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NOTES

Kinetics of Sulfur Oxidation at Suboptimal Temperatures

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Chemolithoautotrophic bacteria were enriched from mine water at incubation temperatures ranging from 4 to 46°C, using elemental sulfur as a substrate in acid mineral salts media. Thiobacillus-type bacteria were successfully enriched for at all test temperatures except 46°C. Changes in pH ($-d$pH/dt) were used to estimate the rate constants for the enrichment cultures. The rate constants yielded a linear Arrhenius plot, an activation energy of 65 kJ/mol, and a temperature coefficient (Q_{10}) of 2.1 for the 4 to 37°C temperature interval.

The microbiological oxidation of iron and sulfur constitutes two key processes in mineral biotechnology. Both mesophilic and thermophilic bacteria have been described which can utilize inorganic compounds of Fe and S as electron donors (3, 5, 6). At present, although thermophiles are characterized by superior oxidation rates, only mesophilic iron- and sulfur-oxidizing acidophiles are used in large-scale leaching processes for metal recovery from sulfide ores. We have previously determined ferrous iron oxidation rates for acidophilic thiobacilli (1). The respective rate constants yielded a linear Arrhenius plot in the range of 4 to 28°C, as also demonstrated for iron-oxidizing isolates from a uranium mine (4). Temperature is a cardinal parameter in biological leaching processes (2, 3, 5), but little information is available to evaluate quantitatively effects of temperature, particularly in the suboptimal range of <20°C, on the biological oxidation of inorganic sulfur. In the present work, rate constants, activation energy, and temperature coefficient were determined for acidophilic bacteria, with elemental sulfur as the electron donor and CO_2 as the source of carbon.

Mine water samples, ranging in pH between 2.5 and 7.8 and in redox potential between 50 and 250 mV (against standard calomel electrode), were collected from a copper mine in eastern Finland (the Keretti mine at Outokumpu) and were used as a composite to provide a uniform inoculum (1). Enrichment experiments under static culture conditions were carried out at 4, 7, 10, 13, 16, 19, 28, 37, and 46°C in mineral salts media [0.4 g each of $(NH_4)_2SO_4$, K_2HPO_4, and $MgSO_4 \cdot 7H_2O$ per liter] supplemented with 5 g of elemental sulfur (flowers of sulfur, S^0) per liter. Shake flask cultures (100-ml cultures in 250-ml shake flasks at 180 rpm) were incubated at 7, 16, 28, and 37°C. The temperature precision was ±1°C. Growth of the cultures with elemental sulfur was monitored by pH measurements. No effort was made to ensure homogeneous dispersion of elemental sulfur during the incubation.

Rate constants (k) for the elemental sulfur cultures were estimated as the absolute values of the slopes of pH curves [i.e., $k = d$pH/dt)], which were fitted to the Arrhenius equation to derive the activation energy (E_a):

$$k = Ae^{-E_a/RT} \quad (1)$$

$$\ln k = \ln A - (E_a/RT) \quad (2)$$

where A is the special constant, R is the universal gas constant, and T is the temperature in kelvin.

Temperature coefficients (Q_{10}) were calculated for the 4 to 37°C interval by using the respective rate constants:

$$Q_{10} = (k_2/k_1)^{10/(T_2 - T_1)} \quad (3)$$

where k_2 and k_1 are the rate constants determined for test temperatures T_2 (37°C) and T_1 (4°C), respectively.

The ultimate end product of the bacterial oxidation of sulfur is the sulfate ion. Therefore, the elemental sulfur oxidation can be presented with the following net equations:

$$2S^0 + 3O_2 + 2H_2O \rightarrow 4H^+ + 2SO_4^{2-} \quad (4)$$

$$2S^0 + 3O_2 + 2H_2O \rightarrow 2H^+ + 2HSO_4^- \quad (5)$$

Because of the eventual liberation of protons upon sulfate formation, the oxidation of sulfur can be monitored by following the increase in hydrogen ion activity (α_{H^+}). Under normal culture conditions, bacterial sulfur oxidation is coupled with growth and the measurement of oxidation based on pH changes (dpH/dt) can therefore also be used to monitor bacterial growth. Exponential growth coupled with sulfur oxidation hence should yield a linear decrease in pH values. Measurement of pH was the method of choice in the present work because homogeneous sampling and direct quantitative measurement of growth by determination of biomass concentration by either cell counts or chemical methods have not been developed for cultures growing with flowers of sulfur.

