

Dependence of the electron transfer capacity on the kinetics of quinone-mediated Fe(III) reduction by two iron/humic reducing bacteria†

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The effects of a series of quinones on goethite reduction by two types of iron/humic reducing bacteria (*Shewanella decolorationis* S12 and *Aeromonas hydrophila* HS01) were investigated in this study. The results showed that the quinones with redox potentials (E_h^0) in the range of -344 to -137 mV (vs. SHE at pH 7) can significantly accelerate Fe(III) reduction by S12 and HS01. Plots of the electron transfer capacities (ETC) of the quinones (including the electron accepting and donating capacities determined using chronoamperometry) vs. E_h^0 were similar to those of the ratios of iron reduction rates with quinones to those without quinones ($k_{\text{cell+Q}}/k_{\text{cell}}$) vs. E_h^0 . The UV/vis diffuse-transmittance absorption spectra of S12 and HS01 suggested the presence of outer membrane *c*-type cytochromes, which are responsible for the extracellular reduction of iron and quinones. The E_h^0 of the different types of cytochromes varies within a broad potential window, which might influence the electron shuttling capacity of various quinones from microorganisms to iron. The $k_{\text{cell+Q}}/k_{\text{cell}}$ of different quinones had a good linear relationship with their ETC, and the difference of the redox equilibria among the different quinones might correlate to their different performance of ETC and mediating microbial iron reduction. This work can help researchers understand the influence of redox properties of quinone compounds on microbial iron transformation in anoxic environments.

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Introduction

Extracellular electron transfer from bacterial cell surfaces to insoluble terminal electron acceptors (*e.g.*, iron(III) oxides) can be facilitated by the presence of exogenous electron shuttles, resulting in significant enhancement of microbial iron reduction under anoxic conditions.^{1–3} Quinones in electron shuttles function as recyclable mediators during the electron transfer process, in which the quinones are microbially reduced to hydroquinones, and these hydroquinones are subsequently oxidised by terminal electron acceptors to the corresponding quinones.^{4–7} The role of quinone-hydroquinone moieties as electron shuttles has drawn considerable interest in the investigation of their properties, such as redox properties and electron transfer capacities (ETC). A strong relationship was found between the redox potentials of electron shuttles and their acceleration of Fe(III) reduction by *Shewanella putrefaciens*,⁸ whereas the redox potentials of most active quinones fall in a narrow range for enhancing Fe(III) reduction by two other

iron reducing bacteria, *i.e.*, *Geobacter metallireducens* and *Klebsiella pneumoniae*.^{9,10} The effectiveness of electron shuttles for the bioreduction of Fe(III) oxide appear to be influenced by various experimental conditions, *e.g.*, bacterial species, electron donors, and the concentrations and redox properties of electron shuttles.

A phylogenetically diverse range of bacteria have been identified as humic-reducing bacteria that are capable of using exogenous electron shuttles as electron acceptors for the anaerobic oxidation of organic and inorganic electron donors.^{11–15} So far, most studies that have examined electron shuttle-mediated extracellular electron transfer from bacteria to insoluble electron acceptors focused on dissimilatory iron-reducing bacteria.^{5,6,8,9,16} The abundance of other humic-reducing bacteria (*e.g.*, fermentative bacteria) is usually one or two orders of magnitude higher than that of dissimilatory iron-reducing bacteria in iron-bearing environments;¹⁷ however, few studies have investigated quinone-mediated iron reduction driven by the other humic-reducing bacteria.

Conversely, the ETC, *i.e.*, electron acceptor (EAC) and donor (EDC) capacities, of synthetic quinones, humic acids, and natural dissolved organic matter have been evaluated using chemical, biochemical, and electrochemical approaches.^{10,18–21} Recently, Li *et al.*¹⁰ reported that the effectiveness of quinone-mediated Fe(III) reduction and current generation by *K. pneumoniae* were strongly dependent on the ETC of the synthetic

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quinones. However, it is unclear how the ETC of electron shuttles affects the microbial iron reduction and what properties control the quinone-mediated extracellular electron transfer processes from microorganisms to iron oxides.

