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Sulfur Production by Obligately Chemolithoautotrophic *Thiobacillus* Species

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Transient-state experiments with the obligately autotrophic *Thiobacillus* sp. strain W5 revealed that sulfide oxidation proceeds in two physiological phases, (i) the sulfate-producing phase and (ii) the sulfur- and sulfate-producing phase, after which sulfide toxicity occurs. Specific sulfur-producing characteristics were independent of the growth rate. Sulfur formation was shown to occur when the maximum oxidative capacity of the culture was approached. In order to be able to oxidize increasing amounts of sulfide, the organism has to convert part of the sulfide to sulfur ($HS^- \rightarrow S^0 + H^+ + 2e^-$) instead of sulfate ($HS^- + 4H_2O \rightarrow SO_4^{2-} + 9\,H^+ + 8e^-$), thereby keeping the electron flux constant. Measurements of the in vivo degree of reduction of the cytochrome pool as a function of increasing sulfide supply suggested a redox-related down-regulation of the sulfur oxidation rate. Comparison of the sulfur-producing properties of *Thiobacillus* sp. strain W5 and *Thiobacillus neapolitanus* showed that the former has twice the maximum specific sulfide-oxidizing capacity of the latter (3.6 versus 1.9 μmol/mg of protein/min). Their maximum specific oxygen uptake rates were very similar. Significant mechanistic differences in sulfur production between the high-sulfur-producing *Thiobacillus* sp. strain W5 and the moderate-sulfur-producing species *T. neapolitanus* were not observed. The limited sulfide-oxidizing capacity of *T. neapolitanus* appears to be the reason that it can convert only 50% of the incoming sulfide to elemental sulfur.

Sulfide-containing waste streams are generally treated by chemical methods which involve high chemical and disposal costs (5, 6). The ability of autotrophic bacteria to oxidize sulfide at high rates has led to the development of biotechnological methods to remove sulfide from effluent streams (8, 13, 19). A biotechnological sulfide-removing process (3), based on the ability of colorless sulfur bacteria to partially oxidize sulfide to elemental sulfur under certain conditions (14), has been developed. A major environmental advantage is the recovery of sulfur from the effluent stream, thereby preventing boosting of the sulfur cycle by the discharge of sulfate-containing wastewater.

Mixed-culture studies of sulfur-producing bacteria have shown that the formation of sulfur is dependent on the sulfide load and the availability of oxygen (2). Fed-batch studies showed that the production of sulfur can be controlled by the amount of oxygen supplied (10). Quantitative measurements of sulfur formation under aerobic conditions with pure cultures of several neutrophilic obligately autotrophic *Thiobacillus* species revealed that the capacity to produce sulfur was also strain dependent (17, 18). The isolation of *Thiobacillus* sp. strain W5, the dominant organism from a sulfur-producing reactor system which is capable of stoichiometrically converting sulfide to elemental sulfur (24), has provided a suitable organism for a detailed study of the formation of sulfur. This paper shows that sulfur formation in this bacterium is directly related to its maximum electron-transporting capacity and explains why *Thiobacillus neapolitanus*, in contrast to *Thiobacillus* sp. strain W5, is not capable of stoichiometrically converting all sulfide to elemental sulfur. A possible role for the in vivo degree of reduction of the cytochrome pool in the process of sulfur formation is considered.

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MATERIALS AND METHODS

Organisms and cultivation. *T. neapolitanus* (type strain, LMD 80.85) and *Thiobacillus* sp. strain W5 (LMD 94.73) were obtained from the Delft Culture Collection. Both organisms were grown in chemostats (pH 7.5; temperature, 25°C) in mineral medium (18) under sulfide limitation (50 mM) at different dilution rates. The dissolved oxygen concentration was maintained at 50% relative to air saturation.

Oxygen uptake experiments. The maximum sulfide- and sulfur-dependent oxygen uptake rates of whole cells, taken directly from chemostat cultures, were measured in a biological oxygen monitor. Oxygen consumption was measured polarographically at 25°C with a Clark-type oxygen electrode. Sulfide (25 μM) and sulfur dissolved in acetone (40 μM) were added to obtain maximum oxidation rates.

