(17) SIMULTANEOUS MICROBIAL SULFUR AND IRON OXIDATION-REDUCTION AND PHOSPHATE RELEASE IN ANAEROBIC-OXIC ACTIVATED SLUDGE

嫌気好気汚泥における硫黄と鉄の生物的酸化還元およびりん放出の相互作用

Edja. K Bosque-Hamilton[1], Ryoko Yamamoto-Ikemoto[2], Tomoaki. Komori[2] and T. Takekuma[1]
エジャコフィボスクハミルトン[1]、池本良子[2]、小森友明[2]、武隈貴裕[1]

Abstract; Simultaneous sulfur and iron oxidation-reduction in anaerobic-oxic activated sludge were examined using activated sludge of a municipal wastewater treatment plants (WWTP) and two laboratory scale plants (AO) and (O). Batch experiments were carried out using artificial wastewater. For the WWTP sludge, the sulfate reducing rates increased with maximum released phosphate but did not show the same relationship with the AO and O sludge. Sulfate reduction rates tended to increase with sulfide oxidation rates for both wastewater treatment and laboratory scale sludge. Under oxic conditions, both sulfide and iron oxidation occurred simultaneously and iron was responsible for lowering sulfide oxidation rates. In anaerobic conditions, sulfate and iron reduction occurred simultaneously with sulfate inhibition by the iron bacteria. Phosphate release tended to increase with a decrease in iron reduction. The results from the electron flow and activity calculations suggested that although the sulfate reducing bacteria utilized more substrate electrons than iron reducing bacteria, the iron reducing activity was enough to cause inhibition of sulfate reduction.

Keywords: Sulfide oxidation, sulfate reduction, iron reduction, iron oxidation, phosphate release

1. Introduction

Iron is a natural constituent of wastewater or is added as an agent for phosphorus removal 1) 2). Nielsen3) showed that iron based respiration might be important mechanisms for acetate production in activated sludge. Iron also reduces phosphorus complexation capacity which results in dissolution of chemically bound phosphate. Ferric iron also plays an important structural role in floc formation in activated sludge by bridging anionic exopolymers3). Although it has also been suggested that sulfate reduction can be an important and previously unrecognized agent for Fe(III) reduction in marine and groundwater environment,4) few studies have looked at simultaneous microbial sulfur and iron processes in the activated sludge process. Sulfate reduction has been reported to be one cause of filamentous bulking in activated sludge due to *Type021N* that can use sulfide as an energy source5) 6)7). Sulfate reduction has also been known to hinder phosphate removal in activated sludge6). The use of iron salts as coagulants has been known to suppress filamentous bulking and sulfate reduction in a continuous laboratory scale activated sludge plant.8) The presence of iron causes precipitation of sulfide and influences the sulfur and iron redox properties. Knowledge of interactions among iron related bacteria and other processes in activated sludge such as sulfide oxidation, sulfate reduction and phosphate release is important.

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1 Student, Graduate School of Natural Science and Technology, Kanazawa University 金沢大学大学院自然科学研究科
2 Department of Civil Engineering, Kanazawa University 金沢大学工学部

This paper therefore investigates interactions between 1) sulfate reducing bacteria (SRB), iron reducing bacteria (IRB) and phosphate release under anaerobic conditions and 2) sulfide oxidizing bacteria (SOB) and iron oxidizing bacteria (IOB) under aerobic conditions using municipal wastewater and a laboratory cultivated activated sludge. The interactions between the SRB and SOB were also investigated.

2. Materials and Methods

2.1 Source of Sludge

The activated sludge that was used in the experiments was collected from the Kanazawa City municipal wastewater treatment plant (referred to as WWTP). Activated sludge was collected from the recycle line from the final clarifier and transported to the laboratory within 45 minutes. The MLSS and SVI at phase 1 were measured soon after sampling.

