B－26 A STUDY OF SULFUR OXIDATION/REDUCTION AND IRON OXIDATION/ REDUCTION IN ANAEROBIC- OXIC SLUDGE OF A MUNICIPAL WASTEWATER PLANT

都市下水処理場嫌気好気性汚泥における硫黄の酸化還元及び鉄の酸化還元の相互作用

○ E.K Bosque-Hamilton (Civil Engineering Dept.Kanazawa University)
R Yamamoto-Ikemoto (same as above)
T. Komori (same as above)
T. Takekuma (same as above)

1. Introduction

Because iron is transported as part of the wastewater or is added as an agent for phosphorus removal, [1)] knowledge of interactions among iron related bacteria and other processes in activated sludge such as sulfate reduction and sulfide oxidation is essential in understanding the complex microbial dynamics of the activated sludge system. For the role of the sulfate reduction bacteria (SRB) in the activated sludge, the authors[1,2)] reported that sulfate reduction was a main cause of filamentous bulking due to Type 021N that can utilize sulfide for an energy source. It is expected that when the iron coagulant is used, sulfides may be precipitated and have an influence on the bulking properties of the sludge and also influence sulfur and iron redox. Few studies have considered interactions between SRB and iron bacteria in activated sludge. The purpose of this study therefore was to examine interactions between 1)sulfate reducing bacteria (SRB) and iron reducing bacteria (IRB) under anaerobic conditions and 2) sulfide oxidizing bacteria (SOB) and iron oxidizing (IOB) under aerobic conditions using municipal wastewater activated sludge.

2. Materials and Methods

Activated sludge used in the batch experiments was collected from the Kanazawa City municipal waste plant between August 6th and November 12th, 1997. For SRB and IRB interactions, one gram of centrifuged activated sludge was put into BOD bottles, and the substrate added and purged by nitrogen gas to ensure anaerobic conditions. Agitation of the reactor contents was done by magnetic stirring. The bottles were then incubated at 20°C, opened at fixed intervals and the samples filtered for analysis. Anthroquinone (10 ppm) was used in inhibiting sulfate reduction while $FeCl_3$ (100mg/l) was added to induce iron reduction. A control setup consisted of no additions, except for the artificial waste water. For SOB and IOB interactions, two grams of centrifuged sludge, 100 ml of mineral substrate (Table 1), 1 ml of sodium sulfide solution (100mg/l) were put into 500ml conical flask and aerated for 9 hrs. 100 mg/l of $FeCl_2 \cdot nH_2O$ was used to induce iron oxidation. Aeration was done by an IWAKI pump with airflow rate fixed at 2 L/min. The activated sludge was sampled at 3 hour intervals and immediately filtered by a 0 45μm filter membrane for analysis. The sulfur and iron oxidizing rates were obtained from the concentration-time profiles. Sulfate was measured by ion chromatography. Acetate and phosphate were analyzed by a HPLC using electron conductive detection with post column pH buffer. The total iron, ferric and ferrous ionic content were measured by the phenanthroline method after pH adjustment of the samples. All the experiments were conducted at 20°C. All substrate combinations are shown in Table 2.

Table 1 Composition of artificial wastewater (mg/l)

	AEROBIC PROCESS	ANAEROBIC PROCESS
CH_3COOK	-	66.7
Polypeptone	-	133.0
Yeast extract	-	13.3
$NaHCO_3$	23.7	23 7
KCl	58.0	58.0
$MgSO_4$ $7H_2O$	105.0	105.0
$CaCl_2.2H_2O$	17.3	17.3
KH_2PO_4	30.7	30.7

Table 2 Substrate combinations
(S=sludge, ant=anthroquinone)

Process	Activated Sludge and substrate combinations
Anaerobic	S,S+$FeCl_3$,S+anth, S+anth+$FeCl_3$,
Aerobic	S,S+$FeCl_2$;S+Na_2S; S+Na_2S+$FeCl_2$

