Reactivity versus flexibility in thiobacilli

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The results of ecophysiological studies on obligately and facultatively chemolithotrophic thiobacilli performed over the past years clearly show that the two types of organisms occupy different ecological niches. Chemostat experiments with cultures of the obligate chemolithotroph *Thiobacillus neapolitanus* and the facultative chemolithotroph *Thiobacillus* A2 have been carried out to explain the competitiveness of *T. neapolitanus* under conditions of strongly fluctuating substrate supply.

Thiobacillus neapolitanus appeared to be very resistant to starvation periods whereafter it could oxidize sulfide (or thiosulfate) almost instantaneously at the original rate. Under alternate supply of 4 h sulfide and 4 h sulfate (or acetate which does not support growth of the organism either) to a chemostat culture of *T. neapolitanus* ($D = 0.05\ h^{-1}$) the sulfide concentration in the growth vessel never reached levels higher than 4μM. This strategy is aimed at maximal reactivity. In contrast to *T. neapolitanus* the facultative chemolithotroph *T.*A2 appeared to be very flexible with respect to its energy generation. Under alternate supply of 4 h sulfide and 4 h acetate ($D = 0.05\ h^{-1}$) *T.*A2 was able to grow continuously since it directed its metabolism to either heterotrophy or autotrophy by rapid induction-repression mechanisms. This flexible strategy seems to be incompatible with a reactive strategy within one organism, since the oxidation capacity for sulfide decreased during the acetate period resulting in accumulation of sulfide during the sulfide period. It is concluded that *T.*A2 needs a continuous supply of an inorganic and an organic substrate to thrive whereas *T. neapolitanus* needs only a continuous supply of a reduced inorganic sulfur source but also will persist in environments with interrupted addition of sulfide provided that the starvation period does not last too long.

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INTRODUCTION

A variety of bacteria is able to oxidize reduced inorganic sulfur compounds, like sulfide, sulfur and thiosulfate under aerobic conditions. These bacteria can be classified into several groups based on their different physiological characteristics (Rittenberg, 1969; Kuenen, 1975) as follows.

1. The obligately chemolithoautotrophic thiobacilli which are able to generate energy only from the oxidation of reduced sulfur compounds (for recent reviews see Smith and Hoare (1977) and Matin (1978)).
2. The facultatively chemolithotrophic thiobacilli which can use reduced sulfur compounds as well as organic compounds as energy sources. Simultaneous oxidation of both the organic and the inorganic substrate is also possible, a process called mixotrophic growth (Matin, 1978; Gottschal and Kuenen, 1980*a*; Smith et al., 1980). Recently it has been shown that also various facultatively chemolithotrophic hydrogen oxidizing bacteria are able to utilize thiosulfate as sole energy source (Friedrich and Mitrenga, 1981).
3. Heterotrophic bacteria some of which are able to gain energy from the oxidation of reduced sulfur compounds (e.g. *Thiobacillus perometabolis*) but which are not able to grow autotrophically (Tuttle and Jannasch, 1972; Gottschal and Kuenen, 1980*b*).
4. Phototrophic bacteria some of which have been shown to grow chemolithotrophically in the dark on reduced sulfur compounds (Kämpf and Pfennig, 1980).

In nature these groups of bacteria often occur in the same habitat and, thus, may compete for the same substrates i.e. reduced sulfur compounds. In a first approach to determine their ecological niches two representatives of physiologically distinctive groups were studied. As a representative of the obligately chemolithotrophic thiobacilli *Thiobacillus neapolitanus* was chosen. *Thiobacillus* A2 was used as a typical example of the facultatively chemolithotrophic thiobacilli. In a previous publication (Gottschal et al., 1979) it was shown that a supply of both inorganic and organic substrates is of clear ecological advantage to the facultatively chemolithotrophic *T.*A2 during competition with specialistic autotrophs and heterotrophs, whereas a supply of thiosulfate only favoured *T. neapolitanus*. The general validity of the results of this type of experiments was confirmed by the outcome of enrichment cultures in the chemostat (Gottschal and Kuenen, 1980*b*). In further studies it was investigated whether the high metabolic flexibility of the facultative chemolithotrophic *T.*A2 does have survival value for this type of organism when competing with specialist thiobacilli (Gottschal et al., 1981*b*). It was pointed out that in contrast to *T.*A2 obligately chemolithotrophic thiobacilli are metabolically rigid since addition of organic compounds to their growth medium never results in induction of enzymes required for the metabolism of these compounds (Smith and Hoare, 1977; Matin, 1978). At first sight this seemed to be of disadvantage for the obli-

