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Mini-Review

Study of the "Sulfur-turf": a Community of Colorless Sulfur Bacteria Growing in Hot Spring Effluent

YŌNOSUKE MAKI

Biological Laboratory, College of Humanities and Social Sciences,
Iwate University, Ueda 3-18-34, Morioka 020, Japan

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Abstract: Sulfur-turf is one of the so-called "sulfureta" which are massive aggregates of colorless sulfur bacteria. Interest in the ecological and physiological aspects of the sulfuretum has strongly increased not only due to the discovery of massive occurrence of colorless sulfur bacteria in an area of hydrothermal vents but also due to the possibility of its significant contribution to the sulfur cycle in the coastal region. Here, I review the types, habitats, and bacterial constituents of the sulfur-turf, and further, reveal the microaerophilic oxidation of elemental sulfur by the sulfur-turf and discuss the possibility of the detoxification of hydrogen sulfide with peroxides. The habitat of the sulfur-turf is regarded as a thermostating, continuous culture enriched with hydrogen sulfide. The massive aggregates including the sulfur-turf are very useful, because they can be harvested directly from their habitats without the need for isolation and mass cultivation. Taking advantage of these massive aggregates, we should design experiments in situ and also in the laboratory to clarify the ecology and physiology of the colorless sulfur bacteria. It is necessary to elucidate the microenvironment caused by bacterial aggregation using microelectrodes, but, on the other hand, more interest should be focused on the bacterial reaction to environmental factors, especially oxygen tension.

Key words: sulfur-turf, colorless sulfur bacteria, hot spring, sulfur oxidation, microaerophily

Introduction

Sulfur-turf is a massive aggregate of colorless sulfur bacteria which has a turf-like appearance because of the adherence of many elemental sulfur particles. It grows in hot spring effluents containing some dissolved sulfide (Maki, 1986). Based on the main bacterial components, the sulfur-turf has been grouped into three types: A-type (mainly constituting of sausage-shaped bacteria), B-type (rod-shaped bacteria), and C-type (filamentous bacteria) (Emoto, 1942). For a long time, such a massive biological aggregate containing sulfur-oxidizing bacteria has been referred to as "sulfuretum" (Baas-Becking, 1925). The ecology and physiology of this massive aggregate, however, remain obscure. For example, 1) what the activities of the sulfuretum under natural conditions are, 2) how the aggregates are physically structured, and 3) how the microenvironment produced by the aggregation influences both the quality and quantities of the bacterial activities?

The discovery of a large-scale biological community developing around hydrothermal vents is one of the most outstanding events in biology in the last few decades (Corliss *et al.*, 1979). The most striking feature of this community is that the large-scale community is supported by an energy supply due to sulfur-oxidizing chemolithotroph (Jannasch and Mottle, 1985). Among a few types or sites of aerobic sulfur-oxidizing chemolithotrophic production, the ecology of microbial mat covering hydrothermal sediment is especially interesting in relation to the sulfuretum.

On the other hand, it has been reported that the major biological transformations of sulfur compounds in the global sulfur cycle are undertaken by saline sediments in shallow water (Trudinger, 1982). It also has been reported that various microorganisms participate in the transformation of sulfur compounds, and sulfur-oxidizing bacteria, including sulfuretum, play significant parts in the aerobic oxidation processes of reduced or partially reduced sulfur compounds (Baas-Becking, 1925 ; Kuenen, 1975 ; Jørgensen, 1982). However, there have been very few qualitative and quantitative studies of these oxidative pathways (Jørgensen, 1988). This is largely due to both the physico-chemical and the biological complexities of coastal sediments.

The formation of the massive aggregate seems to be one of the prominent features of aerobic sulfur-oxidizing bacteria, especially in the group of the so-called "gradient bacteria" (Jørgensen, 1982). Unfortunately, successful techniques for the isolation and mass cultivation of the gradient bacteria have not been developed, so that only few of the species have been isolated (Kuenen, 1989). This is one reason that the study of sulfur-oxidizing bacteria has been delayed.