Transient-state experiments. Short-term sulfur formation was studied with transient-state experiments, which were performed at a dissolved oxygen concentration of 40% air saturation, 25°C, and pH 7.5. Transient-state experiments were started with the effluent from a sulfate-producing, steady-state culture which had been collected overnight at 4°C. After equilibration at 25°C, the cultures were run for 20 min under the original steady-state conditions. The sulfide-limited steady-state cultures were then exposed to increasing sulfide loads for 10-min intervals (18). Sodium sulfide (50 mM) was supplied at the bottom of the chemostat in order to prevent losses to the air. Sulfur production rates were determined from the increase in the sulfur concentration with time. Sulfate production rates were calculated by subtraction of the sulfur production rate from the sulfide load (24). Air was supplied at a rate of 30 liters/h. Oxygen consumption was measured with a Servomex 1100A oxygen analyzer (Servomex Ltd., Sussex, United Kingdom).

In vivo reduction levels of cytochrome *c* pool. The determination of the in vivo reduction levels of the cytochrome pool in growing bacteria was done by coupling a chemostat to a dual-wavelength UV-visible light spectrophotometer (American Instruments Co.; DW-2) with the reference wavelength set at 578 nm as described by Pronk et al. (15). The spectra were recorded between 530 and 570 nm at a scanning speed of 3 nm/s. Cytochrome spectra were dominated by cytochromes *c*, having an absorption maximum at 552 nm. The spectra were recorded in triplicate in order to obtain a representative average spectrum. It was observed that the formation of sulfur influenced the spectra. It was therefore necessary during sulfur formation to measure the fully reduced spectrum directly after the registration of the spectrum at a particular sulfide load in order to calibrate the effect of the increasing sulfur concentration in the chemostat. The reduction levels of the cytochromes were expressed as a percentage of absorbance of fully reduced cytochromes in the alpha region of the spectrum. Fully reduced cyto-

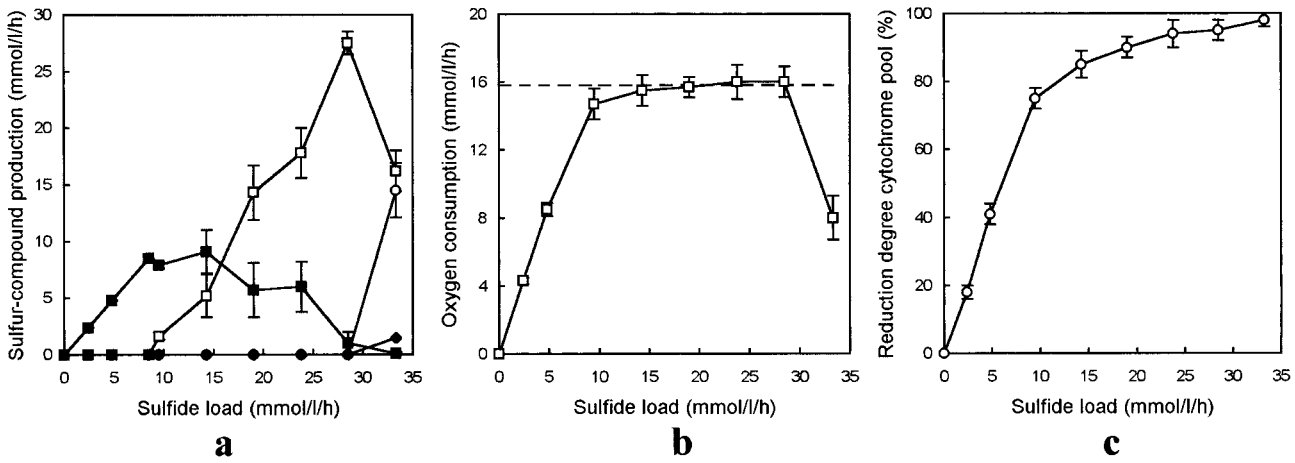

FIG. 1. (a) Production rates of sulfur compounds by *Thiobacillus* sp. strain W5, pregrown at a dilution rate of 0.1 h^{-1}, during transient-state experiments under increasing sulfide loads at 40% air saturation (□, sulfur; ■, sulfate; ○, sulfide; ◆, thiosulfate). (b) Oxygen consumption during transient-state experiments. (c) Degree of reduction of the in vivo cytochrome pool during transient-state experiments.

chromes were obtained by flushing the suspension with pure nitrogen, after which a small amount of sulfide was added. The addition of dithionite did not increase the reduction of the cytochrome pool. For this experimental setup, the absorbance difference between fully reduced and air-oxidized cytochromes at 552 nm was 0.5/g (dry weight).