In this study, *Shewanella decolorationis* S12, as a typical dissimilatory iron-reducing bacterium,^{22,23} was used to investigate the effects of various quinones on the bioreduction of Fe(III) oxide. *Aeromonas hydrophila* HS01 was also selected as a model fermentative humic-reducing bacterium because it has previously been isolated and demonstrated a capacity for humic reduction.^{15,24} With regard to the effectiveness of quinones for the bioreduction of Fe(III) oxide, the relationship of the redox potentials and the ETC of quinones were further discussed. This study is expected to provide more meaningful information on understanding how the redox properties of quinones correlate with their ability to mediate Fe(III) reduction by iron/humic-reducing bacteria.

Experimental

Materials

Shewanella decolorationis S12 is an iron-reducing bacterium that was isolated from the activated sludge of a textile printing wastewater treatment plant in Guangzhou, China.²⁵ *Aeromonas hydrophila* HS01 is a humic-reducing bacterium that was isolated from subterranean forest sediment in Sihui, China.¹⁵ Goethite (α -FeOOH) was synthesised by dissolving hydrated ferric nitrate in potassium hydroxide as described previously.¹⁴ Car (carmines, >97%), AQC (9,10-anthraquinone-2-carboxylic acid, 98%), 2-HNQ (2-hydroxy-1,4-naphthoquinone, 97%) and 5-HNQ (5-hydroxy-1,4-naphthoquinone, 97%) were purchased from Sigma-Aldrich (USA). Ali (alizarin, 97%), AQS (9,10-anthraquinone-2-sulfonic acid, $\geq 98.0\%$) and AQDS (9,10-anthraquinone-2,6-disulfonic acid, $\geq 98.0\%$) were purchased from Fluka (Japan). Other chemicals were of analytical grade and were obtained from Guangzhou Chemical Co. (China). Individual stock solutions (10 mM) of AQS and AQDS were prepared in water and were filter-sterilised (0.22 μm); stock solutions of 2-HNQ and 5-HNQ were prepared in methanol. Stock solutions of AQC, Ali and Car were prepared in acetone.

Fe(III) reduction experiment

Cells of S12 and HS01 were grown in a nutrient broth that consisted of peptone (10 g L⁻¹), beef extract (3 g L⁻¹), and NaCl (5 g L⁻¹) at pH = 7.2 \pm 0.2 (25 °C) under oxic conditions at 25 °C for 16 h. Subsequently, the cells were harvested by centrifugation at 8000 \times g at 5 °C for 10 min. The pellets were washed three times and were resuspended in 50 mM of sterile PIPES (piperazine-1,4-bis(2-ethanesulfonic acid)) buffer at pH 7.0 \pm 0.1 to an optical density of 1.9 to 2.2 (λ = 600 nm). The density of 2.0 corresponded to approximately 7 \times 10⁸ cells mL⁻¹ based on previous experiments that correlated the culture optical density with viable cell counts determined by serial dilution and plating.^{15,26} Glucose (5 mM) was used as the sole electron donor. The medium with 30 mM goethite and 100 μM quinones was dispensed into 20 mL serum bottles purged with O₂-free N₂ for

at least 30 min, capped with butyl rubber closures, and crimp sealed. Based on the results of preliminary experiments (Fig. S1†) which showed that the iron reduction rates increased significantly with increasing AQDS concentrations from 0 to 100 μM , and then reached a plateau from 100 to 1000 μM , the concentrations of different quinones were selected as 100 μM which is coincident with previous studies.^{8,10} All experiments were conducted in triplicate. Inoculation and sampling were conducted using sterile syringes and needles. All vials were incubated in a Bactron Anaerobic/Environmental Chamber II with O₂ concentration <0.01% (Shellab, Sheldon Manufacturing Inc., Cornelius, OR) at 25 °C in the dark. In addition, no significant influence was observed on the microbial Fe(III) reduction when the experiments were conducted with addition of equivalent amount of methanol or acetone without quinone.

