collected and fixed by 2.5% glutaraldehyde in 0.1 M phosphate buffer using the method previously reported (Chen et al., 2014b). The samples after gold coating were observed by a field-emission scanning electron microscope (SEM, Hitachi, SU8020) equipped with an energy dispersive X-ray spectroscopy detector (EDS, X-max80, OXFORD corp.). SEM images were taken at an accelerating voltage of 5 kV in secondary electron mode.

3. RESULTS

3.1. Characteristics of bare and coated Mt NPs

The XRD patterns (Fig. 1A) indicate that only magnetite phase was present in all synthetic NPs. Surface modification by HA or SiO$_2$ did not produce secondary mineral phase, which was consistent with the results of previous studies (Woo et al., 2005; Liu et al., 2008; Hu et al., 2010; Jiang et al., 2014). TEM images at low magnification

![Fig. 1. XRD patterns of solid phase before (A) and after (B) 15-day incubation of defined *G. metallireducens-G. sulfurreducens* co-cultures amended with synthetic bare Mt NPs (blue), Mt-HA (red), Mt-HAES (green), and Mt-SiO$_2$ (black), respectively. The references for vivianite (JCPDS card No. 00-030-0662) and magnetite (JCPDS card No. 01-075-1609) are shown at the bottom. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)](image-url)
(Fig. 1S) showed that both the bare and coated Mt were nearly spherical NPs. The average size of the bare Mt was about 11 nm. Surface modification by HA or SiO$_2$ slightly increased the average particle size, which is consistent with the results of SiO$_2$ or HA coated Mt NPs reported in previous studies (Woo et al., 2005; Liu et al., 2008). The high-magnification TEM images (Fig. 2) showed that the amorphous thin layer (without lattice fringe) was present on the surface of magnetite core (in darker contrast and with lattice fringes) in coated Mt NPs, which confirms the presence of SiO$_2$ or HA coating on Mt surface. However, the porosity of the amorphous surface layers cannot be directly determined from the high-magnification TEM images, because the contrast can also be related to the heterogeneous thickness of amorphous silica or HA coatings.

The binding of HA or SiO$_2$ molecules onto magnetite surface in coated Mt NPs was also proved by FTIR spectra (Fig. 3A). The spectrum of bare magnetite showed a broad peak at 544–582 cm$^{-1}$ that is assigned to Fe–O stretching vibration (Hu et al., 2010; Wang et al., 2010). In the spectra of HA coated Mt NPs, there were two additional peaks at ~1400 cm$^{-1}$ and ~1590 cm$^{-1}$, respectively, which agree well with the reported FTIR spectra of HA coated Mt NPs (Liu et al., 2008; Niu et al., 2011). Because the C=O stretch of free carboxylic acid is above 1700 cm$^{-1}$ (Yantasee et al., 2007), the C=O stretches at the lower numbers (~1400 cm$^{-1}$ and ~1590 cm$^{-1}$) indicate the binding of humic acid to Mt surface in HA coated Mt NPs. In the FTIR spectrum of Mt-SiO$_2$, the strong peak around 1093 cm$^{-1}$ was ascribed to Si–O bond of silica (Hu et al., 2010). The small peak around 800 cm$^{-1}$ suggested the formation of Si–O–Fe bonds in Mt-SiO$_2$ (Haddad et al., 2004).

The SiO$_2$ or HA coating also significantly changed surface charge and zeta potential of Mt NPs. Fig. 3B showed that the surface charge of bare Mt NPs was positive at pH lower than 6.8, but HA or SiO$_2$ coated NPs was negatively charged over the pH range from 3 to 10.4. At pH 7 that was the pH value for all DIET experiments, the zeta potentials of bare Mt, Mt-HA, Mt-HAES, and Mt-SiO$_2$ NPs were ~2.0 mV, ~38.0 mV, ~29.21 mV, and ~18.6 mV, respectively. The significant lower surface charge of the coated Mt was presumably related to the abundant carboxylic groups in HA or the relatively low point of zero
different pH values or Fe\(^{2+}\) concentrations from the stock and coated Mt NPs were exposed to the solutions with suspensions inside the anoxic glovebox. Moreover, when bare and coated Mt slowly changed with time even in the stock and \(C_24\) discussed in this study.

The magnetization curves (Fig. A2) suggest that the coercivity and remanence of coated Mt NPs were similar to those of bare Mt NPs. Thus, the presence of HA or SiO\(_2\) coating on Mt NPs did not obviously change the magnetic response of Mt NPs (see Electronic Annex Section A2 for more details). The Fe(II)/Fe(III) ratio of freshly synthesized Mt NPs, HA coated Mt, and Mt-SiO\(_2\) was \(\sim\)0.54, \(\sim\)0.51, and \(\sim\)0.40, respectively. However, the stoichiometry of bare and coated Mt slowly changed with time even in the stock suspensions inside the anoxic glovebox. Moreover, when bare and coated Mt NPs were exposed to the solutions with different pH values or Fe\(^{2+}\) concentrations from the stock solutions, the stoichiometry could also change easily. Thus, the quantitative relation between the stoichiometry of Mt and the metabolism rates of DIET processes was not discussed in this study.