gate chemolithotrophs during competition with facultatively chemolithotrophic thiobacilli when exposed to an alternating supply of inorganic and organic energy substrates. A series of competition experiments under such conditions showed, however, that coexistence occurred (Gottschal et al., 1981*a*). The results indicated that *T.*A2 was unable to oxidize a substantial portion of the available thiosulfate. These experiments were explained by the assumption that the specialist *T. neapolitanus* was more reactive, in the sense that it retained a higher oxidation potential, allowing for more rapid (initial) growth. As a result *T. neapolitanus* would immediately lower the concentration of thiosulfate to such a low level that *T.*A2 was no longer able to maintain an appreciable thiosulfate oxidizing potential. However likely this might be, this explanation could not be substantiated at that time, since we were unable to measure the actual *in situ* concentration of the growth limiting thiosulfate. This is due to the relative insensitivity of the thiosulfate assay. Therefore it was decided to attempt to grow the organisms at low oxygen concentration on sulfide, which can be measured accurately at much lower concentrations. In this paper we report the successful cultivation of thiobacilli on sulfide. It also appeared possible to follow the transient appearance of sulfide in chemostat cultures of both *Thiobacillus* spp, during alternate supply of sulfide and acetate.

Methods

Organisms and growth conditions

Thiobacillus neapolitanus (strain X) was grown in mineral medium in the chemostat as described previously (Kuenen and Veldkamp, 1973). Cultures were maintained at pH = 6.8, or pH = 7.5 where indicated, by automatic titration with 1 M Na_2CO_3. Special precautions had to be taken to enable growth of *T. neapolitanus* on sulfide. $Na_2S.8H_2O$ was dissolved in hot oxygen free demineralized water and autoclaved separately. Directly after sterilization Na_2S was diluted 15 fold to a final concentration of 80 mM in hot demineralized autoclaved water. This medium was kept under nitrogen pressure to prevent chemical oxidation of sulfide. Since obligately chemolithotrophic thiobacilli derive their carbon from CO_2 it is essential that titration by Na_2CO_3 does occur. Na_2S, however, is very alkaline and to overcome problems of CO_2 limitation it appeared to be essential to administer a 0.08 M H_2SO_4 solution to the growth vessel at the same rate as the administration of the Na_2S solution. The other required minerals were added as a two times concentrated medium to this sulphuric acid solution. This medium contained (final concentration in g/l: K_2HPO_4, 1.0; KH_2PO_4, 1.0; $MgSO_4.7H_2O$, 1.6; NH_4Cl, 0.8). Four ml of a trace element solution was added to a liter of medium (Vishniac and Santer, 1957). Since the sulfide solution and the mineral salts solution containing sulfuric acid are pumped into the culture at the same rate the final concentrations in the

chemostat are half of that reported above.

During growth on Na_2S the dissolved oxygen tension in the chemostat was kept at 5% of air saturation by automatic control. Yields of cultures grown on thiosulfate are equal at oxygen tensions of 5 and 50% of air saturation denoting that oxygen is not growth-limiting at an oxygen tension of 5% of air saturation. The inlet of S^{2-} ended in the culture liquid. During alternate supply of sulfate and sulfide, transitions from one medium to the other medium were done automatically by timed switching of the two pumps feeding the medium to the culture. The starvation medium for *T. neapolitanus* contained an equivalent amount of sulfate instead of thiosulfate at a final concentration of 80 mM. The oxygen tension in the culture during the sulfate period was kept at 5% of air saturation by flushing with mixtures of air and nitrogen. Long term starvation conditions for *T. neapolitanus* were created by switching off the medium supply of a thiosulfate-limited continuous culture. To prevent evaporation during this period the culture was aerated with water saturated air. *T.*A2 was grown under alternate supply of Na-acetate and Na_2S in the chemostat and was maintained at pH 7.5, and pH 8.0 where indicated, by automatic titration with 1 M HCl or 1 M Na_2CO_3. The oxygen tension in the culture was kept also at 5% of air saturation. Medium composition has been described elsewhere (Gottschal et al., 1981*a*). Growth on sulfide was realized as described above for *T. neapolitanus*.