Sulfur pulses. Sulfur pulses were given in order to measure the reduction of cytochromes during the oxidation of externally added sulfur. Sulfur (25 ml/liter) was added as a sulfur-saturated (17 mM) acetone solution, resulting in a final sulfur concentration of 425 μmol of sulfur/liter, high enough to achieve the maximum sulfur oxidation rates for approximately 3 min. Addition of more sulfur solution resulted in inhibition by the acetone, as determined in control experiments.

Protein determination. The protein content of whole cells was measured by a modified biuret method (23).

Analysis of sulfur compounds. Samples from transient-state cultures were filtered over 0.45-μm-pore-diameter nitrocellulose filters (Schleicher & Schuell) and immediately washed with cold water to remove water-soluble inorganic reduced sulfur components. The sulfur-biomass residues were extracted overnight in acetone and analyzed by cyanolysis (1). The filtrate was used for the analysis of soluble sulfur compounds. Thiosulfate, tetrathionate, and trithionate were determined colorimetrically after cyanolysis (12). Sulfite was semiquantitatively determined in the filtrate with commercially available dipsticks (Merck). Sulfide in gas streams was removed by being passed through a zinc acetate solution to precipitate as ZnS. Sulfide in the filtrate was also trapped by the addition of zinc acetate. The ZnS was analyzed by the methylene blue method (21). Sulfide in medium reservoirs was measured by iodometric titration (16).

Activity of sulfide-oxidizing enzyme. Cells collected at 4°C from a sulfide-limited chemostat were centrifuged and resuspended in 50 mM Tris-HCl (pH 7.5). Cell-free extracts were prepared by passage of the cell suspensions three times through a French pressure cell (American Instrument Company, Silver Spring, Md.) at 110 MPa. The broken cells were subsequently treated with DNAse, after which the remaining whole cells and cell debris were removed by centrifugation at 10,000 × *g* for 30 min. The reaction mixture (1 ml) for measuring the activity of the sulfide-oxidizing enzyme contained 50 mM Tris-HCl (pH 7.5), 25 μM horse heart cytochrome *c* (Sigma), 10 μM Na_2S, and cell extract. The measurements were done at room temperature and were started by the addition of sulfide. Reduction of horse heart cytochrome *c* was measured at 550 nm in an HP 8524A Diode array spectrophotometer with an extinction coefficient of 19.6 mM^{-1} cm^{-1}.

RESULTS

Sulfur-producing characteristics of *Thiobacillus* sp. strain W5. When *Thiobacillus* sp. strain W5 was grown in a sulfide-limited chemostat at a dilution rate of 0.10 h^{-1}, oxygen uptake measurements showed a maximum oxidation capacity on sulfide of 15.8 mmol of O_2/liter/h. The results of a typical transient-state experiment are shown in Fig. 1. Up to a sulfide load of 9 mmol/liter/h, sulfate was the only detectable product (Fig. 1a). The oxygen consumption and the in vivo reduction degree of the cytochrome *c* pool increased linearly with the increasing sulfide load (Fig. 1b and c). During the sulfate-producing stage, a constant oxygen/sulfide uptake ratio of approximately 1.8:1 was observed.

Sulfur started to appear in the chemostat when the sulfide load exceeded 9 mmol/liter/h. The oxygen consumption was 15 mmol/liter/h at the onset of sulfur formation, and the cytochrome pool was 70% reduced. A further increase in the sulfide load led to increased sulfur production and decreased sulfate production. During the sulfur-forming stage, in which the sulfide load was increased from 9 to 28.5 mmol/liter/h, oxygen consumption increased by only 8%, reaching its apparent maximum value of 16 mmol/liter/h. This value is in close agreement with the maximum oxidation capacity measured by oxygen uptake experiments (15.8 mmol/liter/h). The oxygen/sulfide uptake ratio decreased during the sulfur-forming stage from 1.8 to 0.56. The in vivo degree of reduction of the cytochrome pool gradually increased from 70% to 95%. At a sulfide load of 28.5 mmol/liter/h, almost all of the incoming sulfide was converted to elemental sulfur (27.5 mmol/liter/h). Below this load, sulfur and sulfate were the only detectable products of sulfide oxidation by *Thiobacillus* sp. strain W5.

