# **Short Communication**

# EFFECT OF TEMPERATURE ON THE BACTERIAL IRON OXIDATION RATE IN A RIVER CONTAMINATED WITH ACID MINE WATER

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The Akagawa River, a tributary of the Kitakami River, has been highly polluted by a large quantity of acid mine water discharged from the Matsuo sulfur and iron-sulfide mine area and therefore has very low pH and high concentrations of soluble ferrous iron (1, 2). In research on the seasonal variations in the number of acidophilic iron-oxidizing bacteria, iron oxidation ratio, and some physical and chemical variables in the river, it was found that water temperature was an important environmental factor affecting the iron-oxidizing activity of the bacteria (2). In this study the effect of water temperature on the rate of bacterial iron oxidation along the river was surveyed quantitatively.

The surveys were conducted during the period from 1974 to 1977. Twelve sampling stations were located along the river as described in a previous paper (I). Water samples were collected in polyethylene bottles at the sampling stations, and transported for analysis to the laboratory in Morioka City. Concentrated HCl was added to the water samples for iron assay at the time of collection, and the assay was carried out within 5 hr after sampling. Iron was measured colorimetrically by the  $\alpha$ ,  $\alpha'$ -dipyridyl method (I). The degree of iron oxidation was evaluated by ferric iron concentration (mg/l) or by the ratio of ferric to total iron (%). Water temperature was measured at the sampling station with a mercury centigrade thermometer, and the pH with a glass electrode pH meter.

As shown in Fig. 1, ferric iron concentrations in the river increased exponentially progressively downstream from sampling stations 4 to 12. When the river was flowing, autooxidation of ferrous iron was ignored because the water pH was less than 2 or so (Table 1). Furthermore, it was found that the difference in water temperature between the two stations was slight or absent during the sampling time.

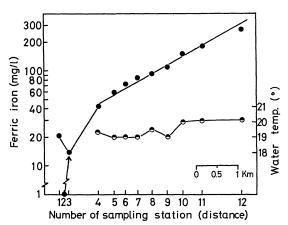


Fig. 1. Progressive changes in ferric iron concentrations and water temperatures in the Akagawa River.

Ferric iron (●); water temperature (●). Aug. 6, 1974.

Table 1. Effect of initial pH on the chemical oxidation of ferrous iron in acid mine water.

Initial pH -	Incubation time (day)					
	1	2	3	4	5	6
1.0	0.6	2.3	3.4	1.7	1.7	0.6
2.0	1.6	2.8	1.6	1.6	0	0
$2.0^{a}$	1.7	2.3	2.8	15.8	88.8	100
3.0	3.3	2.8	3.8	2.8	1.6	1.1
4.0	1.7	3.3	3.9	2.9	2.8	2.8

Acid mine water sampled from a mine drift (Fe<sup>2+</sup>, 1234 mg/l; Fe<sup>3+</sup>, 20 mg/l; pH 1.48) was filtered with a millipore filter (0.2  $\mu$ m) and adjusted to each pH value with either H<sub>2</sub>SO<sub>4</sub> or NaOH solution. Each sample (50 ml) in a 500 ml flask was incubated at 30° with shaking. The values represent ratios of ferric to total iron (%).

<sup>a</sup> Original acid mine water adjusted to pH 2, but not filtered with the millipore filter.

Many specific iron oxidation rates were calculated from the data obtained during the survey period. The rate of increase in the ferric iron concentration (p) in the short time interval (dt) during the flow of the river is given by

$$dp/dt = kp$$

where k is the specific iron oxidation rate (hr<sup>-1</sup>). On integrating we obtain

$$k=1/t(\ln p_{\rm t}/p_{\rm o})$$

where  $p_0$  and  $p_t$  are the concentrations of ferric iron (mg/l) at sampling stations 4 and 12, respectively, and t is the flow time between the two stations (about 1.5 hr).

The effect of temperature on chemical reaction rates is usually depicted by

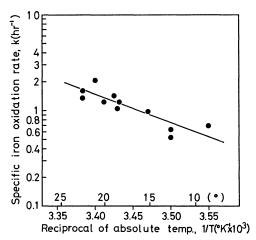


Fig. 2. Arrhenius plot of the specific iron oxidation rate as a function of the reciprocal of the absolute temperature.

 $\log k = 10.0 - 2890/T$  (T=absolute temperature).

### the Arrhenius equation:

$$k = Ae^{-E_a/RT}$$

where k represents the specific reaction rate, A is an empirical constant, R is the gas constant (1.987 cal/mol degree centigrade), T is the absolute temperature (°K), and  $E_a$  is the activation energy (cal/mol). An Arrhenius plot was prepared of the calculated specific iron oxidation rates (k) versus the reciprocal of absolute temperature (i.e., an average of water temperatures at stations 4 and 12 at each sampling time). As shown in Fig. 2, an approximately linear relationship was obtained in the temperature range from 9 to  $23^{\circ}$ .

The activation energy  $(E_a)$  for the bacterial iron oxidation, which was calculated from the slope of the straight line, was found to be 13,200 cal/mol. The value of the temperature coefficient,  $Q_{10}$  (3), calculated from the data was 2.2. These values correspond to those reported for the isolated iron-oxidizing bacterium Ferrobacillus ferrooxidans (4). The  $Q_{10}$  value of 2.2 implies that an increase in water temperature of  $10^{\circ}$  in the range from 9 to  $23^{\circ}$  results in a doubling of the iron oxidation rate in the river. The present experimental results provide more definite information than heretofore available on temperature effects on bacterial iron oxidation in natural water environments.

## REFERENCES

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