Sulfide determination

Sulfide was determined polarographically (Orion Research, 1979). It can be calculated that the culture grown at a dilution rate of 0.05 h^{-1} consumed approximately 0.5 μM of sulfide per liter and per second. As the actual concentrations of sulfide turned out to be in the order of 1–4 μM, a sampling time of less than one second was required. This was accomplished by a rapid sampling and mixing (1: 1 v/v) into a strong alkaline solution containing 80 g/l NaOH, 35 g/l ascorbic acid and 67 g/l Titriplex III (Merck-FRG). This inactivated the bacteria instantaneously.

Protein determination

Protein in cell-free extracts was determined by the Coomassie brilliant blue method using bovine serum albumine as a standard (Bradford, 1976). Protein content of whole cells was quantified with a modification of the microbiuret method (Goa, 1953) as described previously (Kuenen and Veldkamp, 1972).

Miscellaneous methods

D-Ribulose-1,5-bisphosphate carboxylase (EC 4.1.1.39) was measured as ribulose-1,5-bisphosphate-dependent $^{14}CO_2$ fixation in cell-free extracts derived from *T. neapolitanus* as detailed previously (Beudeker et al., 1980). The maximal capacity to respire thiosulfate, sulfide and acetate were measured polaro-

graphically with a YSI Biological Oxygen Monitor. The final concentration of Na_2S in the vessel of the Biological Oxygen Monitor was 0.1 mM. The other substrates were present in concentrations of 5 mM. Chemical oxidation of sulfide was followed by addition of sulfide to the supernatant of the culture. The organic carbon content of washed cell suspensions was measured in a total organic carbon analyzer (Beckman). The dry weight of the cultures were calculated from the organic-carbon content of the cultures assuming that 50% of the dry weight is carbon. The RNA was extracted from the cells as described by Harder and Veldkamp (1968) and the RNA content was determined by the orcinol method (Munro and Fleck, 1966). All cultures were frequently checked for contaminants according to Kuenen (1979).

Results

In order to understand the competitiveness of *T. neapolitanus* under alternating conditions of growth and starvation its capability to survive during starvation was studied with chemostat cultures which had been grown at a dilution rate of 0.07 h^{-1} under thiosulfate limitation. The starvation period was started by turning off the medium supply. *T. neapolitanus* appeared to be very resistant to energy starvation. The maximal capacity to respire thiosulfate ($Q_{O_2}^{max}$-thiosulfate) as well as the activity of the CO_2-fixing enzyme D-ribulose-1,5-bisphosphate carboxylase of thiosulfate-limited cells remained virtually unchanged during at least five days (Fig. 1). The protein and RNA content of the cells did not change during this period whereas the carbon content of the cells decreased slightly, coupled with an increase of the glycollate concentration in the supernatant (not shown). Glycollate is a well-known excretion product of *T. neapolitanus* (Cohen et al., 1979). After five days of energy starvation *T. neapolitanus* cells were able to resume growth without a detectable lag at the same rate as before the onset of the starvation period since the resumption of medium supply at a D of 0.07 h^{-1} did not lead to any observable washout of the culture. Addition of an organic substrate (acetate) to the growth medium during starvation did not influence the measured parameters.

To quantify the apparent reactivity of starved *T. neapolitanus* cells in response to the sudden supply of energy substrate we studied the growth of *T. neapolitanus* during cultivation in the chemostat under alternating growth and starvation conditions. For these experiments sulfide rather than thiosulfate was used as the growth limiting compound. Thiosulfate and sulfide seem to be equivalent substrates for thiobacilli since the yields (per mole of substrate) on both substrates are almost equal and since the maximal oxidation capacities for both substrates are the same. Energy-limited cultures of *T. neapolitanus*, cultivated at a dilution rate of 0.05 h^{-1} and at a dissolved oxygen tension of 5% of air saturation, yielded 4.8 and 5.0 g dry weight per mole sulfide and per