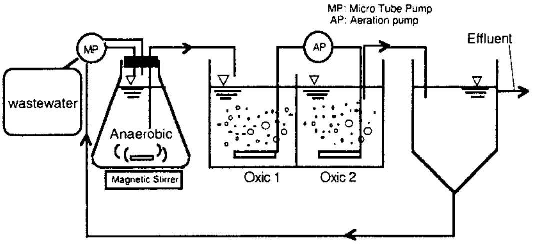


Figure 1 Laboratory setup of Anaerobic-Oxic (AO) sludge

2.2 Continuous Lab experiments.

Two continuous laboratory scale plants were operated. The first unit (Anaerobic-Oxic, referred to as AO) consisted of one 2.5-liter anaerobic flask under constant stirring, two 3-liter aeration tanks and a 6-liter settling tank as shown in Figure 1. Three hundred mls of effluent was withdrawn daily. The artificial wastewater composition used in cultivating the sludge is shown in Table 1. The second unit (referred to as O) consisted of two 3-liter aeration tanks and a settling tank as shown in Figure 2. The artificial wastewater composition was the same as used for the AO unit. Three hundred mls of effluent was withdrawn daily. The O unit was run for about 75 days, after which 0.0021 L/day of poly-ferrous sulfate (PFS) was added to the artificial waste. Activated sludge collected from the plant from this period was used for the storage experiments. Both AO and O units were inoculated with WWTP sludge. The HRT and SRT were 6 hours and 20 days for both units.

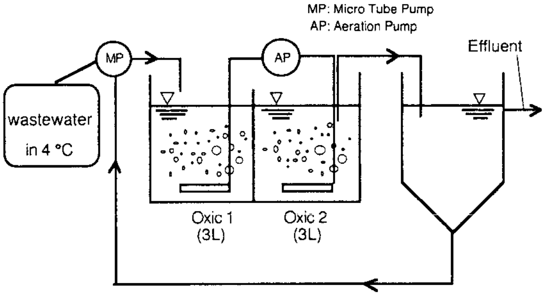


Figure 2 Laboratory setup of Oxic (O) sludge

Table 1 Basic composition of artificial wastewater (mg/l) for batch experiments.

	Aerobic	Anaerobic*
CH_3COOK	-	66.7
poly-peptone	-	133.3
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

* Same composition for both laboratory scale setup.

2.3 Aerobic batch experiments

WWTP, AO and O activated sludge were concentrated by centrifuging at 5,000G, 4°C for 10 minutes. Two grams of centrifuged sludge, 100 ml of substrate (Table 1) and Na_2S (100mg/l) were put into 500ml conical flask and aerated for 9 hrs. 100 mg/l of $FeCl_2.7H_2O$ was added 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 with a 0.45μm membrane filter for chemical analysis. To find out whether the sulfide oxidation was due to either biological or chemical activity, experiments were repeated with sludge which had been concentrated by centrifugation and pasteurized at 130°C for 5 minutes.

2.4 Anaerobic batch experiments

The activated sludge used were first subjected to aeration for 3 hours with KH_2PO_4 for phosphate uptake to occur. In studying sulfate and iron reduction, one gram of centrifuged activated sludge was put into BOD bottles, and artificial wastewater (Table 1) was added. The bottles were stripped with N_2 gas by bubbling to ensure anaerobic conditions. The bottles were stirred by a magnetic stirrer, kept in an incubator and opened at fixed intervals for sampling. Samples were filtered for chemical analysis with 0.45μm membrane filter. Ten mg/l of anthroquinone ($C_{14}H_8O_2$), was added to the artificial wastewater to inhibit sulfate reduction while $FeCl_3$ (100mg/l) was added to induce iron reduction. A control setup consisted of no additions, except for settled sewage. All the experiments were conducted at 20°C.