### 3.2. DIET experiments

As mentioned above, in the defined \(G.\) metallireducens/\(G.\) sulfurreducens co-cultures, only \(G.\) metallireducens can oxidize ethanol, and only \(G.\) sulfurreducens can use fumarate as electron acceptor. Magnetite NPs can promote the syntrophic metabolism by transferring electrons from \(G.\) metallireducens to \(G.\) sulfurreducens. Reversed electron transfer is thermodynamically unfavorable. The ability of bare and coated Mt NPs to promote DIET in defined co-cultures of \(G.\) metallireducens/\(G.\) sulfurreducens was compared by monitoring the concentration change of ethanol and succinate, respectively, as a function of time in the co-cultures with different materials. When no NPs were added to the co-cultures of \(G.\) metallireducens/\(G.\) sulfurreducens, the amounts of ethanol metabolism or succinate production over 15-day study period were negligible (Fig. 4). It was consistent with the previously reported results that more than 30 days were needed for the co-cultures themselves to start adapting for rapid ethanol metabolism (Liu et al., 2012a; Chen et al., 2014b; Liu et al., 2015). However, in the co-cultures with 1.16 g L\(^{-1}\) bare Mt NPs, the amounts of ethanol metabolism or succinate production obviously increased after about two days. The corresponding ethanol metabolism rates \((R_e)\) and succinate production rates \((R_s)\) during the phase that the concentrations of ethanol or succinate changed linearly with time in the co-cultures amended with 1.16 g L\(^{-1}\) bare Mt NPs were 0.48 ± 0.08 and 2.29 ± 0.09 mM d\(^{-1}\), respectively. The results confirmed that Mt NPs can effectively stimulate syntrophic growth of \(Geobacter\) co-cultures via DIET.

Mt-HA was also able to promote syntrophic metabolism of the \(G.\) metallireducens/\(G.\) sulfurreducens co-cultures after about two-day incubation, but to a lesser extent, compared to bare Mt (Fig. 4). Because the percentage of magnetite in the synthetic Mt-HA was 89.9%, the weight concentration of the magnetite core in 1.29 g L\(^{-1}\) Mt-HA was equivalent to that of 1.16 g L\(^{-1}\) bare Mt. \(R_e\) and \(R_s\) of the co-cultures with 1.29 g L\(^{-1}\) Mt-HA NPs were 0.23 ± 0.06 and 0.90 ± 0.13 mM d\(^{-1}\), respectively, which were 50% and 61% less than the values of the co-cultures with 1.16 g L\(^{-1}\) bare Mt (Table 1). In the co-cultures amended with 1.42 g L\(^{-1}\) Mt-SiO\(_2\), the concentrations of ethanol consumed and succinate produced, as well as the corresponding \(R_e\) and \(R_s\), were similar to the values of the co-cultures without added NPs (Fig. 4). Thus, Mt-SiO\(_2\) had no notable stimulatory effect on DIET of \(G.\) metallireducens/\(G.\) sulfurreducens co-cultures. Therefore, the stimulatory efficiency and electron recovery in the co-cultures amended with different NPs were in the order: Mt > Mt-HA > Mt-SiO\(_2\). HA and SiO\(_2\) coating reduced the stimulatory effect of Mt NPs on DIET in \(Geobacter\) co-cultures to varying extents.

To further investigate how HA coating affected the ability of Mt NPs to stimulate DIET, control experiments of \(Geobacter\) co-cultures with pure HA were conducted. Because the weight percentage of HA coating in the synthetic Mt-HA NPs was 10.1%, the equivalent concentration of HA in 1.29 g L\(^{-1}\) Mt-HA NPs was 0.13 g L\(^{-1}\). When the
co-cultures amended with 0.13 g L\(^{-1}\) pure HA (the HA(s) sample in Fig. 4), the measured \(R_e\) and \(R_s\) were similar to those of the co-cultures with 1.29 g L\(^{-1}\) Mt-HA NPs. However, when the concentration of pure HA was 1.16 g L\(^{-1}\) (the HA(t) sample in Fig. 4) that was same as the total weight concentration of bare Mt NPs, the \(R_e\) and \(R_s\) were obviously larger than the values of the co-cultures with 1.29 g L\(^{-1}\) Mt-HA NPs, but similar to the results of the system with 1.16 g L\(^{-1}\) bare Mt NPs (Fig. 4). These results suggest that the rates of syntrophic metabolism in HA-mediated DIET were proportional to the amount of HA added. It agrees well with the trend reported in the previous mineral-mediated DIET studies using bare Mt (Liu et al., 2015), activated carbon (Liu et al., 2012a), and carbon cloth (Chen et al., 2014a). Moreover, the stimulatory effect of Mt-HA NPs on DIET was similar to that of pure HA in the concentration equivalent to HA(s) (the surface HA coating on Mt-HA), rather than HA(t) (the pure HA equivalent to the weight concentration of Mt-HA). It might imply that only HA coating, not the whole Mt-HA NPs, contributed to DIET processes.

