(30) Effect of temperature on sulfate reduction, phosphate release and denitrification and their relation with filamentous sulfur bacteria in an anaerobic-oxic activated sludge of a municipal plant

下水処理場嫌気好気活性汚泥の硫酸塩還元,りん放出,脱窒に及ぼす温度の影響 および糸状性硫黄細菌の増殖との関係

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Abstract; The relations and effect of temperature among the sulfate reducing bacteria (SRB), poly-phosphate accumulating bacteria (PAB), denitrification bacteria (DNB) and the filamentous sulfur bacteria (FSB) were studied at 4, 20, 30 and 37°C using sludge from an anaerobic-oxic plant. The results indicated the coexistence of SRB and FSB in the sludge. Phosphate accumulated sludge tended to decrease sulfate reduction and the number of SRB, sulfide oxidizing rate and filament length tended to increase with sulfate reduction rate. Denitrification completely suppressed SRB and PAB activity over the temperature range while nitrate was present. Results from the application of the Arrhenius equation indicated that the denitrification was the the most sensitive to temperature changes, with a mean Q_{10} value of 2.4, followed by sulfate reduction (1.84) and phosphate release (1.35).

Keywords; Activated sludge; sulfate reduction; phosphate release; denitrification; filamentous sulfur bacteria; Q_{10}

1. Introduction

Knowledge of the interactions among phosphate release and accumulation, sulfate reduction and denitrification processes in the activated sludge is essential for understanding the microbial dynamics of the activated sludge process. Consequently, PAB and DNB have been studied extensively in recent years[1,2]. Yamamoto[3)4)5)6)] examined sulfate reduction in the laboratory scale activated sludge cultivated under several conditions and reported that when sulfate reduction was suppressed by the addition of sodium molybdate or denitrification, filamentous bulking was also suppressed. These results indicated that sulfate reduction enhanced the proliferation of filamentous bacteria in activated sludge because of the provision of reduced sulfur which can be utilized by Type 021N. Using anaerobic-oxic sludge of a municipal plant, Yamamoto[4)] also reported that sulfate reduction was a main trigger of filamentous bulking and that low phosphate release was associated with low sulfate concentration. Yamamoto[5)] further stated that under oxic conditions sulfate reduction and sulfide oxidation occur simultaneously because of a symbiotic relationship between the sulfide oxidizing bacteria (SOB) and SRB.

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There is the need to further investigate other factors on these interactions. An example is temperature, which is an important factor affecting microbial activity, which is critical for maintaining efficient biological wastewater treatment. Other experimental parameters such as the sludge condition can also shed more light on the interactions among these bacteria. The objective of this work was therefore to study the interactions among the FSB, SRB and PAB, DNB at 20°C under shaking conditions in batch experiments, with emphasis on (1) sulfate reduction and phosphate release of a sludge subjected to prior aeration; (2) sulfate reduction and sulfide oxidation, and (3), the effect of temperature changes at 4, 20,30 and 37°C. The arrhenius plot was used to determine and compare their respective temperature coefficients (Q_{10}).

2. Materials and Methods

2.1 Condition of activated sludge

The activated sludge used in the experiments was collected from the Kanazawa City municipal waste treatment plant from the line which feeds the aeration tank with recycled sludge. The plant has a design capacity for 117,500 people. The inflow is mainly domestic waste with a design hydraulic flow of 181,000m^3 of sludge per day. The plant is operated under anaerobic-oxic conditions to control filamentous bulking. The sampling period extended from July to December 1995. The MLSS and SVI at phase 1, (ie diluted SVI measured under the condition that SV_{30} is less than 30%) were measured soon after sampling in the laboratory.

2.2 Experimental procedure

(1) Sulfate reduction, phosphate release and denitrification experiments

The batch reactors used for the experiments were 100ml BOD bottles. Aliquots of activated sludge samples with MLSS equivalent to 1g/l were taken and centrifuged at 5,000 rpm and 4°C. After centrifugation the liquid phase was decanted and the concentrated sludge transferred to the BOD bottles. and Settled sewage with substrate was added. The bottles were then tightly capped to prevent penetration of atmospheric air. The contents of the bottles were kept under constant magnetic stirring at 4, 20, 30 and 37°C. At intervals of 0, 6, 24 and 48 hours, samples were taken from the bottles for analysis. The substrate and their respective concentrations are shown in Table 1.