2.5 Storage Experiments

Two anaerobic storage experiments were carried out using the sludge effluent from the O laboratory unit. As already stated in section 2.2, the unit was operated for about 75 days before PFS was added. In the first storage experiment, sludge was sampled from the unit before PFS addition. After PFS was added to the influent wastewater, activated sludge (referred to as iron amended sludge) was collected again for the storage experiment. The sludge was put into one liter flask mixed with artificial waste water (as shown in Table 1) and covered with a butyl cap. Samples were taken over a 11 day period for analysis.

2.6 Chemical analysis

Samples for chemical analysis were first centrifuged at 5000g, 4°C and filtered with 0.45μm membrane filter paper. Organic acids (acetate and propionate) and phosphate were analyzed by HPLC using electron conductive detection with post column pH buffer (Shimadzu LC 10A). Ferric and ferrous iron were measured by the 1,10 phenanthroline method after acidic adjustment of the samples (pH< 1) by HCl. Total iron was measured by an atomic analyzer (Shimadzu AA6000). TOC determinations were done on a Shimadzu 5000 TOC analyzer. Sulfate was measured on an ion chromatograph (Shimadzu Type HIC6A). The sulfur and iron oxidizing/reducing rates and maximum released phosphate values were obtained from their concentration-time profiles.

2.7 Electron flow calculations

With iron and sulfate reduction occurring simultaneously under anaerobic conditions, the substrate electron flow between SRB and IRB was calculated from the stoichiometric half reactions shown in equations 1 and 2 with the assumption that all organic substrate present was available to both SRB and IRB for heterotrophic metabolism. The reduction of SO_4 to HS^- requires 8 electrons while the reduction of the ferric ion requires one electron. The half reactions[9)] are shown as follows;

$$1/8\ SO_4 + 9/8\ H^+(pH) + e = 1/8\ HS^- + 1/2\ H_2O \qquad p\varepsilon^0 = -3.75 \text{ -----} \textit{for sulfate reduction} \qquad (1)$$

$$FeOOH(s) + HCO_3^-(10^{-3}) + 2\ H^+(pH) + e = FeCO_3(s) + 2H_2O \qquad p\varepsilon^0 = -0.8 \text{ ---} \textit{for iron reduction} \qquad (2)$$

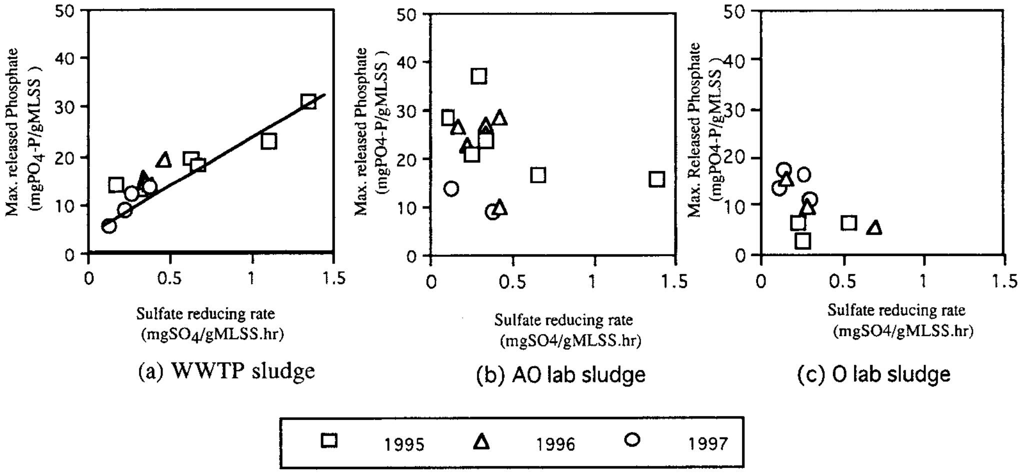
The percentage of electron flow by SRB is given by [A/(A+B)] x 100 where A= electron moles of sulfate reduced. The percentage electron flow by IRB is given by [B/(A+B)] x 100 where B= electron moles of ferrous iron produced.