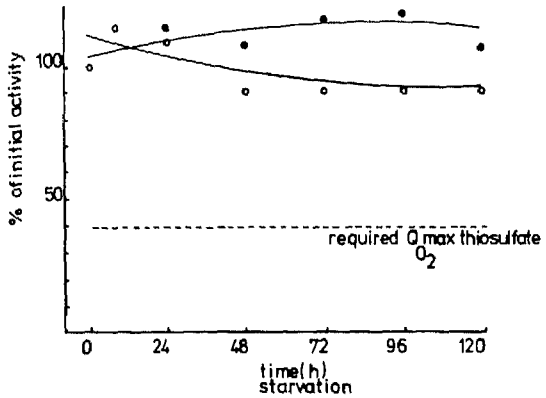

Fig. 1. The maximal oxidation capacity for thiosulfate (O—O) and the activity of D-ribulose-1,5-bisphosphate carboxylase (●—●) as a function of time during energy starvation of a culture of *Thiobacillus neapolitanus* pregrown in the chemostat under thiosulfate limitation (D = 0.07 h^{-1}; dissolved oxygen tension 50% of air saturation). The dotted line represents the $Q\bar{O}_2^{max}$ thiosulfate which would be required by the organisms to grow at the initial rate.

Table 1. The effects of alternate supply of sulfide and sulfate on energy-limited *Thiobacillus neapolitanus* cells grown in continuous culture at a dilution rate of 0.05 h^{-1} at a dissolved oxygen tension of 5% of air saturation. The maximal oxidation capacity for thiosulfate (sulfide), the dry weight of the cells and the actual sulfide concentration in the growth vessel have been determined at optimal pH 6.8 and at pH 7.5, which was used as an intermediate pH during previous competition experiments between *T. neapolitanus* and *T.*A2. It should be noted that during the starvation period the dry weight of the culture decreased, whereas it increased during the growth period. Reservoir concentration of sulfide was 40 mM.

Time after onset of alternation	pH	Dry wt (mg/l)	Measured $Q\bar{O}_2^{max}$ thiosulfate (μl O_2/h.mg dry wt)	Required $Q\bar{O}_2^{max}$ thiosulfate (μl O_2/h.mg dry wt)	Over-capacity (%)	Sulfide (μM)
2h sulfide	6.8	120	1330	746	80	<1
4 h sulfide	6.8	124	1485	723	110	<1
2 h sulfate	6.8	116	1350	773	80	<1
4 h sulfate	6.8	114	1500	786	90	<1
2 h sulfide	7.5	100	1450	896	60	4
4 h sulfide	7.5	110	1500	815	80	4
2 h sulfate	7.5	104	1450	862	70	1
4 h sulfate	7.5	92	1500	974	50	<1

mole thiosulfate, respectively. The use of sulfide rather than thiosulfate as the growth limiting energy source for cultivation of *T. neapolitanus* in the chemostat allowed for the direct measurement of the actual (*in situ*) concentration of sulfide in the chemostat. With aid of a sulfide electrode it appeared possible to measure sulfide quantitatively in concentrations as low as 1 μM. (The detection limit of the colorimetric assay of thiosulfate (Sörbo, 1957) is approximately 50 μM). During alternate supply of sulfide and sulfate to chemostat cultures of *T. neapolitanus* (pH 6.8, D = 0.05 h^{-1}; pO_2 = 5% of air saturation; 4 h sulfide, 4 h sulfate) the organism was alternatively growing and starving. After a 4 h starvation period the organism had retained a high overcapacity to respire sulfide or thiosulfate and appeared to be able to oxidize the added sulfide instantaneously. It can be calculated that if a 40 mM sulfide solution would be administered to a culture at a D = 0.05 h^{-1} the concentration of sulfide in the absence of bacteria would reach up to 33μM after one minute. In the actual experiment the sulfide concentration in the growing culture appeared to be always lower than 1μM (Table 1). Similar experiments were carried out not only at the optimal pH for growth (6.8) of *T. neapolitanus*, but also at pH 7.5 since competition experiments between *T.*A2 and *T. neapolitanus* were done at this intermediate pH (Gottschal et al., 1979; 1981*a*). Comparable results were obtained, although the sulfide concentration measured in the growth vessel reached higher levels (4 μM). This was probably due to the lower capacity of *T. neapolitanus* to oxidize sulfide at pH 7.5 at each stage of the cycle (Table 1). The sulfide concentration was measured routinely at one minute intervals directly after the onset of the sulfide period; since the concentration never was higher than 4 μM these data were not included in Table 1. A lower overcapacity for the oxidation of sulfide at a fixed dilution rate must be expected to result in a higher "*in situ*" concentration of the growth-limiting sulfide.