Further increasing the sulfide load to 33.3 mmol/liter/h led to the accumulation of sulfide in the effluent and the appearance of hydrogen sulfide in the off gas. At the same time, thiosulfate was detected in the effluent. It was observed that the sulfide-oxidizing capacity of the biomass decreased dramatically when sulfide accumulated in the chemostat. Transient-state experiments were terminated when the sulfide load exceeded the sulfide-oxidizing capacity of the biomass.

TABLE 1. Biomass concentration, absolute maximum oxidation capacity ($rO_{2_{max(sulfide)}}$), and specific maximum oxidative capacity ($qO_{2_{max(sulfide)}}$) of *Thiobacillus* sp. strain W5 at different growth rates (dilution rates)[a]

Dilution rate (h^{-1})	Amt of protein (mg/liter)	$rO_{2_{max(sulfide)}}$ (mmol/liter/h)	$qO_{2_{max(sulfide)}}$ (μmol/mg of protein/min)
0.05	120 ± 2	13.5 ± 0.9	1.87 ± 0.12
0.1	135 ± 2	15.8 ± 0.8	1.95 ± 0.10
0.2	164 ± 5	18.2 ± 1.1	1.85 ± 0.11

[a] Results are averages of three measurements.

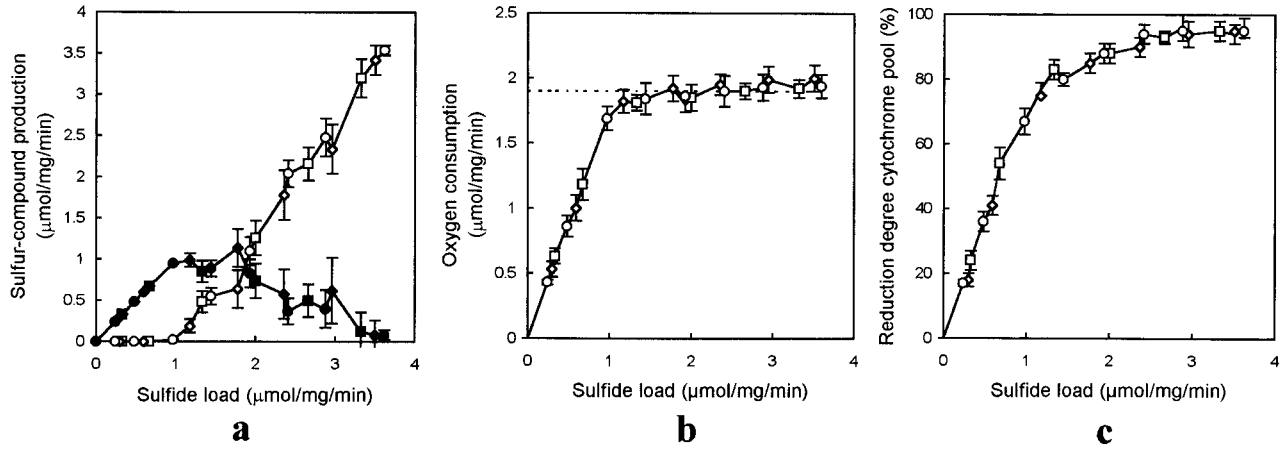

FIG. 2. Results of transient-state experiments performed with *Thiobacillus* sp. strain W5 cells pregrown at dilution rates of 0.05 (□, ■), 0.1 (◆, ◇), and 0.2 (●, ○) h^{-1}. (a) Specific sulfur (□, ◇, ○) and sulfate (■, ◆, ●) production rates. (b) Specific oxygen consumption. (c) Degree of reduction of the in vivo cytochrome pool.

Growth rate dependence of sulfur production by *Thiobacillus* sp. strain W5. The relationship between growth rate and sulfur production was investigated by additional transient-state experiments with cells pregrown at dilution rates of 0.05 and 0.2 h^{-1}. During precultivation, it was observed that the amount of biomass and the absolute maximum oxidative capacity increased with the increasing dilution rate. The specific maximum oxidation capacity, however, remained constant at 1.9 μmol/mg of protein/min (Table 1).

