Biological iron oxidation-reduction and the effects on sulfur oxidation-reduction, denitrification and poly-P accumulation in an anaerobic-oxic activated sludge

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Abstract Iron oxidation and reduction were examined using the activated sludge from a municipal plant. Iron contents of the activated sludge were 1–2%. Iron oxidation rates were correlated with the initial iron concentrations. Iron reducing rates could be described by the Monod equation. The effects of iron reducing bacteria on sulfate reduction, denitrification and poly-P accumulation were examined. Iron reduction suppressed sulfate reduction by competing with hydrogen produced from protein. Denitrification was outcompeted with iron reduction and sulfate reduction. These phenomena could be explained thermodynamically. Poly-P accumulation was also suppressed by denitrification. The activity of iron reduction was relatively high.

Keywords Activated sludge; denitrification; iron oxidation; iron reduction; poly-P accumulation; sulfate reduction

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Introduction

It is known that iron oxidation and reduction occurs in the natural environment such as sediments and soil. Several autotrophic iron oxidation bacteria such as *Ferrobatillus ferroxidans* and *Tobatillus ferroxidance* are well known. Several heterotrophic bacteria such as *Sphaerotilus, Leptothrix and Gallionella* sp. also have iron oxidation activity. Recently, iron-reducing bacteria were also isolated from the sediment. Iron-reducing bacteria (IRB) also grow in the activated sludge (Nielsen, 1996). The authors reported that the sulfur oxidation-reduction cycle was established in the activated sludge, and influenced the microbial population in the sludge (Yamamoto-Ikemoto *et al*., 1994, 1996, 1998a). When iron coagulants are used in order to remove phosphate and improve settling characteristics, IRB grow predominantly (Yamamoto-Ikemoto *et al*., 1998b). Then PAB and SRB are suppressed. It is very important to know the iron cycle in the activated sludge and its interactions with the microbial community. In this study, iron oxidation and reduction were examined using the activated sludge from a municipal plant. Next, the effects of iron reducing bacteria on sulfate reduction, denitrification and poly-P accumulation were examined.

Materials and methods

Activated sludge from a municipal plant operated in the anaerobic-oxic conditions was used in the experiment. Filamentous bulking due to type 021N frequently occurred in the plant. The iron contents in the sludge were analyzed by the atomic analyzer after ultrasonication. Using the sludge the batch experiments were conducted under several conditions. Oxic batch experiments O11 were carried out in a conical flask. Anaerobic and anoxic batch experiments (A11–A16, A21–34) were carried out in BOD bottles.

Results and discussion

Biological iron oxidation and reduction by the activated sludge

Table 1 shows the iron contents in the activated sludge. Influent iron concentrations were very low or were not detected. However the iron was accumulated in the sludge by oxidation. Figure 1 shows the results of the batch experiment in the oxic conditions without sulfide O11. Ferrous iron was immediately oxidized to ferric iron. When the activated sludge was added, iron oxidation did not occur. Figure 2 shows the relationship between the initial iron concentration and the iron oxidation rate. The iron oxidation rates were dependent on the initial iron concentrations. Figure 3 shows the typical results of the anaerobic experiment without sulfate A12. Ferric iron was reduced to ferrous iron. Iron reducing rate could be described by the Monod equation (Figure 4). These results suggested that biological iron oxidation and reduction occurred and the iron cycle was established in the activated sludge. Table 2 summarizes the results of the anaerobic batch experiments using several substrates without sulfate A14–16. When peptone was supplemented in the substrate (A14 and A15), iron reduction occurred at the highest rate. When acetate and propionate were supplemented (A14 and A15), these organic acids decreased by iron reduction. Hydrogen was also utilized by iron reducing bacteria (A16). These results suggested that iron reducing bacteria utilized organic matter and/or hydrogen produced from protein.