To study whether Mt NPs coated with different types of humic acid may have different abilities to stimulate DIET, Mt NPs coated with a soil-derived (Elliott Soil) HA, Mt-HA\(_{ES}\), were also prepared, in addition to the aforementioned Mt-HA NPs with a coal-derived (Aldrich) HA coating. When the weight concentration of magnetite core was same, the amounts of ethanol consumed and succinate produced in the co-cultures with different NPs were also in the order: Mt > Mt-HA\(_{ES}\) > Mt-SiO\(_2\) (Figs. 4 and A3). \(R_e\) and \(R_s\) of the co-cultures with Mt-HA\(_{ES}\) were 42% and 45%, respectively, less than the values of the co-cultures with bare Mt NPs (Table A1). Likewise, the stimulatory effect of Mt-HA\(_{ES}\) on DIET was similar to the pure Elliott Soil HA that had the same weight concentration as the coating on Mt-HA\(_{ES}\) surface (HA\(_{ES}\)(s)). These results indicate that the coal-derived Aldrich HA and the soil-derived Elliott Soil HA influenced the ability of Mt NPs to catalyze DIET in a similar way under the conditions in this study. Considering both Aldrich HA and Elliott Soil HA mainly utilize the redox-active functional groups, quinone–phenol moieties, to transfer electrons (Lovley and Blunt-Harris, 1999), it is quite reasonable that these two types of humic acid influenced magnetite-mediated DIET in the same way. Because Mt-HA and Mt-HA\(_{ES}\) showed the similar stimulatory effect on DIET, in the following sections, only

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**Fig. 4.** Concentrations of ethanol (A) and succinate (B) as a function of time in the *G. metallireducens*/*G. sulfurreducens* co-cultures amended with different materials. The curves of pure SiO\(_2\) almost overlapped with those of the control co-cultures and therefore were not shown in (A) and (B). (C) The comparison of ethanol consumed (blue, 6 times the measured values) and succinate production (orange) after 15-day incubation; (D) the corresponding metabolism rates of ethanol consumed (blue) and succinate production (orange) and electron recovery in the DIET experiments with different materials. HA(t) and SiO\(_2\)(t) represent pure HA and SiO\(_2\) respectively, with the same concentration of bare Mt NPs. HA(s) and SiO\(_2\)(s) represent pure HA and SiO\(_2\) with the same concentration as the coatings on Mt-HA and Mt-SiO\(_2\) NPs, respectively. The error bars represent the standard deviations. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
the results of Mt-HA were discussed in comparison to bare Mt NPs and Mt-SiO₂ NPs. The effect of pure SiO₂ on the syntrophic metabolism of defined *G. metallireducens*/*G. sulfurreducens* co-cultures was also studied. Because the weight percentage of SiO₂ in the synthetic Mt-SiO₂ NPs was ~18.5%, the equivalent concentration of SiO₂ in 1.42 g L⁻¹ Mt-SiO₂ was 0.26 g L⁻¹. The rates and concentrations of ethanol consumed and succinate produced in the experiments with 0.26 g L⁻¹ (SiO₂(s)) or 1.16 g L⁻¹ (SiO₂(t)) pure SiO₂ were close to the values of the co-cultures without any added NPs in 15-day incubation (Fig. 4). It indicates that SiO₂ could not stimulate co-culture metabolism no matter how much SiO₂ was added, probably due to non-conductivity of SiO₂. Therefore, the lack of stimulatory effect of Mt-SiO₂ on DIET could be attributed to the presence of the non-conductive SiO₂ coating on Mt surface.

3.3. Secondary mineral phase

The solid phase after 15-day incubation was separated from the medium by centrifugation and studied by XRD and SEM. The XRD patterns (Fig. 1B) showed that vivianite (Fe₃(PO₄)₂·8H₂O), a secondary mineral phase, was formed in all co-cultures amended with different synthetic NPs. Also, the SEM images (Fig. A4) provided additional evidence for the presence of vivianite particles in the end-products of DIET experiments with bare or coated Mt. The produced vivianite was in a flattened prismatic or blade-like shape with a much larger size (several micrometers along c-axis) compared to the synthetic bare or coated Mt NPs. In addition, the EDS spectra of the microcrystals showed that the primary elements of the secondary phase included phosphorus, iron, and oxygen, in accordance with the chemical composition of vivianite. The formation of vivianite as a secondary mineral phase is quite common in microbial reduction of Fe(III)-containing minerals, when phosphate buffer solution is used in bacterial growth media (Zachara et al., 1998; Dong et al., 2000; Borch et al., 2007). A small amount of Fe²⁺ released from Fe(III) oxide minerals by microbial reduction in phosphate buffer solution may result in the precipitation of vivianite because of the low solubility of vivianite at pH 7. The formation of vivianite was also reported in the recent study of ferrihydrite-facilitated DIET in the *Geobacter-Methanosarcina* co-cultures (Tang et al., 2016).