The absolute sulfur-producing characteristics of cells pregrown at different dilution rates appeared to be different. Cells pregrown at dilution rates of 0.05 and 0.2 h^{-1} started to produce sulfur at sulfide loads of 7.5 and 11 mmol/liter/h, respectively. The loads at which the sulfide-oxidizing capacity was exceeded also differed considerably (27.5 and 37.5 mmol of sulfide/liter/h for cells pregrown at $D = 0.05$ and 0.2 h^{-1}, respectively).

However, when all parameters were converted to biomass-specific loads and rates, there were no differences in the sulfur-producing characteristics of the cultures (Fig. 2). These experiments show that sulfur formation started at a specific sulfide load of 1.0 μmol/mg of protein/min. The maximum specific sulfide-oxidizing capacity was around 3.6 μmol/mg of protein/min. It was also demonstrated that the starting point of sulfur formation was correlated with a degree of reduction of the cytochrome pool of 70% and a specific oxygen consumption rate of 1.75 μmol/mg of protein/min, corresponding to 90% of the maximum value.

Sulfur oxidation by *Thiobacillus* sp. strain W5. Oxygen uptake experiments with *Thiobacillus* sp. strain W5 cells, grown under sulfide limitation at a dilution rate of 0.1 h^{-1}, had a maximum oxidation capacity on sulfur of 1.6 μmol of O_2/mg of protein/min. This activity represents 85% of the activity obtained with sulfide as a substrate. A sulfur pulse to aerobic cells in a fully oxidized state resulted in an approximate 63% ± 3% reduction of the cytochromes. When a sulfur pulse was given to cells which were already loaded with 0.3 μmol of sulfide/liter/h, reduction of the cytochromes increased from 17% to 67% ± 3%. In addition, oxygen consumption increased from 0.55 to 1.6 μmol of O_2/mg of protein/min. This is consistent with the oxygen consumption rate measured during oxygen uptake experiments. The addition of sulfur to sulfur-producing cells being supplied with a sulfide load of 1.8 μmol of sulfide/liter/h did not cause further reduction of the cytochromes or higher

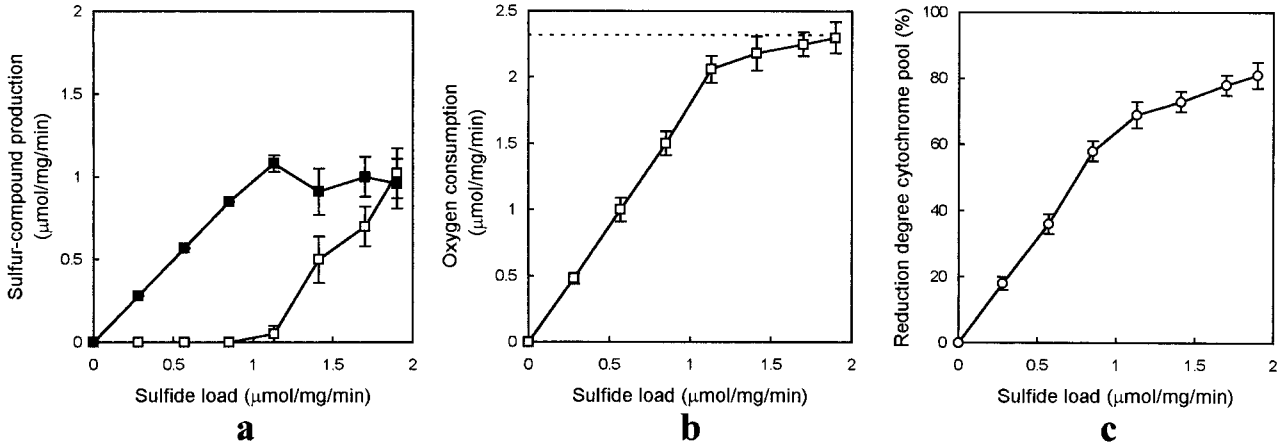

FIG. 3. (a) Specific production rates of sulfur compounds (mmol/liter/h) by *T. neapolitanus*, pregrown at a dilution rate of 0.1 h^{-1}, during transient-state experiments under increasing sulfide loads at 40% air saturation (□, sulfur; ■, sulfate). (b) Specific oxygen consumption during transient-state experiments. (c) Degree of reduction of the in vivo cytochrome pool during transient-state experiments.