The effect of denitrification on phosphate release and sulfate reduction was studied in experiments 2 and 3. Sodium nitrate was used as the source of electron acceptor. Sulfate reduction and phosphate release was studied in experiments 1, 4, 5, 6 and 7. Sodium sulfate was used as electron acceptor.

Under aerobic conditions PAB accumulates phosphates as polyphosphates and releases orthophosphates under anaerobic conditions. For experiments 4, 5, 6 and 7, the relation between phosphate accumulated sludge and sulfate reduction was studied. The sludge was pretreated prior to subjection to the procedure already stated above. 175 mg of KH_2PO_4 was added to a liter of activated sludge and subjected to 3 hours of aeration. Aeration was conducted at 20°C and samples were taken hourly for analysis.

(2) Sulfide oxidation experiments

In experiments 7 and 8, sulfide oxidation experiments were conducted. Activated sludge sample volumes equivalent to 1gMLSS per liter were taken and centrifuged at 5000 rpm and 4°C. After centrifugation the liquid phase was decanted and the concentrated sludge was transferred to 350 ml conical flasks and 100mg/l of sodium sulfide was added. The sludge was then subjected to aeration for 9 hours at 20°C. Samples were taken hourly for analysis.

Table 1. Experimental conditions (Composition of substrate)

Experiment	Date	Substrate	Concentration (mg/l)	Objective
1	1995.07.12	S., $+SO_4$	Na_2SO_4 (75), KH_2PO_4 (175)	SO_4 Reduction, P release
2	1995.07.18	S., $+SO_4+NO_3$	Na_2SO_4 (75), $NaNO_3$ (410)	Denitrification, SO_4 Reduction, PO_4 release
3	1995.08.03	S., $+SO_4+NO_3$	Na_2SO_4 (75) , $NaNO_3$(410)	Denitrification, SO_4 Reduction PO_4 release
4	1995.09.26	S., $+SO_4$	Na_2SO_4 (75), KH_2PO_4 (175)	SO_4 Reduction, PO_4 release
5	1995.10.03	S., $+SO_4$	Na_2SO_4 (75), KH_2PO_4 (175)	SO_4 Reduction, PO_4 release
6	1995.10.17	S., $+SO_4$	Na_2SO_4 (75), KH_2PO_4 (175)	SO_4 Reduction, PO_4 release
7	1995.11.14	S., $+SO_4$ S., $+S^2$	Na_2SO_4,(75), KH_2PO_4 (175) Na_2S (100)	SO_4 Reduction, PO_4 release Sulfide oxidation
8	1995.12.06	S.,$+SO_4$; $S+S^2$	175mg/Na_2SO_4;100mgNa2S/l	SO_4 Reduction, Sulfide oxidation

S is settled sewage.,

2.3 Analytical Methods and calculation of rates

Nitrate and sulfate were analyzed by ion chromatography with phtalic acid as eluent and column temperature of 40°C. Phosphate was analyzed by HPLC using electric conduction detection with post-column pH buffer (Shimadzu Co Ltd, Type LC 6A) and was measured orthophosphate-phosphorus (PO_4-P). The length of filamentous bacteria in the sludge was measured by use of a microscope-video system connected to a personal computer. The number of SRB was enumerated by the MPN method using m-ISA medium after homogenization with an ultrasonic homogenizer. Metabolic rates can provide a basis for comparison of the various processes so the relative denitrification and sulfate reduction rates were estimated as the slope of a linear fit to the measurement data. Released phosphate values were calculated as the difference between the initial value and the maximum value which usually occurred after 24 hours.

3. Results and Discussion

3.1 Behavior of SRB, PAB and DNB in the anaerobic-oxic sludge

Figure 1 shows the general behavior of the SRB and PAB in the activated sludge at 20°C which was typical for experiments 1,4,5,6 and 7. Phosphate release was observed to be in two distinct phases. In the first phase the phosphate release was relatively rapid and reached a concentration of 8.3mgPO_4-P/gMLSS after 6 hours. The second phase after 6 hours was much slower and phosphate release ceased after two days of batch cultivation, attaining a concentration of 12.1mgPO_4-P/gMLSS. When the sludge was aerated with KH_2PO_4 addition, the concentration reached 12.7mgPO_4-P/gMLSS after 6 hours but reached 13.9mgPO_4-P/gMLSS after 48 hours. For the normal sludge, when preaeration was not done, released phosphate levels were lower. The sulfate concentrations were also lower for preaerated samples. This results suggest that PAB outcompeted SRB during the absorption of organic substrate in the preaerated condition.

