Short Communication

Rapid Biological Sulfide Oxidation in the Effluent of a Hot Spring

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The biological oxidation of inorganic reduced sulfur compounds is catalyzed aerobically by the colorless sulfur bacteria, and qualitative and quantitative importance of this process is well recognized[8,18]. Although elemental sulfur seems to be the most important product of aerobic sulfide oxidation[7,9,11], the direct and in situ estimations of sulfide oxidation rate by the so-called gradient bacteria are limited except for a very few cases[4,6].

The gradient bacteria are closely associated with the environments where oxygen and sulfide coexist as a result of the two opposing gradients of oxygen and sulfide. Sulfur-turf is one of the sulfureta growing in hot spring effluents, and consists of the gradient bacteria and sulfur particles adhering to them. It has been revealed experimentally that the sulfur-turf oxidizes dissolved sulfide to elemental sulfur, and further to sulfate via thiosulfate[11,12].

This study was conducted at one of the hot springs of Ganiba Spa in Akita-Ken (Tohoku district, Japan). The major ion concentrations of the hot spring water of the Ganiba Spa were analyzed in 1982 by Nakadate (Table 1 ; Nakadate, unpublished data). Redox potentials (Eh) and dissolved oxygen concentrations were

about -150 mV and 0.6 to 1.0 ppm, respectively[13]. Temperature, pH, and the dissolved sulfide concentrations of the hot spring water were stable throughout one year, and they were $50\pm2°C$, 7.8 ± 0.3, and 4 ± 2 ppm, respectively.

Figure 1 shows the side and top views of the hot spring of the Ganiba Spa. At the two points ((a) and (b), Fig. 1B) of the stream, the following determinations were carried out: 1) dissolved sulfide concentrations when the sulfur-turf grew by adhering to the surfaces of the bed and inside walls of wooden gutter (S_a, value at point (a) ; S_b, value at point (b)) ; 2) dissolved sulfide concentrations when the sulfur-turf was absent from the inside of wooden gutter (control ; s_a and s_b) ; 3) current velocities in 1) and 2) ; and 4) biomass of the

Table 1. The major ion concentrations of the hot spring water at the Ganiba Spa.

Major ions	ppm
Na^+	81.4
K^+	4.3
Ca^{2+}	134.0
Mg^{2+}	0.2
Cl^-	2.9
HCO_3^-	15.1
SO_4^{2-}	441.0

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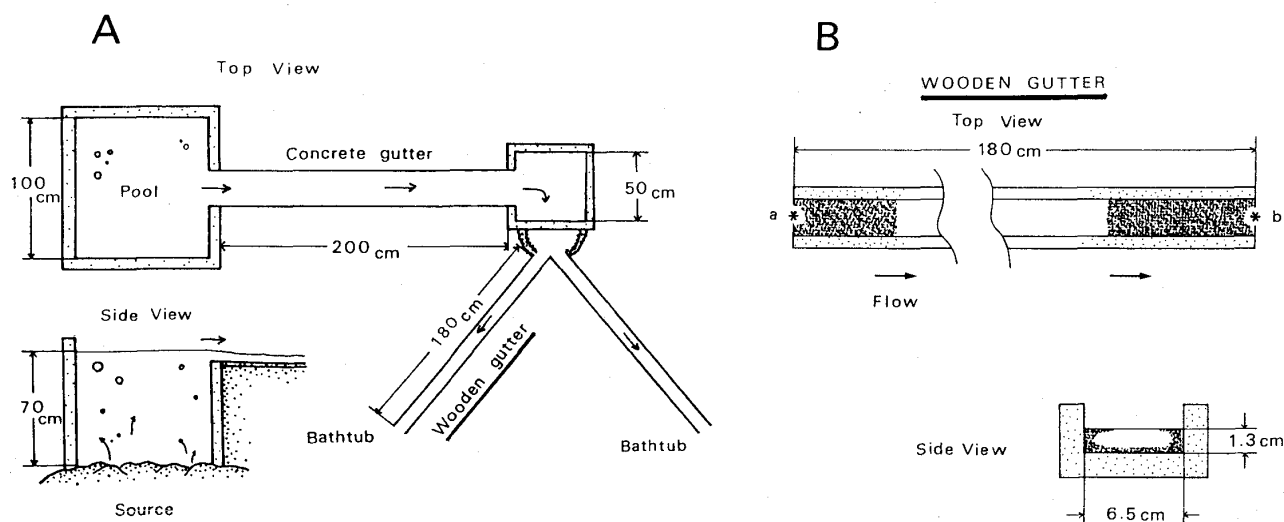
Fig. 1. A : The source of hot spring of the Ganiba Spa. At the source of the hot spring, the water
overflows the pool and runs down a concrete gutter and is distributed to bathtubs through two
wooden gutters. The estimations of the sulfide oxidation rate were made at the wooden gutters to
the bathtubs. B : Top and side views of the wooden gutter. The sulfur-turf grew on the bed and
the inside walls of the wooden gutter (▓). The water samples for the sulfide determinations were
collected at the two points indicated by (a) and (b).