To study the ability of vivianite to promote syntrophic metabolism of *Geobacter* co-cultures via DIET, control experiments amended with 1.16 g L⁻¹ synthetic vivianite particles were conducted. The concentrations of ethanol consumed and succinate produced in the co-cultures with vivianite were similar to the values of the co-cultures without added minerals (Fig. A5), so vivianite could not stimulate DIET in the *G. metallireducens*/*G. sulfurreducens* co-cultures within 14 days. The insignificant effect of vivianite on syntrophic growth of the *Geobacters* might be related to the low conductivity of vivianite or the weak affinity between vivianite and cells (Tang et al., 2016). Thus, vivianite formation during DIET processes in the presence of

| Sample         | Mt | Mt-HA | Mt-SiO₂ | HA (s) | HA (t) | SiO₂ (s) | SiO₂ (t) |
|----------------|----|-------|---------|--------|--------|----------|----------|
| Ethanol consumed (mM) | 42.78 ± 4.44 | 20.34 ± 1.71 | 8.34 ± 0.98 | 42.55 ± 2.33 | 3.51 ± 0.83 | 4.14 ± 0.67 |
| Succinate produced (mM) | 34.29 ± 1.72 | 13.46 ± 1.26 | 3.96 ± 0.98 | 32.78 ± 0.61 | 3.93 ± 0.72 | 4.93 ± 0.64 |
| Electron recovery (%) | 80 | 66 | 48 | 79 | 77 | 81 |
| Electron recovery (mM d⁻¹) | 0.48 ± 0.08 | 0.23 ± 0.06 | 0.22 ± 0.06 | 0.47 ± 0.04 | 0.47 ± 0.04 | 0.47 ± 0.04 |
| Electron recovery (Re) | 2.29 ± 0.09 | 0.90 ± 0.13 | 0.26 ± 0.09 | 1.06 ± 0.07 | 0.47 ± 0.04 | 1.19 ± 0.06 |

- HA(s) or SiO₂(s) represents pure HA or SiO₂ with the same concentration as the coatings on Mt-HA and Mt-SiO₂ NPs.
- HA(t) or SiO₂(t) represents pure HA or SiO₂ with the same concentration of bare Mt NPs.
- Six times the final concentrations of ethanol consumed were compared with the concentrations of succinate produced, according to Eq. (1).
phosphate would not directly influence the efficiency of syntrophic metabolism in the co-cultures via DIET.

3.4. Microbial reduction of bare and coated Mt NPs

The formation of vivianite in the products of the DIET experiments (Figs. 1B and A4) reveals that microbial reduction of magnetite and Fe(II) release from magnetite occurred along with DIET processes. To investigate the effect of microbial Fe(III) reduction on magnetite-mediated DIET, the concentrations of 0.5 M HCl-extractable Fe(II) and dissolved Fe(II) as a function of time during the incubation of the co-cultures amended with different NPs were measured (Fig. 5). In the medium without microbes, bare Mt NPs released a negligible amount of dissolved Fe(II) (Fig. 5B), and the concentration of 0.5 M HCl-extractable Fe(II) fluctuated around 1.36 mM (about 27% of initial Fe(II) in the added Mt NPs) during the 10-day experiment (Fig. 5A). When *G. metallireducens* single culture reacted with bare Mt NPs, the concentration of HCl-extractable Fe(II) continuously increased over time and reached 3.69 ± 0.30 mM on day 10 (Fig. 5C), indicating microbial reduction of Fe(III) in Mt NPs by *G. metallireducens* coupled to ethanol oxidation. On the contrary, the concentrations of HCl-extractable Fe(II) in the experiment of bare Mt NPs with *G. sulfurreducens* single culture fluctuated between 1 and 1.5 mM for ten days, which was similar to the values of bare Mt NPs without microbes (Fig. 5C). It confirms that *G. sulfurreducens* cannot utilize ethanol as the electron donor to reduce magnetite.

