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RESEARCH ARTICLE

# Microbial respiratory quinones as indicator of ecophysiological redox conditions

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**Abstract** The bacterial respiratory quinones and membrane phospholipid fatty acids (PLFA) were measured to test the biochemical responses to the redox conditions after the respiration of diverse electron acceptors by microorganisms. *Shewanella putrefaciens* strain CN32 was examined for its growth with $O_2$, nitrate, ferrihydrite, ferric citrate, and sulfite as electron acceptors. The same parameters were also measured for *Desulfovibrio desulfuricans* strain G-20, *Geobacter metallireducens* strain GS-15, *Thioploca* spp., two strains of magnetotactic bacteria (*Magneteospirilum magnetotactium* marine vibrioid strain MV-1 and *M.* sp. strain AMB-1), and environmental sediments. Microorganisms with aerobic respiratory of oxygen (MV-1 and AMB-1) have high ratios of monounsaturated to saturated straight chain PLFA and ubiquinone to menaquinone ratios; while those that conduct strict anaerobic respirations (G-20 with sulfate and GS-15 with ferric iron) have low ratios of monounsaturated to saturated straight chain PLFA and uniquinone to menaquinone ratios. The facultative respiratory of nitrate (*Thioploca*) has these parameters in the middle. The ratios of menaquinones to ubiquinones in CN32 cells systematically increase according to the increase of redox potential and bioavalibility of electron acceptors. The correlation between $\Sigma$UQ-$n$/$\Sigma$MK-$n$ ratios and redox conditions indicates the structure of respiratory quinone responses sensitively to the microbial ecophysiological conditions.

**Keywords** electron acceptor, redox potential, bacterial metabolism, phospholipid fatty acid (PLFA), respiratory quinone, ecophysiology

## 1 Introduction

Redox is an important ecophysiological parameter reflecting the interplay between geochemical conditions and types of microbial metabolism (Hunter et al., 1998). The dominant bioavailable electron acceptors usually constrain the types of respiration, and as a consequence, the microbial respiration may shift the redox potentials of chemical species and accelerate their geochemical cycles in the environments. The measurement of redox includes two aspects: 1) the composition of oxidized and reduced forms of environmental abundant metals, such as iron or Mn, which is dominant in the behaviors of the other metals, and 2) the Eh and pH of aqueous parts. The measurement of the redox state of environmental minerals, such as those iron-containing minerals may be used to evaluate the trends of geochemical reactions, but it can hardly be used to predict the biologic electron-exchange reactions. The measurements of microbial activity are the meaningful and straightforward methods (Hunter et al., 1998; Hiraishi, 1999). The measurement of environmental molecular is capable of discriminating bacterial metabolism; while the isolation and incubation methods allow the reconstruction of energy and mineral ecophysiological features of the microorganism in the environments. The biochemical methods, such as the measurements of phospholipid fatty acid (PLFA) and respiratory quinones (RespQ) in environments, are characterized by their rapid and culturing-free manner of getting the information on total biomass, community composition, nutritional status, and environmental stress. Several recent studies indicated that the chemical methods also have potential in reflecting the redox status of microbial community, which can be related to the biologic function in the community, the types of respiration, and the reactivity and migration of carbon sources and metals in geochemical environments and contaminated sites (Ringelberg et al., 1997; White et al., 1997; Hunter et al.,1998; Hiraishi, 1999; Li et al., 2007).

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The prokaryotic microorganisms are extremely diverse in respiratory systems as they can use more than 20 inorganic or organic redox pairs for energy production (e.g., Neaslon and Scott, 2006; Ruebush et al., 2006). Most bacterial respiratory systems comprise several dehydrogenases that transfer reducing equivalents to ubiquinones and/or menaquinones, which in turn are reoxidized by the activity of terminal oxidases with a terminal electron acceptor (Søballe and Poole, 1999). Through the cell membrane, microbes metabolize their respiratory chemicals and excrete the reduced byproducts, which subsequently influence the redox zones and even the geochemical environments (Nealson and Saffarini, 1994; Nealson and Little, 1997; Hunter et al., 1998). Direct extraction and identification of cell membrane lipid constituents, including profiling phospholipids fatty acids, and respiratory quinones, are rapid and useful chemotaxonomic methods to detect various respiratory types, environmental stress, nutritional conditions, and community structures (e.g., Hedrick and White, 1986; Hiraishi, 1999; Li et al., 2007).

The major structural groups of bacterial respiratory quinones include ubiquinone (1-methyl-2-isoprenyl-3,4-dimethoxyparabenzoquinone) with side chains abbreviated as UQ-*n*, where n refers to the number of isoprenoid units in the side chain, and menaquinone (1-isoprenyl-2-methyl-naphthoquinone, MK-*n*). The types of quinones and lengths of the side chains vary among different bacteria (Polglase et al., 1966; Whistance and Threlfall, 1968; Collins and Jones, 1981). Previous studies indicated that most Gram-positive bacteria and anaerobic Gram-negative bacteria contain only MK-*n*, whereas the majority of strictly aerobic Gram-negative bacteria contain exclusively UQ-*n* (Collins and Jones, 1981; Hedrick and White, 1986; Søballe and Poole, 1999). Both types of RespQs, however, are found in facultative anaerobic Gram-negative bacteria (Parkes 1987; Søballe and Poole, 1999). As such, quinone profiling is useful as biochemical marker for characterization of microbial community (Parkes, 1987), because the dominant quinone type of a single species is observed to be unchanged for a given respiratory process (Hiraishi, 1999).

According to previous reports, UQ-*n* should be produced preferably for oxygen and nitrate respiratory types because of the large midpoint potential between UQ and UQH2, whereas MK-*n* should be produced preferably for respiratory types with low potential electron acceptors (Holländer, 1976; Gennis and Stewart, 1996; Wissenbach et al., 1992). By combination of the PLFA and RespQ profiles of marine sediments from the Gulf of Mexico, Li et al. (2007) demonstrated that *Beggiatoa*, the sulfide oxidizing bacteria that favors high redox potential electron acceptors, such as nitrate or oxygen as electron acceptors (e.g., McHatton et al., 1996), makes an effective geochemical barrier in exhausting oxygen above the biomats maintaining a highly reduced condition for microbial community below it. *Thioploca* is reported to be similar to *Beggiatoa* in PLFA profile (Zhang et al., 2005); however, its RespQ structure has not been evaluated in respect to the redox and nutritional conditions. Magnetotactic bacteria, which reduce ferric iron through assimilation to precipitate magnetite, need an oxygenic condition to grow but are able to maintain a highly reduced environment within the cell membrane by reducing ferric iron (Bazylinski and Frankel, 2004). The cell membrane of magnetotactic bacteria that may play a role in biochemical redox barrier has not been evaluated by its fatty acid and quinone profiles. As part of the cell membrane, RespQ is expected to be a biologic sensor for redox status of cells because it undertakes the tasks in exchange of chemical energies, nutrients, and byproducts in response to the nature of external chemistry or the interior biochemical processes.