sulfur-turf growing in the area between the two points.

Based on the assumption that decrease in sulfide concentration of the hot spring water was attributed to both oxidation by the sulfur-turf and dispersion from water surface to air, calculation of decrease in dissolved sulfide was done using the following equation :

$$OS = (S_a - S_b) - (s_a - s_b)$$

where OS is the decrease in dissolved sulfide attributed to oxidation by the sulfur-turf, $(S_a - S_b)$ is the total decrease in dissolved sulfide during water flow down the area between the two points when the sulfur-turf grew inside the wooden gutter, and $(s_a - s_b)$ is the decrease in dissolved sulfide when the sulfur-turf was absent and is considered to include the decrease in dispersion to air, chemical oxidation, and others. The hot spring water flowed more slowly when the sulfur-turf grew adhering to the inside surface of the wooden gutter. Therefore, corrections were made according to the assumption that $(s_a - s_b)$ is proportional to the time it took for the spring water to flow from point (a) to point (b).

Sulfide concentrations of the hot spring water were determined as follows ; around 800 ml of the hot spring water was collected in duplicate glass bottles which previously contained enough cadmium chloride to fix dissolved sulfide. After weighing collected water, the resultant cadmium sulfide was dissolved with hydrogen chloride and the liberated cadmium was titrated with ethylenediaminetetraacetic acid using Cu-1-(2-pyridylazo)-2-naphthol as the indicator[15].

All of the sulfur-turfs growing inside the wooden gutter between the two points ((a) and (b), Fig. 1B) were removed completely using a plastic scraper and collected with a nylon net. After fixing with neutralized formaldehyde (final concentration of 2%), the collected sulfur-turf was sedimented for over 1 week in a measuring cylinder (wet volume). The elemental sulfur adhering to the sulfur-turf was dissolved by adding carbon disulfide, and the mixture was filtered with a glass filter (pore size 0.45 μm). The sulfur-turf on the glass filter was dried at 105°C for a few hours and the dry weight was determined. Ignition was then conducted at 500°C on the dried sulfur-turf in order to determine the ignition loss.

The current velocity of hot spring water was determined by measuring the time it took for a plastic float to move from point (a) to point (b) in the gutter.

The growth of the sulfur-turf on the inside surface of the wooden gutter was estimated on the basis of the mass of the sulfur-turf (Fig. 2A). The increase in the biomass (ignition loss) per day peaked on day 2 and then decreased slightly thereafter (Fig. 2B). The mass in the wooden gutter probably attained a maximum value within 3 or 4 days, since the detachment of the sulfur-turf started thereafter.