3. Results and Discussion

3.1 Relation among sulfate reducing, sulfide oxidizing and poly-p bacteria in the sludge.

Aerobic and anaerobic batch experiments were conducted periodically with activated sludge using the WWTP, AO and O activated sludge. Figure 3 shows the relation between the sulfate reducing rates and the maximum released phosphate for the anaerobic batch experiments that was obtained from 1995 to 1997. The maximum phosphate tended to increase with the sulfate reducing rates for the WWTP sludge. The maximum released phosphate ranged from 13.8 to 30.9mgPO$_4$/gMLSS for 1995 whilst the sulfate reducing rates ranged from 0.15 to 1.3mgSO$_4$/gMLSS.hr. In 1997, the maximum released phosphate ranged from 5.7 to 13.5mgPO$_4$/gMLSS and the sulfate reducing rates ranged from 0.12 to 0.38mgSO$_4$/gMLSS.hr. The increase in released phosphate values with increasing sulfate reducing rates can be attributed to the generation of acetate by SRB for metabolism by poly-p bacteria.

The generation of acetate by SRB was confirmed by the experiments in which anthroquinone was used for inhibition of SRB. Inhibition of SRB caused a decrease in produced acetate (Fig 5). However in the laboratory scale setup (AO and O), the sulfate reducing rates did not increase with increase in released phosphate. This is probably due to the availability of acetate in the artificial wastewater. The AO sludge tended to have higher released phosphates, ranging from 9.4 to 36.9mgPO$_4$-p/gMLSS.hr than the O sludge which ranged from 2.5 to 17.2 mgPO$_4$-P/gMLSS.hr.



(a) WWTP sludge (b) AO lab sludge (c) O lab sludge

Figure 3 Relationship between the maximum released phosphate and the sulfate reducing rate in the activated sludge.

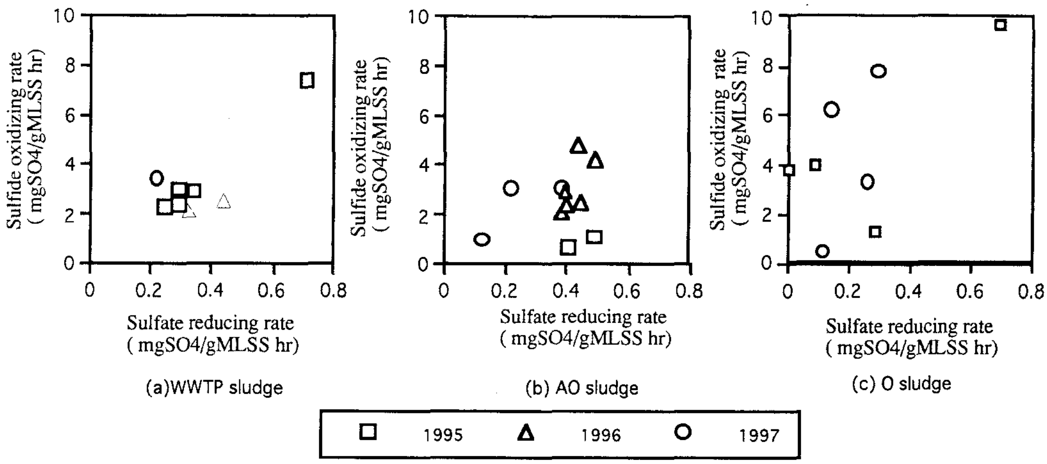


Figure 4 Relationship between sulfate reducing rate and sulfide oxidation rate in the activated sludge

Figure 4 shows the relation between the sulfate reducing rates and sulfide oxidation rates in the activated sludge. The WWTP sludge tended to have lower sulfur reducing/oxidation rates than the laboratory setup sludge although for the AO and O sludge, the sulfide oxidation rates tended to increase with increase in sulfate reducing rates. This suggests the formation of a sulfur cycle in the activated sludge. Figure 5 shows an example of results obtained from the batch experiment using anthroquinone as an inhibitor of sulfate reduction with sludge from the laboratory sludge (O). Anthroquinone prevented sulfate reduction as there was no change in sulfate concentrations and also lowered acetate production. Propionate concentrations were also reduced with anthroquinone addition. The results showed that anthroquinone had an ability to inhibit sulfate reduction. However the amount of released phosphate did not change significantly with sulfate reduction inhibition. This may be due to the availability of acetate for poly-p bacteria metabolism in the activated sludge.