In *G. metallireducens/G. sulfurreducens* co-cultures amended with bare Mt NPs, the concentrations of dissolved Fe(II) and HCl-extractable Fe(II) significantly increased within two days and then kept constant around 0.79 ± 0.07 mM and 2.18 ± 0.12 mM, respectively (Figs. 5A and 6B). Likewise, the concentrations of HCl-extractable Fe(II) and dissolved Fe(II) in the co-cultures amended with Mt-HA NPs also increased in two days and then fluctuated around 1.86 ± 0.13 mM and 0.53 ± 0.07 mM, respectively (Fig. 5A). In the co-cultures, no further increase of HCl-extractable Fe(II) after two-day incubation might be related to the establishment of syntrophic metabolism, which will be discussed further in Section 4.1. Moreover, similar to the experiment between bare Mt and *G. metallireducens* single culture, the HCl-extractable Fe(II) concentration in the *G. metallireducens* single culture with Mt-HA also continuously increased with time and reached 2.82 ± 0.22 mM on day 10. The equilibrium concentration of dissolved Fe(II) in the co-cultures with Mt-SiO₂ was around 0.08 ± 0.04 mM that was much lower than the values of bare Mt (0.79 ± 0.07 mM) and Mt-HA (0.53 ± 0.07 mM). Moreover, the concentrations of HCl-extractable Fe(II) in the co-cultures with Mt-SiO₂ were similar to the results of bare Mt NPs without bacteria. In the reaction of Mt-SiO₂ NPs with *G. metallireducens* single culture, the concentration of dissolved Fe(II) also gradually increased with time, but the rate was much slower than those of bare Mt or Mt-HA NPs (Fig. 5C).

Fig. 5. The concentrations of 0.5 N HCl-extractable Fe(II) (A) and dissolved Fe(II) (B) on bare Mt NPs (black), Mt-HA (red), and Mt-SiO₂ (blue), respectively, during the incubation with the *G. metallireducens/G. sulfurreducens* co-cultures; (C) The corresponding concentrations of HCl-extractable Fe(II) on bare Mt NPs (blue for NPs with *G. sulfurreducens*; purple for NPs with *G. metallireducens*), Mt-HA with *G. metallireducens* (Brown), or Mt-SiO₂ with *G. metallireducens* (yellow) as a function of time. No obvious increases of HCl-extractable or dissolved Fe(II) were observed on bare Mt NPs without microbes (Cyan). Error bars represented the standard deviations. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
3.5. Aggregation state of NPs with cells

The close spatial proximity of syntrophic co-cultures can facilitate interspecies electron transfer (Summers et al., 2010), so the aggregation state of cells in the presence of different NPs might also influence the efficiency of DIET processes. The hydrodynamic sizes of the aggregates in the co-cultures amended with different NPs after 8-day DIET experiments were compared in Fig. 6. The average hydrodynamic diameter of aggregates in the co-cultures with bare Mt NPs was about 6 and 20 times, respectively, larger than the values of the co-cultures with Mt-SiO$_2$ and Mt-HA NPs. The larger and denser aggregates of bare Mt NPs with cells might be attributed to the lower surface charge. The zeta potential (Fig. 3B) of bare Mt NPs in the medium at pH 7 was close to zero, but the surface of HA or SiO$_2$ coated Mt NPs was negative charged under the same conditions. The negatively charged surface of coated Mt NPs provided the larger electrostatic repulsion between NPs, which may inhibit aggregation behavior. Moreover, the zeta potentials of Mt-HA at pH 7 was $-38.0$ mV that was more negative than the value ($-18.6$ mV) of Mt-SiO$_2$ (Fig. 3B). As a result, the hydrodynamic diameter ($717 \pm 97$ nm) of aggregates in the case of Mt-HA was much smaller than the value ($2545 \pm 642$ nm) of Mt-SiO$_2$ (Fig. 6). These results show that surface charge of magnetite NPs could obviously impact aggregation state of syntrophic bacteria with particles during magnetite-mediate DIET. In addition, SEM images of cell-NP aggregates after DIET experiments also confirm the dependence of aggregation structure on the surface properties of NPs (Fig. A6). Although sample preparation for SEM imaging might change the aggregation state of cell-NP aggregates to certain extent, the SEM images still could provide insights into the differences of aggregation state between these samples. In all samples, cells were commonly attached to the added NPs, indicating that NPs did play a role in connecting cells via aggregation. However, the aggregates of cells with Mt-HA NPs exhibited the more loose structure and smaller sizes than the other samples, which agreed well with the DLS results (Fig. 6).

4. DISCUSSION

4.1. Effects of microbial reduction on DIET

Most previous studies of mineral-mediated DIET considered minerals as a conductor to transfer electrons between microbial cells of different species (Shrestha and Rotaru, 2014; Cheng and Call, 2016). Thus, changes of mineral properties during mineral-mediated DIET have rarely been investigated thoroughly. In this study, the