Analytical methods

The Fe(II) concentration, as HCl-extractable Fe(II), was determined by extracting Fe(II) from the suspensions using 0.5 M HCl for 1.5 h and assaying the extract using 1,10-phenanthroline colorimetry, which was not affected by the presence of the tested quinones.²² Chronoamperometry measurements were employed to determine the ETC of the quinones using a potentiostat (CHI660D, Chenhua Co. Ltd, Shanghai, China) with a conventional three-electrode cell at ambient temperature as described previously.¹⁰ Briefly, a graphite plate (3.5 cm \times 5.0 cm), platinum mesh (4 cm \times 4 cm), and saturated calomel electrode (SCE) were used as the working, counter, and reference electrodes, respectively. A phosphate buffer solution (0.1 M, pH = 7.0) with 0.1 M KCl was used as the electrolyte at applied potentials of -0.8 and +0.5 V to evaluate the EDC and EAC of the quinones under constant stirring. The cell was purged with O₂-free N₂ for 15 min before the electrochemical measurements. The final concentration of quinones was 6.7 μM , in which all the tested quinones were completely dissolved and the ETCs measured were not influenced by the solubility of the quinones.⁹ The UV/vis diffuse-transmittance absorption spectra of S12 and HS01 were measured using an UV/visible spectrophotometer (TU-1901 Beijing, China) equipped with an IS19-1 integrating sphere reflectance attachment. The optical length of the cuvette for the measurement was 10 mm. The cell suspensions in sterile PIPES buffer were purged with O₂-free N₂ for 30 min before the cuvettes were capped with butyl rubber closures. The spectra of the cell suspensions obtained in the cuvettes were considered to be under anoxic conditions. The spectra of cell suspensions obtained in cuvettes without N₂-purging were considered to be under oxic conditions.

Results and discussion

Effects of quinones on goethite bioreduction

The kinetics of goethite reduction by S12 or HS01 in the absence and presence of quinones are shown in Fig. 1. The Fe(II) concentrations increased as time elapsed for all treatments. The Fe(II) concentration was 4.22 mM in the vials inoculated with S12 in the absence of quinones after a 30-day incubation;

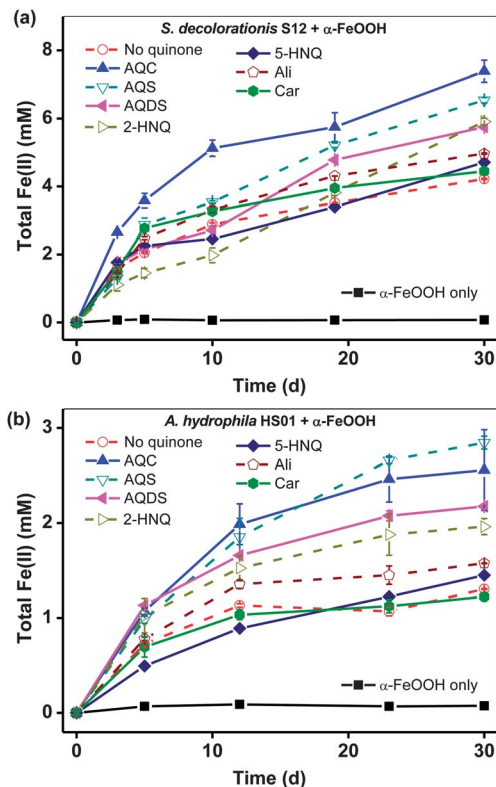


Fig. 1 Concentration of 0.5 M HCl extractable Fe(II) during the bio-reduction of goethite (30 mM) by 10^7 cells mL^{-1} of (a) S12 and (b) HS01 provided with 5 mM glucose in the presence and absence of 100 μM quinones. Lines are a visual aid only. Data are mean \pm SD ($n = 3$).

