The Journal of Microbiology, December 2001, p.273-278 Vol. 39, No. 4

Growth Properties of the Iron-reducing Bacteria, *Shewanella putrefaciens* IR-1 and MR-1 Coupling to Reduction of Fe(III) to Fe(II)

Doo Hyun Park[1] and Byung Hong Kim[2]*

[1]*Department of Biological Engineering, Seokyung University, Seoul 136-704, Korea*
[2]*Environment Research Center, Korea Institute of Science and Technology, Seoul 136-791, Korea*

(Received August 7, 2001 / Accepted October 13, 2001)

Shewanella putrefaciens **IR-1 and MR-1 were cultivated by using various combinations of electron donor-acceptor, lactate-Fe(III), lactate-nitrate, pyruvate-Fe(III), pyruvate-nitrate, H_2-acetate-Fe(III) and H_2-acetate-nitrate. Both strains grew fermentatively on pyruvate but not on lactate without an electron acceptor. In culture with Fe(III), both strains grew on pyruvate and lactate but not on H_2-acetate-CO_2. In cultivation with nitrate, both strains grew on pyruvate, lactate and on H_2-acetate-CO_2. The growth yields of IR-1 on pyruvate, pyruvate-Fe(III) and lactate-Fe(III) were about 3.4, 3.5, and 3.6 (g cell/M substrate), respectively, but the yields on lactate-nitrate, pyruvate-nitrate and H_2-acetate-CO_2-nitrate were about 6.8, 5.9, and 9.4 (g cell/M substrate), respectively. From the growth properties of both strains on media with Fe(III) as an electron acceptor, the bacterial growth was confirmed not to be increased by addition of Fe(III) as an electron acceptor to the growth medium, which indicates a possibility that the dissimilatory reduction of Fe(III) to Fe(II) may not be coupled to free energy production.**

Key words: anaerobic respiration, electron sink, iron-reducing bacterium, growth yield, *Shewanella putrefaciens*

Dissimilatory Fe(III) reduction can be defined as the use of Fe(III) as an external electron acceptor in bacterial respiration (11). A distinction of dissimilatory Fe(III) reduction from assimilatory reduction is that, under normal physiological conditions, significant quantities of Fe(II) are accumulated outside the cell during growth (8). The organisms that can obtain the energy for growth by the oxidation of organic compounds coupled to reduction of Fe(III) have been described as Fe(III)-reducing bacteria (8, 14). At least three Fe(III)-reducing bacteria, *Geobacter metallireducens* (10), *Desulfuromonas acetoxidans* (3, 10) and *Shewanella putrefaciens* (17) are known to conserve energy for anaerobic growth by coupling the oxidation of organic compounds to the dissimilatory reduction of Fe(III). Loveley (8) reported that hydrogen and formate oxidation is coupled to the dissimilatory reduction of Fe(III) or Mn(IV) by *Alteromonas putrefaciens*. However, in that study, *A. putrefaciens* was confirmed not to grow when amino acids, e.g., L-arginine, L-glutamine and DL-serine, were omitted (15).

S. putrefaciens is an obligate respiratory bacterium that is capable of using a variety of oxidized compounds, e.g., nitrate, nitrite, fumarate, Mn(IV) and Fe(III), as terminal electron acceptors in the absence of oxygen (4, 20, 23). Myer and Nealson (14) reported that *S. putrefaciens* MR-1 grown anaerobically with fumarate or nitrate as the electron acceptor translocate protons in response to Fe(III), Mn(IV), thiosulfate or oxygen, but aerobically grown cells translocate protons only in response to oxygen (1). They suggested that anaerobic reduction of Mn(IV) and Fe(III) by *S. putrefaciens* MR-1 is coupled to respiration-linked proton translocation. Metal-reducing bacteria which use solid substrates such as Fe(III) or Mn(IV) as the terminal electron acceptor for anaerobic respiration are presented with a unique problem: they must somehow establish an electron transport across the outer membrane between large particulate metal oxides (e.g., Fe_2O_3 or MnO_4) and the electron transport chain in the cytoplasmic membrane (15). However, there are no reports describing the reduction mechanism of Fe(III) or Mn(IV) by metal-reducing bacteria and which describe the localization of electron carrier components to the outer membrane of the metal reducer. Myers and Myers (17) reported that when *S. putrefaciens* MR-1 is grown under anaerobic conditions, approximately 80% of its membrane-bound cytochromes are localized in its outer membrane, which is confirmed by using a bioelectrochemical method (6, 7). This cytochrome distribution plays a key role in the ability to mediate Fe(III) or Mn(IV) reduction in the anaerobic respiration of the metal reducer. Several researchers (2, 19, 18, 24) have reported that Fe(III) was used as an