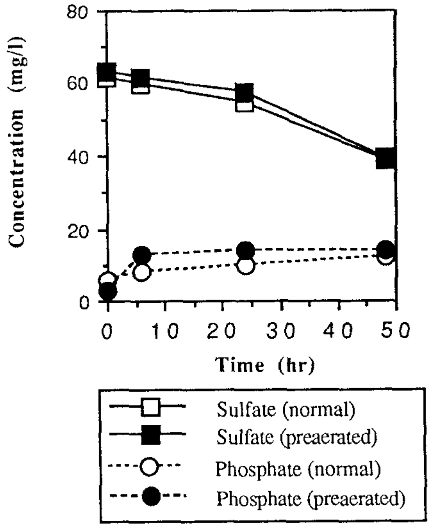

Fig.1 Behavior of SRB and PAB in the activated anaerobic-oxic sludge (Exp.1 at 20°C).

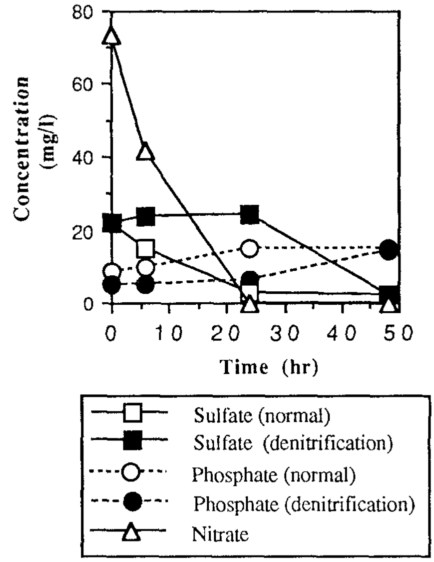

Fig.2 Behavior of SRB,PAB and DNB in the activated anaerobic-oxic sludge. (Exp.2 at 20°C)

Figure 2 shows the behavior among SRB, PAB and DNB in the sludge which was typical for experiment 2 and 3 at 20°C. For the normal sludge, when nitrate was not added the phosphate concentration reached 15.1mgPO_4-P/gMLSS after 24 hours and stabilized afterwards at about 15.19mgPO_4-PO4-P/gMLSS after 48 hours. When nitrate was added to the sludge, the concentration reached 6.35mgPO_4-P/gMLSS after 24 hours but reached 14.77mgPO_4-P/gMLSS after 48 hours. When nitrate had been nearly consumed at 0.27mg NO_3/gMLSS after 24 hours, phosphate release resumed. Sulfate reduction did not occur under denitrification conditions when nitrate was present. Sulfate concentrations were nearly constant at about 23.7mgNO_3/gMLSS. When nitrate levels became depleted at about 24 hours, sulfate reduction resumed afterwards and rapidly dropped to 2.1mgSO_4/gMLSS after 48 hours. This suggests that DNB outcompeted and suppressed PAB and SRB activity for organic substrates when nitrate was present.

3.2 Relationship among number of SRB, sulfate reduction, filament length and sulfide oxidation.

Figure 3 shows the relationship between the number of sulfate reducing bacteria and sulfate reduction at 20°C. Sulfate reducing rates tended to increase with increase in the number of SRB. This suggests that high numbers of SRB will contribute to high sulfate reduction rates. This is likely to lead to generation of high levels of sulfide which is used by the filamentous sulfur bacteria for metabolism. Figure 4 shows the filament length variation with sulfate reduction with extra data from a previous experiment under stirring conditions at 1gMLSS/l. Although the plot shows some scatter, filament length tended to increase with increase in sulfate reduction rates. The reason for this may be that the filament length data is the total of all filamentous microorganisms which includes FSB. Therefore the likelihood of sludge bulking due to sulfur bacteria in activated sludge is high when sulfate reduction rates are high in the sludge. Conditions that are likely to promote filamentous sulfur bacteria growth in activated sludge include low DO, increased organic loads and/or sulfide containing wastewaters[9]. Figure 5 shows the relationship between sulfate reduction and sulfide oxidation. Sulfate reduction rates increased with sulfide oxidizing rates.

