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Reductive Formation of Palladium Nanoparticles by *Shewanella oneidensis*: Role of Outer Membrane Cytochromes and Hydrogenases

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Production of catalytic palladium (Pd) nanoparticles using dissimilatory metal-reducing bacteria such as *Shewanella oneidensis* has a great potential for applications in the treatment of environmental pollutants and in chemical synthesis. The objective of this study was to investigate the role of outer membrane cytochromes and hydrogenases in bioreductive formation of Pd nanoparticles in *S. oneidensis*. Our results showed that i) the reduction of tetrachloropalladate (Pd(II)) by *Shewanella oneidensis* can be well described by a first-order model; ii) outer membrane *c*-type cytochromes MtrC/OmcA do not play an essential role in Pd(II) reduction under our tested conditions; iii) [NiFe]-hydrogenase HyaB facilitates Pd(II) reduction with the presence of lactate or formate as electron donors. Our findings suggest that [NiFe]-hydrogenase HyaB may serve as a potential target for molecular manipulation in *S. oneidensis* towards an improved performance in reductive formation of Pd nanoparticles.

Introduction

Dissimilatory metal-reducing bacteria (DMRB) are a group of microorganisms capable of using various metal(loid)s as terminal electron acceptors for anaerobic respiration [1, 2]. In natural environments, DMRB-mediated reduction of iron and other metal(loid)s in the Earth's crust plays an important role in biogeochemical cycles of these metals and in the fate of organic matter [1-3]. In engineered settings, there have been many attempts at harnessing the power of DMRB for various applications including bioremediation of environmental contaminants, generation of power or high value products in bioelectrochemical devices [4-6].

DMRB are capable of changing the oxidation state of metals and these microbial processes have opened up a new window for novel applications including biosynthesis of metal nanomaterials [7, 8]. Because the synthesis of nanomaterials using DMRB usually occurs in water under gentle and environmentally benign conditions, it has become one attractive research focus in current green nanotechnology [9, 10]. Among the DMRB most often used in biosynthesis of nanomaterials, *Shewanella oneidensis* has attracted great interest because it is able to reduce a diverse range of metal ions and the extracellular electron transport chains responsible for transferring electrons across cell membranes have been relatively well characterized [6, 11, 12]. *S. oneidensis* has been used to synthesize various nanomaterials including catalytic palladium (Pd) nanoparticles with great potentials for applications in the treatment of environmental pollutants and in chemical synthesis [13-15]. However, the primary molecular sites responsible for Pd reduction have not been elucidated, which limits further enhancement of Pd reduction and the design of controllable and reproducible bioprocesses.

S. oneidensis MR-1 genome possesses 42 *c*-type cytochromes many of which are known to be involved in extracellular electron transfer [6]. The metal-reducing (MTR)-pathway consisting of a set of proteins including *c*-type cytochromes CymA and MtrC/OmcA is a well-characterized extracellular electron transport chain responsible for transferring electrons across cell membranes [16, 17]. CymA is required to electrically charge the outer membrane cytochromes (OMCs), i.e., MtrC and OmcA. Extensive studies have suggested an essential role of MtrC/OmcA in the reduction of various metals including Fe(III), Cr(VI), Tc(VII) and U(VI) [18, 19]. In addition to *c*-type cytochromes, hydrogenases comprise another class of redox proteins that play a central role in microbial reduction of metals in *S. oneidensis*. The genome of *S. oneidensis* MR-1 encodes both a [FeFe]-hydrogenase HydAB and a [NiFe]-hydrogenase HyaAB [12]. HydA functions as a hydrogen-forming hydrogenase, while HyaB can function either in formation or oxidation of hydrogen as a bidirectional hydrogenase [20]. Previous studies have shown that the [NiFe]-hydrogenase of MR-1 was involved in H_2-driven reduction of Tc(VII) to Tc(IV)/Tc(V) [21, 22]. However, the contribution of OMCs and hydrogenases to Pd bioreduction in *S. oneidensis* has not been well elucidated.