* To whom correspondence should be addressed.
(Tel) 82-2-958-5831; (Fax) 82-2-958-5839
(E-mail) bhkim@kist.re.kr

electron sink or minor electron sink in a primarily fermentative metabolism of *Clostridium beijerinckii* and NADH-dependant Fe(III) reductase activity was localized to the bacterial membrane.

In this paper, we report that studies were made to confirm growth properties of *S. putrefaciens* IR-1 and *S. putrefaciens* MR-1 under various cultivation conditions. We report here on the possibility that Fe(III)-reducing bacteria, *S. putrefaciens* IR-1 and *S. putrefaciens* MR-1 may use Fe(III) as an electron sink, and not as an electron acceptor.

Materials and Methods

Organisms and growth

Strain IR-1 was isolated from a paddy field and identified as *Shewanella putrefaciens* by using a biochemical method and 16s rDNA sequence (5), and strain MR-1 was used as a model strain that was kindly donated by Dr. Myers. Both strains were cultivated on carbonate buffered basal medium (CBBM) (22) with 35 mM of lactate, 1.0 g/l of yeast extract and 10.0 g/l of FeOOH as a source of Fe(III). The strain IR-1 was the first iron-reducing bacterium isolated and identified in Korea, which can dissimilatorily reduce Fe(III) to Fe(II) under anaerobic growth conditions and has electrochemical activity because its cytochrome (respiration enzyme) is located on the outer membrane (6).

Cultivation and measurement of growth yields (13)

The electron donors used in the growth test of the metal-reducing bacteria were lactate, pyruvate and acetate-CO_2-H_2. In addition, FeOOH (10 g/l), nitrate (100 mM) and O_2 (aeration rate, 15 l/min/l) were used as the electron acceptors. Variable combinations of electron donors and electron acceptors were used to measure the growth yield of IR-1 and MR-1 as follows: lactate-FeOOH, pyruvate-FeOOH, acetate-CO_2-H_2-FeOOH, lactate-nitrate, pyruvate-nitrate and acetate-CO_2-H_2-nitrate. CBBM with lactate or pyruvate and without the electron acceptors was used as the control. The headspace of acetate-CO_2-H_2-FeOOH and acetate-CO_2-H_2-nitrate medium was pressurized to 30 psig with H_2-CO_2 mixture (4 : 1). The others' headspace was filled with N_2-CO_2 (4 : 1). Growth was made using serum vials (Wheaton Scientific, NJ, USA). The medium was warmed up to 26 ± 1.0°C before being inoculated with 5% of culture, which was pre-cultivated on lactate-FeOOH for 96 hrs and incubated at the same temperature. FeOOH was made from $FeCl_2$ with a neutralization processed by using 10N-NaOH. Growth yield (Yx/s) was determined from Linweaver-Burke plots based on equation [1] (13).

$$rs/X = 1/Yx/s * rx/X + m_s \quad (1)$$

where

rs : substrate consumption rate (mol substrate h^{-1})
rx : cell growth rate (g cell $l^{-1}h^{-1}$)
Yx/s : yield coefficient for biomass on substrate (g cell mol substrate^{-1})
m_s : maintenance constant
x : cell mass (g cell)

Analysis

Organic acids were analyzed by HPLC (Waters Model 510, Milford MA USA) equipped with an Aminex HPX-87H ion exchange column (Bio-Rad, CA, USA). A solution of 0.005 N H_2SO_4 was used as the mobile phase at a flow rate of 0.6 ml per min. Fe(II) was determined by the Ferrozine method (21). Dry cell weight was determined by using a predetermined calibration curve in a function of the absorbance at 660 nm. Dry cell weight (g/l)= 0.365 × Ab_{660}. Nitrate and nitrite were analyzed using an auto-analyzer (Altken Model Slow Solution O^{IV}, Chicago, USA) and the concentration was calculated using a standard calibration curve that was previously made.