The facultatively chemolithotroph *T.*A2 appeared to be very flexible with respect to its energy generation since it was able to grow continuously under alternate supply of sulfide and acetate at pH 8.0. The maximal capacity to oxidize sulfide was induced during the supply of sulfide to the growth vessel but decreased again during the acetate period (Table 2), whereas the $Q_{O_2}^{max}$ acetate showed less variation. This confirmed earlier experiments carried out with alternate supply of thiosulfate and acetate (Gottschal et al., 1981*a*). The organism did not possess any overcapacity to oxidize sulfide after the acetate period. The concentration of sulfide in the growth vessel increased up to 8 μM (Fig. 2).

At suboptimal pH 7.5 we were unable to grow *T.*A2 for a prolonged number of 4 h cycles under alternate supply of sulfide and acetate. Since in cultures of *T.*A2 a transient increase of sulfide concentration can be observed, some chemical oxidation of the sulfide occurred resulting in the formation of small amounts of elemental sulfur. This phenomenon was only observed at pH 7.5 which is the lower pH limit for growth of *T.*A2. Since *T.*A2 can oxidize elemen-

Table 2. The effect of an alternate supply of sulfide and acetate on energy-limited *Thiobacillus* A2 cells grown in continuous culture at a dilution rate of 0.05 h^{-1}. The dissolved oxygen tension was kept at 5% of air saturation. Besides the parameters mentioned in the legend to Table 1, the maximal oxidation capacity for acetate was also determined. Experiments were carried out at the optimal pH 8.0 and at pH 7.5 (see Table 1.)

Time after onset of alternation	pH	Dry wt (mg/l)	Measured $Q_{O_2}^{max}$ thiosulfate (μl O_2/h.mg dry wt)	Required $Q_{O_2}^{max}$ thiosulfate	Over-capacity (%)	$Q_{O_2}^{max}$ acetate (μl O_2/h.mg dry wt)
2 h sulfide	8.0	172	595	520	10	132
4 h sulfide	8.0	176	635	509	20	128
2 h acetate	8.0	166	605	540	10	136
4 h acetate	8.0	166	560	540	0	150
2 h sulfide	7.5	149	542	602	−10	123
4 h sulfide	7.5	149	610	602	0	121
2 h acetate	7.5	149	570	602	−10	125
4 h acetate	7.5	149	474	602	−20	129

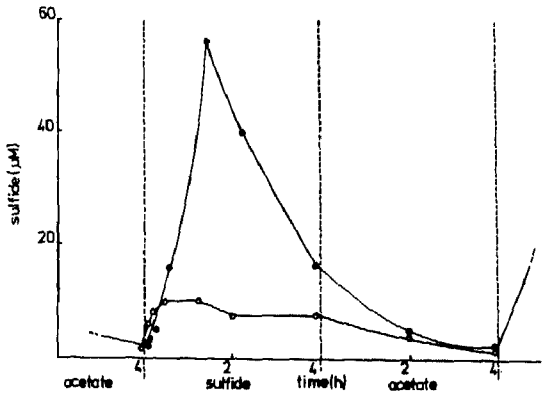

Fig. 2. The accumulation of sulfide under alternate supply of 4 h acetate 4 h sulfide to an energy-limited continuous culture of *Thiobacillus* A2. Sulfide concentration in the culture at pH 8.0 (O–O); at pH 7.5 (●–●).