Fig. 6. Distribution of hydrodynamic diameters of bare Mt(A), Mt-HA(B), and Mt-SiO$_2$(C) aggregates after 8-day incubation with the co-cultures under the similar conditions for DIET experiments.
changes of dissolved Fe(II) and HCl-extractable Fe(II) during DIET experiments were monitored. It is worth mentioning that, in the co-cultures with bare Mt or Mt-HA, dissolved Fe(II) and HCl-extractable Fe(II) reached equilibrium concentrations after about two days, when the co-cultures coincidently started to grow syntrophically via DIET (Fig. 5). Thus, syntrophic metabolism of the co-cultures via DIET after two-day incubation might inhibit further microbial reduction of Mt NPs by *G. metallireducens* and also change electron acceptor from Mt NPs to *G. sulfurreducens*. On the other hand, microbial reduction of Mt or Mt-HA mainly occurred before syntrophic metabolism of the co-cultures was established, indicating that magnetite-mediated DIET was a more favorable pathway for substrate metabolism than microbial reduction of magnetite in the *Geobacter* co-cultures. Because Mt-SiO₂ could not stimulate DIET in defined *G. metallireducens/G. sulfurreducens* co-cultures, the inhibition of microbial Fe(III) reduction due to the establishment of syntrophic metabolism was not observed in the case of Mt-SiO₂.

As shown in Fig. 5 and Table A2, the equilibrium concentrations of dissolved Fe(II) and HCl-extractable Fe(II) in the co-cultures with Mt-HA were obviously lower than the values of bare Mt. Moreover, in the experiments with *G. metallireducens* single culture, less HCl-extractable Fe(II) was measured in the case of Mt-HA compared to bare Mt. These results suggest that HA coating might slightly hinder electron transfer from *G. metallireducens* to magnetite core in Mt-HA. Although Mt-SiO₂ NPs did not stimulate syntrophic metabolism of *G. metallireducens/G. sulfurreducens* co-cultures during the 15-day incubation, the concentration of dissolved Fe(II) gradually increased with time in the reaction with *G. metallireducens* single culture. It indicates that the SiO₂ coating did not fully cover the surface of magnetite core, and microbial Fe(III) reduction by *G. metallireducens* single culture did happen on Mt-SiO₂. However, the much lower concentrations of HCl-extractable Fe(II) in the experiments of Mt-SiO₂ with the co-cultures or *G. metallireducens* single culture suggest that the non-conductive SiO₂ coating on Mt-SiO₂ significantly inhibited electron transfer between Mt core and *Geobacters*.

### 4.2. Electron transfer in the *G. metallireducens*/G. sulfurreducens* co-cultures amended with bare or coated Mt NPs

In the syntrophic metabolism of the *G. metallireducens/G. sulfurreducens* co-cultures via DIET, electrons from ethanol metabolism were partly transferred to fumarate for succinate production (Liu et al., 2012a). No accumulation of acetate was observed in all experiments (data not shown), so the DIET processes in defined co-cultures can be described as (Chen et al., 2014b):

\[
\text{CH}_3\text{CH}_2\text{OH} + 6\text{COOHCHCHCOOH} + 3\text{H}_2\text{O} \\
\rightarrow 2\text{CO}_2 + 6\text{COOHCH}_2\text{CH}_2\text{COOH}
\]  

(1)

It shows that the oxidation of one mole ethanol should be concomitant with the production of six moles succinate for electron balance. Thus, the concentrations of succinate produced and six times the concentrations of ethanol consumed after the 15-day incubation of the co-cultures amended with different materials were compared in Fig. 4C to study the electron recovery in the DIET processes. In the co-cultures amended with 1.16 g L⁻¹ bare Mt, 80% of electrons were recovered from ethanol consumption to succinate production (Table 1). The 20% loss of electrons was consistent with the results of previous studies, which has been attributed to microbial respiration or biomass formation (Chen et al., 2014b; Liu et al., 2015). However, the increasing concentrations of HCl-extractable Fe(II) and dissolved Fe(II) (Fig. 5), as well as the formation of vivianite (Fig. 1B and S3), in the magnetite-mediated DIET experiments suggest that the 20% loss of electrons were at least partly related to the reduction of structural Fe(III) in Mt NPs. In the DIET experiment with 1.16 g L⁻¹ bare Mt NPs, totally 8.49 mM electrons were not recovered in succinate production (Table 1). The concentrations of HCl-extractable Fe(II) and dissolved Fe(II) in the co-cultures with bare Mt NPs were, respectively, 0.82 and 0.79 mM more than the values in the control experiments of bare Mt NPs without bacteria (Fig. 6). This portion of electrons only accounted for ~1.9% of total electrons produced from ethanol metabolism in the 15-day incubation (Table 1). In addition, a part of the consumed electrons might induce the formation of vivianite, but it was difficult to precisely quantify the amount of vivianite formed at the end of DIET experiments based on the XRD results of end-products. In NBF medium for DIET experiments, there were 3.1 mM KH₂PO₄ and 1.3 mM K₂HPO₄. If all phosphate were converted into vivianite, 6.6 mM dissolved Fe(II), i.e. 15% of the total electrons produced from ethanol metabolism, was needed according to the stoichiometry of vivianite. Considering a portion of phosphate might be consumed by microbes for microbial respiration or biomass formation, the amount of vivianite in end-products could be less than 2.2 mM. Therefore, in syntrophic metabolism of defined *Geobacter* co-cultures amended with bare Mt NPs, 80% of the electrons from ethanol metabolism were recovered in succinate production, ~1.9% of them were transferred into bioavailable Fe(II) (most were dissolved Fe(II)), and less than 15% of them were consumed in the formation of vivianite (Fig. 7A). The rest of electrons might be consumed for microbial respiration or biomass formation.