The objective of the current study was to investigate the role of hydrogenases, *i.e.* HydA and HyaB, and OMCs, *i.e.*, MtrC and OmcA, in bioreductive formation of Pd nanoparticles by *S. oneidensis* MR-1. We compared the rates of Pd reduction the wild-type (WT) and the mutants lacking OMCs and [NiFe]-hydrogenase. We demonstrated that the reduction of Pd by *S. oneidensis* can be well described by the first-order model. Our results showed that there is no significant difference in Pd

reduction rates of WT and mutant lacking OMCs, while the reduction rate was significantly lower in the mutant lacking [NiFe]-hydrogenase HyaB than in the WT. Taken together, we report for the first time that outer membrane *c*-type cytochromes MtrC/OmcA do not play an essential role in Pd(II) reduction, while [NiFe]-hydrogenase HyaB facilitates Pd(II) reduction, suggesting that HyaB may serve as a potential target for molecular manipulation in *S. oneidensis* towards an improved performance in reductive formation of Pd nanoparticles.

Experimental

Bacterial strains and growth conditions: *S. oneidensis* MR-1 wild-type (WT) and mutants lacking selected c-type cytochrome or hydrogenase (Table 1) were used in this study.

Table 1. *S. oneidensis* strains used in this study.

Strains	Description	Reference
MR-1[a]	Manganese-reducing strain	[11]
Δ*mtrC*Δ*omcA*	Deletion of SO1778 (*mtrC*)-SO1779 (*omcA*)	[23]
Δ*cymA*	SO4591 (*cymA*) deletion derivative of MR-1	[21]
Δ*hydA*	SO3920 (*hydA*) deletion derivative of MR-1	[20]
Δ*hyaB*	SO2098 (*hyaB*) deletion derivative of MR-1	[20]
Δ*hyaB*Δ*hydA*	Deletion of SO2098 (*hyaB*)-SO3920 (*hydA*)	[21]

[a] Wild-type (WT) strain.

Stock cultures were maintained in LB medium with 20% glycerol at -80°C. The bacterial cells were grown in LB medium or modified M1 medium under aerobic or anaerobic conditions. The modified M1 medium (pH ~7.0) consisted of 3.00 mM PIPES, 7.50 mM NaOH, 26.04 mM NH_4Cl, 1.34 mM KCl, 4.35 mM NaH_2PO_4 and 0.68 mM $CaCl_2$ supplemented with trace amounts of minerals, vitamins, and amino acids [24]. For bacterial growth in M1 medium, 20 mM sodium lactate or sodium formate was used as an electron donor and the sole carbon source. For growth under anaerobic conditions, the medium containing 10 mM ferric nitrilotriacetate (Fe(III)-NTA) as an electron acceptor was bubbled with nitrogen gas for 5 min and the headspace in the culture was less than 3% of the total volume.

Pd(II) reduction by resting cells: All chemicals were from Sigma Aldrich, unless otherwise stated. Anaerobic cultures of various *S. oneidensis* strains (Table 1) were incubated as an electron acceptor for 16 hours at 30°C with shaking at 200 rpm. Cells were harvested through centrifugation (6,000×g for 30 min), washed twice with HEPES buffer (30 mM, pH 7.0), and resuspended in the same buffer. Ten ml of the cell suspension was mixed with 5 ml of HEPES buffer (30 mM, pH 7.0) in an anaerobic tube capped with butyl rubber stopper, and bubbled with nitrogen gas for 5 min. Sodium formate and sodium tetrachloropalladate (Na_2PdCl_4) (Pd(II)) were introduced into the anaerobic tube through syringe needles to a final concentration of 20 mM and 1 mM, respectively. Aliquots of each cell suspension were incubated at 90°C for 10 min and were used as controls containing heat-killed cells. Each experiment was carried out in triplicates.