tal sulfur only very slowly this compound gradually accumulated during consecutive cycles. The sulfide introduced combined with the elemental sulfur to give polysulfides which led to inhibition of the growth of *T.*A2 and, thus, to even further accumulation of sulfur compounds in the culture. In order to determine the sulfide accumulation at the onset the sulfide period, *T.*A2 was grown initially with alternate supply of thiosulfate and acetate at pH 7.5. Under such conditions *T.*A2 was able to grow continuously. In fact, the observed $Q_{O_2}^{max}$ thiosulfate was too low to account for the growth rate (Table 2). This can be explained by the fact that washing of the cells leads to a reduction of their oxidative capacity by 15–25% (Gottschal et al., 1981*a*). It appeared that in thiosulfate-grown cells of *T.*A2 the capacity to oxidize sulfide was the same as for thiosulfate. Once growth under these conditions was established we supplied the culture with sulfide instead of thiosulfate for one 4-h cycle only. In this first cycle the formation of sulfur was insignificant and the build up of the concentration of sulfide could conveniently be measured (Fig. 2). The concentration of sulfide in the growth vessel increased up to 56 μM. After about 2 h the sulfide concentration decreased again due to the induction of the capacity to oxidize sulfide during this period (Table 2). At the start of the acetate period a small concentration of sulfide had remained and disappeared during the acetate period.

Discussion

The differences between metabolically versatile and specialized *Thiobacillus* spp. have been summarized in Table 3. Some of these differences will be detailed below and the consequences of these differences for both types of bacteria will be discussed in relation to their survival in nature.

The specialized chemolithotroph *Thiobacillus neapolitanus* retained its high respiration potential for thiosulfate and sulfide under all growth conditions and even during starvation (Fig. 1) over a period of at least several days. Its reactivity is high since it can respond rapidly to the appearance of a reduced sulfur compound. This strategy of high reactivity is accompanied by a low flexibility with respect to energy generation and to the assimilation of organic compounds as carbon sources. At first sight the lack of response to the presence of exogenously supplied organic carbon compounds might be considered as a competitive draw back for such organisms during competition with flexible facultatively chemolithotrophs. It has been shown, however, that an alternate supply of acetate and thiosulfate ($D = 0.05\ h^{-1}$; 4 h acetate, 4 h thiosulfate; pH 7.5) to mixed cultures of *T.*A2 and *T. neapolitanus* resulted in coexistence (Gottschal et al., 1979). The most likely explanation for this observation is that *T. neapolitanus* metabolized all available thiosulfate and *T.*A2 grew mainly heterotrophically. This explanation can now be substantiated on the basis of di-

Table 3. Differences between a metabolically versatile and a specialized chemolithotrophic thiobacillus

Specialist *T. neapolitanus*	Versatile *T.*A2
1. Few energy substrates utilized (S^{2-}, S^0, $S_2O_3^{2-}$, $S_4O_6^{2-}$ etc.).	Many different inorganic and organic energy substrates utilized (Gottschal et al., 1981*b*).
2. High specific growth rate on single substrate (μ_{max} on thiosulfate = 0.35 h^{-1}).	Low specific growth rate on single substrate (μ_{max} on thiosulfate = 0.10 h^{-1}). Relatively hig specific growth rate on mixed substrates (μ_{max} on thiosulfate plus acetate = 0.22 h^{-1}).
3. High affinity for reduced sulfur compounds (Gottschal et al., 1979; this paper).	Relatively low affinity for reduced sulfur compounds (Gottschal et al., 1979; this paper).
4. High overcapacity of respiratory capacity during substrate-limited growth (this paper).	Low overcapacity of respiratory capacity during substrate-limited growth (this paper).
5. Low "flexibility" with respect to energy generation and organic carbon assimilation; "constitutive" enzymes (Beudeker et al., 1981*c*; this paper).	High "flexibility"; "inducible" enzymes for energy generation and carbon assimilation (Gottschal et al., 1981*a*; this paper).
6. High "reactivity" towards few substrates (this paper).	Low "reactivity" towards many substrates (Gottschal et al., 1981*a*; this paper).
7. Metabolic "lesions" (see Smith and Hoare, 1977; Matin, 1978).	Many pathways, often overlapping (see for example Wood and Kelly, 1980).
8. Low endogenous respiration (Smith and Hoare, 1977, unpublished observations).	High endogenous respiration in autotrophically and heterotrophically grown cells (unpublished observations).
9. Very resistant to starvation (this paper).	Less resistant to starvation (Gottschal et al., 1981*b*).
10. Ecological niche: In environments with continuous or fluctuating supply of reduced sulfur compounds and a low turnover of organic compounds.	Ecological niche: In environments with simultaneous presence of both inorganic and organic substrates. ("mixotrophic" conditions).

rect measurements of the growth limiting concentration of sulfide.