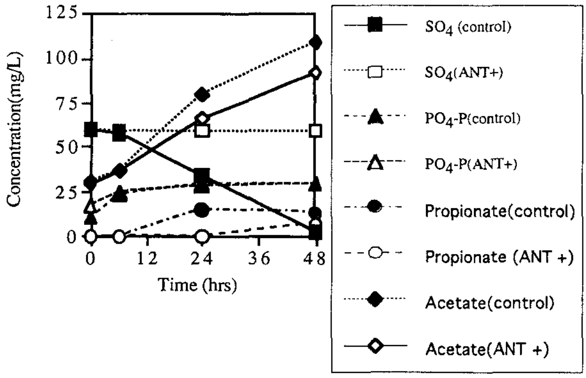


Figure 5. Example of results from batch experiments using anthroquinone. Control: No anthroquinone was used. ANT+ Anthroquinone was added

Table 2 Initial total iron concentration (WWTP sludge)

Date (1997)	Total iron (mg/l)
Aug 6	7.8
June 29	9.16
Oct 22	10.6
Oct 23	9.8
Nov 4	12.5
Nov 12	18.5

3.2 Microbial sulfide and ferrous oxidation

In order to elucidate whether sulfide oxidation was due to the metabolic activities of sulfur oxidizing bacteria rather than a chemical reaction, pasteurized sludge (WWTP) samples were used in this aerobic batch experiment. Figure 6 shows the concentration-time profile of sulfide oxidation under iron oxidation using pasteurized samples. It is evident that sulfide oxidation did not occur with the pasteurized samples. The results in Figure 6 when compared to Figure 7 where unpasteurized sludge was used indicated that much of the sulfide oxidation was due to biological action. According to Fig 6, the sulfate concentration of sludge with only NaS_2 addition for unpasteurized sludge ranged between 3.4mgSO_4/gMLSS and 3.5mgSO_4/gMLSS. Addition of $FeCl_2$ also did not have much effect on sulfide oxidation. Figure 7 shows an example of concentration time profile of unpasteurized activated sludge samples. The amount of sulfate produced by sulfide oxidation (Na_2S addition) only was about 17mgSO_4/gMLSS. Iron oxidation reduced this value to 9.1mgSO_4/gMLSS indicating that the introduction of ferrous oxidation reduced the activity of sulfide oxidizing bacteria in the unpasteurized sludge. When the same experiment was repeated with a freshly sampled activated sludge sample (WWTP), iron oxidation reduced the produced sulfate from 34mgSO_4/gMLSS to 27 mgSO_4/gMLSS. The results show the ability of ferrous oxidation to lower sulfide oxidation rates in the activated sludge. The use of iron salts in full-scale activated sludge plants has also been known to inhibit and lower other metabolic rates in activated sludge such as nitrification and denitrification [10].

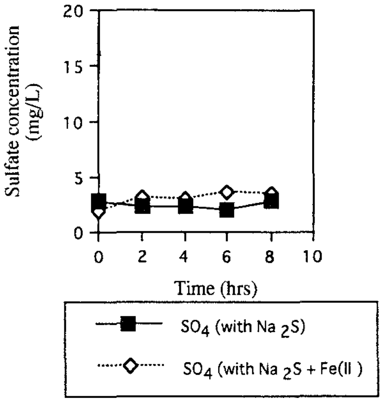


Figure 6 Concentration-time profile of sulfide and iron oxidation using pasteurized sludge (WWTP)

