CHAPTER 9

Interactions between obligately and facultatively chemolithotrophic sulphur bacteria

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Although Darwin developed his thesis of survival of the fittest on the selection and survival of higher animals, microbiologists have long been aware that, if anywhere, this principle certainly holds for the selection and survival of microorganisms in an ecosystem. The survival of any species will depend upon how well its physiology is adapted to the conditions existing in that environment, and on the duration of those conditions within the system.

Over the last few years the use of continuous culture has allowed the artificial amplification of selective pressures to be exerted on microbial populations, and thus permitted the study of selection and survival of microorganisms under carefully chosen conditions, and thus in defined physiological states. (For reviews see Harder *et al.*, 1977 or Parkes, 1982.)

Undoubtedly, one of the most important environmental pressures is nutrient limitation, and this is particularly suitable for simulation in a continuous flow system. Insight into the mechanisms of selection by nutrient limitation has been gained from the pioneering work such as that reviewed by Veldkamp & Jannasch (1972) which showed the occurrence, in nature, of many bacteria able to grow relatively rapidly on very low concentrations of nutrients. The properties of such organisms differ from those of bacteria normally found in batch cultures which grow relatively rapidly with nutrient excess, but, relatively, more slowly as nutrients become limiting.

Attention over the last decade or so has focussed mainly on growth limitation by one single nutrient. However, nature normally provides a mixture of substrates and, according to several theoretical treatments (Frederickson, 1977, Taylor & Williams, 1974, Yoon *et al.*, 1977, de Freitas & Frederickson, 1978), this would be one of the explanations for the co-existence of many species in

one habitat. (For review see Bull & Slater, 1982.) In order to provide experimental support for such an explanation, studies in our laboratory have been made on the effect mixed substrates have on the competition, selection and co-existence of a number of suitable bacterial species in the chemostat. The purpose of the study was:

1. To find any general principles of competition between bacterial species during mixed substrate supply.
2. To verify theoretical predictions made from mathematical models in our, and other laboratories.
3. To understand the co-existence in nature of the colourless sulphur bacteria, a group comprising a wide spectrum of physiological types.

The colourless sulphur bacteria play a crucial role in the aerobic oxidation of reduced inorganic sulphur compounds in the sulphur cycle (Kuenen, 1975, Kuenen & Beudeker, 1982). In a single habitat, such as sediment, one can find co-existing populations comprising at least three metabolic types (Table 1). These include first, the highly specialised, obligately chemolithotrophic sulphur oxidizers which use inorganic sulphur compounds as their sole source of energy, and carbon dioxide for their major supply of carbon under all growth conditions (Smith & Hoare, 1977). Second, there are the versatile facultative chemolitho-(auto)trophs which are able to grow as chemoorganoheterotrophs, or in the same way as their obligate counterparts, albeit more slowly. Under appropriate conditions these bacteria can also grow mixotrophically, that is, they can use both modes of growth simultaneously (Matin, 1978, Smith *et al.*, 1980, Gottschal & Kuenen, 1980a). Finally, there are the chemolithotrophic heterotrophs. These are essentially heterotrophic species which can utilize organic sulphur compounds as an additional energy source for growth (Tuttle & Jannasch, 1972, Gottschal & Kuenen, 1980b).

Table 1
The metabolic definitions of some reduced sulphur compound-oxidizing bacteria

	Energy source		Carbon source	
Metabolic type	Inorganic sulphur	Organic compounds	CO_2	Organic compounds
Obligate chemolithotroph	+	−	+	−
Facultative chemolithotroph	+	+	+	+
Chemolithotrophic heterotroph	+	+	−	+

The ecological niche occupied by the facultative chemolithotrophs (also known as mixotrophs) has long been under question. It was suggested that their ability to use both organic and inorganic substrates might give the mixotrophs a selective advantage (Rittenberg, 1969), but it has since been argued that a facultative species should either be a very good heterotroph or a very good autotroph in order to succeed in competition for substrates with specialist organisms (Whittenbury & Kelly, 1977). In general, specialist species are known to possess a much higher maximum specific growth rate than the versatile types.