Most redox processes in sedimentary environments and groundwater are microbially mediated. As an important supplementary to geochemical redox consideration, ester-linked PLFA is a chemical way to characterize microbial communities. PLFA provides measurements on viable microbial biomass, microbial community composition, and community nutritional status (White et al., 1997). Microbial metabolism directly linked the redox of organic and inorganic matters in environments (e.g., Nealson and Scott, 2006; Li et al., 2007) which may also have fingerprints on PLFA because of its sensitive response to the change of environmental redox (Ludvigsen et al., 1999; Li et al., 2007).

*Shewanella putrefaciens* is capable of metabolizing a wide spectrum of electron acceptors (Nealson et al., 1995; Moser and Nealson, 1996; Nealson and Scott, 2006). *S. oneidensis* strain MR-1 uses different groups of genes when exposed to metal and nonmetal electron acceptors, showing its complex respiratory chains (Beliaev et al., 2005). Among all the electron acceptors strain CN32 can use oxygen, nitrate, sulfite, and metal oxides which are of high importance of biochemical cycles in sedimentary environments (e.g., Perry et al., 1993; Nealson and Little, 1997; Neaslon and Scott, 2006). The highly flexible strategy in respiration may enable *Shewanella* to thrive in a wide range of environments that contain different redox pairs with either high or low potentials. The RespQ profiles from microbial membranes are expected to indicate the different respiratory features of aerobic/anaerobic, obligately/ facultatively anaerobic, and different types of microbial communities. As an example, *S. putrefaciens* constitutes a major part of microbial populations at the redox-clines in the Baltic Sea and is found to be a redox-interface organism (Nealson and Saffarini, 1994). Frolova et al. (2005) reported the UQ-7 and UQ-8 dominated quinone profiles as taxonomic criteria for *Shewanella* from sediments of Far Eastern Seas, which identified their functions as chemotaxonomies. However, the possible response of the intracellular compositions, such as RespQ and fatty acids to the redox conditions resulted from the reduction of varied electron acceptors, though predicated to be sensitive (Nealson and Little, 1997), has not been examined under experimental conditions in its entirety.

In this study, I report quinone- and PLFA compositions of geobiologically important microorganisms that thrive in a wide spectrum of environments with a variety of terminal electron acceptors. The results indicate that the microbial RespQ and PLFA respond sensitively to the changing of redox conditions and can be used to evaluate the major microbial electron-exchange conditions in biogeochemical environments.

## 2 Bacteria and methods

For all the culturing experiments, the originally prepared inoculum was transferred four times to eliminate the possible influence from the residue of electron acceptor. As only 2.5% (v/v) of the previous culture was used to inoculate the next batch, after four times of transfer, the left-over of electron acceptor from inoculum was negligible. For example, 40 mM of ferric citrate was used as the electron acceptor to prepare the inocula, and the left-over of ferric citrate from the first inoculum was less than 2 nM.

### 2.1 Bacterial cultures

*S. putrefaciens* strain CN32 was originally isolated from an anaerobic aquifer in north-western New Mexico (Myers and Nealson, 1988). CN32 is a Gram-negative bacterium with a highly versatile electron transport chain that grows favorably with $O_2$ or nitrate as electron acceptors; however, it can also grow under anoxic conditions with a variety of organic, inorganic, soluble, and insoluble electron acceptors (Nealson and Little, 1997; Nealson and Scott, 2006). Among those electron acceptors, transition metals ferric iron oxides, nitrate, oxygen, and sulfide are of great importance in biogeochemical cycles of carbon and metals in sedimentary environments. *Desulfovibrio desulfuricans* strain G-20, an obligately anaerobic sulfate-reducing bacterium (Li et al., 2006), was cultured anaerobically with sulfate and lactate as the electron acceptor and donor. A marine, vibrioid, *Magnetospirilum magnetotactium* strain MV-1 was first isolated from mud and water from a salt marsh pool at the Neponset River Estuary, Massachusetts, that grows favorably under microaereobically atmosphere (Bazylinski et al., 1988). *Magnetospirillum* sp. strain AMB-1 was first isolated from fresh water sediments of a natural spring at Koganei (Matsunaga et al., 1991), which was oxygen tolerant and incubated in microoxygenic condition. *Thioploca* is a facultative chemolithoautotroph capable of mixotrophic growth (Otte et al., 1999) and is thought to play a major role in coupling the biogeochemical cycles of nitrogen and sulfur in upwelling areas (Fossing et al., 1995).

### 2.2 Growths of CN32, G-20, and AMB-1 cultures

The two-line ferrihydrite ($Fe_5HO_8 \cdot 4H_2O$, FHO), ferric citrate, oxygen, nitrate, sulfite, and carbon source lactate were used to culture CN32. The growth of CN32 was also tested by using $SO_4^{2-}$ and $CO_2$ as putative electron acceptors. Sulfate and lactate were used as electron acceptor and donor, respectively, for G-20. The media for cultivating CN32, GS-15, and G-20 contained the following ingredients per 1 L deinoized distilled $H_2O$: 7.2 g HEPES organic buffer, 0.1 g KCl, 0.05 g $CaCl_2 \cdot H_2O$, 1.5 g $NH_4Cl$, 0.1 g NaCl, 0.1 g $MgCl_2 \cdot 6H_2O$, 0.5 g yeast extract, 1 mL vitamin solution, 10 mL minerals solution (Li et al., 2006), and resazurin (1 mg/L) for indication of redox. The medium was boiled while being degassed with $N_2$, and 10 ml was dispensed into 26 mL tubes. The tubes were sealed with butyl rubber stoppers and aluminum crimp caps and autoclaved at 121°C for 20 min. A sterile stock solution of $NaH_2PO_4$ was added to each bottle to 2 mM before inoculation, which would serve as the source of phosphate for bacterial growth. Final pH of the media was 7.2. $N_2$ was used as headspace gas for all cultures amended with soluble electron acceptors. In the FHO-amended media, 2.5 mM of Fe(II) was added to exhaust the potential oxygen, and 5 mM of nitrilotriacetic-Fe(III) was added as the water-soluble Fe(III) to initiate the growth; 10 mL air was filtered by 0.2 μm filter before injected to the tubes with oxygen as electron acceptor. The duplicate or triplicate tubes of the fourth time growth were harvested after 48 h incubation at 30°C. AMB-1 was cultured at 26°C under microoxygenic atmosphere with medium described in ATCC® 700264. The biomass of the pure culture of a crenarchaeota strain G12 was cultured under strict anaerobic conditions so as to compare with the biomass from a hot spring of Kamchatka, Russia.