1. During the starvation (acetate) period *T. neapolitanus* was washed out and consequently was reduced in numbers by 20%. However, since it had an almost two fold overcapacity to respire either sulfide or thiosulfate (Fig. 1), the total population of *T. neapolitanus* after the starvation period still possessed a high overcapacity. This allowed *T. neapolitanus* to maintain a concentration of sulfide lower than 4 μM, which is more than 10 fold lower than that found in cultures of *T.*A2 (56 μM) under similar conditions. This implies that *T. neapolitanus* can grow much faster than *T.*A2 during the thiosulfate period.
2. *T. neapolitanus* has been shown to excrete glycollate during thiosulfate limitation (Cohen et al., 1979; Beudeker et al., 1981*c*) and during energy starvation. If *T. neapolitanus* and *T.*A2 are grown in mixed culture *T.*A2 will be

exposed to, and consume, the glycollate excreted by *T. neapolitanus*. From previous experiments it was concluded that the induction of the autotrophic potential of *T.*A2 is a function of the thiosulfate to organic carbon ratio in the culture (Gottschal and Kuenen, 1980*a*). Due to the immediate oxidation of thiosulfate (sulfide) by *T. neapolitanus* after the acetate period, the concentration of reduced sulfur compounds available to *T.*A2 will be very low (Table 1). Thus, it is likely that *T.*A2 is confronted with a thiosulfate to glycollate ratio too low to induce substantially the autotrophic potential of *T.*A2.

These considerations lead to the conclusion that under the conditions described, *T.*A2 was forced to grow as a heterotroph. In this light it is not surprising that the versatile organism was completely outcompeted in a comparable three-membered mixed culture of *T. neapolitanus, T.*A2 and a specialized heterotroph (Gottschal et al., 1981*a*). It should be emphasized here that adaptation of *T.*A2 to the changing environment renders the organism less reactive than it would have been if it synthesized its acetate and thiosulfate oxidizing potential constitutively. On the other hand the inability of *T. neapolitanus* to respond to the presence of organic compounds ensures its high reactivity to inorganic sulfur compounds. It seems that the specialistic type of metabolism has evolved during evolution as a strategy for survival whereby its versatility and flexibility have been given up only with respect to the utilization of exogenous organic substrates. Recent work in our laboratory has shown that *T. neapolitanus* is as flexible and versatile with respect to the assimilation of nitrogenenous compounds as many heterotrophs (Beudeker et al., 1981*d*) and also exhibits flexible characteristics with regard to the fixation of CO_2 (Beudeker et al., 1981*a*). Even with respect to energy generation *T. neapolitanus* possesses some flexibility since under starvation conditions it can also derive energy from the metabolism of intracellular polyglucose, which is formed under nitrogen limitation in the chemostat (Beudeker et al., 1981*b*).

In conclusion, Table 3 shows us the profile for a specialist organism such as *T. neapolitanus*. Its properties seem to make it well suited to function competitively in an environment with either a continuous supply of thiosulfate, and at the same time, low turnover rates or organic compounds, or under conditions where the supply of inorganic and organic compounds strongly fluctuate or even alternate. The latter situation in particular demands that the specialist survives starvation conditions and as a consequence that it possesses a low endogenous respiration and a high resistance to starvation.

Table 3 also clearly depicts the characteristics of a typical versatile organism able to cope with a spectrum of situations where mixtures of inorganic and organic energy sources are available. In particular its ability to grow mixotrophically seems to be its competitive strength (Gottschal et al., 1979). As a consequence the organism must possess inducible enzymes for many substrates. In addition its potential to respond to different energy substrates demands higher

turnover rates of enzymes during starvation conditions, and consequently a higher endogenous respiration.

The general picture emerging is that the specialistic and versatile mode of life as a whole can never be combined in one and the same organism. Apparently the constraints put on the evolution of bacteria only allow the combination of a limited number of properties.

The studies described in this and previous papers have been carried out with "model organisms", and on enrichment cultures in the chemostat. It must be stressed that at its best the conclusions drawn from these experiments can indicate trends. Many questions remain to be answered. First of all the role of heterotrophic sulfur oxidizing bacteria which seem to occur very commonly in nature should be included in future studies. Furthermore it will be crucial to develop methods by which the activities of the different types of metabolism can be measured in the field. In the years to come we will focus our research on these topics.

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