Quantification of Pd(II): Samples (~1 ml each) were taken using a syringe needle at pre-determined time points. Each sample was centrifuged at 10,000×g for 5 min to remove the cells and the concentration of Pd(II)was quantified using a colorimetric method [25]. Briefly, 100 µl of sample was mixed with 500 µl of EDTA-NaOH buffer (100 mM, pH 10.4) and 50 µl 4-(2-pyridylazo)-resorcinol (0.1%, pH 10.5) followed by 10 min incubation at 80°C and then at 4°C for 20 min. The mixture was then diluted 10 times using MilliQ water and the optical density at 515 nm was measured.

Epifluorescence microscopy: One drop of the cell suspension was placed on a glass microscope slide and dried in an oven at 70˚C. The dried sample was then stained using 4'-6-diamidino-2-phenylindole (DAPI) [26]. Briefly, 5 µl of DAPI solution (1 mg/mL) was dropped onto the sample and incubated at room temperature, in the dark, for 45 min. The glass slide was then rinsed with ultrapure water to remove excess DAPI. The stained samples were examined using an inverted epifluorescence microscope (Microscope Axio Observer.Z1, Carl Zeiss).

Transmission electron microscopy (TEM): For TEM imaging, samples was fixed, dehydrated and embedded in LR white (Electron Microscopy Sciences, Hatfield, PA) following protocols reported elsewhere [18]. Briefly, the sample was fixed in 2.5% glutaraldehye overnight and centrifuged at 5,000×g for 5 min, and the supernatant was discarded. The cells were resuspended and washed three times with 10 mM PIPES buffer (pH 6.8) at 5,000×g for 5 min. Gradual dehydration was carried out in an ethanol series of 30, 50, 75, and 90% for 30 min each and then 100% ethanol three times for 1 h each. The cell pellet was further washed for 30 min in a 50:50 mixture of ethanol and LR white (Electron Microscopy Sciences, Hatfield, PA). Finally, the cell pellet was washed three times using resin for 1 h each, and centrifuged at 5,000 rpm for 5 min. After the final wash with resin, the resin was discarded, and the Eppendorf tube containing the cell pellet was filled with fresh resin to about 75% of the tube's volume and incubated at 60˚C overnight. The polymerized block of cell pellets was sectioned with a diamond knife (Diatome, Biehl, Switzerland) to a thickness of 70 nm and mounted on a Cu grid and sputtered with carbon. These sections were examined using a Jeol JEM-1230 TEM instrument operated at an accelerating voltage of 120 kV.

Results and discussion

Upon exposure to Pd(II), cell suspension of the WT gradually turned black, suggesting that Pd(II) was reduced by *S. oneidensis* to Pd(0), which was further confirmed by the decrease in Pd(II) concentration in the aqueous phase and the formation of Pd(0) crystals in the cultures revealed by energy-dispersive X-ray spectroscopy and X-ray diffraction (Fig. S1). Experiments conducted with various initial Pd(II) concentrations showed that the Pd(II) reduction rate by MR-1 cell suspension increased with increasing initial Pd(II) concentrations (Fig. 1).

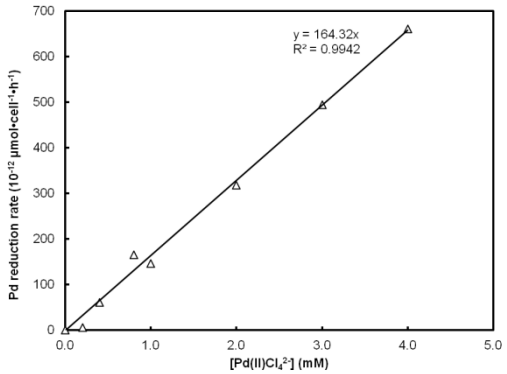

Fig. 1. The rate of Pd(II) reduction by *S. oneidensis* with formate as an electron donor measured as a function of initial Pd(II) concentration at 30°C. The solid line represents the best fit. The rate of Pd reduction can be well described by a first-order model $-d[Pd(II)]/dt = k_1 \times [Pd(II)] \times X$ where the parameter k_1 was determined to be 164.3 $h^{-1} \bullet cell^{-1}$ in the reduction of Pd(II) within the concentration range of 0-4 mM.