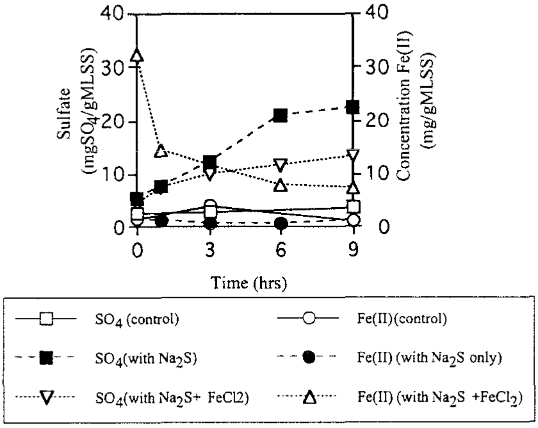


Figure 7 An example of concentration-time profile of sulfide oxidation with FeCl2 using unpasteurized sludge (WWTP).

3.3 Microbial sulfate and iron reduction

Batch experiments were carried out to investigate the influence of iron reduction on sulfate reduction. An example of the effect of iron reduction on sulfate reduction in the activated sludge is shown in Figure 8. Fe(II) values generally increased while Fe(III) values decreased indicating the occurrence of iron reduction in the sludge. Table 3 shows a comparison of the rates of anaerobic experiments using Fe(III) and anthroquinone. The sulfate reducing rate decreased from 0.25 to 0.14mgSO_4/g MLSS.hr in batch Run 1 with iron reduction. In batch run 2, a decrease in sulfate reducing rate was also observed from 0.13 to 0.06mgSO_4/gMLSS.hr except for batch Run 3 where the effect was not pronounced. This suggests that IRB inhibited SRB. However the electron flow between IRB and SRB suggested that SRB utilized more substrate electrons than IRB in the control experiments. In Run 1, SRB utilized 85.9% of substrate electrons than IRB (14.1%) in the control run. When $FeCl_3$ was added, the SRB electron flow dropped to 53.7% while IRB increased to 46.3%. The trend was observed for Runs 2 and 3. In the AO sludge, the sulfate reducing rates for the control experiment was 0.73mgSO4/gMLSShr with an electron flow of 98%. With Fe(III) addition, the sulfate reducing rate dropped to 0.37mgSO4/gMLSS.hr and the electron of SRB also dropped to 74.8%. The results show the importance of iron reduction on sulfate reduction in the sludge and its effect on substrate utilization by SRB. Anthroquinone was used in combination with $FeCl_3$ to study the effect of SRB inhibition on iron reduction. The results in Table 3 showed that the inhibition of SRB also caused a decrease in the Fe(II) producing rates. In Run 1, the rate of Fe(II) production decreased from 0.56 to 0.44mgFe/gMLSShr when sulfate reduction was induced. The trend was also observed for Runs 2 and 3.

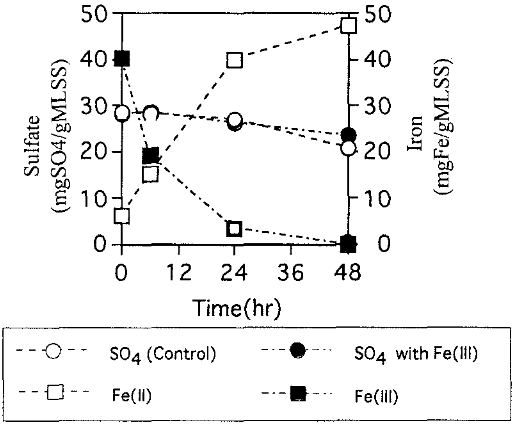


Figure 8 An example of the effect of iron reduction on sulfate reduction (AO sludge). Control: Without Fe(III) addition.