3.Results and Discussion

Table 3 shows the measured total iron content of waste plant. During the sampling period the iron concentration ranged from 7.8 to 18.5 mgFe/gMLSS. These concentrations were smaller in value as compared to those reported in the literature[4)] Since the plant is operated as an anaerobic-oxic plant, the low Fe concentrations may not be significant enough for iron oxidation or reduction although the potential do exists. Fig1 shows the typical concentration-time profile of aerobic batch experiments (SO_4 and iron profile). The addition of $FeCl_2$ decreased the sulfate concentration. The sulfate produced by Na_2S addition only was about 17mg/l. Fe oxidation reduced this value to 9.1mgSO4/gMLSS implying that the introduction of ferrous oxidation reduced the activity of sulfide oxidizing bacteria. On Oct 21, when the same experiment was repeated with a freshly sampled sludge sample from the waste plant, iron oxidation reduced the produced sulfate from 34 to 27mgSO_4/gMLSS. This implies that iron in reasonable quantities in the aeration basin of an activated sludge plant can somewhat suppress sulphide oxidation. The consumption of Fe^{2+} corresponded to the generation of Fe^{3+} In the Oct 21 experiment, 24.8mgFe^{2+}/gMLSS was oxidized while 4.18mgFe^{3+}/gMLSS was reduced. In the second experiment, 11mgFe^{2+}/gMLSS was oxidized while 2.5mgFe^{3+}/gMLSS was reduced. The discrepancy in the ferrous and ferric values may be accounted for by other factors in the iron oxidation system. Table 4 show the results of generated sulfate an and for the sulfide oxidation experiments conducted on Oct 21 and Oct 28 at 20°C IOB are able to obtain their energy for respiration from the oxidation of sulfur and iron. Therefore these bacteria are expected to be quite active when significant amounts of iron and sulfide are present in the sludge. The iron oxidation system of Thiobacillus ferroxidans is inhibited during growth on sulfur [5)] . In our experiments however, iron inhibits sulphide oxidation.

Table 3 Iron content in activated sludge.

Date	Total iron (mgFe/gMLSS)
Aug 6	7.8
June 29	9.16
Oct 22	10.6
Oct 23	9 8
Nov 4	12.5
Nov 12	18.5

Table 4 Results of aerobic experiments <Oct 21>

	ΔS	ΔFe^{2+}	ΔFe^{3+}
blank	0.02	-	-
Na_2S	1.8	-	-
Na_2S + $FeCl_2$	1.01	2.75	0.45

Table 4 Results of aerobic experiments < Oct 28>

	ΔS	ΔFe^{2+}	ΔFe^{3+}
blank	0.01	-	-
Na_2S	3.8	-	-
Na_2S + $FeCl_2$	3.0	1.22	0.3

Units: ΔS=mgSO_4/gMLSS hr,
ΔFe^{2+}(mgFe^{2+}/gMLSS hr)
ΔFe^{3+}=(mgFe^{3+}/gMLSS hr)

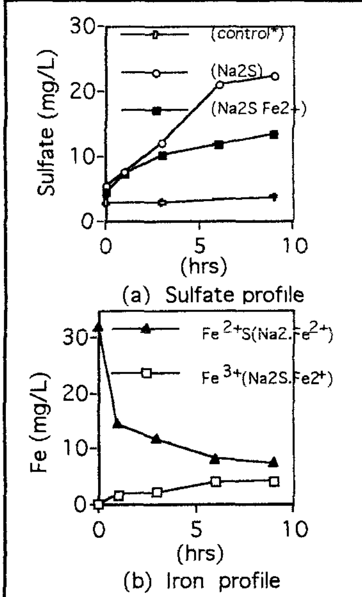

Figure 1 Concentration-time profile of aerobic batch experiments (SO_4 and Fe profile)

3.1 Interactions between SRB and IRB

Table 5 shows the results of the metabolic rates obtained from the anaerobic batch experiments. The values presented are the net produced or consumed species after 48 hours of batch testing. It is obvious that both iron reduction and sulfate inhibition and an effect on acetate and general metabolic activity. Because different sludges were used for the batch tests, the obtained values fluctuated but generally a pattern was established. In the first experiment (Sept 29), iron reduction caused a reduction of produced of acetate from 0.41 to 0.21 mg Acetate/gMLSS.hr. The phosphate releasing rate also dropped from 0.12 to 0.08mgPO_4/gMLSS.hr. The sulfate reducing rate also dropped. Similar results were obtained for the rest of the batch experiments. This suggests that IRB probably outcompeted SRB for organic substrates.

The substrate electron flow between IRB and SRB was evaluated in terms of the results are presented in Table 6. The calculated electron flow percentages for SRB ranged from 18.7 to 31% and IRB ranged from 68.9 to 83.3% This showed that IRB outcompeted SRB in all the anaerobic experiments. Although IRB outcompeted SRB, it is possible that SRB had an influence on IRB.