On the other hand, the formation of the massive aggregate is very advantageous for field and ecological investigations. That is we can detect them in the field with the naked eye, and use them in both field and laboratory experiments without going through the steps of isolation and mass cultivation. This means that we can apply the results obtained from our experiments more directly to nature. Furthermore, the cell density of the aggregate is very high and hence the microbiological reactions are intensive. Therefore, we can detect the outputs of the reactions with ease, even under natural conditions. The comparative simplicity of the bacterial constituents of the aggregate is another advantage.

The sulfur-turf grow massively in running hot spring water, where molecular oxygen and hydrogen sulfide are continuously supplied from air and hot spring water. Temperatures, pH, and the chemical ingredients of the hot spring water are stable throughout the year (Maki, 1986). Accordingly, the habitat of the sulfur-turf is regarded as a thermostating, continuous culture enriched with hydrogen sulfide. Taking advantage of the massive aggregates including the sulfur-turf, we should design experiments in situ and also in laboratory to clarify the ecology and physiology of the colorless sulfur bacteria.

In this article, I describe the history and the present state of the study of the sulfur-turf. I hope that this review will stimulate other investigators to study this interesting microbial community.

Historical review

The first study on the sulfur-turf was done in 1897 by Miyoshi who had researched Yumoto Spa in Nikko and reported the occurrence of the sulfur-turf (Miyoshi, 1897). Miyoshi described that the sulfur-turf is one kind of the "barĕgine" occurring in sulfur spring in Europe. According to Winogradsky, the barĕgine was a white slimy mass which precipitated from water and was thought to be a lifeless organic substance for a long time (Winogradsky, 1887). Miyoshi labelled the bacterial aggregates together with many elemental sulfur particles "Schwefelrasen" (sulfur-turf)[1], and described the bacterial constituents of the sulfur-turf. The dominant bacterial component was "Vibrio"-type bacteria and was named "sensenförmige Bacterien" (sickle-formed bacteria). Furthermore, through experimental means in situ,

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[1] Sulfur-turf: Jimbo translated "Schwefelrasen" into the word "sulphur-turf" (Jimbo, 1937). The author used the word "sulfur-turf" according to Jimbo's translation (Maki, 1986).

he demonstrated that the elemental sulfur particles adhering to the aggregates were formed through the chemical oxidation of sulfide contained in the hot spring water. According to Miyoshi, the amount of the adhered sulfur particles was determined by the ventilating condition of the hot spring water. This indicates the importance of oxygen concentration in the production of elemental sulfur by the biological and/or chemical oxidation of sulfide contained in hot spring water.

Molisch (1926) had also examined the Yumoto Spa and confirmed the occurrence of the sulfur-turf. Molisch named the whole bacterial constituents of the sulfur-turf as "Schwefelrasenbacterien". He had noted the possibility of biological oxidation of sulfide to elemental sulfur by these bacteria.

Emoto is one of the earliest investigators who studied sulfur-oxidizing bacteria inhabiting in hot spring water. He had researched the distribution of the sulfur-turf all over Japan with Hirose and Yoneda, and reported its occurrence in a series of papers published under the title of "Studien über die Thermalflora von Japan" (Emoto und Hirose, 1940a, 1940b, 1940c, 1940d, 1940e, 1941, 1942a, 1942b, 1942c, 1943, 1948; Emoto und Yoneda, 1940, 1942). Emoto summarized the papers reporting the occurrence of the sulfur-turf (Emoto, 1941). Based on the dominant bacterial components, he proposed three types of the sulfur-turf: A (sickle-formed or crescent-shaped bacteria), B (rod-shaped bacteria), and C (filamentous bacteria).

Re-examining the bacterial components of the sulfur-turf, Emoto gave the tentative name of "*Thiovibrio miyoshii*" for the sickle-formed bacteria in the A-type sulfur-turf, and "*Thiothrix miyoshii*" for the filamentous bacteria constituting of the C-type sulfur-turf (Emoto, 1942).

Growth conditions of the sulfur-turf

A close relationship was found between the types of the sulfur-turf and their environmental conditions (Maki, 1986); the A-type sulfur-turf grew under a condition in which temperature is between 50 and 72°C, the pH ranges from 6 to about 8, and the dissolved sulfide concentration is below 40 ppm (Table 1). Although the ranges of temperature, pH, and the dissolved sulfide concentration for the C-type sulfur-turf (Table 2) were similar to those of the A-type, the C-type sulfur-turf inhabited only in pools while the A-type occurred in streams. In contrast, the B-type sulfur-turf was supposed to grow under more acidic conditions, because it was known that the B-type sulfur-turf consisted predominantly of acidophilic rods, probably *Thiobacillus thiooxidans* (Emoto, 1928, 1929, 1933, 1941, 1942; Vishniac, 1974).