### 2.3 Marine sedimentary samples from Gulf of Mexico

Marine sedimentary samples GC234D4426C4, GC233D4425C1 and NBP 2B represent sedimentary columns containing gas hydrate cold seep without gas hydrate, and background area without gas hydrate or cold seep. Generally, GC234D4426C4 represents the sedimentary column with rich sulfate-reducing bacteria in the depth and *Beggiatoa* on the surface of sediments; GC233D4425C1 represents the column with rich *Beggiatoa* on the surface but no gas hydrate underneath; while NBP 2B represents the control area with low microbial activity. The PLFA data has been reported in Li et al. (2007). The PLFA data of these sedimentary columns (Li et al., 2007) and respiratory quinone profiles reported in this study are used to test their response to the redox conditions indicated by geochemical parameters.

### 2.4 Total lipid extraction and the analysis of PLFA

The cells in growth cultures were destroyed by two minutes ultrasonication at room temperature. A modified Bligh and Dyer (1959) single-phase solvent system (White et al., 1979) was used for total lipid extraction. As the pH of all growing cultures has been buffered by HEPES ($C_8H_{17}NO_3S$) to around 7.2, cultures were used for total lipid extraction directly by adding methanol and chloroform according to the ratio of 1:2:0.9 of chloroform: methanol: culture (equals to the volume of $NaH_2PO_4$ buffered $H_2O$ at pH = 7.2). After an overnight extraction, equal volume of water was added to split the phase. The bottom layer (organic solvent) was transferred to a new tube, and the same procedure was repeated by adding same amount of chloroform again for a further extraction. The $N_2$-blow dried organic portion was separated into neutral-, glycol-, and polar lipids by silicic acid column chromatography with chloroform, acetone, and methanol as the elution solvents, respectively (Guckert et al., 1985). The polar lipid fraction (eluted by methanol) with ester-linked fatty acids were prepared for gas chromatography-mass spectrometric analysis after a mild alkaline transesterification (Guckert et al., 1985). The fatty acid methyl esters were identified and quantified with a Hewlett Packard 5890 Gas-Chromatography in constant flow mode coupled to a HP5973 quadrupole mass selective detector. Nomenclatures of PLFA could be found elsewhere (White et al., 1979; Guckert et al., 1985; Ringelberg et al., 1997).

### 2.5 Analysis of Quinones

The neutral organic fraction was analyzed for the content and structure of major RespQs without further purification by high-performance liquid chromatography coupled to atmospheric pressure chemical ionization tandem mass spectrometry, as described by Geyer et al. (2004). An Agilent-1100 quaternary high-performance liquid chromatography system delivered a constant flow of methanol-chloroform (4:1 in volume) to the atmospheric pressure chemical inlet for tandem quadrupole mass spectrometry. The tandem mass spectrometer was a Sciex365 triple quadrupole mass spectrometer (MDS SCIEX, Concord, Ontario, Canada) that worked in the positive mode with the nebulizer probe maintained at 425°C. The protonated molecular ion $[M + H]^+$ and the ion fragment of 1-methyl-2-isoprenyl-3,4-dimethoxyparabenzoquinone (UQ with *m/z* = 197.1) or 1-isoprenyl-2-methyl-naphthoquinone (MK with *m/z* = 187.0) were used to determine the corresponding quinones with different side-chains at high accuracy (Fig. 1). For example, the base peak in the spectrum of a Q1 scan $[M + H]^+$ at *m/z* 597.1 and a base peak at *m/z* 197.1 scanned by Q3 make a pair to determine UQ6 as one and the only isoprenoid quinone (Fig. 1); similarly, MK4 was determined as the one and the only quinone by its protonated molecule at *m/z* 445.6 and a major ion in the product ion with *m/z* equals to 187.0 (Fig. 1). Because different quinones had varied sensitivities, the concentration of each quinone was calculated by its own standard set with a range of concentrations (4.8, 48, 480, and 4800 ppb).

## 3 Results

### 3.1 The PLFA profiles

The total biomass represented by PLFA of CN32 grown with $SO_3^{2-}$, FHO, $O_2$, ferric citrate, and $NO_3^-$ were significantly higher than those measured in cultures with

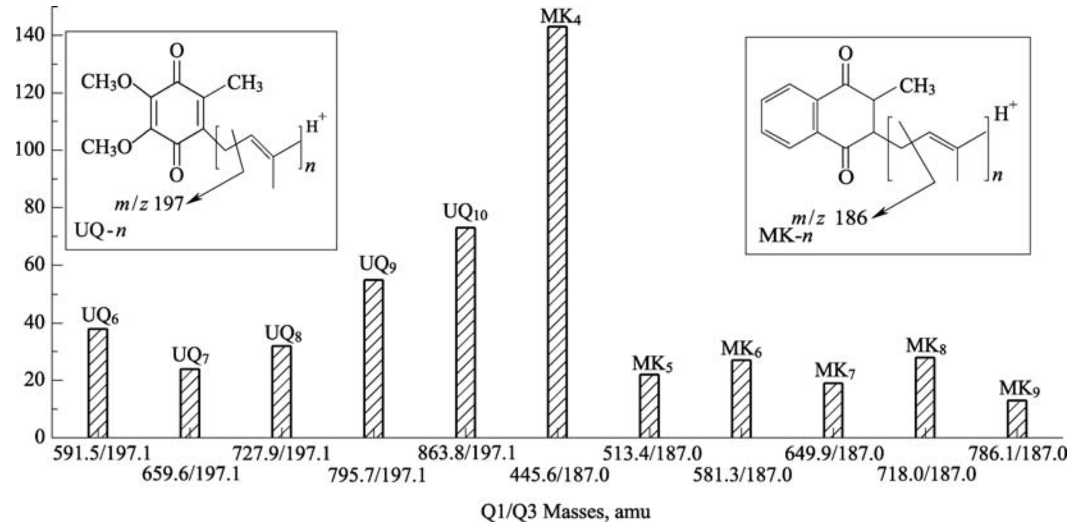

**Fig. 1** Schematic of the pairs of ubiquinones-molecular ions, and the pairs of menaquinone quinones-molecular ions used to determine the specific quinones by the Tandem Mass Spectrometry

$SO_4^{2-}$ and $CO_2$ as putative electron acceptors (grossly 18 to 210 times). Correspondingly, the total amounts of RespQ of $SO_3^{2-}$, $FHO^-$, $\mathbf{O}_2^-$, ferric citrate-, and $NO_3^-$-amended cultures were 6 to 53 times higher than those in $SO_4^{2-}$ and $CO_2$-amended cultures. This implied that the cells in $SO_4^{2-}$ and $CO_2$-amended cultured did not have substantial growth. Total biomass in $SO_3^{2-}$-amended culture represented by PLFA was lower than the growths with $NO_3^-$, ferric citrate, $O_2$, and FHO as electron acceptors.