The total decrease in the dissolved sulfide became greater than 1 ppm within 2 days (Table 2). The decrease in sulfide due to oxidation by
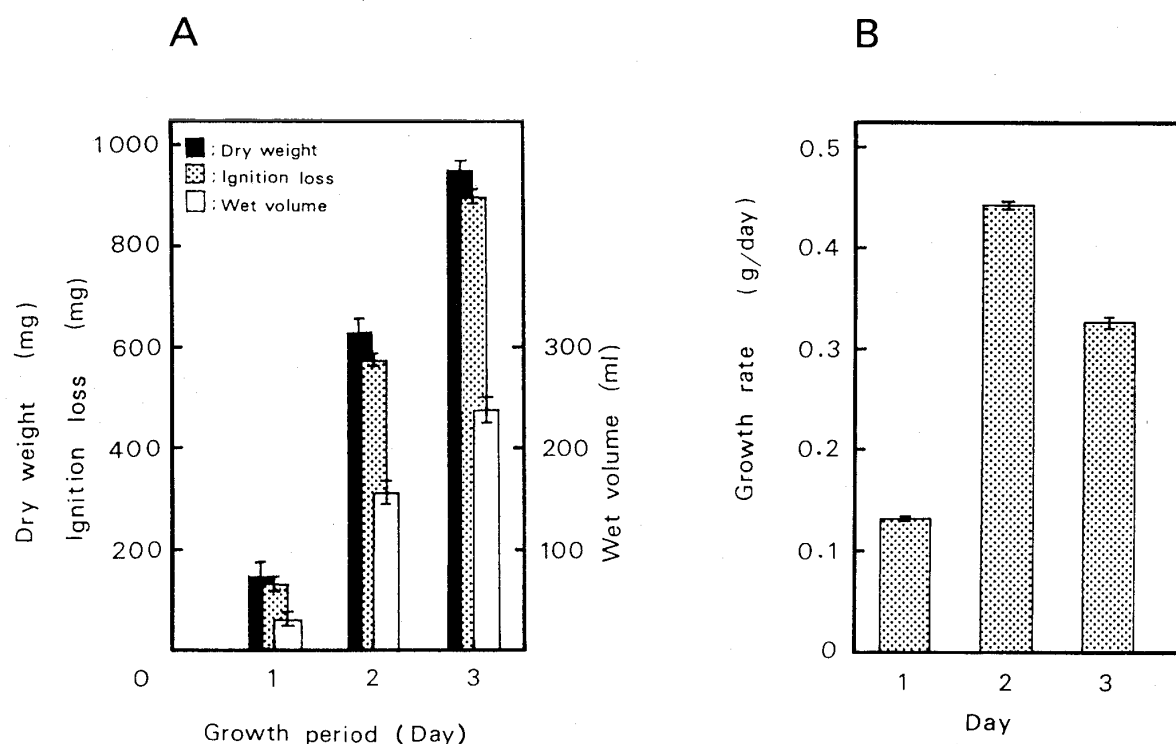

Fig. 2. A : The growth of the sulfur-turf. The growth was expressed in three different terms ; dry weight (■, mg), ignition loss (⊟, mg), and wet volume (□, ml). B : The growth rate (g/day) of the sulfur-turf in term of ignition loss.

Table 2. The water velocity and the decrease in the dissolved sulfide accompanied by the growth of the sulfur-turf.

Growth (day)	Sulfur-turf present (+) or absent (−)	Velocity (cm/sec±SD[a])	Flow-time (sec/m±SD)	Total decrease of sulfide (ppm±SD)	Sulfide decrease due to the sulfur-turf	
					(ppm±SD)	(%±SD)
1	+(n[b]=2)	34.6±1.5	2.90±0.13	0.68±0.09	0.27±0.00	39.9±4.9
2	+(n=2)	30.3±0.4	3.26±0.05	1.14±0.09	0.68±0.09	59.2±3.4
3	+(n=2)	27.4±1.1	3.66±0.15	1.01±0.16	0.82±0.12	80.6±3.0
0(control)	−(n=6)	46.0±1.2	2.18±0.05	0.24±0.11	—	—

[a] Standard deviation
[b] Number of measurements

the sulfur-turf was 40% on day 1, but it increased rapidly to 60% on day 2 and attained 80% on day 3 (Table 2). This rapid increase in the oxidation attributed to sulfur-turf formation and indicates the significance of biological sulfide oxidation in this thermal effluent system.