3.4 Electron flow between SRB and IRB

The substrate electron flow between IRB and SRB were evaluated from the values of Fe(III) reducing and control rates (Table 3). The results showed that the calculated electron flow percentages for SRB ranged from 57.8 to 77.5% and IRB ranged from 22.5 to 42.29% for the WWTP sludge. For the AO sludge, SRB electron flow ranged from 58.7 to 79.8% and IRB ranged The electron flow for IRB also increased with SRB inhibition as shown in Run 2. This suggests that SRB may be indirectly involved to an extent in iron reduction since the inhibition of SRB by IRB also reduces the iron reducing rate. The likely involvement of SRB in reduction of Fe(III) in anaerobic sediments has been reported[4]. The activities of SRB and IRB were calculated based on Table 3, using the control values where sulfate reduction was predominant and the (ANT + Fe(III)) condition where sulfate reduction was inhibited but iron reduction was predominant with the addition of $FeCl_3$.

The sulfate reducing activity ranged from 0.09 to 0.80 mgO_2/gMLSShr and IRB ranged from 0.06 to 0.1mgO2/gMLSS.hr. The results showed that SRB had higher activities than IRB. The results (Table 4) also show that although for this particular sludge and batch experiments, the activated sludge had a low initial iron concentration (Table 2), the IRB activity was enough in this batch experiments to inhibit sulfate reduction.

TABLE 3: Comparison of metabolic rates of anaerobic batch experiments using Fe(III) and anthroquinone. R refers to rate expressed in mg/gMLSS.hr. $R_{Acetate}$=rate of acetate generation per unit gMLSS.hr, R_{SO4}=rate of sulfate reduction per unit gMLSS.hr, $R_{Fe(II)}$ =rate of ferrous iron produced per unit gMLSS.hr.

Run (WWTP)	Substrate Condition (aim)	R_{SO4}	$R_{Fe(II)}$	*ELECTRON FLOW (%)*		$R_{Acetate}$
				(SRB)	*(IRB)*	
1	CONTROL (SR)	0.25	0.19	85.9	14.1	0.76
	Fe(III) (SR + IR)	0.14	0.56	53.7	46.3	0.46
	ANT (SR inhibition)	0.04	0.20	48.2	51.8	0.51
	ANT+ Fe(III) (SR inhibition + IR).	0.09	0.44	48.7	51.3	0.40
2	CONTROL (SR)	0.13	0.09	87.0	13.0	0.34
	Fe(III) (SR + IR)	0.06	0.45	38.3	61.7	0.30
	ANT (SR inhibition)	0.05	0.12	65.9	34.1	0.31
	ANT+ Fe(III) (SR inhibition + IR).	0.05	0.43	35.0	65.0	0.11
3	CONTROL (SR)	0.57	0.16	94.3	5.7	1.38
	Fe(III) (SR + IR)	0.56	0.86	75.2	24.8	1.04
	ANT (SR inhibition)	0.06	0.13	68.2	31.8	1.35
	ANT+ Fe(III) (SR inhibition + IR).	nd	0.81	nd	nd	0.91
AO sludge 4	CONTROL (SR)	0.73	0.07	98.0	2.0	2.5
	Fe(III) (SR + IR)	0.37	0.58	74.8	25.2	1.86
	ANT (SR inhibition)	0.02	0.01	90.3	9.7	2.24
	ANT+ Fe(III) (SR inhibition + IR).	0.03	0.73	16.0	84.0	2.18
5	CONTROL (SR)	1.2	0.05	99.1	0.9	1.62
	Fe(III) (SR + IR)	0.98	0.54	89.4	10.6	0.73
	ANT (SR inhibition)	0.03	0.01	93.3	6.7	1.29

Substrate conditions for Table 3 → Control – no $FeCl_3$ and Anthroquinone additions were made. Fe(III) → only $FeCl_3$ additions were made. ANT + Fe(III) → anthroquinone and $FeCl_3$ additions were made. Nd →not determined. SR refers to sulfate reduction, IR refers to iron reduction.