Monod model and its derivatives under no-growth conditions have been used to describe the bioreduction kinetics for metals such as Fe(III), Co(II), U(VI), Cr(VI) and Tc(VII) [27]. Reductive formation of Pd(0) nanoparticles by *S. oneidensis* has been previously reported [28, 29] and the biogenic Pd(0) nanoparticles have been characterized and applied to various applications [13, 14]. However, bioreduction kinetics for Pd(II) reduction has never been elucidated. Here, we demonstrated that Pd(II) reduction by *S. oneidensis* could be well described by a first-order model derived from the Monod model. No inhibition effects were incorporated in the first-order model, suggesting that the (by)products of the Pd(II) reduction have no significant effects on the bioreduction activity. Although the first-order model fits our macroscopic Pd(II) reduction results well, a fundamental understanding of the reduction mechanisms is required for a better interpretation of the kinetics. Bioreduction of Pd(II) by microorganisms in the presence of an electron donor is believed to occur in three concomitant steps: (i) biosorption of Pd(II) onto cell surfaces, (ii) bioreduction of Pd(II) to Pd(0), and (iii) autocatalytic reduction of Pd(II) on Pd(0) nuclei [25]. Thus, the macroscopic reduction results are most likely a combination of enzyme-mediated bioreduction and Pd(0)-based autocatalytic reduction. Enzymatic reduction is expected to play an important role, especially at the initial stage and under conditions with high cell-to-Pd(II) ratios. The complex biochemical and redox properties of bacterial surfaces pose a significant challenge to comprehensively uncover the underlying Pd(II) bioreduction mechanisms.

The cell suspension was further examined using light microscopy by overlay of bright-field and fluorescence imaging of DAPI-stained cells as well as using TEM. Representative images are shown in Fig. 2. In Fig. 2A, the black precipitates with a size of hundreds nm could be the aggregates of nanoparticles. The black precipitates were further extracted and the particle size distribution was analyzed. We found that, after ultrasonication, the size of the nanoparticles were in the range of 8-50 nm with the majority in the range of 10-20 nm in diameter (Fig. S2). Fig. 2B shows that the nanoparticles could be formed in the cell membrane.

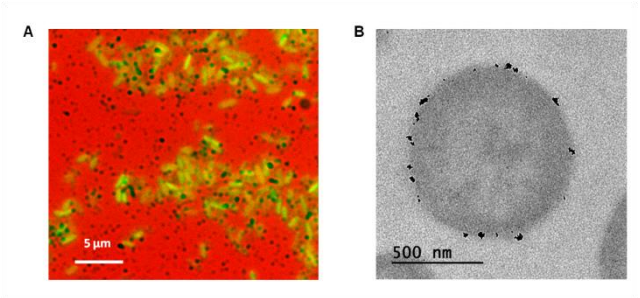

Fig. 2. (A) Dispersion of Pd(0) nanoparticles (black) in a suspension of cells (green). Cells were stained with DAPI. For enhanced clarity, the image was pseudo-colored. (B) TEM image showing the association of Pd(0) nanoparticles with *S. oneidensis* MR-1.

A significant amount of Pd(0) nanoparticles were found in the cell exterior and associated with cell membrane, suggesting an important role of extracellular and/or membrane-bound proteins in Pd(II) reduction.

Previous studies have shown that OMCs, *i.e.*, MtrC and OmcA, play an essential role in the reduction of various metals including Fe(III), Cr(VI), Tc(VII) and U(VI) [18, 19]. To elucidate whether the OMCs, *i.e.*, MtrC and OmcA, facilitate Pd(II) reduction, we compared Pd(II) reduction by *S. oneidensis* MR-1 WT and the cytochrome mutants with lactate or formate as electron donors and the results are shown in Fig. 3.