Results

Anaerobic growth of metal-reducing bacteria with Fe(III) has been previously reported. In studies using the oxidant pulse technique, the bacterial reduction of Fe(III) was reported to be coupled to the respiratory metabolism (16). However, such studies did not provide unequivocal evidence for energy generation from Fe(III) or Mn(IV) reduc-

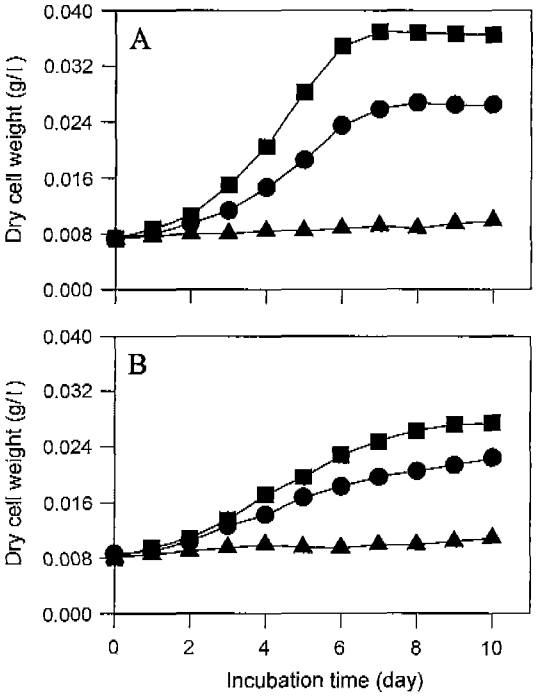

Fig. 1. Growth of *Shewanella putrefaciens* IR-1 (A) and MR-1 (B) on lactate-Fe(III) (●), pyruvate-Fe(III) (■) and H_2-acetate-Fe(III) (▲).

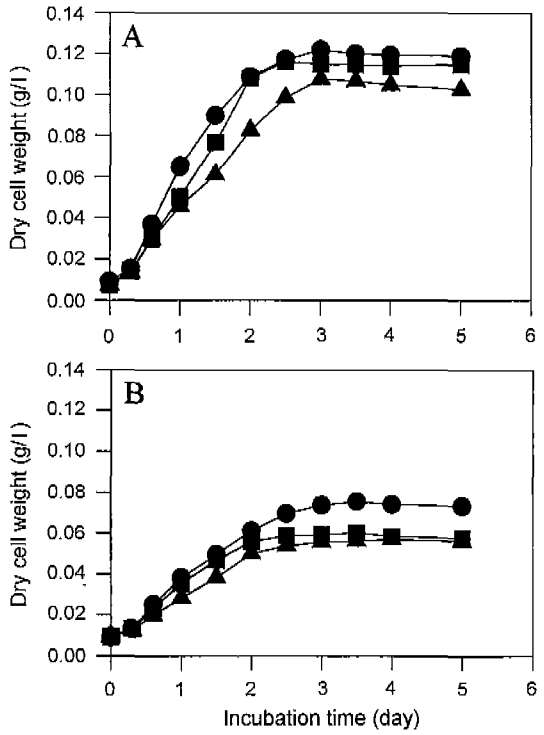

Fig. 2. Growth of *Shewanella putrefaciens* IR-1 (A) and MR-1 (B) on lactate-nitrate (●), pyruvate-nitrate (■) and H_2-acetate-nitrate (▲).

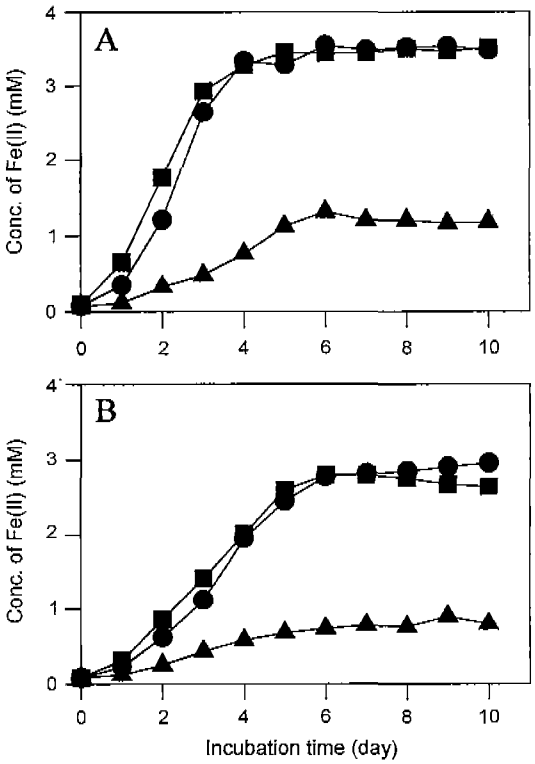

Fig. 3. Reduction of Fe(III) to Fe(II) by growing cells of *Shewanella putrefaciens* IR-1 (A) and MR-1 (B) on lactate-Fe(III) (●), pyruvate-Fe(III) (■) and H_2-acetate-Fe(III) (▲).