Table 1. Physico-chemical factors of the hot spring water which was inhabited by the A-type sulfur-turf (Maki, 1988a).

Name of Spa[1]	Temp. (°C)	pH	Dissolved sulfide (S^{2-}, ppm)	Total Residue[2] ($g \cdot l^{-1}$)
Dai	68	7.7	5	—[3]
Ganiba	50–52	7.9–8.0	3–5	0.759
Geto	54–72	6.0–6.4	1–3	4.73–4.85
Magoroku	58–63	6.7–6.8	1–3	—
Naka-no-yu	66–72	8.1	2–4	—
Tsuru-no-yu	57–60	6.3–6.6	24–40	2.69

[1] Spas are listed in alphabetical order.
[2] "Total Residue" indicates the total weight of dissolved and particulate matters in hot spring water.
[3] "—" indicates that the determinations were not made.

Table 2. Physico-chemical factors of the hot spring water which was inhabited by the C-type sulfur-turf (Maki, 1988a).

Name of Spa	Temp. (°C)	pH	Dissolved sulfide (S^{2-}, ppm)	Total Residue ($g \cdot l^{-1}$)[1]
Geto	56–68	6.0–6.4	1–3	4.73–4.85
Magoroku	55–58	6.9–7.0	2	—

[1] "Total Residue" indicates the total weight of dissolved and particulate matters in hot spring water.
[2] "—" indicates that the determinations were not made.

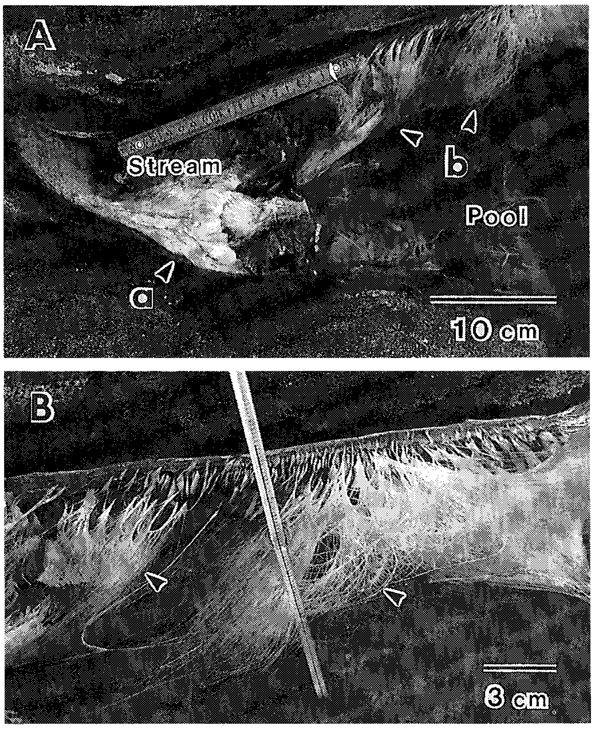

Fig. 1. Growth of the A- and C-type sulfur-turf at the Mukai-no-yu Spring in the Geto Spa. A : The A-type sulfur-turf (a) grew only in the stream overflowing from the pool. In contrast, the growth of the C-type (b) occurred only in the pool. B : The C-type sulfur-turf was attached to the walls of the pool within a few centimeters from the water surface and extended its filaments (arrows) (Maki, 1986).