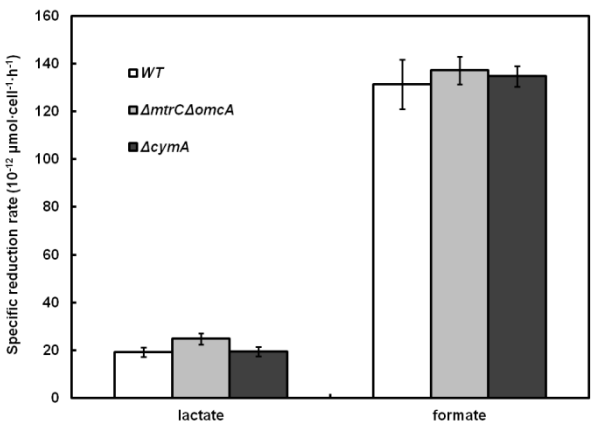

Fig. 3. Specific rates of Pd(II) reduction (determined for the first 3 h) by *S. oneidensis* MR-1 WT and OMC mutants with 20 mM lactate or formate as electron donors in 30 mM HEPES buffer (pH 7.0) under no-growth conditions. Data represent the mean and standard deviation (n=3).

The experimental data demonstrated that no significant Pd(II) reduction occurs in the absence of live cells, suggesting an important role of enzymatic reduction. Intriguingly, our results showed that the mutants lacking MtrC/OmcA have an comparable capability of Pd(II) reduction with that of the WT, implying that the OMCs, MtrC/OmcA, do not play an essential role in Pd(II) reduction under our tested conditions. In a recent study, Bucking et al. [30] reported a OMCs-independent reduction of extracellular electron acceptors in *S. oneidensis*, where two point mutations in MtrA and MtrB were found to regain the ability of the mutant lacking OMCs to grow as a dissimilatory metal reducer. Hence, we further tested the mutant lacking CymA, a cytochrome transferring electrons to MtrA, in Pd(II) reduction. Interestingly, the mutant Δ*cymA* reduced Pd(II) as fast

as the WT, suggesting that the MTR-pathway does not play an essential role in Pd(II) reduction by *S. oneidensis*. As one well-characterized extracellular electron transport chain in *S. oneidensis*, the MTR-pathway getting electrons from the quinone pool through CymA has been reported to be responsible for transferring electrons across cell membranes to a wide variety of extracellular electron acceptors, especially insoluble electron acceptors such as ferrihydrite [17, 31]. Here we show that the MTR-pathway is not essential for Pd(II) reduction, implying that the electrons for Pd(II) reduction are taken from the upstream from the electron carrier CymA, possibly at the quinone pool level.

In addition to OMCs, hydrogenases comprise another class of redox proteins that can take electrons from the quinone pool and they have also been shown to be involved in the reduction of Tc(VII) to Tc(IV)/Tc(V) [21, 22]. The redox potential of the $[Pd(II)Cl_4]^{2-}/Pd(0)_{(s)}$ couple is 600 mV at the standard state [32]. The [4Fe-4S] clusters of the [NiFe]-hydrogenase have mid-point potentials of around -300 mV [33], indicating that it is thermodynamically feasible for hydrogenases to reduce Pd(II) to Pd(0).

To test the involvement of hydrogenases in Pd(II) reduction in *S. oneidensis*, the capability of the mutants lacking hydrogenases in reducing Pd(II) in the presence of formate as electron donors was compared with that of the WT (Fig. 4).

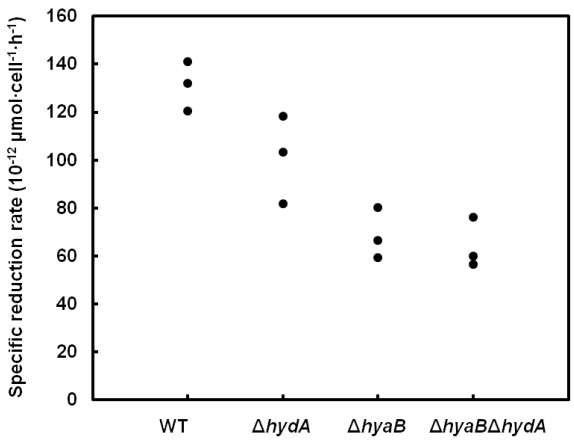

Fig. 4. Specific rates of Pd(II) reduction by *S. oneidensis* MR-1 WT and hydrogenase mutants with 20 mM formate as electron donors in 30 mM HEPES buffer (pH 7.0) under no-growth conditions. Specific reduction rate was determined for the first 3 h of Pd(II) reduction.