Table 4 Comparison of SRB and IRB activities in terms of COD units (O_2/gMLSShr)

Run	Sulfate reduction	Iron reduction
1	0.17	0.06
2	0.09	0.06
3	0.38	0.01
4	0.49	0.1
5	0.80	0.2

3.5 Role of SRB and IRB in acetate production in the activated sludge.

The role of SRB in acetate production is clear from the decrease in acetate production when SRB was inhibited with anthroquinone as seen in the values of acetate before and after anthroquinone addition in Table 3. For example in Run 1, the acetate producing rates decreased from 0.76 to 0.51mg Acetate/gMLSS.hr with anthroquinone addition. Similar trends was observed for the other runs. Secondly, iron reduction also decreased acetate production. The results are not in agreement with Nielsen,[1, 2, 3] who stated that iron reduction bacteria play a major role in acetate production, in other words, iron reduction in activated sludge caused acetate production. The results rather showed the ability of iron reduction to reduce acetate production therefore further investigations are therefore necessary for a better understanding of the role of iron reduction on acetate production in activated sludge.

3.6 Iron reduction and phosphate release

The relationship between iron reduction and phosphate release was investigated. Figure 9 shows the relation between the amount of ferrous reduced and the amount of phosphate released in both sludges. The results showed that increase in amount of reduced ferrous tended to decrease with increase in amount of released phosphate. These results show that high iron reduction in the activated sludge can lower or inhibit phosphate release.

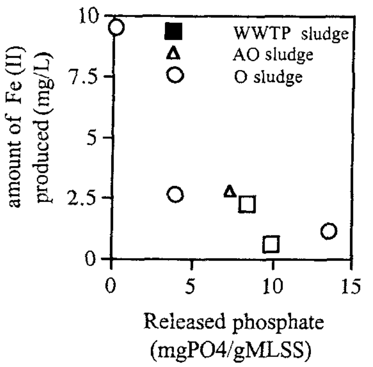


Figure 9 Effect of iron reduction on released phosphate.

3.7 Effect of iron coagulant on O sludge

Sulfate reduction was examined under storage conditions over a period of 11 days using sludge from lab setup in which an iron coagulant had been used to control filamentous bulking8). Figure 10 shows the results of the batch storage experiments for normal and iron-amended sludge. The results showed that sulfate reducing rate was lower for iron amended sludge than the normal sludge. The same phenomenon was observed for phosphate release concentrations and the organic acids.

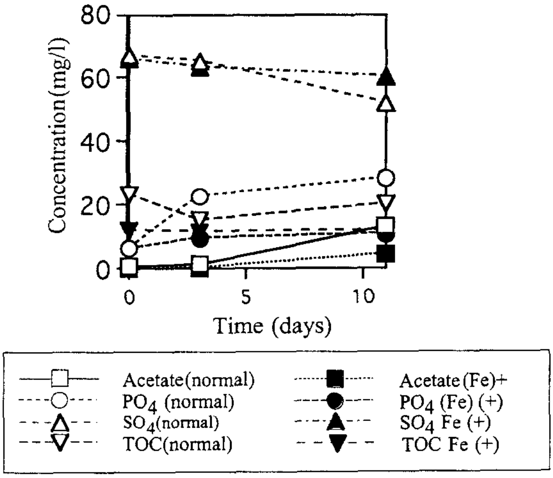


Figure 10 Results of storage batch experiments for normal O sludge and iron amended sludge. Normal: sludge without iron coagulant, (Fe)+ iron amended sludge.

4. Conclusions

The following conclusions were drawn and summarized as follows:

1) The phosphate release increased with sulfate reducing rates for the wastewater treatment plant sludge but the same phenomenon was not observed with the laboratory cultivated sludge (AO and O).
2) Iron oxidation generally reduced sulfide oxidation.
3) Iron reduction was responsible for lowering sulfate reduction and phosphate release activities.
4) SRB was inhibited by anthroquinone and also led to a decrease in iron reducing ability implying that SRB may play a role in iron reduction processes.
5) SRB utilized more substrate electrons than IRB and had higher activities although the activity of the IRB was high enough to affect the sulfate reducing process.

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