The PLFA profiles of *Thioploca*, MV-1, AMB-1, G-20, and GS-15 are listed in Table 1. MV-1 had high amounts of C18:1ω7c (46.6%) and C16:1ω7c (34.3%) followed by C16:0 (13.0%). Similarly, AMB-1 had high amounts of C18:1ω7c (64.6%) and C16:1ω7c (19.2%) followed by C16:0 (7.0%) and C14:0 (6.5%). *Thioploca* also contained high amounts of C16:1ω7c (32.7%) and C18:1ω7c (27.4%) with a relatively high amount of C16:0 (18.5%). AMB-1 had significant amount of "*trans*" fatty acids (7.1%). G-20 had high amounts of *i*C17:0 (51.8%), *i*C15:0 (16.7%), and C18:0 (9.7%). GS-15 had high amount of C16:0 (32.9%) followed by *i*C15:0 (21.8%), aC15:0 (13.8%), and C16:1ω7c (14.6%); the sum of those PLFA (83.5%) in GS-15 biomass was highly consistent with previous report (83%–87%) with poorly crystalline Fe(III) oxide and nitrate as electron acceptors under strict anaerobic conditions (Lovley et al., 1993).

The PLFA profiles of CN32 could be classified into three groups (Table 2): 1) culture with solid-state electron acceptor (ferrihydrite) that was characterized by low C16:0 and undetectable monounsaturated fatty acids; 2) cultures with preferred electron acceptors ($NO_3^-$, ferric citrate and $O_2$) were characterized by their moderate C16:0 percentages (26.7%–35.9%) and high of C16:1ω7c; and 3) cultures with poor electron acceptor ($SO_3^{2-}$) or without bioavailable electron acceptors (amended with $SO_4^{2-}$ and $CO_2$) were characterized by their high C16:0 (40.1%–48.7%).

### 3.2 Profiles of respiratory quinones

Microoxygenic iron-reducing bacteria (MV-1 and AMB-1) were all high in UQ-*n* (Table 3). MV-1 contained 47.0% of UQ-9 and 15.8% of UQ-8, and 31.9% MK-8; whereas AMB-1 was dominated by UQ-10 (91.8%) and contained

**Table 1** Percentage compositions of PLFA of microorganisms conduct aerobic, facultative, and obligate anaerobic respirations. Except C15:0 and C17:0, trace amount ( < 0.1%) of saturated straight chain-, monounsaturated-, double unsaturated-, and OH-branched fatty acids were omitted from the table

| sample | MV-1 | AMB-1 | *Thioploca* | GS-15 | G-20 |
|---|---|---|---|---|---|
| electron acceptor | $O_2$ | $O_2$ | $NO_3^-$ | $Fe^{3+}$-citrate | $SO_4^{2-}$ |
| saturated | | | | | |
| C14:0 | 0.9 | 6.5 | 1.3 | 12.4 | |
| *i*C15:0 | | | 2.4 | 21.8 | 16.7 |
| aC15:0 | | | 1.7 | 13.8 | 3.5 |
| C15:0 | 0.1 | | 0.4 | | |
| *i*C16:0 | | | 0.2 | | 0.7 |
| C16:0 | 13.0 | 7.0 | 18.5 | 32.9 | 4.5 |
| *i*C17:0 | | | 0.5 | | 51.8 |
| aC17:0 | | | 0.6 | | 5.8 |
| C17:0 | | | 0.3 | | 0.3 |
| C18:0 | 0.2 | 0.6 | 1.3 | 4.5 | 9.7 |
| sum of saturated/% | 14.2 | 14.1 | 27.2 | 85.4 | 94.3 |
| unsaturated | | | | | |
| 14:1ω5c | | 1.2 | | | |
| C16:1ω7c | 34.3 | 19.2 | 32.7 | 14.6 | 0.7 |
| C16:1ω7t | 2.0 | 0.1 | 4.1 | | |
| C16:1ω5c | 2.8 | 0.1 | 1.3 | | |
| *i*C17:1 | | | 0.4 | | 3.1 |
| C18:1ω9c | | 0.1 | 0.6 | | 0.4 |
| C181ω7c | 46.6 | 64.6 | 27.4 | | 2.7 |
| C18:1ω7t | | 0.1 | 3.0 | | |
| C18:1ω5c | 0.2 | | 1.8 | | |
| sum of unsaturated/% | 85.9 | 84.2 | 71.3 | 14.6 | 6.9 |

**Table 2** Percentage composition of PLFA compositions of *Shewanella putrefaciens* strain CN32 with a variety of electron acceptors. PLFAs of less than 0.1% for all cultures were omitted from the table

| electron acceptor | FHO | ferric citrate | $NO_3^-$ | $O_2$ | $SO_3^{2-}$ |
|---|---|---|---|---|---|
| saturated | | | | | |
| C14:0 | 1.3 | 4.8 | 1.7 | 2.0 | 2.3 |
| *i*C15:0 | 35.3 | 4.8 | 10.5 | 9.5 | 6.4 |
| aC15:0 | 9.3 | 0.4 | 0.5 | 0.8 | 0.6 |
| C15:0 | 4.1 | 0.3 | 0.4 | 1.9 | 0.5 |
| *i*C16:0 | 4.3 | | | | |
| C16:0 | 16.3 | 35.9 | 26.7 | 31.1 | 40.1 |
| C17:0 | 0.6 | | 0.7 | 2.3 | 0.5 |
| *i*C17:0 | 8.4 | | 2.0 | 1.1 | 0.8 |
| aC17:0 | 7.1 | | 1.6 | 3.9 | 0.8 |
| cyC17:0 | 4.1 | 0.4 | 0.1 | 0.3 | 0.2 |
| C18:0 | 8.7 | 3.8 | 3.7 | 2.3 | 4.6 |
| sum of saturated | 100 | 50.4 | 47.9 | 55.2 | 56.8 |
| unsaturated | | | | | |
| C16:1ω9c | | 2.7 | 2.0 | 1.8 | 1.5 |
| C16:1ω7c | | 32.2 | 32.3 | 31.0 | 26.5 |
| C18:2 ω6,9c | | 0.7 | 0.1 | 0.1 | 1.2 |
| C18:1ω9c | | 5.7 | 8.2 | 6.6 | 7.7 |
| C181ω7c | | 6.4 | 9.4 | 5.4 | 5.5 |
| sum of unsaturated | | 47.7 | 52.0 | 44.9 | 42.2 |

no MK-*n* (Table 3). Facultative sulfide oxidizer *Thioploca* contained substantial percentages of both MK-*n* and UQ-*n*. In *Thioploca*, UQ-8 was 27.2% and MK-4, MK-6, MK-7, and MK-8 were all higher than 13%. The quinone profiles of those obligate anaerobes (G-20 and GS-15) were 100% MK-*n* (Table 3). GS-15 was dominated by MK-7 (81.7%) and MK-4 (18.3%), while G-20 was dominated by MK-6 (84.9%) followed by MK-4 (11.0%) and MK-5 (3.8%).