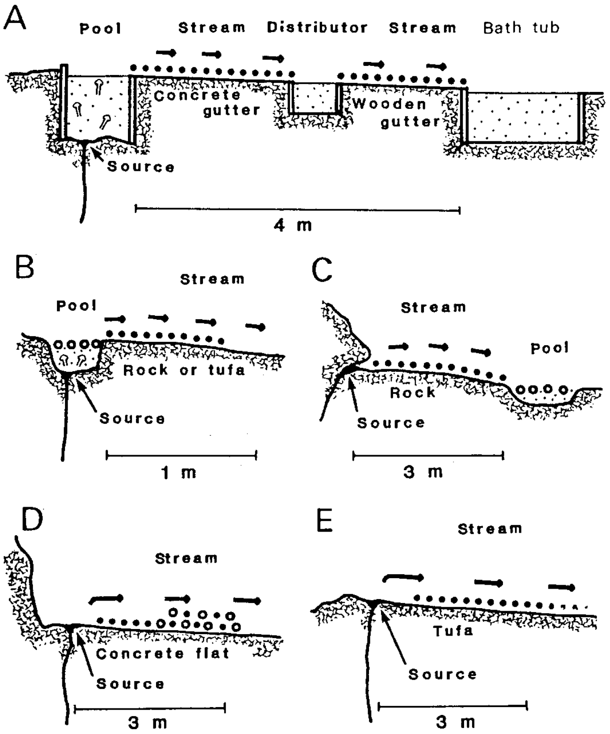

Fig. 2. Schematic representations of the side views of the hot spring and the occurrence of the A- (●●●) and C-type (○○○) sulfur-turfs. This figure shows the arrangements of the source, the pool, and the stream, relative to the occurrence of the sulfur-turfs. The Teizo-no-yu and the Mukai-no-yu had the same arrangement. A ; Ganiba Spa, B ; Teizo-no-yu and Mukai-no-yu Springs in the Geto Spa, C ; No. 2 Spring in the Magoroku Spa, D ; Karematsusawa Spring in the Geto Spa, E ; No. 2 Spring in the Tsuru-no-yu Spa (Maki, 1986)

It is very interesting that the A- and C-type sulfur-turfs grew in separate locations according to the difference in current velocity (Fig. 1). Figures 2B and 2C show the occurrence of these two types of the sulfur-turf in one effluent from one spring source. In the case of Fig. 2D (Karematsuzawa Spring), the current velocity was slow and small pools were formed along the stream. The range of dissolved oxygen concentration where the C-type grew was considerably wider than that for the A-type (Maki, 1986). Accordingly, it is postulated for this separate inhabitation that the A-type sulfur-turf has a lower and stricter requirement for dissolved oxygen than that of the C-type sulfur-turf (Maki, 1986). As molecular oxygen is supplied only from air to hot spring water originallycontaining no oxygen, it is reasonable to think that the continuous flow of hot spring water from the source controlls the oxygen content of the habitat resulting in a microaerophilic environment suitable for the A-type sulfur-turf.

Bacterial constituents of the sulfur-turf and their taxonomic position

The A-type sulfur-turf is dominantly consisted of large and curved bacteria. Miyoshi coined the name of "sensenförmige Bacterien" for these bacteria (Miyoshi, 1897). He described that these organisms have round ends, curved like a sickle, and flagellated peritricously. On the other hand, Emoto called Miyoshi's "sensenförmige Bacterien" crescent-shaped bacteria (Emoto, 1941, 1942). According to Emoto, the cell length of the crescent-shaped bacteria ranged from 6 to 28 μm and averaged 20 μm, in addition, the mean cell width was 1.34 μm (Emoto, 1941, 1942). I adopted here the name of "sausage-shaped bacteria" for the large and curved bacteria constituting the A-type sulfur-turf (Maki, 1986), as the bacteria have round ends and the non-tapered shape of cells (Fig. 3). Judging from the descriptions by Miyoshi and Emoto, it seems that both the "sensenförmige Bacterien" and the "crescent-shaped bacteria" are identical with the "sausage-shaped bacteria" (Maki, 1986).

Comparative studies on the distribution of the cell length of the sausage-shaped bacteria collected from different hot springs revealed that there are a few morphological types of the sausage-shaped bacteria (Maki, unpublished data).

As stated in the section of historical review, Emoto gave the tentative scientific name of "*Thiovibrio miyoshii*" to his crescent-shaped bacteria constituting the A-type sulfur-turf (Emoto, 1942). However, his data on their morphological characteristics are insufficient for identification of the bacterium. We must wait for further investigations to judge the taxonomic position of the sausage-shaped bacteria.