tion. Therefore, experiments were conducted to examine the mechanism of Fe(III) reduction that was linked to bacterial metabolism by using the growth properties of IR-1 and MR-1. Growth of IR-1 and MR-1 was measured and compared by using a medium with various combinations of electron donors and electron acceptors. Neither IR-1 and MR-1 grew on lactate or acetate-CO_2-H_2 without an electron acceptor, but fermentatively grew on pyruvate without the electron acceptor (Fig. 1). When nitrate was used as an electron acceptor instead of FeOOH both of the strains grew more actively on lactate, pyruvate and even acetate-CO_2-H_2 (Fig. 2). These results are not consistent with the results previously reported in which Fe(III) acts as an electron acceptor coupled to the energy production of metal-reducing bacteria. When FeOOH was used as an electron acceptor, both strains grew on lactate and pyruvate, but they did not grow on acetate-CO_2-H_2. Reduction of Fe(III) to Fe(II) was shown to be related to the growth properties. About 3.5 and 2.8 mM of Fe(III) was reduced by growing cells of IR-1 and MR-1 on lactate-FeOOH or pyruvate-FeOOH, respectively, but approximately 1.1 mM and 0.8 mM of Fe(III) was reduced to Fe(II) on acetate-CO_2-H_2 (Fig. 3). However, the Fe(III) was not reduced to Fe(II) under an abiotic control test (data not shown).

Growth yields of both IR-1 and MR-1 on lactate-Fe(III) and pyruvate-Fe(III) were 3.5 to 3.7, which are similar to the growth yields of 3.3 to 3.4 when fermentatively growing on pyruvate but much lower than the growth yield of 5.9 to 9.5 on when respiratorily growing on lactate-nitrate, pyruvate-nitrate and acetate-CO_2-H_2-nitrate as shown in Table 1.

Table 1. Growth yield of *Shewanella putrefaciens* IR-1 and MR-1 on different combinations of electron donor and accceptor

e^-donors	e^-acceptors	Carbon sources	[a]Growth Yield (g cell/mole edonor)	
			IR-1	MR-1
Lactate	-	Lactate	No growth	No growth
Pyruvate	-	Pyruvate	3.4±0.3	3.3±0.3
Lactate	FeOOH	Lactate	3.6±0.2	3.6±0.2
Pyruvate	FeOOH	Pyruvate	3.5±0.2	3.7±0.2
H_2	FeOOH	Acetate/CO_2	No growth	No growth
Lactate	NO_3^-	Lactate	6.8±0.3	6.6±0.2
Pyruvate	NO_3^-	Pyruvate	5.9±0.2	6.6±0.1
H_2	NO_3^-	[b]Acetate/CO_2	9.4±0.3	8.5±0.2

[a]Growth yield (g cell/mol e^--donor) corresponds to $Y_{substrate}$
[b]calculated based on the amount of acetate consumption

In dissimilatory nitrate reduction, nitrate is reduced to nitrogen via the intermediates nitrite, nitric oxide and nitrous oxide. The nitrate-reducing bacteria can use nitrate or nitrite as an electron acceptor but can't use nitric oxide and nitrous oxide because they are metabolic intermediates, which are very unstable and bound to special enzymes. For determination of the dissimilatory nitrate

Table 2. The variation of nitrate and nitrite concentration before and after cultivation of *Shewanella putrefaciens* IR-1 and MR-1 which were incubated for 48 hr

Strains	e⁻donors	e⁻acceptors	Concentration of nitrate and nitrite (mM)[a]	
			Before cultivation	After cultivation
IR-1	Lactate	NO_3^-	102.7 (0.8)	13.5 (0.5)
	Pyruvate	NO_3^-	104.1 (0.4)	14.2 (0.6)
	H_2	NO_3^-	101.9 (0.9)	5.9 (0.7)
MR-1	Lactate	NO_3^-	103.3 (1.1)	14.0 (0.8)
	Pyruvate	NO_3^-	101.6 (0.9)	12.4 (0.5)
	H_2	NO_3^-	100.5 (1.0)	7.4 (1.1)