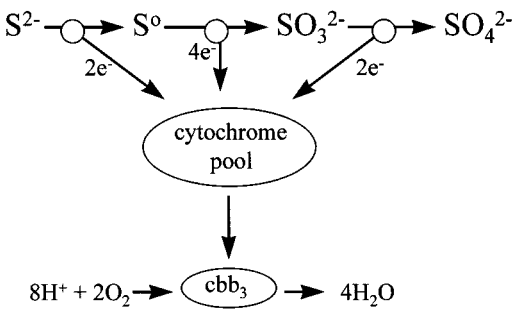

FIG. 4. Schematic representation of the sulfide-metabolizing pathway in obligately autotrophic thiobacilli. It is proposed that sulfide is oxidized to sulfate via intermediary sulfur and sulfite. Electrons enter the respiratory chain at the level of cytochrome *c* and are coupled to oxygen via a cbb_3-type oxidase (25).

oxygen consumption. A sulfur pulse given to cells under anaerobic conditions reduced the cytochromes to 85% ± 3%, indicating that externally added sulfur was not capable of totally reducing all cytochromes present in *Thiobacillus* sp. strain W5.

Sulfur-producing characteristics of *T. neapolitanus*. Transient-state experiments were done with the moderate-sulfur-producing species *T. neapolitanus* in order to determine quantitative differences from sulfur production by *Thiobacillus* sp. strain W5. Oxygen uptake experiments with *T. neapolitanus* cells, grown under sulfide limitation at a dilution rate of 0.1 h^{-1}, showed a specific maximum oxidation capacity on sulfide of 2.3 μmol of O_2/mg of protein/min. During the transient-state experiments, *T. neapolitanus* produced sulfate up to a specific sulfide load of 1.1 μmol/mg of protein/min (Fig. 3). The oxygen consumption rate and degree of reduction of the cytochromes increased linearly during the sulfate-producing stage. This was similar to the observations during transient-state experiments with *Thiobacillus* sp. strain W5. Sulfur formation started again when the cytochromes were reduced by 70% and the oxygen consumption had reached 90% of its maximum value. However, *T. neapolitanus* reached its maximum sulfide-oxidizing capacity at the much lower specific load of 1.9 μmol of sulfide/mg of protein/min, and a further increase in the sulfide load led to the accumulation of sulfide. The sulfide-oxidizing capacity of *T. neapolitanus* was therefore approximately half that of *Thiobacillus* sp. strain W5. A consequence of this limited sulfide-oxidizing capacity was that *T. neapolitanus* could convert a maximum of 50% of the sulfide to sulfur, the rest being oxidized to sulfate. During the sulfur-forming stage, oxygen consumption still reached the maximum activity found during oxygen uptake experiments. The degree of reduction of the cytochromes, however, only increased to 80%.

In vitro sulfide-oxidizing capacities of *Thiobacillus* sp. strain W5 and *T. neapolitanus*. The in vitro sulfide-oxidizing capacities of cell extracts from *Thiobacillus* sp. strain W5 and *T. neapolitanus* were 25 ± 3 and 11 ± 2 nmol of cytochrome *c*/mg/min, respectively. The difference between these values supports the observation that *Thiobacillus* sp. strain W5 has double the sulfide-oxidizing capacity of *T. neapolitanus*.

DISCUSSION

Two physiological phases of sulfide oxidation by *Thiobacillus* sp. strain W5. The transient-state experiments revealed that *Thiobacillus* sp. strain W5, the dominant organism from a sulfur-producing microbial community, was able to convert the sulfide nearly stoichiometrically to sulfur under aerobic conditions. The sulfur-producing characteristics of *Thiobacillus* sp. strain W5 were shown to be independent of the growth rate and seemed to be determined by its specific maximum oxidation capacity. Transient-state experiments revealed that sulfide oxidation by *Thiobacillus* sp. strain W5 can be split into two phases, after which sulfide toxicity occurs.

During the first phase, sulfide is completely oxidized to sulfate ($HS^- + 2O_2 \rightarrow SO_4^{2-} + H^+$). The observed oxygen/sulfide ratio of 1.8 indicates that 10% of the electrons from the oxidation of sulfide were required for the fixation of carbon dioxide via reversed electron flow. This is in good agreement with the results of Stefess (17), who found that 9% of the electrons were used for carbon dioxide reduction by *T. neapolitanus*. The degree of reduction of the cytochromes and the oxygen consumption rate were found to increase linearly with increasing sulfide loads.