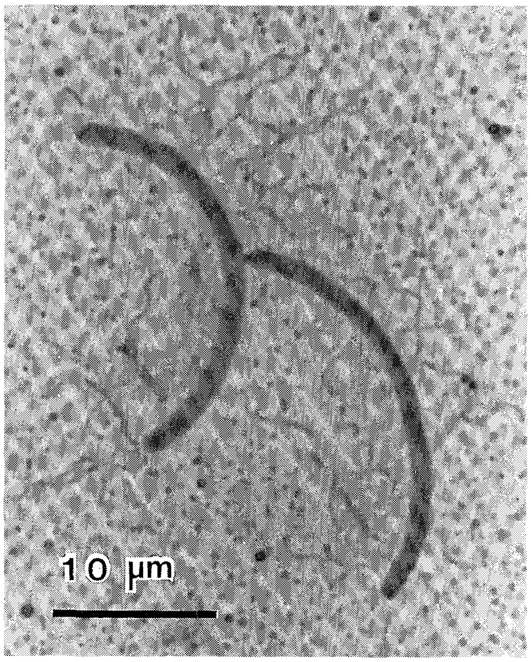

Fig. 3. Peritrichous flagella of the sausage-shaped bacteria stained by the Toda method (Maki, 1981).

Emoto isolated four rod-shaped bacteria from the B-type sulfur-turf or from the mud to which the B-type sulfur-turf was attached. He named them "*Thiobacillus thermitanus*", "*Thiobacillus lobatus*", "*Thiobacillus crenatus*", and "*Thiobacillus umbonatus*" (Emoto, 1928, 1929, 1933). However, these names given by Emoto are all abandoned, since they have been reported but are no longer available in pure culture for evaluation (Skerman, *et al.,* 1980 ; Kelly and Harrison, 1989).

The filamentous bacteria constituting dominantly the C-type sulfur-turf had given the name of "*Thiothrix miyoshii*" by Emoto. However, it resembles *Thermothrix* more closely, although the rod-shaped growth had not been confirmed (Caldwell *et al.,* 1976, 1984 ; Caldwell, 1989 ; Maki, unpublished data). The similarities between the filamentous bacteria and *Thermothrix* are as follows : (a) The cells depositing elemental sulfur extracellularly are frequently observed in natural sulfur-turf, but there is no grobules of elemental sulfur inside the cell ; (b) The trichomes of the filamentous bacteria lack sheaths, and the rosette-shaped growth has not been confirmed ; (c) They live in hot spring having a high temperature and a neutral pH as shown in Table 2.

The sulfur-turf contains various kinds of bacteria in addition to the dominant bacteria above mentioned. They are spirochaete-shaped bacteria, various rods, cyanobacteria, and others. They can be easily distinguished each other using light microscopy, although they could not still be isolated from the spot (Miyoshi, 1897 ; Emoto, 1941, 1942 ; Maki, 1980, 1981, 1986).

The taxonomic positions of each dominant bacteria constituting above three types of the sulfur-turf are still uncertain. Judging from the environmental conditions of their habitats and the results of our labratory experiments, it is likely that they derive metabolically useful energy and/or other any benefits from the oxidation of sulfide or elemental sulfur (Maki, 1986, 1987a). They look like colorless sulfur bacteria.

Colorless sulfur bacteria comprise many taxonomically different groups of bacteria having an ability to oxidize reduced or partially reduced sulfur compounds, and contain many unisolated sulfur-oxidizing bacteria (Kuenen, 1989). A powerful means to clarify the taxonomic or phylogenic relationship of the colorless sulfur bacteria is to investigate directry natural mixed populations without going through the step of isolation. Recent technical advances in chemotaxonomy make it possible to determine the sequence of the ribosomal RNA nucleotides of mixed microbial populations without the need for isolation (Pace *et al.,* 1986 ; Ward *et al.,* 1990). While the nucleotide sequence of 5S ribosomal RNA obtained from natural microbial communities has yielded valuable information concerning the taxonomic and phylogenic relationships among sulfur-oxidizing bacteria (Stahl *et al.,* 1985), a more recent and improved method of 16S ribosomal RNA sequencing is expected to yield more reliable and precise information when applied to colorless sulfur bacteria, including the sulfur-turf (Pace *et al.,* 1986 ; Oyaizu *et al.,* 1989).