[a]The values in parentheses indicate nitrite concentration.

reduction, nitrate and nitrite concentration were measured before and after cultivation of IR-1 and MR-1 but nitrogen concentration was not measured. As shown in Table 2, the nitrate concentration after cultivation was consumed to below 20% of the values before cultivation and the nitrite concentration after cultivation was similar to or the same as the values before cultivation, which shows the nitrate was not produced from bacterial metabolism.

Discussion

Dissimilatory Fe(III) reduction can be defined as the use of Fe(III) as an external electron acceptor in bacterial metabolism. However, there has been no evidence showing that growth of the metal reducing bacteria was stimulated or ATP production was increased when Fe(III) was offered to the growth medium as an electron acceptor (8, 12). The growth yield of IR-1 or MR-1 fermentatively grown on pyruvate was 3.3 to 3.4 (g cell/mol substrate) which was very similar to the growth yield 3.6 to 3.7 (g cell/mol substrate) of the cells grown on pyruvate-FeOOH or lactate-FeOOH but the growth yield of the cells grown on pyruvate-nitrate and lactate-nitrate was 5.9 to 6.8 (g cell/mol substrate). Neither strain IR-1 nor MR-1 can fermentatively grow on lactate without an electron acceptor because the produced reducing power coupled to oxidation of lactate to pyruvate has to be reoxidized through the electron transport system but can grow on lactate-FeOOH because the reducing power produced coupled to oxidation of lactate to pyruvate can be coupled to reduction of Fe(III) to Fe(II) through the electron transport system as shown in pathway [2] of Fig. 4. The differences of average values of growth yields among the cultures on pyruvate, pyruvate-FeOOH and lactate-FeOOH seemed to be caused by an experimental error because the differences were less than 0.2, which is less than one tenth of Y_{ATP}. Since one ATP is synthesized by acetate kinase in pyruvate oxidation metabolism (Fig. 4), the $Y_{pyruvate}$ 3.5 (g cell/mol substrate) corresponds to the Y_{ATP} of IR-1. The growth yield on cultures with nitrate was more than 5.9 (g cell/mol substrate), which corresponds to about 1.7 ATP that was 0.7 higher than that on the cultures with FeOOH. This result shows that IR-1 and MR-1 can produce ATP through the oxidative phosphorylation coupled to reduction of nitrate as an electron acceptor (Table 2). Nitrate functions as an electron acceptor for the electron transport system but Fe(III) functions as an electron sink. Both IR-1 and MR-1 fermentatively grew on pyruvate but not on lactate. The lactate can not be metabolically oxidized to pyruvate without an electron acceptor because a redox protein reduced by lactate dehydrogenase must be reoxidized through an electron transport system but the reducing power (e.g., Fd. H_2) that produced coupling to the oxidation of pyruvate to acetyl-CoA can be oxidized through the phosphoroclastic reaction without the electron acceptor (Fig. 4). H_2 produced in the phosphoroclastic reaction can act as an electron donor. Given acetate and CO_2 as a carbon source and the proper material as electron acceptors, *Shewanella* sp. is recognized to use H_2 as an electron donor (8). The growth yield of IR-1 and MR-1 grown on acetate-CO_2 with H_2 as the electron donor and nitrate as the electron acceptor was about 9.4 and 8.5 (g cell/mol acetate), respectively, but neither strain grew on acetate-CO_2-H_2 without the electron acceptor nor with FeOOH as an electron acceptor. This result shows that the bacterium uses acetate and CO_2 as its sole carbon source, and that ATP and NAD(P)H can be obtained from oxidation of H_2 coupled to the reduction of nitrate. The reason why bacterium could not grow with FeOOH in the