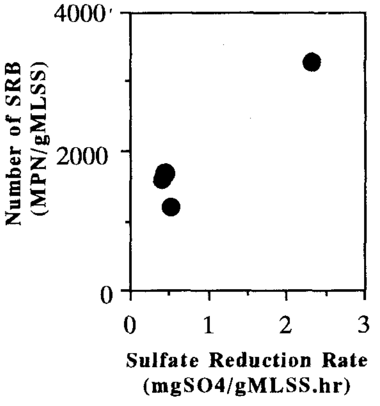

Fig.3 Relationship between the number of SRB and sulfate reduction at 20°C.

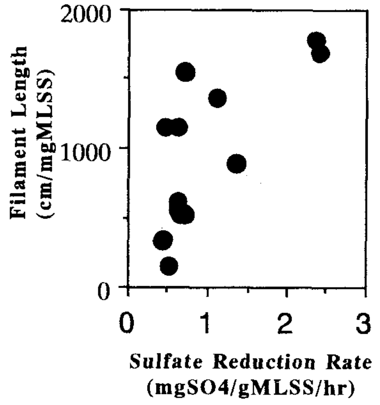

Fig.4 Filament length variation with sulfate reduction rate at 20°C, with data from previous experiment.

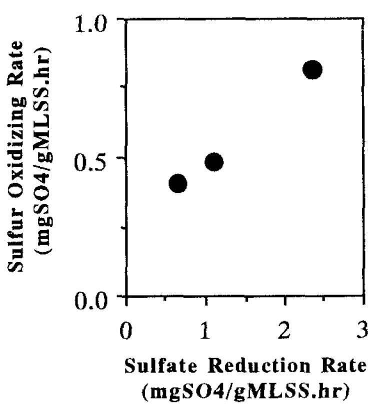

Fig.5 Variation of sulfur oxidizing rate with sulfate reduction rate at 20°C.

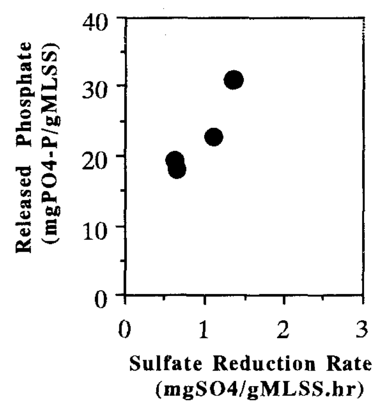

Fig.6 Variation of the released phosphate with sulfate reduction rate at 20°C (Exp 4, 5,6,7)

3.3 Phosphate release and Sulfate Reduction Rate

Figure 6 shows variation of phosphate release with sulfate reduction. Phosphate release increased with sulfate reduction. Released PO_4-P ranged from 18.01mg/l to 30.9mg/l while sulfate reduction rates ranged from 0.66mg/l to 1.34 mg/l. In an earlier experiment[6], sulfate reduction inhibition by sodium molybdate caused phosphate release and acetate concentration to drop. This suggests that PAB utilizes acetate produced by SRB. Any increase in sulfate reductions is likely to be associated with an increase in released phosphate.

3.4 Rate variation as function of temperature

Figure 7 shows the variation of the rate of sulfate reduction and phosphate release as a function of temperature in the 4 to 37°C range. The results and graph profiles were typical for the other experiments in which sulfate reduction and phosphate release were studied. The normal sludge had a phosphate release rate of 0.39mgPO4-P/gMLSS.hr at 4°C and increased gradually over the temperature range to a concentration of 0.75mgPO_4-P/gMLSS.hr at 37°C. When the sludge was aerated, phosphate release rates increased with levels of 1.39mgPO_4-P/gMLSS.hr at 4°C, 1.54mgPO_4-P/gMLSS.hr at 20°C, 1.68mgPO_4-P/gMLSS.hr at 30°C and 1.94mgPO_4-P/gMLSS.hr at 37°C. The effect of phosphate accumulation on sulfate reduction in the sludge can be observed in the 20 to 37°C range when the sulfate reduction rate dropped from 1.15 to 0.95mgSO_4/gMLSS.hr at 30°C and 1.08 to 0.95mgSO_4/gMLSS.hr at 37°C. The tendency of the phosphate accumulated to lower sulfate reduction rates was observed over the entire temperature range.