meanwhile, the Fe(II) concentration in the presence of AQC was 7.39 mM (Fig. 1a). For better comparison, the reaction rates of Fe(III) reduction (k) were calculated by linear regression of the Fe(II) production data from day 0 to day 30 (Table 1). The Fe(III) reduction rates by S12 with and without quinones followed a descending order: AQC > AQS > AQDS > Ali > 2-HNQ > Car \approx No quinone > 5-HNQ, in which the k value with AQC was nearly three-fold higher than that without quinone. Only 1.31 mM Fe(II) was detected in the vials inoculated with HS01 at the end of incubation, whereas the Fe(II) concentrations increased

significantly in the presence of all the tested quinones except Car (Fig. 1b). The Fe(III) reduction rates by HS01 with and without quinones were ranked as: AQC > AQS > AQDS > 2-HNQ > Ali \approx 5-HNQ \approx Car > No quinone, in which the k value with AQC was four-fold higher than that without quinone. As one example of fermenting iron/humic reducing bacterium,¹⁵ HS01 showed weak capability of Fe(III) reduction because fermentative iron reducing bacteria can only transfer less than 5% of electron equivalents to Fe(III) in fermentative metabolism, whereas most of the electron equivalents are recovered in organic fermentation products and hydrogen.^{6,27} AQC, AQS, and AQDS showed strong accelerating effects on the bioreduction of goethite by both S12 and HS01, whereas Ali and 2-HNQ showed small accelerating effects, and the presence of 5-HNQ and Car slightly inhibited and increased Fe(III) reduction by S12 and HS01, respectively, which is similar to the observations in previous studies.^{8–10}

Dependence of the redox potentials of quinones

Because quinones can mediate electron transfer from micro-organism to iron oxide in the system of S12/HS01 + quinone + goethite, the redox potential of quinones is a critical parameter to thermodynamically control the kinetics of microbial iron reduction.^{8,9} The ratios of the Fe(III) reduction rates of the cell + quinone to the cell without quinone (*i.e.*, $k_{\text{S12+Q}}/k_{\text{S12}}$ or $k_{\text{HS01+Q}}/k_{\text{HS01}}$) were plotted *versus* the redox potential (E_{h}^0 vs. SHE, pH 7) of the respective quinone (Table 1). The results (Fig. 2) suggested that the quinones with positive effects on goethite reduction by both S12 and HS01 have E_{h}^0 values ranging from -344 mV (Ali) to -137 mV (2-HNQ), within which AQC ($E_{\text{h}}^0 = -254$ mV) showed the most positive effects. This result is similar to that reported by Li *et al.*¹⁰ when investigating the effects of different quinones on goethite reduction by a fermentative iron-reducing bacterium (*i.e.*, *K. pneumonia* L17). Wolf *et al.*⁹ also reported a similar relationship between E_{h}^0 and the iron reduction rates by *G. metallireducens*, though only AQS, AQDS and 2-HNQ showed significantly positive kinetic effects on ferrihydrite reduction.

The extracellular electron transfer from iron-reducing bacterium to Fe(III) oxide is mediated by *c*-cytochromes (*c*-Cyts)

Table 1 Fe(III) reduction rates (k) by S12 and HS01 with and without the addition of quinones, standard redox potentials (E_{h}^0) and electron transfer capacities (EAC and EDC) of quinones

Quinones	Goethite reduction					ETC ¹⁰ $\text{mmol e}^- (\text{mol Q})^{-1}$	
	<i>S. decolorationis</i> S12		<i>A. hydrophila</i> HS01		E_{h}^0 (mV) ⁹	EAC	EDC
	k (mM d ⁻¹)	R^2	k (mM d ⁻¹)	R^2			
AQC	0.454 ± 0.008	0.628	0.156 ± 0.006	0.828	-254	488	470
AQS	0.275 ± 0.002	0.938	0.112 ± 0.001	0.969	-225	380	361
AQDS	0.228 ± 0.001	0.788	0.076 ± 0.001	0.593	-184	249	233
2-HNQ	0.200 ± 0.003	0.995	0.071 ± 0.003	0.924	-137	164	142
5-HNQ	0.130 ± 0.025	0.842	0.045 ± 0.006	0.924	-3	162	132
Ali	0.210 ± 0.000	0.956	0.050 ± 0.000	0.968	-344	52.4	42.8
Car	0.159 ± 0.001	0.932	0.046 ± 0.001	0.894	-500	19.0	12.4
No Quinone	0.159 ± 0.001	0.912	0.039 ± 0.000	0.869	—	—	—