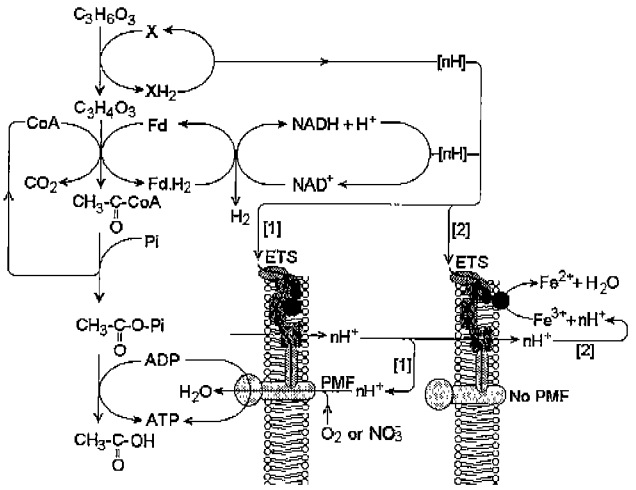

Fig. 4. A schematic diagram of the respiratory pathway [1] and the electron sinking pathway [2] in energy metabolism of *Shewanella putrefaciens*. It is very possible that the ATP may not be produced from a respiratory metabolism in a growing cell by using Fe(III) as an electron acceptor since protons may be consumed outside of the bacterial cell for reduction of Fe(III) to Fe(II). PMF, proton motive force; ETS, electron transport system.

same condition might be caused by the bacterium's inability to synthesize ATP through the respiratory phosphorylation with FeOOH as an electron acceptor as shown in Fig. 4. This is more evidence that Fe(III) functions as an electron sink.

S. putrefaciens and *Pseudomonas* sp. serve as pure culture models for the oxidation of H_2 coupled to Fe(III) reduction. The H_2-oxidizing and Fe(III)-reducing microorganism have been reported to be incapable of autotrophic growth (8). The *Pseudomonas* sp. was reported to require 0.025% yeast extract for growth and *S. putrefaciens* was known to require an addition of 0.02 g each of L-argining hydrochloride, L-glutamine, and L-serine per liter (14). *S. putrefaciens* can also metabolize a variety of organic compounds with O_2 as the electron acceptor but unfortunately, it is very restricted in the organic compounds which can be oxidized with Fe(III) as the sole electron acceptor (23). *Clostridium beijerinckii* was reported to have NAD(P)H-dependent Fe(III) reductase and to utilize Fe(III) as an electron sink in the dissimilatory Fe(III) reduction metabolism (2). These results show that ATP cannot be produced by the H_2-oxidation coupled to Fe(III) reduction in growing cells of the metal reducers. In the growing cells of IR-1 or MR-1 under a condition with Fe(III) as an electron acceptor, all of the reduction potential produced from oxidation of lactate to pyruvate, pyruvate to acetyl-CoA or H_2 to $2H^+ + 2e^-$ was thought to be consumed to reduce Fe(III) to Fe(II) without producing ΔG. Therefore, the ΔG for growth of IR-1 or MR-1 may be produced depending upon only the substrate level phosphorylation under a growth condition with Fe(III) as the sole electron acceptor. When one mole of NADH is oxidized through the electron transport system the $\Delta G_o'$ generated is identified as -206.12 ($KJ/2NO_3^-$) and -209.46 (KJ/Fe(III)) and in cases using NO_3^- and Fe(III) as an electron acceptor, respectively. Based on these data, the growth yield of IR-1 or MR-1 on a medium with NO_3^- or Fe(III) as an electron acceptor may be theoretically similar to each other. However, the growth yield on Fe(III) was indeed much lower than on NO_3^- as shown in Table 1. From these results, it can be proposed that the metal-reducing bacteria, IR-1 and MR-1 use Fe(III) as an electron sink but not as an electron acceptor.