In the co-cultures amended with pure HA in the same weight concentration as bare Mt NPs (HA(t)), both metabolism rates and electron recovery of pure HA were similar to the values of bare Mt. It indicates that the stimulatory effect of pure HA and bare Mt NPs on DIET was comparable, though HA and Mt transfer electrons between syntrophic partners in different ways. HA transfers electrons mainly by redox reactions of quinone moieties, while Mt exchanges electrons via electron hopping between Fe(II) and Fe(III) on neighboring octahedral sites. The analogue for quinone moieties in HA, anthraquinone-2,6-disulfonate (AQDS), has been reported to promote DIET in defined co-cultures of *Geobacter metallireducens*/*Geobacter sulfurreducens* (Liu et al., 2012a; Smith et al., 2015). The results of DIET experiments (Table 1 and S1) showed that...
both the coal-derived (Aldrich) HA and the soil-derived (Elliott Soil) HA exhibited high efficiency in promoting DIET. Considering the ubiquity of HA in natural environment, it may play an important role in accelerating electron exchange in syntrophic microbial communities.

However, HA coating on Mt NPs restrained the ability of Mt to catalyze DIET in Geobacter co-cultures. The metabolism rates and the amount of ethanol consumed in the co-cultures amended with Mt-HA were only comparable to the values of the co-cultures with pure HA at the weight concentration equivalent to the HA coating on Mt-HA (HA(s)). It might suggest that, in the Mt-HA mediated DIET, most electrons from ethanol metabolism were transferred to G. sulfurreducens selectively through the HA coating (Fig. 7B). Similarly, in the reduction reactions of Cr(VI) by HA-coated Mt, Cr(III) generation was observed on the surface of HA-coated by X-ray absorption near edge structure spectroscopy (XANES), but the valence state of the magnetite core was unchanged (Jiang et al., 2014). Accordingly, it was proposed that the reactivity of HA-coated Mt in Cr(VI) reduction is dominated by the characteristics of humic acid, but not obviously influenced by properties of

Fig. 7. Different pathways of electron transfer via bare Mt (A), Mt-HA (B), and Mt-SiO₂ (C) NPs, respectively, in the syntrophic metabolism of the G. metallireducens/G. sulfurreducens co-cultures.
magnetite core. Likewise, in Mt-HA mediated DIET, electrons might also be transferred mainly through the HA coating on Mt surface and bypassed the Mt core. Although HA was fixed on the surface of Mt-HA NPs, the diffusion of Mt-HA NPs or electron transfer between neighboring NPs in the aggregates of NPs and microbes can facilitate interspecies electron transfer. On the other hand, both in the DIET experiments and the experiment with G. metallireducens single culture, the concentration of HCl-extractable Fe(II) and metabolism rates in the system with Mt-HA was lower than the values with bare Mt under the same conditions (Fig. 6), indicating the inhibition of HA coating on electron transfer between Mt core and G. metallireducens. In addition, the electron recovery of the co-cultures with Mt-HA (66%) was lower than the value (79%) of pure HA(s), probably because of the consumption of electrons produced from ethanol metabolism for the reduction of Mt core in Mt-HA. As shown in Table A2, the concentration of dissolved Fe(II) or HCl-extractable Fe(II) in the co-cultures with Mt-HA was about 0.50 mM more than the values of bare Mt NPs in the medium without bacteria (Table A2). Thus, in DIET experiments with Mt-HA, 66% of electrons from ethanol metabolism were transferred via the HA coating to G. sulfurreducens for succinate production, ~2.5% of them reduced magnetite core for the production of bioavailable Fe(II) (most were dissolved Fe(II)), and the rest were consumed for vivianite precipitation, as well as microbial respiration or biomass formation (Fig. 7B).

Different from HA, SiO₂ coating was non-conductive and could not stimulate DIET in defined co-cultures of G. metallireducens/G. sulfurreducens (Fig. 4). Nevertheless, the amount of ethanol consumed in the co-cultures with Mt-SiO₂ was almost two times higher than the values of the co-cultures without any added material or with pure SiO₂ (Table 1). Moreover, the presence of dissolved Fe(II) and vivianite indicate that the SiO₂ coating might be porous or incompletely cover Mt surface, which allowed G. metallireducens to reduce the Mt core in Mt-SiO₂ to a certain degree. However, the low electron recovery (48%) and the small amount (3.96 ± 0.98 mM) of succinate produced in the co-cultures with Mt-SiO₂ suggest that the electrons from G. metallireducens could hardly be transferred further to G. sulfurreducens through Mt-SiO₂ due to the non-conductive SiO₂ coating (Fig. 7C). These results reveal that HA and SiO₂ coatings influenced the ability of Mt NPs to stimulate DIET in totally different ways and to different extents. It is worth to mention that the zeta potentials of the samples were in the order of bare Mt > Mt-SiO₂ > Mt-HA, but the metabolism rates were bare Mt > Mt-HA > Mt-SiO₂. Although surface potential of minerals might impact electron transfer at the mineral-microbe interface, no correlation between surface potentials and metabolism rates was observed in this study.