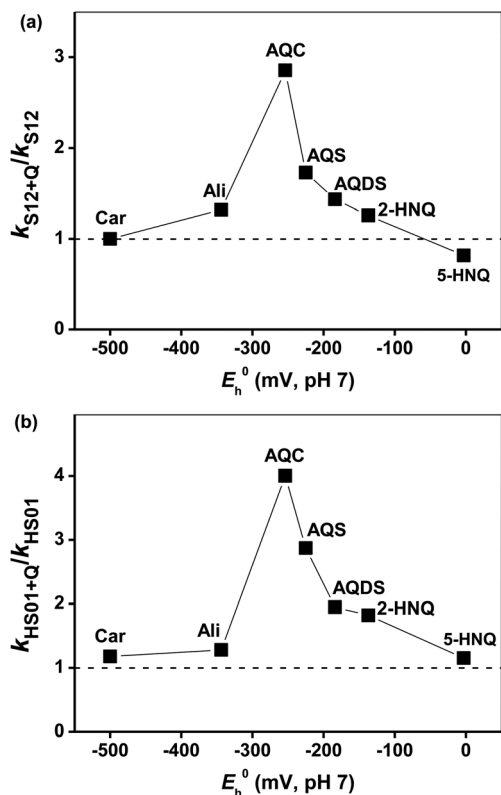


Fig. 2 (a) Ratios of k_{S12+Q}/k_{S12} and (b) k_{HS01+Q}/k_{HS01} vs. standard redox potentials (E_h^0) of the tested quinones. The k_{S12+Q}/k_{S12} and k_{HS01+Q}/k_{HS01} were calculated from the ratios of the iron reduction rates with quinones divided by the iron reduction rate without quinones.

in the outer membrane of a cell.¹ Because the active centre of *c*-Cyts, heme irons, have a large molar absorption coefficient, spectroscopic methods have been employed to investigate their *in vivo* properties.²⁸ The diffuse-transmittance absorption spectra of cell suspensions of S12 and HS01 were measured under both oxic and anoxic conditions. The results of S12 in Fig. 3a show that a peak at 410 nm was observed under oxic conditions, whereas two peaks at 419 nm and 550 nm were observed under anoxic conditions, reflecting the presence of the oxidised state (410 nm) and the reduced state (419 nm and 550 nm) of *c*-Cyts in the cell surface (or outer membrane) of S12 under different conditions. In Fig. 3b, HS01 showed similar oxidised peaks of *c*-Cyts at 410 nm and a reduced peak at 418 nm. However, the absorbance of individual peaks obtained from S12 was higher than that from HS01 with the same cell density, suggesting that the content of *c*-Cyts in S12 was much higher than that in HS01. The *c*-Cyts-mediated iron reduction can be described as reaction (1)



where Cyt_{red} and Cyt_{ox} represent reduced cytochromes and oxidised cytochromes, respectively, and Fe(II) includes both dissolved and adsorbed ferrous species. Based on this reaction, the reduced cytochromes should dominate the iron reduction by microorganisms. Hence, the discrepancy of content of *c*-Cyts

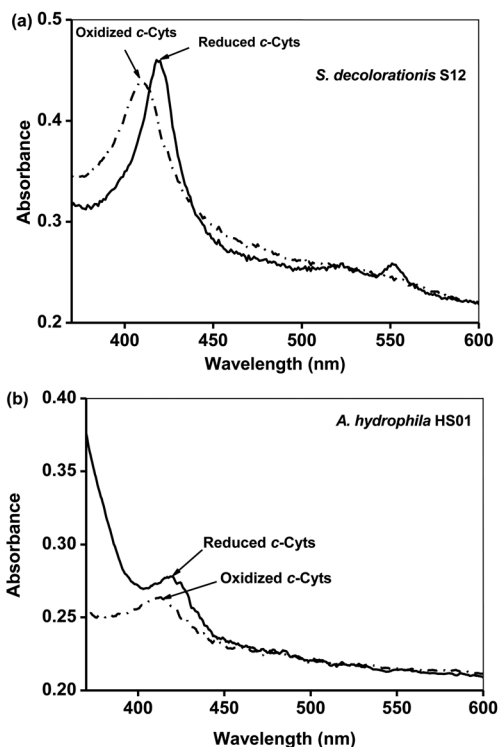
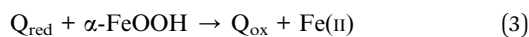
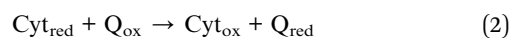


Fig. 3 UV/vis diffuse-transmittance absorption spectra of the cell suspension ($\text{OD}_{600} = 2.0$) of (a) S12 and (b) HS01; the optical length of the cell was 10 mm. The spectrum was taken under oxic conditions for the oxidised form and anoxic conditions for reduced form.

between S12 and HS01 might account for the different iron reducing capacity of these two bacteria.