References

1. Coleman, M.L., D.B. Hedrick, D.R. Lovley, D.C. White, and K. Pye. 1993. Reduction of (III) in sediments by sulfate-reducing bacteria. *Nature* 361, 436-438.
2. Dobbin, P.S., J.P. Carter, C.G.D. San Juan, M. von Hobe, A.K. Powell, and D.J. Richardson. 1999. Dissimilatory Fe(III) reduction by *Clostridium beijerincki* isolated from freshwater sediment using Fe(III) maltol enrichment. *FEMS Microbiol.* 176, 131-138.
3. Eric, E.R. and D.R. Lovely. 1993. Dissimilatory Fe(III) reduction by the marine microorganism *Desulfuromonas acetoxidans*. *Appl. Environ. Microbiol.* 59, 734-742.
4. Gossett, J.M. 1987. Measurement of Henry's low constants for C1 and C2 chlorinated hydrocarbons. *Environ. Sci. Technol.* 21, 202-208.
5. Hyun, M.S., B.H. Kim, I.S. Chang, H.S. Park, H.J. Kim, G.T. Kim, and D.H. Park. 1999 Isolation and identification of an anaerobic dissimilatory Fe(III)-reducing bacterium, *Shewanella putrefaciens* IR-1. *J. Microbiol.* 37, 206-212.
6. Kim, H.J., M.S. Hyun, I.S. Chang, and B.H. Kim. 1999. A microbial fuel cell type lactate biosensor using a metal-reducing bacterium, *Shewanella putrefaciens*. *J. Microbiol. Biotechnol.* 9, 365-367.
7. Kim, B.H., H.J. Kim, M.S. Hyun, and D.H. Park. 1999. Direct electrode reaction of Fe(III)-reducing bacterium, *Shewanella putrefaciens*. *J. Microbiol. Biotechnol.* 9, 127-131.
8. Lovley, D.R. 1991. Dissimilatory Fe(III) and Mn(IV) reduction. *Microbiol. Rev.* 55, 259-287.
9. Lovley, D.R., M.J. Baedecker, D.J. Lonergan, I.M. Cozzarelli, E.J.P. Phillips, and I. Siegel. 1989. Oxidation of aromatic contaminants coupled to microbial iron reduction. *Nature* 339, 297-299.
10. Lovley, D.R., S.J. Givannoni, D.C. White, J.E. Champine, E.J.P. Phillips, Y. Gorby, and S. Goodwin. 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, 336-344.
11. Lovely, D.R. and E.J.P. Philips. 1988. Novel mode of microbial energy metabolism: Organic carbon oxidation coupled to dissimilatory reduction of iron or manganese. *Appl. Environ. Microbiol.* 54, 1472-1480.
12. McInerney, M.J. and P.S. Beaty. 1988. Anaerobic community structure from a nonequilibrium thermdynamic perspective. *Can. J. Microbiol.* 34, 487-493.
13. McNei, B. and L.M. Harvey. 1990. Fermentation: A practical approach. IRL Press at Oxford University Press, Oxford.
14. Myers, C.M. and K.H. Nealson. 1990. Respiration-linked proton translocation coupled to anaerobic reduction of manganese (IV) and iron (III) in *Shewanella putrefaciens* MR-1. *J. Bacteriol.* 172, 62632-6238.
15. Myers, C.M. and K.H. Nealson. 1988. Bacterial manganese reduction and growth with manganese oxide as the sole electron acceptor. *Science* 240, 1319-1321.
16. Myers, C.M. and K.H. Nealson. 1988. Microbial reduction of manganese oxides: interactions with iron and sulfur. *Geochem. Cosmochim. Acta* 52, 2727-2732.
17. Myers, C.R. and J.M. Myers. 1992. Localization of cytochromes to the outer membrane of anaerobically grown *Shewanella putrefaciens* MR-1. *J. Bacteriol.* 174, 3429-3438.
18. Munch, J.C. and J.C.G. Ottow. 1977. Modelluntersuchungen zum Cechnanismus der bakteriellen eisenreduktion in hydromorphen boden. *Z. Pflanzenernaehr. Bodenkd.* 140, 549-562.
19. Munch, J.C. and J.C.G. Ottow. 1983. Reductive transformation mechanism of ferric oxides in hydromorphic coils. *Environ. Biogeochem. Ecol. Bull* 35, 389-394.
20. Nealson, K.H. and C.R. Myers. 1992. Microbial reduction of manganese and iron: new approaches to carbon cycling. *Appl. Environ. Microbiol.* 54, 439-443.
21. Phillips, E.J.P. and D.R. Lovley. 1987. Determination of Fe(III)

and Fe(II) in oxalate extracts of sediment. *Soil Sci. Soc. Am. J.* 51, 938-941.
22. Schink, B. and M. Bomer, 1991. The Prokaryotes, 2nd ed. p 1923-1936, Springer Verlag, New York.
23. Semple, K. M. and D.W.S. Westlake. 1987. Characterization of iron-reducing *Alteromonas putrefaciens* strains from oil field fluids. *Can. J. Microbiol.* 33, 366-371.
24. Timble, R.B. and H.L. Ehrlich. 1968. Bacteriology of manganese nodules. III. Reduction of MnO_2 by two strains of nodule bacteria. *Appl. Microbiol.* 16, 695-702.