The [FeFe]-hydrogenase mutant (Δ*hydA*) exhibited a comparable reduction rate to the WT, while both deletions of the [NiFe]-hydrogenase (Δ*hyaB* and Δ*hyaB*-Δ*hydA*) have significantly lower reduction rates than the WT. The results suggested that the [NiFe]-hydrogenase HyaB is a key enzyme facilitating Pd(II) reduction by *S. oneidensis*.

The involvement of unspecified hydrogenases in Pd(II) reduction by *Desulfovibrio desulphuricans* has been implicated by the inhibition of the bioreduction with 0.5 mM Cu^{2+}, a hydrogenase inhibitor [34]. However, the presence of Cu^{2+} could lead to conformational changes in proteins in general [35]; hence, the inhibition might not be specific for hydrogenases. The direct evidence supporting the involvement of hydrogenases in bioreduction of Pd(II) by *D. fructosivorans* and *E. coli* was reported using mutant strains lacking hydrogenases [36, 37]. Here in this study, we showed that the periplasmic [NiFe]-hydrogenase HyaB played an important role in mediating Pd(II) reduction. It should be noted that, although [NiFe]-hydrogenase HyaB plays an important role in facilitating Pd(II) reduction, other enzymes may also mediate Pd(II) reduction as suggested by ~50% of reduction capability remaining in the mutant strain lacking HyaB (Fig. 4).

Based on our results, we proposed a working model for the mechanisms of hydrogenase-facilitated Pd(II) reduction in *S. oneidensis* (Fig. 5).

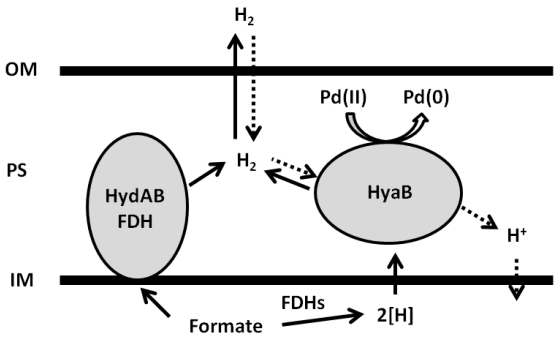

Fig. 5. Model of hydrogenase-facilitated Pd(II) reduction by *S. oneidensis* with formate as electron donors.

The hydrogenases of *S. oneidensis* are located in the periplasm [20-22]. The [FeFe]-hydrogenase HydAB and a putative formate dehydrogenase FDH form a formate-hydrogen lyase (FHL) complex, mediating the conversion of formate to hydrogen, while the [NiFe]-hydrogenase HyaB can function bidirectionally in both formation and oxidation of hydrogen [20]. Using formate as electron donors, the cells may produce hydrogen via the FHL activity as well as the FDHs and HyaB activity. Hydrogen can be oxidized by HyaB coupling to the reduction of Pd(II). The reduced Pd products, presumably Pd(0) nanoparticles, are most likely accumulated in the periplasm. The released protons are then transported into the cytoplasm by proton transporters such as ATP synthase located in the inner membrane.

Conclusions

The reduction of Pd(II) by *Shewanella oneidensis* can be well described by a first-order model. The OMCs, i.e., MtrC/OmcA, do not play an essential role in Pd(II) reduction under our tested conditions, while [NiFe]-hydrogenase (HyaB) facilitates Pd(II) reduction using lactate or formate as electron donors. Our findings suggest that [NiFe]-hydrogenase HyaB may serve as a potential target for molecular manipulation in *S. oneidensis* towards an improved performance in reductive formation of Pd nanoparticles.