Oxidation of hydrogen sulfide and elemental sulfur

Miyoshi (1897) tested the capacities of various artificial materials to hold elemental sulfur particles in the flowing hot spring water. Based on these results, he considered that the oxidation of dissolved sulfide to elemental sulfur is completely due to chemical reaction, and mucilagenous bacterial aggregates contribute as a "glue" for holding elemental sulfur particles on the surface. He supposed that the bacterial constituent of the A-type sulfur-turf oxidized hydrogen sulfide directly to sulfate without intermediating elemental sulfur.

However, it was found that the sausage-shaped bacteria which are the dominant bacteria of the A-type sulfur-turf oxidize hydrogen sulfide to elemental sulfur and then elemental sulfur is oxidized to sulfate via thiosulfate (Maki, 1987a). An addition of the sulfide solution to the medium previously inoculated with the A-type sulfur-turf caused a milky cloudiness of elemental sulfur particles. At the same time, the sausage-shaped bacteria which deposited elemental sulfur along their long axis of the outer surface appeared in the culture vessel (Fig. 4). These results indicate that the sulfide was oxidized to elemental sulfur by the sausage-shaped bacteria.

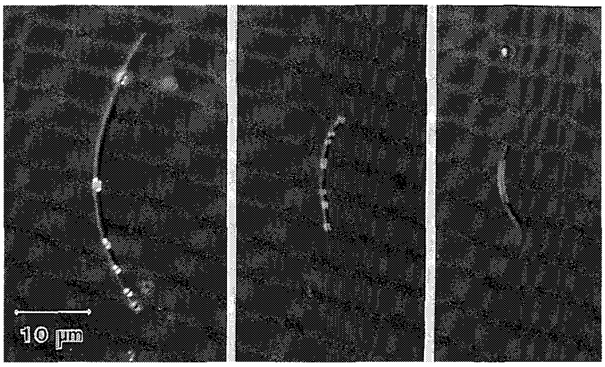

Fig. 4. The sausage-shaped bacteria carrying elemental sulfur outside the cell. These bacteria often observed in the A-type sulfur-turf which caused a milky cloudiness of the medium (Maki, unpublished data).

Figure 5 shows the decrease of sulfide ion concentration under different oxygen conditions. Sulfide decreased more rapidly under high dissolved oxygen. Concomitant with the increase of dissolved oxygen in the medium, both the production of elemental sulfur and the appearance of the sausage-shaped bacteria bearing elemental sulfur were accelerated.

A possible mechanism of this rapid formation of elemental sulfur is that the sulfide reacts with peroxide produced in the cell as postulated in the case of *Beggiatoa* (Burton and Morita, 1964 ; Joshi and Hollis, 1977). If the production of peroxides were activated in response to the increase in dissolved oxygen, both the more rapid sulfide decrease and the more accelerated production of elemental sulfur under high oxygen conditions are elucidated (Maki, 1987b).

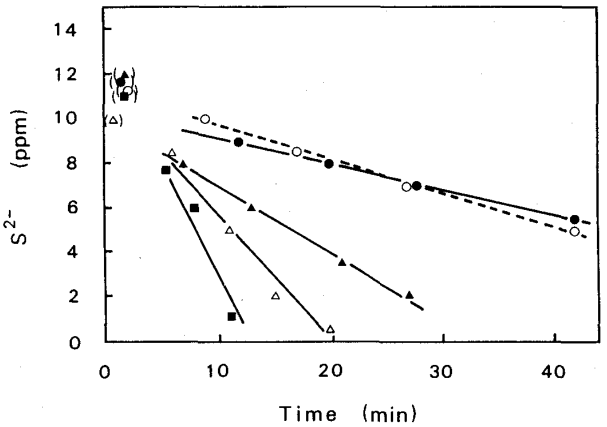

Fig. 5. The decrease of sulfide concentrations in the media. Filtered hot spring water enriched with micronutrient solution was inoculated with the A-type sulfur-turf. Dissolved oxygen concentrations in the media were 0.1 ppm (●), 1 ppm (▲), 2 ppm (△), and 6 ppm (■). The control (○) contained no sulfur-turf, and the dissolved oxygen concentration was 6 ppm. The initial points on parentheses were not included in the regression lines (Maki, 1987b).