CN32 amended with $NO_3^-$, $O_2$, ferric citrate, and FHO had MK-7 plus MK-8 of 60.2%–83.3% as major isoprenologues; however, those amended with $SO_3^{2-}$ and those with $SO_4^{2-}$ and $CO_2$ as putative electron acceptors were characterized by low MK-7 + MK-8 (9.7%–38.7%) but high UK-8 (47.2%–61.1%) (Table 3).

Same as the PLFA structures (Li et al., 2007), RespQ profiles of marine sediments from Gulf of Mexico showed their consistency with the redox conditions. All the biomats from the seabed had high UQ-*n* while deep in the column changed to high in MK-*n*. The RespQ profile of sediment from hot spring sediment from hot spring of Kamchatka, Russia was similar to that of a pure culture of a strain of Crenachaeota strain G12 incubated under strict anaerobic condition.

Figure 2 showed a wide range of ratios of monounsaturated to saturated straight chain PLFA from strict anaerobic to aerobic microorganisms; while a wide range of ratios of UQ-*n*/MK-*n* could be observed for CN32 when electron acceptors with different redox potential were provided.

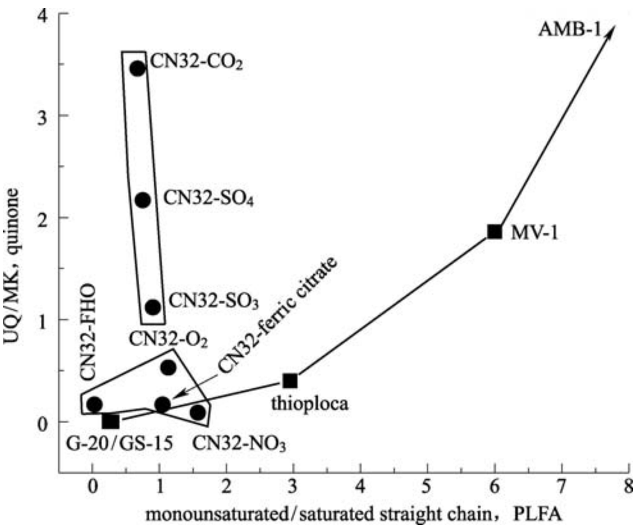

**Fig. 2** The ratio of monounsaturated to saturated straight chain PLFA versus the ratio of ΣUQ-*n*/ΣMK-*n*. Note CN32 amended with $CO_2$ and $SO_4^{2-}$ as putative electron acceptors did not have substantial growths

### 3.3 UQ-*n*/MK-*n* ratio versus redox potential of electron acceptors

The ratios of UQ-*n*/MK-*n* (Table 3) were plotted versus the redox potentials of electron acceptors amended in CN32 cultures (Fig. 3). The redox potentials ($E_0'$, mv) calculated at pH 7 and 25°C were cited from Nealson and Myers

**Table 3** Percentage compositions of UQ-*n* and MK-*n* of *Thioploca*, MV-1, AMB-1, GS-15, G-20, marine sediments from Gulf of Mexico, sediment from a volcanic hot spring of Kamchatka, and CN32 with a variety of electron acceptors

| sample | UQ6 | UQ7 | UQ8 | UQ9 | UQ10 | MK4 | MK5 | MK6 | MK7 | MK8 | MK9 | ΣUQ-*n*/ ΣMK-*n* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| AMB-1 | 1.3 | 2.0 | 1.9 | 3.0 | 91.8 | | | | | | | 100:0 |
| MV-1 | 0.6 | 1.1 | 15.8 | 45.0 | 0.5 | | | | 3.1 | 31.9 | | 65:35 |
| *Thioploca* | 0.3 | 0.6 | 27.2 | 0.5 | 0.2 | 14.0 | | 13.1 | 15.5 | 28.6 | | 29:71 |
| GS-15 | | | | | | 18.3 | | | 81.7 | | | 0:100 |
| G-20 | | | | | | 11.0 | 3.8 | 84.9 | 0.3 | | | 0:100 |
| CN32 with various electron acceptors | | | | | | | | | | | | |
| $^*CO_2$ | 1.9 | 8.8 | 59.9 | 5.3 | 2.6 | 10.5 | 2.4 | | 9.6 | | | 78.5:21.5 |
| $^*SO_4^{2-}$ | 1.0 | 4.9 | 61.1 | 1.4 | 0.2 | 5.5 | 1.1 | 1.9 | 19.7 | 3.1 | 0.2 | 69:31 |
| $SO_3^{2-}$ | 0.6 | 3.5 | 47.2 | 1.5 | 0.2 | 4.7 | 1.3 | 2.5 | 35.8 | 3.0 | | 53:47 |
| ferrihydrite | | 1.2 | 13.5 | 0.1 | | 10.7 | 3.8 | | 54.0 | 16.7 | | 14:86 |
| $Fe^{3+}$-citrate | 0.1 | 0.3 | 14.2 | 0.1 | | 12.9 | 3.9 | 8.4 | 43.3 | 16.9 | | 15:85 |
| $NO_3^-$ | 0.1 | 0.1 | 8.2 | 0.1 | | 5.4 | 1.8 | 2.7 | 66.0 | 15.4 | 0.2 | 8.5:91.5 |
| $O_2$ | 0.1 | 0.2 | 5.4 | 0.1 | | 6.3 | 2.0 | 2.9 | 59.4 | 23.9 | | 6:94 |
| sediment from a hot spring of Kamchatka | | | | | | | | | | | | |
| KM04078 | 0.1 | 0.3 | 0.9 | 0.1 | 0.1 | 44.68 | 7.29 | 11.52 | 15.68 | 17.43 | 1.92 | 1.5:98.5 |
| G12 | 0.3 | 0.0 | 0.3 | 0.1 | 0.4 | 19.8 | 14.4 | 18.1 | 27.7 | 14.3 | 4.5 | 1:99 |
| marine sediments of Gulf of Mexico | | | | | | | | | | | | |
| NBP-2B (without visible biomats and gas hydrate) | | | | | | | | | | | | |
| 0–2 cm | | | 100.0 | | | | | | | | | 100:0 |
| 8–10 cm | 10.1 | 5.4 | 2.5 | 1.9 | 1.4 | 43.0 | | 24.4 | 4.8 | 3.4 | | 66:34 |
| GC233D4425C1 (with visible biomats but no gas hydrate) | | | | | | | | | | | | |
| 0–2 cm | 5.4 | 6.0 | 43.7 | 1.6 | 2.2 | 14.7 | | 23.1 | 1.9 | 1.2 | | 74:26 |
| 8–10 cm | 6.0 | 4.8 | 9.1 | 1.2 | 1.3 | 40.0 | | 28.7 | 5.5 | 2.9 | | 63:37 |
| GC2344426C4 (with visible biomats and gas hydrate below) | | | | | | | | | | | | |
| 0–2 cm | 4.0 | 5.0 | 43.1 | 1.1 | 3.0 | 7.6 | | 33.7 | 1.5 | 0.8 | | 64:36 |
| 8–10 cm | 3.1 | 3.4 | 12.0 | 0.9 | 1.4 | 15.4 | | 59.4 | 3.1 | 1.2 | | 36:64 |