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Notes and references

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1. D. R. Lovley, *Annu Rev Microbiol*, 1993, **47**, 263-290.
2. K. H. Nealson, *Annu Rev Earth Pl Sc*, 1997, **25**, 403-434.
3. D. R. Lovley and R. T. Anderson, *Hydrogeol J*, 2000, **8**, 77-88.
4. D. R. Lovley, *Annu Rev Microbiol*, 2012, **66**, 391-409.
5. K. H. Nealson and S. E. Finkel, *Mrs Bull*, 2011, **36**, 380-384.
6. J. K. Fredrickson, M. F. Romine, A. S. Beliaev, J. M. Auchtung, M. E. Driscoll, T. S. Gardner, K. H. Nealson, A. L. Osterman, G. Pinchuk, J. L. Reed, D. A. Rodionov, J. L. M. Rodrigues, D. A. Saffarini, M. H. Serres, A. M. Spormann, I. B. Zhulin and J. M. Tiedje, *Nature Reviews Microbiology*, 2008, **6**, 592-603.
7. J. Lloyd, J. Byrne and V. Coker, *Curr Opin Biotechnol*, 2011, **22**, 509-515.
8. G. M. Gadd, *Microbiology*, 2010, **156**, 609-643.
9. D. Mandal, M. Bolander, D. Mukhopadhyay, G. Sarkar and P. Mukherjee, *Appl Microbiol Biotechnol*, 2006, **69**, 485-492.
10. T. Hennebel, B. De Gusseme, N. Boon and W. Verstraete, *Trends Biotechnol*, 2009, **27**, 90-98.
11. C. R. Myers and K. H. Nealson, *Science*, 1988, **240**, 1319-1321.
12. J. F. Heidelberg, I. T. Paulsen, K. E. Nelson, E. J. Gaidos, W. C. Nelson, T. D. Read, J. A. Eisen, R. Seshadri, N. Ward, B. Methe, R. A. Clayton, T. Meyer, A. Tsapin, J. Scott, M. Beanan, L. Brinkac, S. Daugherty, R. T. DeBoy, R. J. Dodson, A. S. Durkin, D. H. Haft, J. F. Kolonay, R. Madupu, J. D. Peterson, L. A. Umayam, O. White, A. M. Wolf, J. Vamathevan, J. Weidman, M. Impraim, K. Lee, K. Berry, C. Lee, J. Mueller, H. Khouri, J. Gill, T. R. Utterback, L. A. McDonald, T. V. Feldblyum, H. O. Smith, J. C. Venter, K. H. Nealson and C. M. Fraser, *Nature Biotechnology*, 2002, **20**, 1118-1123.
13. T. Hennebel, S. De Corte, W. Verstraete and N. Boon, *Curr Opin Biotech*, 2012, **23**, 555-561.
14. S. De Corte, T. Hennebel, B. De Gusseme, W. Verstraete and N. Boon, *Microbial Biotechnology*, 2012, **5**, 5-17.
15. C. Ng, K. Sivakumar, X. Liu, M. Madhaiyan, L. Ji, L. Yang, C. Tang, H. Song, S. Kjelleberg and B. Cao, *Biotechnol Bioeng*, 2013, **110**, 1831-1837.
16. D. J. Richardson, J. N. Butt, J. K. Fredrickson, J. M. Zachara, L. Shi, M. J. Edwards, G. White, N. Baiden, A. J. Gates, S. J. Marritt and T. A. Clarke, *Mol Microbiol*, 2012, **85**, 201-212.
17. L. Shi, K. M. Rosso, T. A. Clarke, D. J. Richardson, J. M. Zachara and J. K. Fredrickson, *Front Microbiol*, 2012, **3**, 50.
18. S. M. Belchik, D. W. Kennedy, A. C. Dohnalkova, Y. Wang, P. C. Sevinc, H. Wu, Y. Lin, H. P. Lu, J. K. Fredrickson and L. Shi, *Appl Environ Microb*, 2011, **77**, 4035-4041.