Table 1 Iron contents in the anaerobic-oxic activated sludge

Date	6.29.97	8.6.97	10.22.97	10.23.97	11.4.97	11.12.97	9.8.99	9.20.99	10.18.99	11.4.99	11.18.99	12.2.99	12.13.99	12.16.99	12.22.19	1.12.00	1.17.00	Average
Iron content (mg/gMLSS)	9.2	7.8	10.6	9.8	12.5	18.5	11.7	8.5	8.8	9.5	10.5	9.5	9.7	10.7	7.5	13.7	13.7	10.7

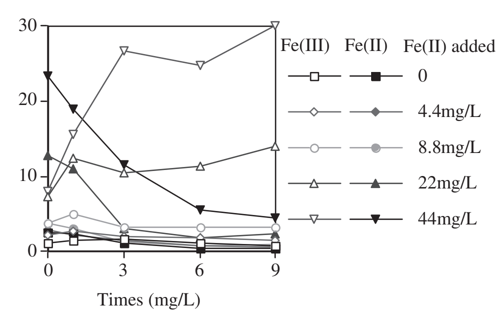

Figure 1 Results of the iron oxidation experiment (O11) rate

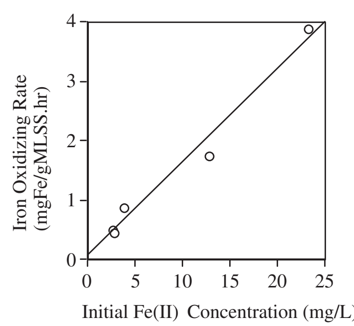

Figure 2 Effects of initial Fe(II) concentration on iron oxidizing rate

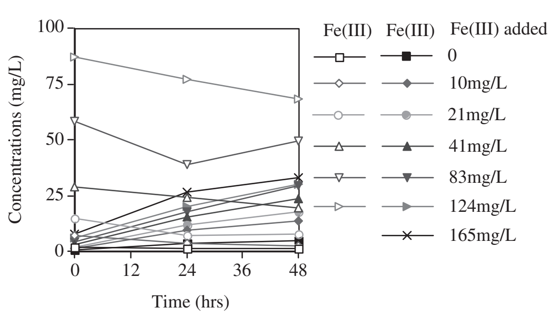

Figure 3 Results of the iron reduction experiment (A12)

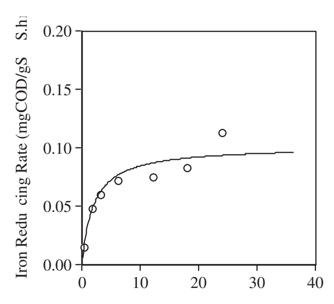

Figure 4 Effects of initial Fe(III) concentration on iron reducing rate

Table 2 Reduced iron, produced acetate and produced propionate

Exp. No.	Substrate	Decreased Fe(II) (mg/L)	Increased acetate (mg/L)	Increased propionate (mg/L)
A14	I + Ace	4.8		13.7
	I + Ace + Fe	13.3	−29.0	5.7
	I + Pro	3.2	12.9	−9.4
	I + Pro + Fe	13.0	9.2	−18.2
	I + Peptone	2.7	24.2	12.0
	I + Peptone + Fe	17.0	25.3	13.3
A15	I	3.5	2.3	2.4
	I + Fe	8.5	2.7	0.0
	I + Ace	2.3	−14.5	6.2
	I + Ace + Fe	8.8	−1.5	7.6
A16	I + H_2	−0.5	2.7	2.5
	I + H_2 + Fe	4.6	0.0	0.0
	I + N_2	−1.0	0.0	−0.8
	I + N_2 + Fe	−.5	0.0	−0.9

Effects of iron reduction on sulfate reduction

Figure 5 shows the typical results of the anaerobic batch experiments without nitrate (A33). Sulfate reduction occurred when only sulfate was added to the substrate as an electron acceptor. Ferric iron accumulated in the sludge was reduced to ferrous iron. When both sulfate and ferric iron was added, sulfate reduction and iron reduction occurred simultaneously. Sulfate reduction rate was lower than that when iron was not added. When sulfate reduction was suppressed by sodium molybdate, iron reduction also occurred, meaning that biological iron reduction was the main reaction. Since iron reduction was accelerated with