In order to shed light on these conflicting views, a study was made of model organisms representing some of the metabolic types mentioned, and it soon became clear that both views hold some truth. The species chosen are listed in Table 2. *Thiobacillus neapolitanus* is a specialist chemolitho(auto)troph able to grow rapidly in mineral thiosulphate media. *Thiobacillus* A2 is a very versatile facultative chemolithotroph able to grow autotrophically, heterotrophically or mixotrophically on reduced sulphur compounds, acetate and many other organic compounds. *Spirillum* G7 was chosen as a typical, specialist heterotroph able to grow rapidly on an acetate mineral medium.

Table 2

Maximum specific growth rates (μ_{max}) of the three model organisms during growth on thiosulphate (T), acetate (A) and both substrates (T+A). (From Gottschal & Kuenen, 1981.)

Organism	Metabolic type	Maximum specific growth rate μ_{max} (h^{-1})		
		T	A	T+A
T. neapolitanus	obligate chemolithotroph	0.35	0	0.35
Thiobacillus A2	facultative chemolithotroph	0.10	0.22	0.22
Spirillum G7	obligate heterotroph	0	0.43	0.43

To facilitate a direct comparison of specific growth rates under nutrient limitation, competition experiments were carried out between the versatile *Thiobacillus* A2 and each of the two specialists. As expected, each of the specialists out-competed the versatile organism under the appropriate specialist substrate limitation, not only at their μ_{max} but also at lower dilution rates. An example of the results obtained during competition between *T. neapolitanus* and *Thiobacillus* A2 for thiosulphate is shown in Fig. 1A and B. *Thiobacillus* A2 was, however, able to maintain its presence in low numbers (less than 10% of the population) by means of heterotrophic growth on glycollate excreted by the dominant species (Gottschal *et al.*, 1979). Even when the versatile species was given an intial major numerical advantage, the specialist outnumbered *Thio-*

bacillus A2 after only two volume changes (not shown). However, at an extremely low dilution rate (0.004 h^{-1}) the *Thiobacillus* A2 population steadily increased, and the *T. neapolitanus* numbers fell. This turned out to be due to a rapid loss of viability of *T. neapolitanus* at this low dilution rate (Gottschal *et al.*, 1979). Essentially similar results were obtained when *Thiobacillus* A2 was grown in a chemostat with an obligate heterotroph, *Spirillum* G7, when acetate was the limiting factor, although in this case *Thiobacillus* A2 washed out completely. Again, at an extremely low dilution rate, the positions were reversed and *Thiobacillus* A2 was able to predominate. The conclusion to which these experiments lead is that the μ-s curves of the specialists lie above that of the versatile organism over a whole range of substrate concentrations and/or growth rates, as long as only one substrate is present.

To investigate the consequences of supplying mixed rather than single substrates, a pure culture of *Thiobacillus* A2 was initially studied. Provided that both substrates were limiting, it was possible to demonstrate the simultaneous utilization of thiosulphate and acetate. Fig. 2 shows the different growth parameters of chemostat grown *Thiobacillus* A2 with different mixtures of thiosulphate and acetate in the feed. It should be noted that in these experiments neither substrate was detectable in the chemostat. The bacterium gradually adapted its enzymic machinery to the turnover rate required for each respective substrate, and also adjusted its ability to fix carbon dioxide according to the amount of organic carbon available in the mixture. Very similar observations have been made for mixed substrate utilization by *Pseudomonas oxalaticus* (Dijkhuizen *et al.*, 1978) and, for example, *Escherichia coli* (Silver & Mateles, 1969).

When the two-membered competition experiments were repeated using mixed substrates, the picture changed. The addition of acetate or glycollate to the autotrophic cultures (Fig. 3), and thiosulphate to the heterotrophic cultures (not shown), resulted in an increase in the numbers of *Thiobacillus* A2 which depended on the concentration of the additive. This eventually allowed the versatile species to outnumber either specialist. Smith and Kelly (1979) obtained similar results using mixtures of thiosulphate and glucose rather than acetate. It is important to note from Fig. 3 that, in addition to selection, the possibility of the co-existence of two species competing for the same substrate has been demonstrated. For the survival of versatile organisms, such as *Thiobacillus* A2, substrate limitation is essential and, since in nature nutrients are probably often limiting, this would indicate an explanation for the survival of versatile species in association with specialists in the wild. In the presence of excess substrate, such as is found in batch culture, the specialists have an advantage both because of their superior maximum specific growth rate and also because the versatile organism often cannot grow mixotrophically if the substrates are not limiting. The versatile species would therefore use the substrates sequentially, showing diauxic growth (Gottschal & Kuenen, 1980a).