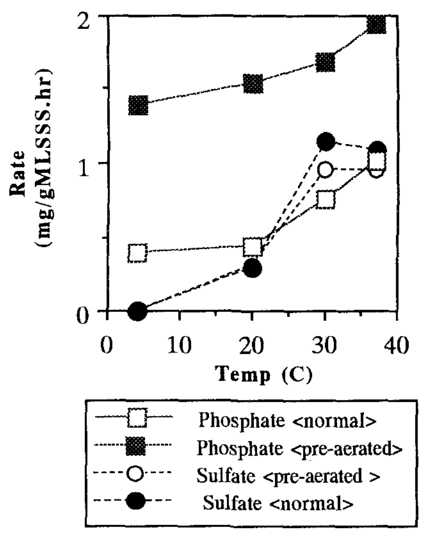

Fig.7 Variation of rate as a function of temperature (Exp.1)

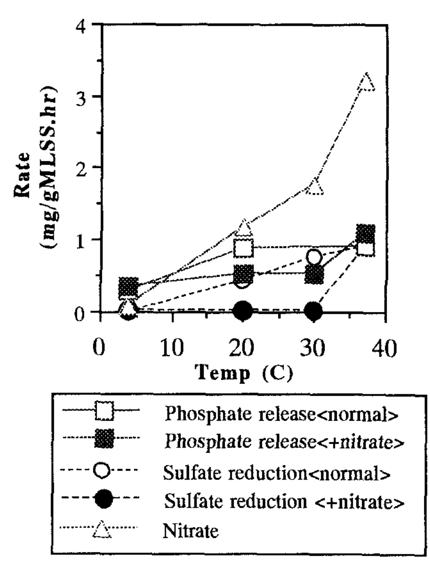

Fig.8 Variation of rate as function of temperature (Exp.3)

Figure 8 shows the variation of the rate of sulfate reduction, phosphate release and denitrification as a function of temperature in the 4 to 37°C range. The results and graph profiles were typical for the denitrification experiments. For the normal sludge, which had no nitrate addition, the phosphate release rate profile was similar to Fig.7. The phosphate release increased steadily from 0.52mgPO4-P/gMLSS.hr at 4°C to 1.09mgPO4-P/gMLSS.hr at 37°C. Under denitrification conditions, phosphate release rates were suppressed and did not increase significantly from 4 to 30°C. The effect of denitrification on sulfate reduction in the sludge can be observed in the 4 to 30°C range when the sulfate reduction rate barely changed from 0.025mgSO_4/gMLSS.hr at 4°C to 0.04mgSO_4/gMLSS.hr at 30°C. The strong dependence of denitrification on temperature can be seen in the nitrate profile. The denitrification rates increased sharply from 0.1mg NO3/gMLSS.hr to 3.22NO3/gMLSS.hr . At 37°C, nitrate consumption by DNB was very rapid so sulfate reduction and phosphate release were able to take place after the nitrate levels were depleted. This probably accounts for the low rates in the 4 to 30°C temperature range.

3.5 Temperature coefficient (Q_{10})

In order to quantitatively describe the effect of temperature on each process, the Arrhenius equation was applied. The Arrhenius equation gives a quantitative description of the effect of temperature on each microbial process. Therefore this equation was applied to this work to investigate the sensitivity of the various processes to temperature changes.

The Arrhenius equation is given as

$k = A\exp(-E_a / RT)$

The temperature coefficient Q_{10} is defined as the ratio of the rates for a temperature difference of 10°C, where $Q_{10} = k_{t+10} / k_t = \exp(10E_a / RT_1T_2)$ kt is the metabolic rate expressed as μmol/gMLSS.hr at a constant temperature, t, where t is in degree Celsius; A is the Frequency factor; Ea is the apparent activation energy expressed in *kcalmol/K*; R is the gas constant ; T is the absolute temperature (in Kelvin). By plotting the logarithm of the rate against the reciprocal of the absolute temperature (T), both A and Ea were estimated from the gradient of the slope which is ($-Ea/RT$) and the constant which is $\log A$. The Q_{10} was then calculated from the estimated gradient and constant. Figures 9 and 10 show typical examples of the Arrhenius plots that were obtained.