Figure 1 shows the time course of pH changes in shake flask cultures at each of the four test temperatures. A change in the rate of sulfur oxidation around the pH equivalent of the respective pK_a value of 1.91 could not be discerned with certainty, although it was a typical feature that the rates of pH changes declined toward the end of the incubation period. Some decrease in the dpH/dt at the lower range of

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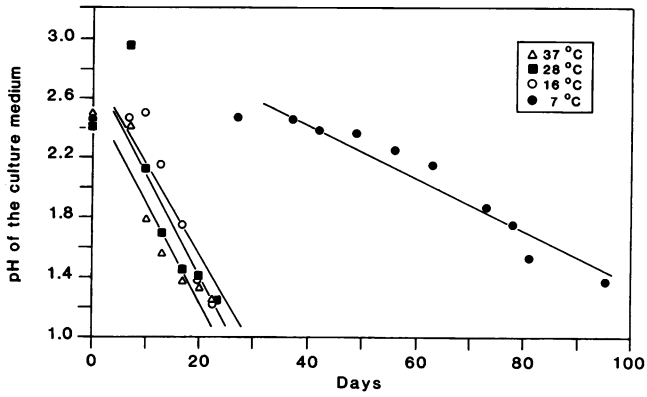

FIG. 1. Growth curves of enrichment cultures with elemental sulfur based on pH measurements of cultures incubated in shake flasks.

pH values is to be expected not only because of the relative distribution of the SO_4^{2-} and HSO_4^- species, but also because the amount of the substrate, S^0, decreased concomitantly with the oxidation and thus may have gradually become a rate-limiting factor. An additional consideration is that a low pH, <1.3, is increasingly inhibitory to the bacteria, thereby causing a decrease in the rate during the lower pH phase of the growth curve.

In shake cultures the three higher temperatures (16, 28, and 37°C) yielded pH curves with more or less similar slopes (Fig. 1; Table 1). Sulfur oxidation at 7°C was slower by a factor of approximately 4 compared with the three higher incubation temperatures. The α_{H^+} values increased from 0.003 (pH 2.5) to almost 0.1 (pH 1), indicating that almost 0.1 mol of elemental sulfur per liter was oxidized, equalling approximately two-thirds of the initial amount of the initial 5 g of S^0 per liter. Caution should be exercised, however, in data analysis because the concentration changes of soluble species are not directly comparable with the respective α_{H^+} changes. Because the biphasic character of the pH curves was not clearly discerned, the pH profiles of the sulfur cultures were linearized throughout the time course to calculate the rate constants. The respective correlation coefficients indicated a good fit (Table 1). The linear regression lines are indicated in Fig. 1 for each shake culture. Microscopic examination of the test cultures indicated the presence of rod-shaped cells resembling in size and shape those of *Thiobacillus ferrooxidans* and *T. thiooxidans*. No attempt was made to identify the cultures taxonomically.

TABLE 1. Rate constants of elemental sulfur oxidation by acidophilic chemolithotrophic bacteria in static and shake cultures in the temperature range of 4 to 37°C

Culture condition	Incubation temp (°C)	Rate constant, $-d\text{pH}/dt$ (day^{-1})	Correlation coefficient (r)
Shake cultures	7	0.01775	0.9619
	16	0.06113	0.9059
	28	0.06641	0.9410
	37	0.06725	0.8539
Static cultures	4	0.00168	0.8814
	7	0.00253	0.9801
	10	0.00402	0.9822
	13	0.00483	0.9754
	16	0.00668	0.9686
	19	0.00847	0.9707
	28	0.01590	0.9907
	37	0.02140	0.9882