3.2 Effect of Fe reduction on p phosphate release

Although observed high potential Fe(III) reduction rate might also be responsible for a significant chemical phosphate release due to reduction of Fe(III) to Fe(II) in clarifiers[4], sludge storage tanks and anaerobic tanks with biological P removal; our results showed that Fe reduction tended to give poor phosphate release. In the anaerobic experiments, phosphate values were depressed, for example in experiment 2, phosphate releasing rate dropped from 0.28 to 0.07mgPO$_4$/gMLSS hr so the only possible reason may be due to phosphate precipitation by Fe(III) since iron reduction has been associated with higher phosphate release. It is obvious from the various experimental results that there exists some kind of interactions among the iron based bacteria and SRB, SOB and poly p bacteria. In activated sludge where both reduction and reoxidation took place in the treatment plant. The results show that iron based activities oxidation and reduction can suppress other processes in the activated sludge system. Further investigation is necessary for a better understanding.

TABLE 5 Metabolic rates of anaerobic batch experiments

DATE	Exp No	SUBS-TRATE ADDED	ΔAcetate (mgAcetate/gMLSS hr)	ΔPO4 (mgPO$_4$/g MLSS hr)	ΔSO_4 (mgSO_4/g MLSS hr)	ΔFe^{2+} (mgFe^{2+}/g MLSS hr)
Sept29	1	CONT	0 41	0.12	0 13	nd
		Fe^{2+}	0.21	nd	0 12	nd
		Fe^{3+}	0.18	0 08	0.11	nd
Oct 21	2	CONT	0.76	0 28	0.25	-
		Fe^{3+}	0.46	0.07	0.14	0 44
		ANT	0.51	0.28	0 04	0 20
Oct 28	3	CONT	0.31	0.21	0.13	0.09
		Fe^{3+}	0.30	0.10	0.06	0.45
		ANT	0.34	0.18	0.05	0.12
Nov11	4	CONT	1.35	0.16	0.57	0.16
		Fe^{3+}	1.04	0.12	0.56	0 86
		ANT	1.38	0.16	0 06	0.13

Cont- Control experiment. No $FeCl_3$ was added, Fe^{3+}- $FeCl_3$ was added, S=sludge, ANT - anthroquinone, nd= not determined

Table 6 Electron flow between SRB and IRB

Exp	*SO_4	% flow (SRB)	*Fe^{2+}	% flow (IRB)
1	nd	nd	nd	nd
2	0.12	26.64	0.33	73.35
3	0.06	18.73	0.28	81.26
4	0.28	31.06	0.62	68 93

* concentration in moles

4. Conclusion

Interactions among iron reduction-oxidation, sulfate reduction and phosphate release was studied by batch tests using sludge from a municipal waste plant. The following conclusions were drawn: 1) In the aerobic experiments, iron oxidation also reduced the sulfide oxidation capacity, 2) Iron reduction was responsible for lowering sulfate reduction and phosphate release activities. 3) Inhibition of SRB led to a decrease in iron reducing ability implying that SRB may play a role in iron reduction processes. 4)These results indicated that the iron bacteria in the presence of sulfur in activated sludge is important and further tests are necessary.

5. References

1) Luedecke C, Hermanowicz S W and Jenkins D Precipitation of ferric phosphate in activated sludge a chemical model and its verification Wat Sci Tech, Vol 21 pp325-337, 1989

2) Yamamoto Ikemoto R., Komori T And Matsui S. 1992 Influence of sulfate reduction on filamentous bulking in activated sludge cultivated with acetone and peptone Proceedings of Environmental and Sanitary Engineering Research Vol 32, pp 93-103. Committee on Environmental Engineering Japan Society of Civil Engineers (In Japanese)

3) Yamamoto Ikemoto R, Komori T and Matsui S 1991 Filamentous bulking and hindrance of phosphate removal due to sulfate reduction in activated sludge. Water Science Technology, Vol 23 927 - 935.

4) Nielsen P H, . The significance of microbial Fe(III) reduction in the activated sludge process Wat Sci Tech Vol 34, pp 129-136, 1994

5) Kulpa C F, MjoliN, Rosky M T (1986) Comparison of Iron and Sulfur Oxidation in thiobacillus ferrooxidans, Inhibition of Iron Oxidation by growth on Sulfur.Biotech Bioeng. Symp No 16