$^*SO_4^{2-}$ and $CO_2$ are putative electron acceptors, and there were not substantial growths in these two cultures

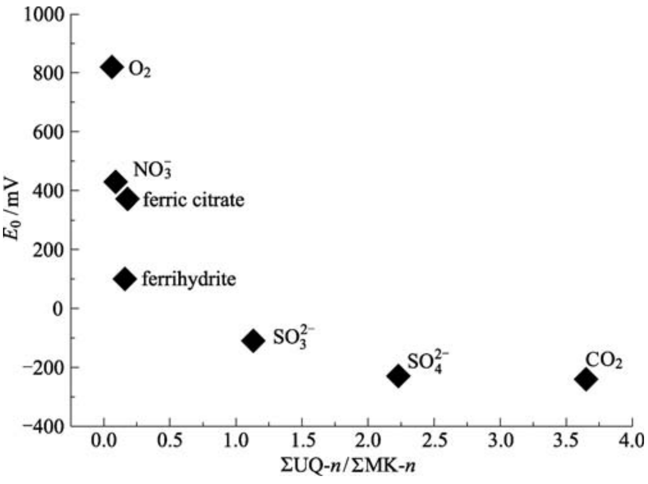

**Fig. 3** ΣUQ-*n*/ΣMK-*n* versus standard redox potential of terminal electron acceptors amended in CN32 cultures

(1992), except those of ferrihydrite and ferric citrate which were cited from Straub et al. (2001).

## 4 Discussion

The content of PLFA has been demonstrated to be valid in representing the microbial biomass (e.g., Guckert et al., 1985; Ringelberg et al., 1997) and the total concentration of quinones was highly correlated to the microbial biomass and the total cell counts (Hiraishi et al., 1998; Hiraishi, 1999). Significantly low amount of PLFA and RespQ in $SO_4^{2-}$ and $CO_2$-amended CN32 cultures, and to some extent, the $SO_3^{2-}$ -amended culture indicated little growth of cells when compared to the growth with FHO, $O_2$, ferric citrate, or $NO_3^-$ as electron acceptors. These results, therefore, corroborated previous reports that $SO_4^{2-}$ and $CO_2$ cannot be reduced by CN32 for its growth (e.g., Nealson and Saffarini, 1994; Beliaev et al., 2005; Neaslon and Scott, 2006); $SO_3^{2-}$, however, is a poor electron acceptor for CN32 though the substantial growth of cell can be observed.

Upon the observation of PLFA profiles of estuarine sediments and laboratory enrichments, Guckert et al. (1985) and Parkers and Taylor (1983) found that the saturated straight chain and the branched chain *iso* and *anteiso* PLFA of enrichments of marine sediments increase with the expense of monounsaturated fatty acids when the redox condition shifted from aerobic to anaerobic condition and correspondingly has an increase in MK-*n* at the expense of UQ-*n* (Hedrick and White, 1986; Wissenbath et al., 1992). This shift of redox can also be reflected by the electron redox-pairs used by microbes. *Thioploca* species thrive in bottom waters that are poor in oxygen but rich in nitrate, and the cells harbor large liquid vacuoles as storage for high concentrations of nitrate as an electron acceptor for sulfide oxidation (Jørgensen and Gallardo, 1999; Schulz and Jørgensen, 2001; Zopfi et al., 2001; Fossing et al., 1995). The PLFA profile was consistent with previous reports (e.g., McCaffrey et al., 1989), which indicated a facultative respiratory type of *Thioploca* (Parkes and Taylor, 1983). The UQ-*n*/MK-*n* ratio of *Thioploca* is close to biomass of CN32 harvested from $NO_3^-$-amended culture. MV-1 and AMB-1 have significantly high monounsaturated fatty acids (C16 plus C18) and high concentration of UQ-*n* (Fig. 2), reflecting their aerobic respiratory feature (Guckert et al., 1985; Baird et al., 1985). On the contrary, the obligate anaerobes G-20 and GS-15 are characterized by low monounsaturated PLFA and MK-*n* dominated quinone profiles (Tables 2 and 3 and Fig. 2), which can be explained by the fact that biogenic Fe(II) and $H_2S$ are highly effective sink for free oxygen and may lead to highly reduced conditions. The consistency of RespQ profile of G12 that was incubated under strict anaerobic condition with the hot spring sediment implies the strict anaerobic condition of hot spring and the simple and archaeol-dominated community in the hot spring sediment.

The significantly high content of ubiquinone, especially UQ-8 (61.1% and 59.9%, respectively) in CN32 cultures amended with $SO_4^{2-}$ and $CO_2$ but low in MK-7 + MK-8 ($<23\%$) indicated the lack of electron acceptor induced stress effect. Inversely, those cultures with favored electron acceptors contain more than 80% of MK7 + MK-8 and less UQ-8 (5.4%–14.2%).