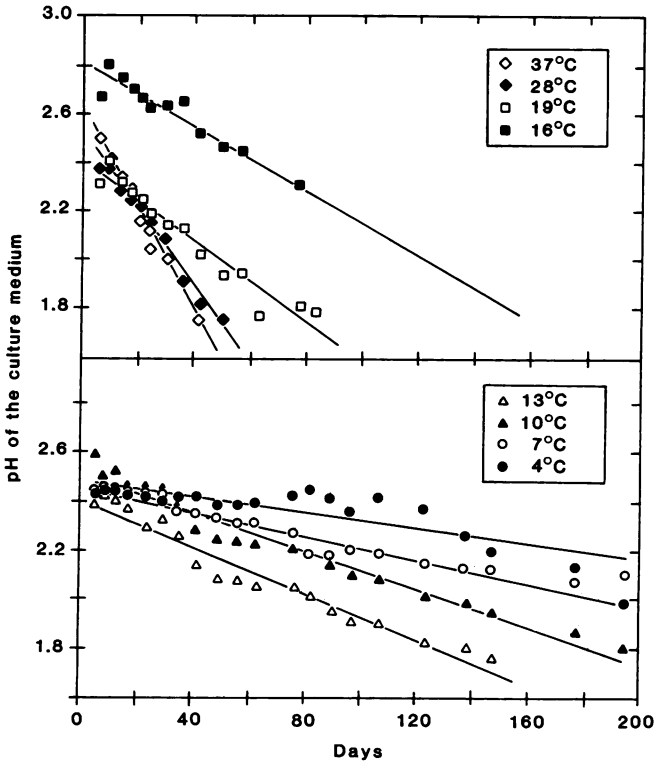

FIG. 2. Growth curves of enrichment cultures with elemental sulfur based on pH measurements of cultures incubated under static conditions.

Changes in pH in the static cultures also displayed linearity (Fig. 2). Because of the static incubation conditions, the pH changes were now much slower than in shake cultures. Under the static culture conditions, the fastest oxidation was achieved at 37°C (Table 1).

The rate constants (Table 1) thus derived were used to

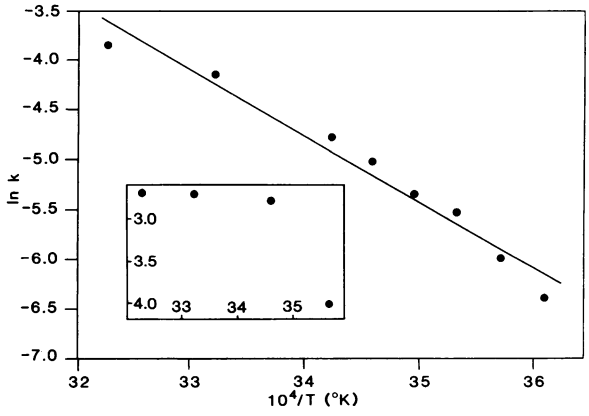

FIG. 3. Arrhenius plot of rate constants determined for elemental sulfur cultures incubated under static conditions. The straight line was based on linear regression (r^2 = 0.963). (Inset) Arrhenius plot of rate constants for elemental sulfur cultures in shake flasks.

construct Arrhenius plots (Fig. 3). For shake cultures, a discrete linear relationship was not apparent. For the static cultures, the Arrhenius plot was linear in the temperature range tested (4 to 37°C) (Fig. 3) and yielded a value of 65 kJ/mol for the activation energy. For comparison, the E_a for Fe^{2+} oxidation by cultures enriched from the same composite mine water sample was determined to be 83 kJ/mol (1). Thus, the E_a values for ferrous iron and elemental sulfur oxidation indicate that the rates of these *Thiobacillus*-type bacteria were controlled primarily by the temperature of incubation. Transition temperatures were not apparent for static cultures with elemental sulfur because the correlation coefficient indicated a good linear fit. Although the temperature profiles are very different for the static and shake cultures, the interpretation is complicated due to the fact that, in addition to a potential involvement of diffusion control, the sulfur oxidation involves bacterial attachment, which is affected by culture shaking and may also be a temperature-sensitive phenomenon.

The Q_{10} value was 2.1 for the static cultures, based on the 4 to 37°C temperature interval. The Q_{10} value for ferrous iron oxidation was 1.9, based on previously presented rate constants (1).

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