Although a variety of *c*-Cyts (such as OmcA, OmcB, OmcS, OmcZ, and MtrC) within a broad potential window spanning from ~ -400 mV to ~ 0 mV have been identified as being responsible for microbial Fe(III) reduction in different iron-reducing bacteria,^{6,29} it is still unclear which types of *c*-Cyts contribute to iron and quinone reduction by S12 and HS01. Based on the thermodynamic considerations, the electron transfer from the microorganism to the electron shuttle can only occur when the E_h of the electron shuttle is higher than that of the specific *c*-Cyts on the cell surface. Therefore, most of the tested quinones could be readily reduced by iron-reducing bacteria except Car, which has an E_h^0 (-500 mV) lower than the lowest value of the *c*-Cyts reported (-400 mV). The quinones with higher E_h may be more favourably reduced by *c*-Cyts followed by quinone-mediated iron reduction; however, AQS, AQDS, and 2-HNQ, with redox potentials even higher than that of AQC, showed lower accelerating effects on the goethite reduction by S12 and HS01. The elemental reactions from *c*-Cyts to goethite *via* quinone cycling are shown as reactions (2) and (3).



wherein Q_{ox} represents all the oxidised quinones, and Q_{red} includes all the reduced quinones.²⁰ Generally, the oxidised

quinone (Q_{ox}) accepts electrons from reduced cytochromes (Cyt_{red}) in reaction (2), whereas the reduced quinone (Q_{red}) acts as an electron donor for goethite reduction forming Q_{ox} again in reaction (3). Electrons can be shuttled *via* the redox cycle of Q_{ox}/Q_{red} , and the electron transfer efficiency from microorganism to iron oxide controlled by both reactions (2) and (3). The EAC of Q_{ox} and the EDC of Q_{red} are consequently two pivotal factors controlling the kinetics of quinone-mediated microbial iron reduction. Particular consideration is given below to the ETC of quinones.

Dependence of the electron transfer capacity of quinones

The EAC and EDC of different quinones determined using chronoamperometry measurement reflected the number of electrons transferred from the working electrode to individual dissolved quinones and *vice versa*, which followed a descending order as $AQC > AQS > AQDS > 2\text{-HNQ} \approx 5\text{-HNQ} > Ali > Car$ (Table 1). In addition, the EAC and EDC values were close, though the individual quinone exhibited a little higher EAC value than that of its EDC, which is similar to the results of previous studies, suggesting that electron transfer in the quinones was nearly reversible.^{21,30,31} Based on the concept of EAC and EDC, the total accepting electrons ($n_{e^{-},A}$) of quinones should include all the reduced quinone species, *i.e.*, H_2Q , HQ , HQ^- , Q^- and Q^{2-} ,^{20,32,33} which can be expressed as eqn (4). Similarly, the total donated electrons ($n_{e^{-},D}$) of the quinones should include all the oxidised quinone species, *i.e.*, Q , HQ and Q^- , which can be expressed as eqn (5).

$$EAC = \frac{n_{e^{-},A}}{[Q]_{tot}} = \frac{2[H_2Q] + 2[HQ^-] + [HQ] + 2[Q^{2-}] + [Q^-]}{[Q]_{tot}} \quad (4)$$

$$EDC = \frac{n_{e^{-},D}}{[Q]_{tot}} = \frac{2[Q] + [HQ] + [Q^-]}{[Q]_{tot}} \quad (5)$$