4.3. Effects of aggregation on mineral-mediated DIET

In addition to changing pathways of electron transfer in minerals during DIET processes, the surface coating on Mt also impacted the aggregation state of Mt NPs with cells. At pH 7, HA or SiO₂ coating changed the surface of Mt NPs from nearly neutral to negatively charged (Fig. 3B), which increased electrostatic repulsive force and inhibited the aggregation of NPs. Also, surface charge of cells at near-neutral pH is commonly negative, so the negatively charged surface of HA or SiO₂ coated Mt is theoretically less favorable for cell adhesion because of electrostatic repulsion. Fig. 6 and 86 indicate that bare Mt NPs formed the larger and more compact aggregates with cells after DIET experiments. It is known that Mt NPs readily aggregate in aqueous suspensions due to a combination of Lifschitz–van der Waals and magnetic forces (Vikesland et al., 2016). Moreover, the nearly neutral surface of Mt NPs could facilitate the attachment of cells to Mt. The formation of compact aggregates of cells and bare Mt NPs could promote the efficiency of DIET by increasing the contact area or facilitating electron transfer between NPs and cells, or between the cells of different species. In addition to (semi)conducting minerals, extracellular c-type cytochromes in extracellular polymeric substances (EPSs) can also promote the interspecies electron transfer between syntrophic partners in large aggregates (Summers et al., 2010). Thus, the formation of large and compact aggregates of Mt NPs with cells might also contribute to the high efficiency of syntrophic metabolism in magnetite-mediated DIET.

However, in the DIET experiments with coated Mt NPs, the positive correlation between aggregation state and metabolism rates was not observed. The average hydrodynamic diameter of cell-NP aggregates in the co-cultures with Mt-HA NPs was smaller than the value in the case of Mt-SiO₂ (Fig. 6). Moreover, SEM images (Fig. A6) also show that the aggregate structure of Mt-HA NPs with cells was relatively less compact. Nevertheless, Mt-HA exhibited the stronger stimulatory effect on DIET than Mt-SiO₂. It suggests that the electron transferring capacity and surface properties of minerals influenced DIET processes more significantly than aggregation state. However, DLS only provides an estimation of aggregation state of NPs, based on the assumption that all aggregates are spherical, so the quantitative correlation between the factors mentioned above and metabolism rates could not be established. On the other hand, the VSM results show that magnetic properties of bare and coated Mt NPs were similar, but the different abilities of these NPs to catalyze syntrophic metabolism indicate that no direct correlation between magnetic properties and the ability of Mt NPs to catalyze DIET was observed in this study.

Magnetite-mediated DIET in syntrophic microbial communities is not only a new way for magnetite to participate in biogeochemical cycles of carbon and iron, but also a promising method to stimulate syntrophic metabolism in engineered systems for energy recovery and environmental treatments. However, magnetite particles in natural environments commonly possess the surface properties that are quite different from those of pristine magnetite, resulting in the different efficiencies of magnetite-mediated DIET in laboratory and natural settings. In this study, Mt NPs were modified by HA or SiO₂ coating, which is common for naturally occurring magnetite particles or engineered Mt NPs in natural environment. Studying the ability of
these coated Mt NP can help us to understand the actual roles of magnetite-mediated DIET in natural environment. To the best of our knowledge, this is the first study to investigate the relationship between surface properties and the stimulatory effect of magnetite on DIET in defined co-cultures of Geobacter metallireducens and Geobacter sulfurreducens. The findings in this study reveal that the ability of magnetite to catalyze syntrophic metabolism was obviously affected by the properties of surface coatings. The changes of surface properties of minerals by surrounding environment need to be considered for evaluating the role of mineral-mediated DIET in bacterial metabolism and biogeochemical cycling of elements. Also, the results in this study indicate that microbial reduction of magnetite and the formation of vivianite occurred along with Mt-mediated DIET. The slow transformation of mineral phases from conductive magnetite to non-conductive vivianite might also impact the efficiency of the mineral-mediated DIET in long-term processes. Thus, to comprehensively understand the mechanisms and role of mineral-mediated DIET in biogeochemical cycles, further studies need to be conducted to investigate the transformation of minerals during DIET processes in complex natural or engineered systems and the dominating properties of minerals for promoting DIET in syntrophic consortia.

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APPENDIX A. SUPPLEMENTARY MATERIAL

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.gca.2018.02.009.

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