Figure 3 shows the changes in both oxidation rate and oxidation activity (sulfide oxidation rate per unit biomass) of the sulfur-turf accompanied by growth. The oxidation rate of dissolved sulfide by the sulfur-turf increased gradually and attained $3 \, mol/m^2/day$ at 2 or 3 day's growth, while the oxidation activity was high on the first day and then decreased rapidly to a level of about half the maximum. It is presumed that the decrease in oxidation activity was due to the increase in inactive constituents (dead cells, excreted organic materials, etc.) of the sulfur-turf.

These biological sulfide oxidation rates obtained in this study are provably overestimated. Because that the values of the flow-time (Table 2) using in

the calculation of sulfide oxidation rate were the flow-time near the surface of the water and not the mean flow-time of the hot spring water. Since the values of the surface flow-time are considered to be less than that of the mean flow-time, it is supposed that the values of biological sulfide oxidation rate exceed the true value within a limit of 50%. However, this level of the sulfide oxidation rate is in accord with the rate estimated by laboratory experiment (about $4 \, mol/m^2/day$) using the sulfur-turf harvested from the Ganiba Spa[13].

The values are over hundred times the rates so far reported. The rate of sulfide oxidation in the chemocline in the Black sea, the Solar Lake, and in the mats of *Beggiatoa* were estimated at a level of 10 to $30 \, mmol/m^2/day$[4,6,17].

The ratio of sulfide ions to molecular oxygen dissolved in the hot spring water ranged from 1 to 5 in situ[13]. These values are much larger than the optimum stoichiometric proportion (0.5)[2], and indicate the presence of excess sulfide. However, the supplies of sulfide and molecular oxygen by the swift flow (about 30 cm per second, Table 2) of the hot spring water probably exceed the requirement of biological oxidation, although the sulfur-turf grows densely inside the wooden gutter. It can therefore be presumed that the level of this sulfide oxidation rate ($mol/m^2/day$) might be a maximum under natural conditions.

Mosser *et al*.[16] reported that elemental sulfur in the sulfur-containing pool at a high temperature ($>65°C$) was oxidized by *Sulfolobus* at rates which ranged from 67 to 190 grams (2.0 to 5.3 mol) per square meter per day. These values are comparable to our values, and indicate the possibility of a high rate of biological oxidation of sulfur compounds at high temperatures.
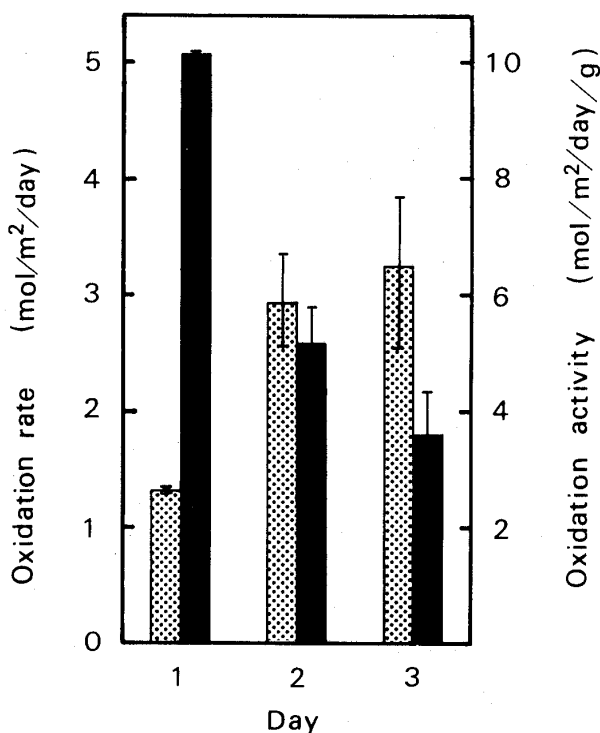

Fig. 3. The gradual increase in the sulfide oxidation rate (▨, $mol/m^2/day$) by the sulfur-turf in contrast to the rapid decrease in the oxidation activity (■, $mol/m^2/day/g$).

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