The CN32 culture with solid-state electron acceptor FHO as an electron acceptor has different PLFA structure from those with soluble-electron acceptors, which may likely be due to a different respiratory pathway the microbe used (e.g., Lovley et al., 1996; Schröder et al. 2003; Myers and Myers, 2004; Beliaev et al., 2005; Ruebush et al., 2006). The ferric iron reducing cultures would produce high concentration of ferrous iron that would exhaust oxidants with high redox potential (e.g., Nealson and Saffarini, 1994), and menaquinones are synthesized and play a major role in Fe(III)-reduction (Søballe and Poole, 1999; Nealson and Scott, 2006). Except CN32 cultures without usable electron acceptors, those with preferred electron acceptors and other genus showing increased monounsaturated to saturated straight chain PLFA ratios and ubiquinone to menaquinone ratios along with the increase of redox potentials of electron acceptors (Fig. 3). The ratios of MK-*n*/UQ-*n* in CN32 cultures decrease consistly with the decrease of redox potentials (Nealson and Myers, 1992; Straub et al., 2001) in the order of $O_2 \rightarrow NO_3^- \rightarrow$ferric citrate$\rightarrow$ferrihydrite$\rightarrow SO_3^{2-} \rightarrow SO_4^{2-} \rightarrow CO_2$. This relation clearly indicates the interplay between the major microbial metabolisms in the community and its chemical environment. This indicated a potential use of UQ-*n*/MK-*n* ratio of environmental sample, such as the subsurface or ground water to indicate the redox zone. Because RespQ also reflect the dynamics of cell growth, it may reflect the actual redox condition when compared to the geochemical signatures, such as the redox state of authigenic minerals, which may not be sensitive and quick enough to reflect the redox of the microbial environments.

## 5 Conclusions

The strict anaerobic microbes (G-20 and GS-15) are characterized by their low content of monounsaturated relative to saturated straight chain PLFAs and low content of ubiquiones relative to menaquinones. On the contrary, aerobic magnetotactic bacteria (MV-1 and AMB-1) are characterized by their high content of monounsaturated PLFA and ubiquinones. *Thioploca* has its PLFA and Respiratory quinone compositions between the extremely anaerobic and the aerobic microorganisms due to its facultative respiratory type. The PLFA profiles of all CN32 cultures fall in a narrow range, while their quinone profiles showed influences from redox and/or stress effect. CN32 cultures with $SO_4^{2-}$ and $CO_2$ as putative electron acceptors and the culture with poor electron acceptor $SO_3^{2-}$ showed remarkably high in ubiquinones, showing a stress effect; while the cultures with preferred electron acceptors, which have high redox efficiencies, demonstrated to be high in menaquinones. These results indicate that the bacterial membrane PLFA and the quinone profiles are sensitive to the availability and type of electron acceptors in the environments. Moreover, the metabolisms and the microbial metabolism will subsequently have impacts on the redox chemistry of its environment.

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## References

Baird H H, Nivens D E, Parker J H, White D C (1985). The biomass, community structure, and spatial distribution of the sedimentary microbiota from a high-energy area of the deep sea. Deep-Sea Res, 32 (9): 1089–1099

Bazylinski D A, Frankel R B (2004). Magnetosome formation in prokaryotes. Nat Rev Microbiol, 2 (3): 217–230

Bazylinski D A, Frankel R B, Jannasch H W (1988). Anaerobic magnetite production by a marine magnetotactic bacterium. Nature, 334 (6182): 518–519

Beliaev A S, Klingeman D M, Klappenbach J A, Wu L, Romine M F, Tiedje J M, Nealson K H, Fredrickson J K, Zhou J (2005). Global transcriptome analysis of *Shewanella oneidensis* MR-1 exposed to different terminal electron acceptors. J Bacteriol, 187 (20): 7138–7145

Bligh E G, Dyer W J (1959). A rapid method of total lipid extraction and purification. Can J Biochem Physiol, 97: 911–917

Collins M D, Jones D (1981). Distribution of isoprenoid quinone structural types in bacteria and their taxonomic implication. Microbiol Rev, 45 (2): 316–354

Fossing H, Gallardo V A, Jørgensen B B, Hüttel M, Nielsen L P, Schulz H, Canfield D E, Forster S, Glud R N, Gundersen J K, Küver J, Ramsing N B, Teske A, Thamdrup B, Ulloa O (1995). Concentration and transport of nitrate by the mat-forming sulfur bacterium *Thioploca*. Nature, 374 (6524): 713–715

Frolova G M, Pavel' K G, Shparteeva A A, Nedashkovskaia O I, Gorshkova N M, Ivanova E P, Mikhaĭlov V V (2005). Lipid composition of novel *Shewanella* species isolated from far Eastern seas. Mikrobiologiia, 74 (6): 766–771

Gennis R B, Stewart V (1996) Respiration in *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. 2nd edition. Neidhardt F C, eds. Washington D C: American Society for Microbiology, 217–261

Geyer R, Peacock A D, White D C, Lytle C, Van Berkel G J (2004). Atmospheric pressure chemical ionization and atmospheric pressure photoionization for simultaneous mass spectrometric analysis of microbial respiratory ubiquinones and menaquinones. J Mass Spectrom, 39 (8): 922–929

Guckert J B, Antworth C P, Nichols P D, White D C (1985). Phospholipid, ester-linked fatty acid profiles as reproducible assays for changes in prokaryotic community structure of estuarine sediments. FEMS Microbiol Letters, 31: 147–158

Hedrick D B, White D C (1986). Microbial respiratory quinones in the environment: a sensitive liquid chromatographic method. J Microbiol Methods, 5 (5–6): 243–254

Hiraishi A (1999). Isoprenoid quinones as biomarkers of microbial populations in the environment. J Biosci Bioeng, 88(5): 449–460

Hiraishi A, Ueda Y, Ishihara J (1998). Quinone profiling of bacterial communities in natural and synthetic sewage activated sludge for enhanced phosphate removal. Appl Environ Microbiol, 64(3): 992–998

Holländer R (1976). Correlation of the function of demethylmenaquinone in bacterial electron transport with its redox potential. FEBS Lett, 72 (1): 98–100

Hunter K S, Wang Y, van Cappellen P (1998). Kinetic modeling of microbially-driven redox chemistry of subsurface environments: coupling transport, microbial metabolism and geochemistry. J Hydrol (Amst), 209 (1–4): 53–80

Jørgensen B B, Gallardo V A (1999). *Thioploca* spp: filamenteous sulfur bacteria with nitrate vacuoles. FEMS Microbiol Ecol, 28 (4): 301–313

Li Y L, Peacock A, White D C, Geyer R, Zhang C L (2007). Spatial patterns of bacterial signature biomarkers in marine sediments of the Gulf of Mexico. Chem Geol, 238 (3–4): 168–179

Li Y L, Vali H, Yang J, Phelps T J, Zhang C L (2006). Reduction of iron oxides enhanced by a sulfate-reducing bacterium and biogenic $H_2S$ production. Geomicrobiol J, 23 (2): 103–117