19. M. J. Marshall, A. S. Beliaev, A. C. Dohnalkova, D. W. Kennedy, L. Shi, Z. Wang, M. I. Boyanov, B. Lai, K. M. Kemner, J. S. McLean, S. B. Reed, D. E. Culley, V. L. Bailey, C. J. Simonson, D. A. Saffarini, M. F. Romine, J. M. Zachara and J. K. Fredrickson, *PLoS biology*, 2006, **4**, e268.
20. G. Meshulam-Simon, S. Behrens, A. D. Choo and A. M. Spormann, *Appl Environ Microb*, 2007, **73**, 1153-1165.
21. M. J. Marshall, A. E. Plymale, D. W. Kennedy, L. Shi, Z. M. Wang, S. B. Reed, A. C. Dohnalkova, C. J. Simonson, C. X. Liu, D. A. Saffarini, M. F. Romine, J. M. Zachara, A. S. Beliaev and J. K. Fredrickson, *Environ Microbiol*, 2008, **10**, 125-136.
22. L. Shi, S. M. Belchik, A. E. Plymale, S. Heald, A. C. Dohnalkova, K. Sybirna, H. Bottin, T. C. Squier, J. M. Zachara and J. K. Fredrickson, *Appl Environ Microb*, 2011, **77**, 5584-5590.
23. C. L. Reardon, A. C. Dohnalkova, P. Nachimuthu, D. W. Kennedy, D. A. Saffarini, B. W. Arey, L. Shi, Z. Wang, D. Moore, J. S. McLean, D. Moyles, M. J. Marshall, J. M. Zachara, J. K. Fredrickson and A. S. Beliaev, *Geobiology*, 2010, **8**, 56-68.
24. B. Cao, L. Shi, R. N. Brown, Y. J. Xiong, J. K. Fredrickson, M. F. Romine, M. J. Marshall, M. S. Lipton and H. Beyenal, *Environ Microbiol*, 2011, **13**, 1018-1031.
25. A. E. Rotaru, W. Jiang, K. Finster, T. Skrydstrup and R. L. Meyer, *Biotechnol Bioeng*, 2012, **109**, 1889-1897.
26. M. Bunge, L. S. Sobjerg, A. E. Rotaru, D. Gauthier, A. T. Lindhardt, G. Hause, K. Finster, P. Kingshott, T. Skrydstrup and R. L. Meyer, *Biotechnol Bioeng*, 2010, **107**, 206-215.
27. C. X. Liu, Y. A. Gorby, J. M. Zachara, J. K. Fredrickson and C. F. Brown, *Biotechnol Bioeng*, 2002, **80**, 637-649.
28. W. De Windt, P. Aelterman and W. Verstraete, *Environ Microbiol*, 2005, **7**, 314-325.
29. W. De Windt, N. Boon, J. Van den Bulcke, L. Rubberecht, F. Prata, J. Mast, T. Hennebel and W. Verstraete, *Antonie van Leeuwenhoek*, 2006, **90**, 377-389.
30. C. Bucking, A. Piepenbrock, A. Kappler and J. Gescher, *Microbiology*, 2012, **158**, 2144-2157.
31. L. Shi, T. C. Squier, J. M. Zachara and J. K. Fredrickson, *Mol Microbiol*, 2007, **65**, 12-20.
32. P. Atkins, T. Overton, J. Rourke, M. Weller and F. Armstrong, *Shriver & Atkins Inorganic Chemistry*, Oxford University Press, 2006.
33. M. Teixeira, I. Moura, A. V. Xavier, J. J. G. Moura, J. Legall, D. V. Dervartanian, H. D. Peck and B. H. Huynh, *J Biol Chem*, 1989, **264**, 16435-16450.
34. J. R. Lloyd, P. Yong and L. E. Macaskie, *Appl Environ Microb*, 1998, **64**, 4607-4609.
35. Z. Vardanyan and A. Trchounian, *Cell Biochemistry and Biophysics*, 2010, **57**, 19-26.
36. K. Deplanche, I. Caldelari, I. P. Mikheenko, F. Sargent and L. E. Macaskie, *Microbiology*, 2010, **156**, 2630-2640.
37. I. P. Mikheenko, M. Rousset, S. Dementin and L. E. Macaskie, *Appl Environ Microb*, 2008, **74**, 6144-6146.