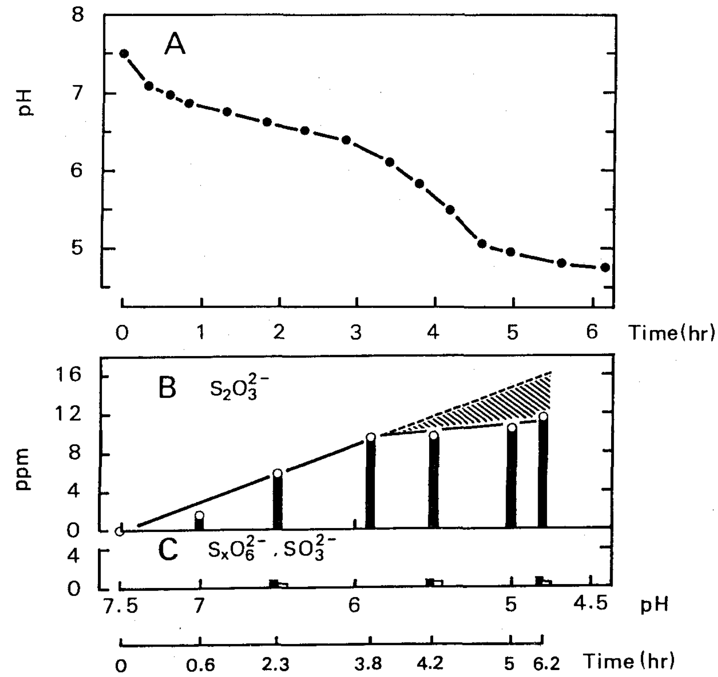

Fig. 6. Relationship between the decrease in pH and the production of sulfur compounds in the range of the start of experimental run to about pH 5. Filtered hot spring water enriched with micronutrient solution was inoculated with the A-type sulfur-turf. No hydrogen sulfide was added. A : The time-course of pH decrease in the medium containing the A-type sulfur-turf. B : A plot of the concentrations of thiosulfate versus pH decrease. Crosshatched area indicates the leveling off of increase in thiosulfate. Plotted points are shown by (○). Dotted line is extrapolation from the first 4 points. C : The changes of concentrations of polythionates (■) and sulfite (□). At pH 7.5, both polythionates and sulfite were not detected, but no determinations were made at pH 7, 5.9, and 5. The time scales in Figs. 6B and 6C are not equivalent (Maki, 1987b).

When no hydrogen sulfide is added to the culture medium, the elemental sulfur adhering to the A-type sulfur-turf is at first oxidized to thiosulfate and then to sulfate (Maki, 1987a). Figure 6 represents the relationships between the changes of the composition of sulfur compounds in the medium and the pH decrease ranging from pH 7.5 to about 5 (Maki, 1987a). It is worth noting that the increase of thiosulfate in the medium sufficiently explained the pH decrease from 7.5 to about 6, but below pH 6 the rate of thiosulfate increase decreased markedly. Based on the results shown in Fig. 6, it is conceivable that elemental sulfur is oxidized exclusively to thiosulfate in the pH range of 7.5 to 6 while thiosulfate oxidation to sulfate starts at below pH 6 (pH-dependent oxidation of thiosulfate) (Maki, 1987a).

Effects of dissolved oxygen concentrations on the oxidation of elemental sulfur are shown in Fig. 7. As the oxygen conditions of the A-type sulfur-turf in hot spring effluent are estimated at about 1 ppm (Maki, 1986), it is very interesting that the optimum dissolved oxygen tension (1.5 ppm) on the oxidation of elemental sulfur lies at a similar level. It can be assumed that this microaerophilic oxidation of elemental sulfur gives a physiological basis for the microaerophilic habitat preference of the A-type sulfur-turf (Maki, 1986).