Lovley D R, Coates J D, Blunt-Harris E L, Phillips E J P, Woodward J C (1996). Humic substances as electron acceptors for microbial respiration. Nature, 382(6590): 445–448

Lovley D R, Giovannoni S J, White D C, Champine J E, Phillips E J P, Gorby Y A, Goodwin S (1993). *Geobacter metallireducens* gen. nov. sp. nov., a microorganism capable of coupling the complete oxidation of organic compounds to the reduction of iron and other metals. Arch Microbiol, 159(4): 336–344

Ludvigsen L, Albrechtsen H-J, Ringelberg D B, Ekelund F, Christensen T H (1999). Distribution and composition of microbial populations in a landfill leachate contaminated aquifer. Microbial Ecology, 37 (3): 197–207

Matsunaga T, Sakaguchi T, Tadokoro F (1991). Magnetite formation by a magnetic bacterium capable of growing aerobically. Appl Microbiol Biotechnol, 35 (5): 651–655

McCaffrey M A, Farrington J W, Repeta D J (1989). Goechemical implications of the lipid composition of *Thioploca* spp. From the Peru upwelling region 15°S. Org Geochem, 14 (1): 61–68

McHatton S C, Barry J P, Jannasch H W, Nelson D C (1996). High nitrate concentrations in vacuolate, autotrophic marine *Beggiatoa* spp. Appl Environ Microbiol, 62 (3): 954–958

Moser D P, Nealson K H (1996). Growth of the facultative anaerobe *Shewanella putrefaciens* by elemental sulfur reduction. Appl Environ Microbiol, 62 (6): 2100–2105

Myers C R, Myers J M (2004). *Shewanella oneidensis* MR-1 restores menaquinone synthesis to a menaquinone-negative mutant. Appl Environ Microbiol, 70 (9): 5415–5425

Myers C R, Nealson K H (1988). Bacterial manganese reduction and growth with manganese oxide as the sole electron acceptor. Science, 240 (4857): 1319–1321

Nealson K H, Little B (1997). Breathing manganese and iron: solid-state respiration. Adv Appl Microbiol, 45: 213–239

Nealson K H, Moser D P, Saffarini D A (1995). Anaerobic electron acceptor chemotaxis in *Shewanella putrefaciens*. Appl Environ Microbiol, 61 (4): 1551–1554

Nealson K H, Myers C R (1992). Microbial reduction of manganese and iron: new approaches to carbon cycling. Appl Environ Microbiol, 58 (2): 439–443

Nealson K H, Saffarini D (1994). Iron and manganese in anaerobic respiration: environmental significance, physiology, and regulation. Annu Rev Microbiol, 48 (1): 311–343

Nealson K H, Scott J (2006). Ecophysiology of the Genus *Shewanella*. The Prokaryotes, 6: 1133–1151

Otte S, Kuenen J G, Nielsen L P, Paerl H W, Zopfi J, Schulz H N, Teske A, Strotmann B, Gallardo VA, Jørgensen BB (1999). Nitrogen, carbon, and sulfur metabolism in natural *Thioploca* samples. Appl Environ Microbiol, 65 (7): 3148–3157

Parkes R J, Taylor J (1983). The relationship between fatty acid distributions and bacterial respiratory types in contemporary marine sediments. Estuar Coast Shelf Sci, 16 (2): 173–174

Parkes R J (1987). Analysis of microbial communities within sediments using biomarkers. 141–177. In: Fletcher M, Gray T R Gand Jones J, eds. Ecology of Microbial Communities. Cambridge University Press: Cambridge

Perry KA, Kostka J E, Luther G W, Nealson K H (1993). Mediation of sulfur speciation by a Black Sea facultative anaerobe. Science, 259 (5096): 801–803

Polglase W J, Pun W T, Withaar J (1966). Lipoquinones of *Escherichia coli*. Biochim Biophys Acta, 118 (2): 425–426

Ringelberg D B, Sutton S, White D C (1997). Biomass, bioactivity and biodiversity: microbial ecology of the deep subsurface: analysis of ester-linked phospholipid fatty acids. FEMS Microbiol Rev, 20 (3–4): 371–377

Ruebush S S, Icopini G A, Brantley S L, Tien M (2006). In vitro enzymatic reduction kinetics of mineral oxides by membrane fractions from *Shewanella oneidensis* MR-1. Geochim et Cosmochim Acta, 70 (1): 56–70

Schröder I, Johnson E, de Vries S (2003). Microbial ferric iron reductases. FEMS Microbiol Rev, 27 (2–3): 427–447

Schulz H N, Jørgensen B B (2001). Big bacteria. Annu Rev Microbiol, 55 (1): 105–137

Søballe B, Poole R K (1999). Microbial ubiquinones: multiple roles in respiration, gene regulation and oxidative stress management. Microbiology, 145 (8): 1817–1830

Straub K L, Benz M, Schink B (2001). Iron metabolism in anoxic environments at near neutral pH. FEMS Microbiol Ecol, 34 (3): 181–186

Whistance G R, Threlfall D R (1968). Effect of anaerobiosis on the concentrations of demethylmenaquinone, menaquinone and ubiquinone in Escherichia freundii, Proteus mirabilis and Aeromonas punctata. Biochem J, 108 (3): 505–507

Wissenbach U, Ternes D, Unden G (1992). An *Escherichia coli* mutant containing only demethylmenaquinone, but no menaquinone: effects on fumarate, dimethylsulfoxide, trimethylamine N-oxide and nitrate respiration. Arch Microbiol, 158 (1): 68–73

White D C, Davis W M, Nickels J S, King J D, Bobbie R J (1979). Determination of the sedimentary microbial biomass by extractible lipid phosphate. Oecologia, 40 (1): 51–62

White D C, Ringelberg D B, MacNaughton S J, Alugupalli S A, Schram D (1997) Signature lipid biomarker analysis for quantitative assessment in situ of environmental microbial ecology. In: Molecular markers in environmental geochemistry. Eganhouse R P, ed. ACS Symposium Series 671, American Chemical Society, Washington D C, 22–34

Zhang C L, Huang Z Y, Cantu J, Pancost R D, Brigmon R L, Lyons T W, Sassen R (2005). Lipid biomarkers and carbon isotope signatures of a microbial (*Beggiatoa*) mat associated with gas hydrates in the gulf of Mexico. Appl Environ Microbiol, 71 (4): 2106–2112

Zopfi J, Kjaer T, Nielsen L P, Jørgensen B B (2001). Ecology of *Thioploca* spp.: nitrate and sulfur storage in relation to chemical microgradients and influence of *Thioploca* spp. on the sedimentary nitrogen cycle. Appl Environ Microbiol, 67 (12): 5530–5537