When the cytochromes reached 70% reduction and the oxygen consumption rate approached its maximum, the second sulfur-producing phase set in. The oxygen consumption rate is also a direct and quantitative measure of the flow of electrons passing through the respiratory chain towards the terminal oxidase ($O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$). Further increasing the sulfide load beyond the point where *Thiobacillus* sp. strain W5 reaches its maximum electron-transporting capacity leaves two possible outcomes. If sulfate continues to be the sole product, additional incoming sulfide will accumulate in the culture. Because of the toxic nature of sulfide, this can be expected to be unfavorable to the bacterium. The second option is to redistribute the electrons from the increasing sulfide flux by converting some of the sulfide to sulfur ($HS^- \rightarrow S^0 + H^+ + 2e^-$) instead of sulfate ($HS^- + 4H_2O \rightarrow SO_4^{2-} + 9H^+ + 8e^-$). This prevents the accumulation of sulfide and is therefore more favorable. The decrease of the oxygen/sulfide uptake ratio from 1.8 to 0.56 during the sulfur-producing phase was a consequence of the fact that the oxygen consumption rate (electron flux) remained constant while the sulfide load increased considerably. The observation that sulfur production was maximal at an oxygen/sulfide uptake ratio of around 0.56 is in close agreement with the theoretical expectations ($HS^- + 0.5O_2 \rightarrow S^0 + OH^-$) and the results obtained with mixed cultures (10). The specific sulfide load at which *Thiobacillus* sp. strain W5 started to produce sulfur is also very close to the reported specific value of 1.04 μmol of sulfide/mg of protein/min obtained with mixed cultures (4). It should also be noted that sulfide could not be detected in the medium during the sulfur-producing stage, indicating that chemical oxidation of sulfide does not play an important role under these circumstances.

When the sulfide load becomes so high that it exceeds the capacity of the sulfide-oxidizing system, even when all sulfide is converted to elemental sulfur, sulfide accumulates in the culture. The appearance of sulfide in the medium led to a decrease of the oxidative capacity of the biomass, thereby accelerating the accumulation of sulfide and eventually resulting in the total deterioration of the sulfur-producing system. This decrease in oxidative capacity is probably caused by the toxic effects of sulfide, which has been reported to inhibit thiobacilli at concentrations above 150 μM (9). The appearance of thiosulfate during this phase was caused by chemical oxidation of sulfide, which has been described as the main product of chemical sulfide oxidation (7, 10).

Redox-related regulation of the activity of sulfur production? Figure 4 shows a schematic drawing of the hypothetical sulfide-metabolizing pathway in *Thiobacillus* sp. strain W5. Sulfur is bound to accumulate when the conversion of sulfur to sulfite is slower than the conversion of sulfide to sulfur. Transient-state experiments indicated that sulfur formation begins

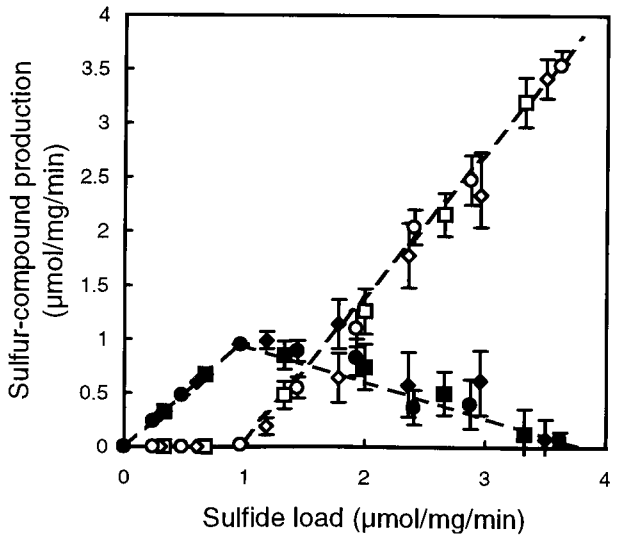

FIG. 5. Calculation of sulfur-producing characteristics on the basis of the maximum oxidative capacity. The markers represent the results as shown in Fig. 3. The dotted line connects the calculated point at which sulfur formation starts (0.95 μmol of sulfide/mg of protein/min) and the point at which sulfide starts to accumulate (3.8 μmol of sulfide/mg of protein/min).

when the cytochromes started to become saturated. It is therefore tempting to speculate that the physiological signal for the regulation of sulfur oxidation is related to the degree of reduction of the cytochrome chain.