Although direct contact of the cell surface with elemental sulfur is a prerequisite for the oxidation of elemental sulfur (Weise, 1973 ; Takakuwa *et al.*, 1979), no direct contact of the cell surface with elemental sulfur particles has been observed using light microscope. This suggests the possibility that the enzyme(s) pertaining to the oxidation of

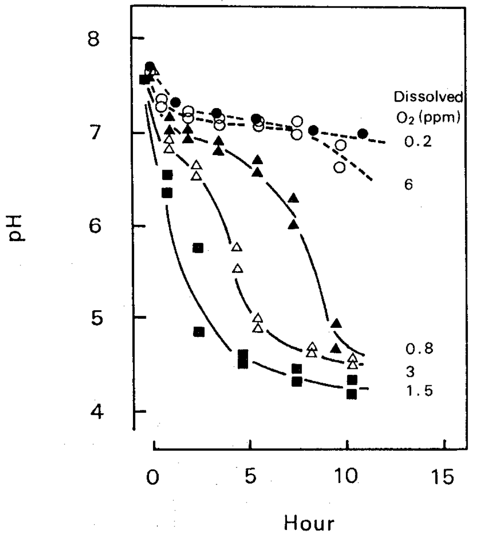

Fig. 7. The differences in the pH decrease in the culture media under different oxygen conditions. The pH decrease in the media containing the A-type sulfur-turf were measured at five oxygen conditions: 6 ppm (○), 3 ppm (△), 1.5 ppm (■), 0.8 ppm (▲), and 0.2 ppm (●) of dissolved oxygen. Dissolved oxygen concentrations were obtained by changing the partial pressure of oxygen in the gas mixture (nitrogen, oxygen, and carbon dioxide of 0.03%) which was supplied to the head space of the culture vessels. The pH was measured after the addition of the A-type sulfur-turf to the culture media containing no hydrogen sulfide (Maki, 1988b).

elemental sulfur are excreted by the A-type sulfur-turf. More extensive studies are required for understanding the mechanisms of elemental sulfur oxidation by the A-type sulfur-turf.

Under natural conditions, hydrogen sulfide dissolved in hot spring water is probably oxidized by the sausage-shaped bacteria at the surface of the bacterial aggregates. This active sulfide oxidation results in an accumulation of sulfur particles and covering the bacterial aggregates. These sulfur crusts are thought to obstruct water exchange inside the bacterial aggregates. This indicates the possibility of formation of microenvironment inside the crusted filament. If this were true, thiosulfate production by the sausage-shaped bacteria readily lowers the pH below 6, and leads to the oxidation of thiosulfate to sulfate by acidophilic thiobacilli included in the A-type sulfur-turf (Maki, 1987a).

Application of microelectrode method to microbial ecology was an epoch-making step in this field. It made possible to measure pH, Eh, temperature, and dissolved oxygen concentration in a precisions of micrometer scale (Revsbech and Jørgensen, 1986). Studies using microelectrodes are expected to clarify the microenvironments formed by the A-type sulfur-turf and help to determine the relationships between the physical structure of the filament and the mechanism of the sulfur oxidation process under natural conditions.

Concluding Remarks

The pure culture of the sausage-shaped bacteria consisting mainly of the A-type sulfur-turf has not yet been accomplished. Even in the case of relatively well-studied *Beggiatoa,* a large collection of isolates has only recently been obtained and comparative studies have begun (Larkin and Strohl, 1983). However, many obstacles relating to the isolation and cultivation of colorless sulfur bacteria must be overcome by technical developments in the near future. The success of obtaining pure culture and mass cultivation will clarify the presence of various kinds of sulfur-oxidizing bacteria.

Using several types of microelectrode, physicochemical factors around microorganisms can be determined in a scale of micrometer precissions (Jørgensen, and Des Marias, 1986). In contrast, the microbial response to the microenvironmental factors seems to remain comparatively obscure. In relation to the sulfur cycle, coastal sediments are very complicated both physico-chemically and biologically (Jørgensen, 1988). In response to environmental oxygen tensions, for example, some microorganisms switch their mode of energy-yielding metabolism and others migrate to optimal locations (Møller and Jørgensen, 1985; Kelly, 1988). In this regard, the concentration of molecular oxygen seems to be a primarily important environmental factor determining the activities and distribution of microorganisms pertaining to sulfur transformation.

Therefore, the next step in our investigation should be focused on the physiological reactions or the tolerance of microorganisms to molecular oxygen in order to determine both the maximum and minimum oxygen-requiring concentration. This type of investigation is called a "stress test" in which organisms are subjected to the range of experimental conditions carried out in the laboratory or field (Odum, 1971). Such a physiological approach will help us to understand the relationships between the distribution and the physiological bases of microorganisms.

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