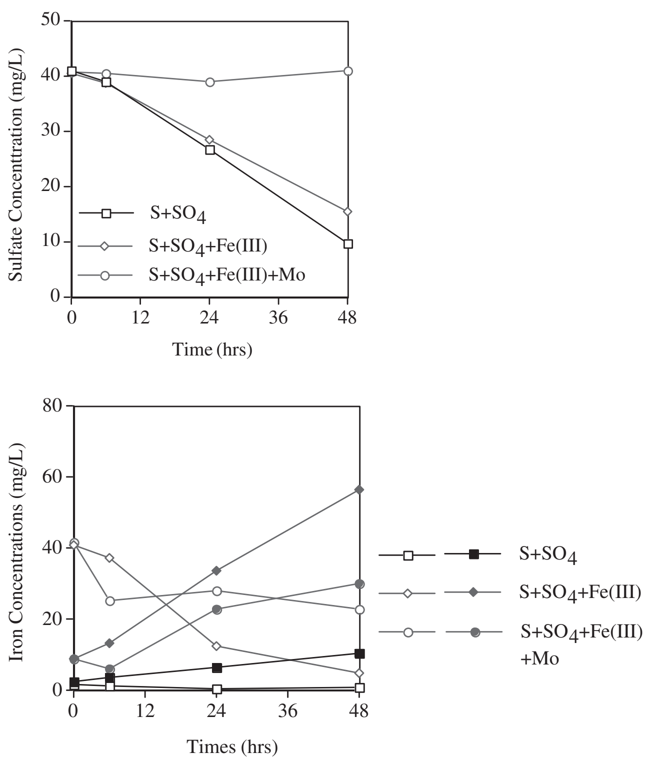

Figure 5 Results of the batch experiments in the anaerobic conditions (A33)

Table 3 Reduced sulfate and Fe(III) and produced organic acids

Exp. No.	Substrate	Decreased SO_4 (mg/L)	Decreased Fe(II) (mg/L)	Increased acetate (mg/L)	Increased propionate (mg/L)
A27	$I + SO_4$	0.4	0.5	8.1	1.7
	$I + SO_4 + Fe$		22.6		
	$I + SO_4 + Fe + Mo$	0.0	6.1	2.5	7.9
A28	$I + SO_4 + Pro$	26.6	3.3	20.1	1.7
	$I + SO_4 + Pro + Fe$	0.6	14.9	15.1	1.8
	$I + SO_4 + Pro + Fe + Mo$	0.0	7.8	17.9	5.1
A29	$I + SO_4$	20.6	4.7	27.4	10.2
	$I + SO_4 + Fe$	20.3	30.2	34.2	11.3
	$I + SO_4 + Fe + Mo$	2.2	15.3	21.1	16.8
A30	$I + Peptone + SO_4$	31.7	2.8		28.8
	$I + Peptone + SO_4 + Fe$	34.3	31.7	60.9	31.7
	$I + Peptone + SO_4 + Fe + Mo$	0.0	18.2	54.5	39.3
A31	$I + H_2 + SO_4$	29.1	7.8	3.7	3.7
	$I + H_2 + SO_4 + Fe$	18.3	17.5	3.5	3.5
	$I + H_2 + SO_4 + Fe + Mo$	0.3	10.6	7.0	7.0
A32	$I + N_2 + SO_4$	6.1	1.2	8.5	3.8
	$I + N_2 + SO_4 + Fe$	9.9	13.0	16.9	7.0
	$I + N_2 + SO_4 + Fe + Mo$	0.0	7.0	8.8	6.8
A33	S + SO4	31.2	7.7	52.2	33.7
	$S + SO_4 + Fe$	25.0	44.2	54.3	34.5
	$S + SO_4 + Fe + Mo$	0.0	23.6	37.7	39.0
A34	$I + Ace + SO_4$	16.3	6.2	24.0	13.3
	$I + Ace + SO_4 + Fe$	17.7	53.6	33.1	20.4
	$I + Ace + SO_4 + Fe + Mo$	0.0	17.5	18.2	22.9

sulfate reduction, a part of iron might be reduced by chemical reaction with sulfide. Suppression of sulfate reduction was much greater than we assume that the sulfide was re-oxidized to sulfate by iron reduction. Table 3 summarizes the results of the anaerobic batch experiments using several substrates. When iron reduction occurred, the sulfate reducing rate decreased using the substrate supplemented with propionate and peptone (A28, A30, A33). Acetate increased with iron reduction. When hydrogen was added as the electron donor (A31), sulfate reduction was also suppressed by the iron reduction. However, production of organic acids was not recognized. On the other hand, when the mineral substrate and the substrate supplemented with acetate was used (A27, A29, A32, A34), iron reduction did not affect sulfate reduction. Since the electron donor was not added to the substrate in these conditions, iron reducing bacteria and sulfate reducing bacteria might utilize electron donor accumulated in the sludge. Therefore the competition of electron donor might not occur. These results suggest that iron reduction suppressed sulfate reduction by competing with small molecular organic substances and/or hydrogen produced from peptone.