The theoretical total electrons can be calculated *via* the complete transformation from quinone (Q) to hydroquinone (H_2Q) or from H_2Q to Q , which is equal to $2[Q]_{tot}$, hence the overall conversion factor (r) can be expressed as eqn (6), which is just half of the ETC.²⁰

$$r = \frac{n_{e^{-}}}{n_{e^{-},tot}} = \frac{n_{e^{-}}}{2[Q]_{tot}} = \frac{1}{2} ETC \quad (6)$$

The r values for different quinones reflect the maximum electron accepting or donating ratios, which are mainly determined by the equilibrium speciation of the quinones. The ETC and r values *vs.* E_h^0 of each quinone were plotted in Fig. 4, and these plots were similar to that of the k_{cell+Q}/k_{cell} *vs.* E_h^0 (Fig. 2). The results suggested that the quinones with high ETC and r values have E_h^0 values ranging from -344 mV (Ali) to -137 mV (2-HNQ), within which AQC ($E_h^0 = -254$ mV) was the highest.

Based on the Nernst equation, the E_h of a quinone in solution is not only determined by its standard redox potential (E_h^0) but is also strongly correlated to the ratios of its reduced forms to oxidised forms (*i.e.*, equilibrium speciation). The following equation shows the quantitative correlation between E_h and ETC.²⁰

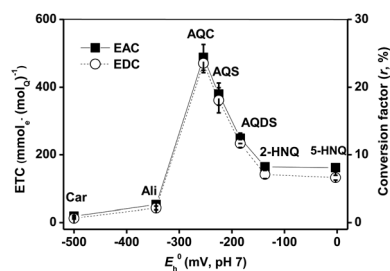


Fig. 4 ETC and the conversion factor (r) *vs.* standard redox potentials (E_h^0) of the tested quinones. ETC (EAC and EDC) data were from Table 1, and the conversion factor (r) was calculated according to eqn (6).

$$ETC = \sum_i \frac{10 \frac{FE_{h,i}^0}{2.3RT} C_i}{\left(10 \frac{FE_h}{2.3RT} + 10 \frac{FE_{h,i}^0}{2.3RT} \right) [Q]_{tot}} \quad (7)$$

where C_i is the concentration of each reduced moiety, E_h (V) and $E_{h,i}^0$ (V) are the reduction potential and the standard reduction potential, respectively, and R ($J K^{-1} mol^{-1}$), T (K) and F ($C mol^{-1}$) are the universal gas constant, absolute temperature and Faraday constant, respectively. From eqn (7), the ETC of quinones are not only related to the E_h but also to the concentrations of reduced moieties (*i.e.*, equilibrium speciation of quinones). Although the detailed speciation and experimental E_h/E_h^0 of individual quinones are not provided here, the

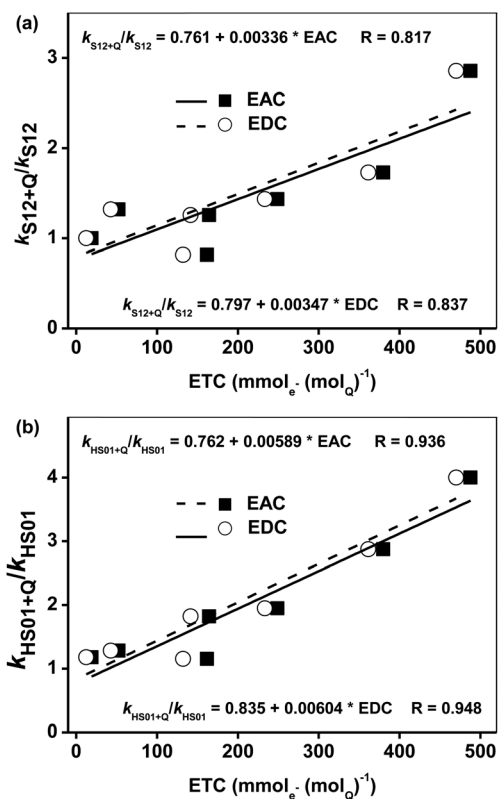


Fig. 5 Ratios of k_{cell+Q}/k_{cell} *vs.* ETC (EAC and EDC) for (a) S12 and (b) HS01. The fitting lines in the figures are the linear regression curves with parameter estimates.

equilibrium speciation of quinones as a function of solution pH and E_h vary from one type of quinone to another,^{20,34} resulting in significant discrepancy of their EAC and EDC values.