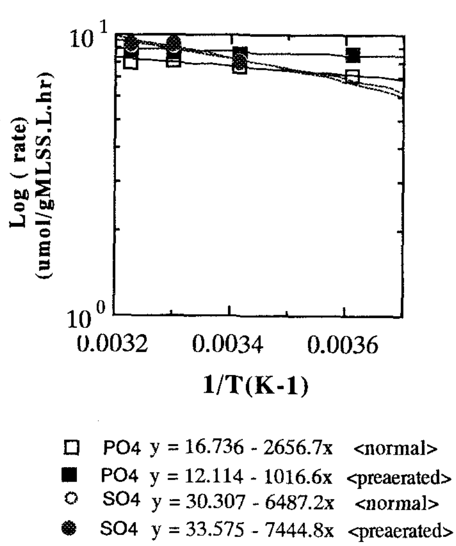

Fig.9 Arrhenius plot obtained from Exp.4 showing regression equations

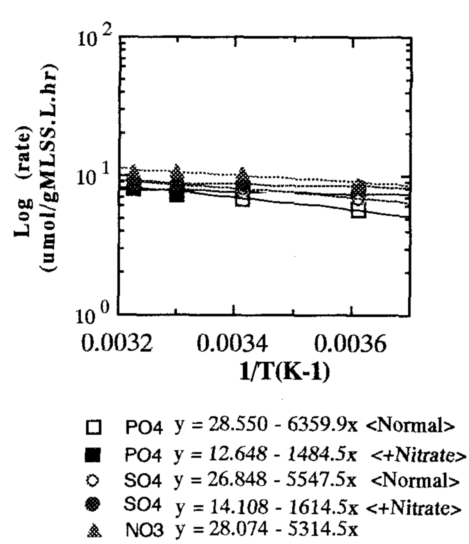

Fig.10 Arrhenius plot obtained from Exp.3 showing regression equations

Table 2 shows the results of regression analysis of sulfate reduction, phosphate release and denitrification. The Q_{10} values for phosphate release ranged from 1.14 to 1.74, with a mean coefficient of regression of 0.9. For sulfate reduction, the Q_{10} values ranged from 1.63 to 2.26, with a mean coefficient of regression of 0.91. The Q_{10} values for denitrification ranged from 1.8 to 2.6, with a mean coefficient of regression of 0.98. Theoretically, the activation energy and the temperature coefficient for an enzyme-catalyzed reaction are constant[10]. However the results show variations in the activation energy (E_a) and the Q_{10} . This is because activated sludge sampled on different days with varying microbial content were used for each experiment.

The Q_{10} values show that phosphate release is the least sensitive to temperature changes, followed by sulfate reduction and then denitrification. However, under different experimental and sludge conditions the sensitivity might vary.

Table 2 Summary of results of regression analysis of metabolic rates according to Arrhenius equation

	Log A	-Ea/R	r^2	E_a	Q_{10}
PAB	1) 18.2 2) 13-25.4 3) 8.0	1) 1571 2) 1214-5079 3) 1607	1) 0.9 2) 0.6-1.0 3) 0.2	1) 2503 2) 2351-9839 3) 2603	1) 1.35 2) 1.14-1.74 3) 0.2
SRB	1) 24.8 2) 22.6-33.5 3) 9.8	1) 5408 2) 3902-7444 3) 1090	1) 0.91 2) 0.68-1.0 3) 1.0	1) 11103 2) 8651-14420 3) 1881	1) 1.84 2) 1.63-2.26 3) 0.2
DNB	1) 33.8 2) 28-39 3) ND	1) 7077 2) 5314-8840 3) ND	1) 0.98 2) 0.95-1.0 3) ND	1) 10878 2) 9190-12566 3) ND	1) 2.4 2) 1.8-2.6 3) ND

1) mean value, 2) range, 3)standard deviation r^2 =coefficient of regression, constant =Log A, slope= -Ea/R
ND - not determined

4. Conclusion

The following conclusions were drawn and are summarized as follows:

(1) Phosphate accumulated sludge tended to decrease sulfate reduction and the number of SRB, sulfide oxidizing rate and filament length tended to increase with sulfate reduction rate.

(2) Denitrification completely suppressed SRB and PAB activity while nitrate was present.

(3) The effect of temperature on each microbial process indicated that denitrification was the most sensitive to temperature changes, with a mean Q_{10} value of 2.4, followed by sulfate reduction (1.84) and phosphate release (1.35).

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