Interactions among sulfate reduction, iron reduction, denitrification and poly-P accumulation

Figure 6 shows the typical results of the anoxic batch experiments A26. When nitrate was added to the substrate as the electron acceptor (anoxic condition), denitrification occurred predominantly, and iron reduction was inhibited. After nitrate disappeared, iron reduction occurred. The authors reported that sulfate reduction was also suppressed in the anoxic conditions (Yamamoto-Ikemoto *et al.*, 1996). These results indicated that the interactions among denitrification, iron reduction and sulfate reduction could be explained thermodynamically. Poly-P accumulation was also suppressed in anoxic conditions. Since denitrification bacteria completely oxidized organic substances, poly-P bacteria could not utilize organic acids.

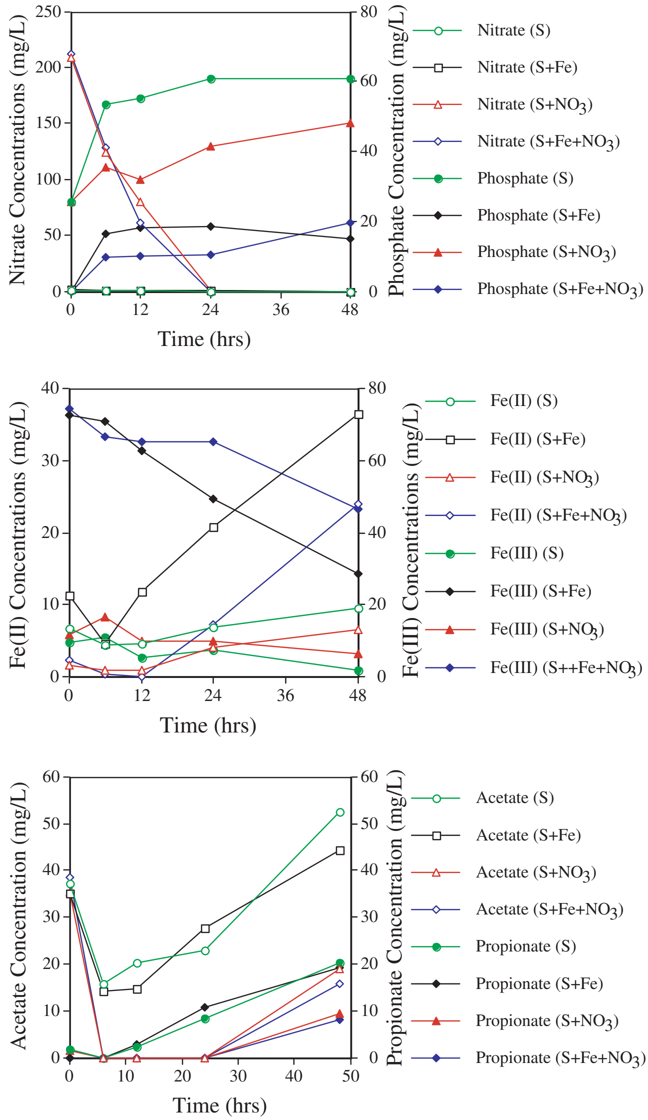

Figure 6 Results of the batch experiments in the anoxic conditions (A26)

The iron reducing activity and the sulfate reducing activity in the activated sludge were 0.05~0.2 mgCOD/gMLSS.hr and 0.2~0.8mgCOD/gMLSS.hr respectively. Although the iron concentration in the influent sewage was much lower than sulfate concentration, the iron reducing activity was almost 1/4 of sulfate reducing activity. These results indicated that when the iron was added in the aeration tank to improve phosphate removal and settling characteristics, iron reducing activity increased in the activated sludge.

Conclusions

Iron contents of the activated sludge in a municipal plant were 1–2%. They were accumulated in the sludge by microbial oxidation. The iron oxidation rates were correlated with the initial iron concentrations. The iron reducing rates could be described by the Monod equation. Iron reduction suppressed sulfate reduction by competing with hydrogen produced from protein. Denitrification was outcompeted with iron reduction and sulfate reduction. These phenomena could be explained thermodynamically. Poly-P accumulation was also suppressed by denitrification. The activity of iron reduction was relatively high.

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