One possibility is that the dropping redox poise of the cytochromes would still enable electrons from the sulfide-sulfur couple to enter the respiratory chain, while the entry of the electrons from the sulfur-sulfite couple becomes more and more difficult. The standard midpoint potential of the sulfide-sulfur couple is indeed lower ($E_0' = -260$ mV) than that of the sulfur-sulfite couple ($E_0' = -45$ mV) (20). The observation that the addition of sulfur to anaerobic cells does not lead to a total reduction of the cytochrome pool is in agreement with this option. Another possibility is that the sulfur-oxidizing enzyme itself is redox sensitive and is controlled by the redox level of the respiratory chain.

The suggestion that the activity of the sulfur-oxidizing enzyme is regulated by redox is supported by the observation that sulfur formation occurs under oxygen limitation (10, 18, 22). The limited availability of oxygen will result in an increase of the degree of reduction of the cytochrome pool, thereby blocking the conversion of sulfur to sulfite. However, detailed studies of the enzymes involved in the oxidation of sulfide to sulfate and the components of the respiratory chain involved in transporting the electrons to the terminal oxidase are obligatory to determine the exact regulatory and rate-determining mechanisms involved in the production of sulfur.

Limited capacity of the sulfide-oxidizing enzyme as a cause for a lower sulfur-producing capacity of *T. neapolitanus*. Transient-state experiments with *T. neapolitanus* showed that this organism is capable of converting only 50% of the sulfide to elemental sulfur under aerobic conditions. This is in reasonable agreement with the previously reported sulfur-producing capacity of 42% (18). The sulfate-producing phase is comparable to that of *Thiobacillus* sp. strain W5. Sulfur formation started at a slightly higher specific sulfide load, probably because *T. neapolitanus* has a higher maximum oxidation capacity. However, *T. neapolitanus* accumulated sulfide at a load of 1.9 μmol of sulfide/liter/h, indicating that this is its maximum sulfide-oxidizing capacity. This suggests that the sulfide-oxidizing enzyme is the limiting factor. In *Thiobacillus* sp. strain W5, the electron-transporting capacity (or oxygen consumption rate) limits the sulfide oxidation rate. The differences between the capacities of the two sulfide-oxidizing systems are supported by the fact that the in vitro sulfide-oxidizing capacity of *Thiobacillus* sp. strain W5 is twice as high as that of *T. neapolitanus*. The profiles of the cytochrome reduction levels and oxygen consumption rates in the two organisms were comparable up to the point at which the sulfide load exceeded the sulfide-oxidizing capacity of *T. neapolitanus*. From this work, it can be concluded that the causes of sulfur formation in the high-sulfur producer *Thiobacillus* sp. strain W5 and the moderate-sulfur producer *T. neapolitanus* appear not to be significantly different. The higher sulfide-oxidizing capacity of *Thiobacillus* sp. strain W5 also confers on the organism a competitive advantage under sulfide limitation conditions. Preliminary results from sulfide-limited chemostat cultures of a mixture of *Thiobacillus* sp. strain W5 and *T. neapolitanus* showed that the latter was rapidly outcompeted by *Thiobacillus* sp. strain W5.

Determination of sulfur-producing characteristics on the basis of a single parameter: maximum oxidative capacity. When the formation of sulfur depends only on the maximum oxidation capacity, as is the case for *Thiobacillus* sp. strain W5, the onset of sulfur formation and the point at which sulfide starts to accumulate can be easily calculated. The sulfide load at which sulfur production starts can be determined by division of the maximum oxidation capacity by 2 (1.9 μmol of O_2/liter/h/2 = 0.95 μmol of S^{2-}/liter/h), while the maximum sulfide load can be calculated by multiplication of the maximum oxidation capacity by 2 (1.9 μmol of S^{2-}/liter/h × 2 = 3.8 μmol of O_2/liter/h). Figure 5 shows that the sulfur production rates increased almost linearly between these two points. The percentage of sulfur formation can thus be predicted for any sulfide load when the maximum oxidation capacity is known.

ACKNOWLEDGMENTS

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