To clearly illustrate the relationship between the effectiveness of quinones for microbial iron reduction and their ETC, the ratios of $k_{\text{cell+Q}}/k_{\text{cell}}$ as a function of ETC were plotted in Fig. 5. The results showed that the ratios of $k_{\text{S12+Q}}/k_{\text{S12}}$ and $k_{\text{HS01+Q}}/k_{\text{HS01}}$ correlated well with both the EAC and EDC of the quinones, as indicated by the correlation coefficients (R) ranging from 0.817 to 0.948 for the linear regression fitting. These results indicated that the effectiveness of different quinones for accelerating Fe(III) reduction by S12 and HS01 are positively associated with the EAC and EDC of the quinones. This finding is consistent with the previous results, which suggested that quinone-mediated iron reduction by *K. pneumoniae* L17 was strongly dependent on the ETC of the quinones.¹⁰ Based on the aforementioned discussion about ETC and E_h , the equilibrium speciation of quinones is considered to play an important role in controlling their ETC and E_h , which might associate with the reactivity of quinone-mediated iron reduction.

Some quinones, such as Car, Ali and 5-HNQ, reveal low solubility and high adsorption on the surface of Fe(III) oxide,⁹ and the introduction of these quinones into goethite reduction vials may result in lower concentrations of dissolved quinone than expected (100 μM) due to precipitation or adsorption. The good relationship between the ETC of quinones and their effectiveness for accelerating microbial Fe(III) reduction suggests that the dissolved quinones might be more important in controlling extracellular electron transfer from microorganisms to Fe(III) oxide. However, the presence of solid-phase humic substance has been demonstrated to be capable of accelerating Fe(III) oxide reduction by some iron/humic reducing bacteria,³ which implies that both dissolved and solid-phase quinones may be able to mediate the extracellular electron transfer from the tested microorganisms to goethite, although the contributions of dissolved and solid-phase quinones on accelerating microbial Fe(III) oxide have not been verified. In addition, some quinones, such as 5-HNQ, have antibiotic properties,³⁵ and the association of the tested quinones with the goethite surface may vary with their structural properties, such as the number of aromatic molecules and substituents,³² which may also affect their performance of accelerating microbial Fe(III) reduction.

Environmental implication

A number of quinones and humic substances have been reported to be involved in many redox reactions not only responsible for the geochemical cycling of redox active elements but also for contaminant transformations in laboratory and field studies.^{7,36,37} The finding that the presence of quinones could also significantly facilitate Fe(III) oxide reduction by these two iron/humic reducing bacteria (*i.e.*, *Shewanella* and *Aeromonas*), which are ubiquitous in natural environments, suggested that quinone-mediated extracellular electron transfer may commonly occur, particularly in anoxic environments. The

discussions about ETC, E_h and equilibrium speciation of quinones also provide some fundamental insight into the effects of quinone-bearing substances on the microbial iron transformation processes. Although the mechanistic aspects of the relationship between ETC and the speciation of quinones still need to be elucidated in detail, the recognition that quinones with higher ETC can result in higher electron transfer reactivity for microbial iron reduction highlights the importance of investigating ETC properties of humic substances, particularly in natural anoxic soils and sediments.

Conclusions

This study determined that quinones with E_h^0 in the range of -344 to -137 mV (*vs.* SHE at pH 7) can significantly accelerate the Fe(III) reduction by two types of iron reducing bacteria. The relation of the ETC of quinones *vs.* E_h^0 showed a similar manner to those of $k_{\text{cell+Q}}/k_{\text{cell}}$ for Fe(III) reduction *vs.* E_h^0 . The presence of outer membrane *c*-Cyts was observed in S12 and HS01, and the electron shuttling capacity of different quinones for iron reduction might be influenced by the E_h^0 of different types of *c*-Cyts. The $k_{\text{cell+Q}}/k_{\text{cell}}$ of quinones exhibited a good linear relationship with their ETC, and the different redox equilibria of various quinones might account for both the ETC and iron reduction rates.

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