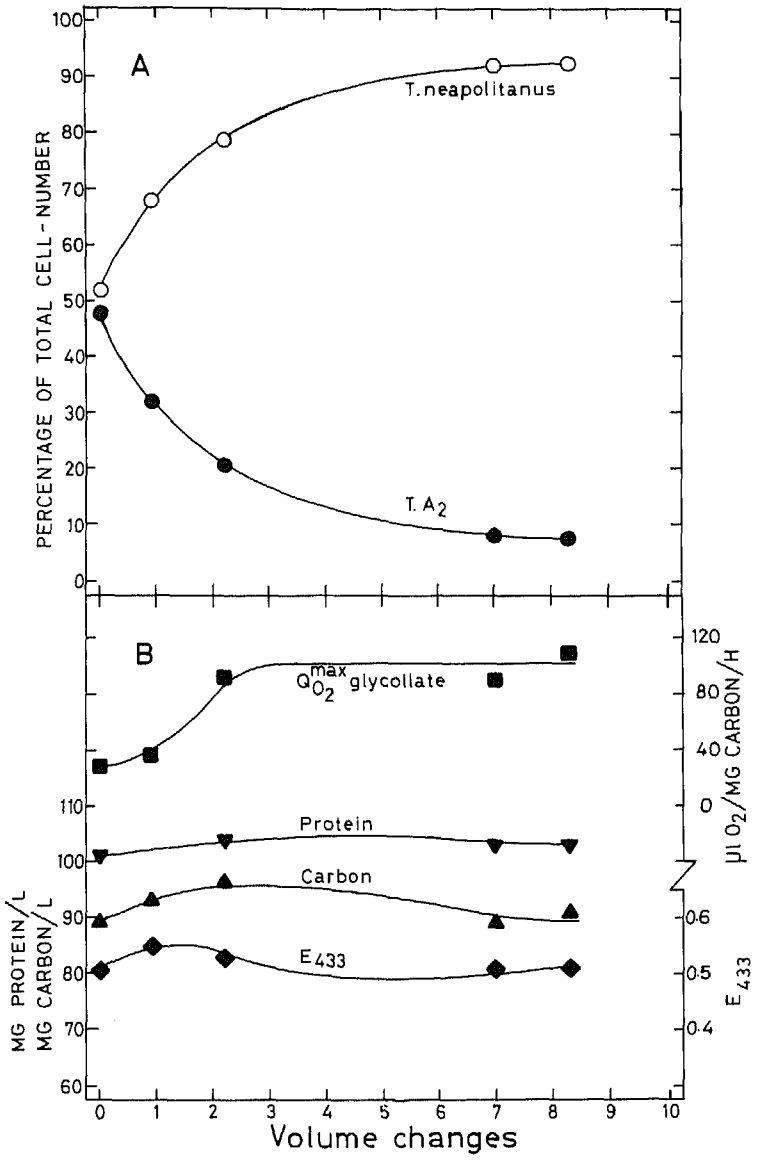

Fig. 1 A+B – Competition in a chemostat between *Thiobacillus* A2 and *T. neapolitanus* for growth limiting thiosulphate at a dilution rate of 0.05 h^{-1} with 40 mM thiosulphate in the feed. The organisms were pregrown in separate chemostats and mixed in a 1:1 ratio at $t = 1$. A: Relative numbers of *Thiobacillus* A2 (●—●) and *T. neapolitanus* (○—○) vs the number of volume changes. B: Absorbance (◆—◆), organic cell carbon (▲—▲), protein (▼—▼), maximum gycollate-oxidizing capacity of *Thiobacillus* A2 (■—■) expressed per mg cell carbon.

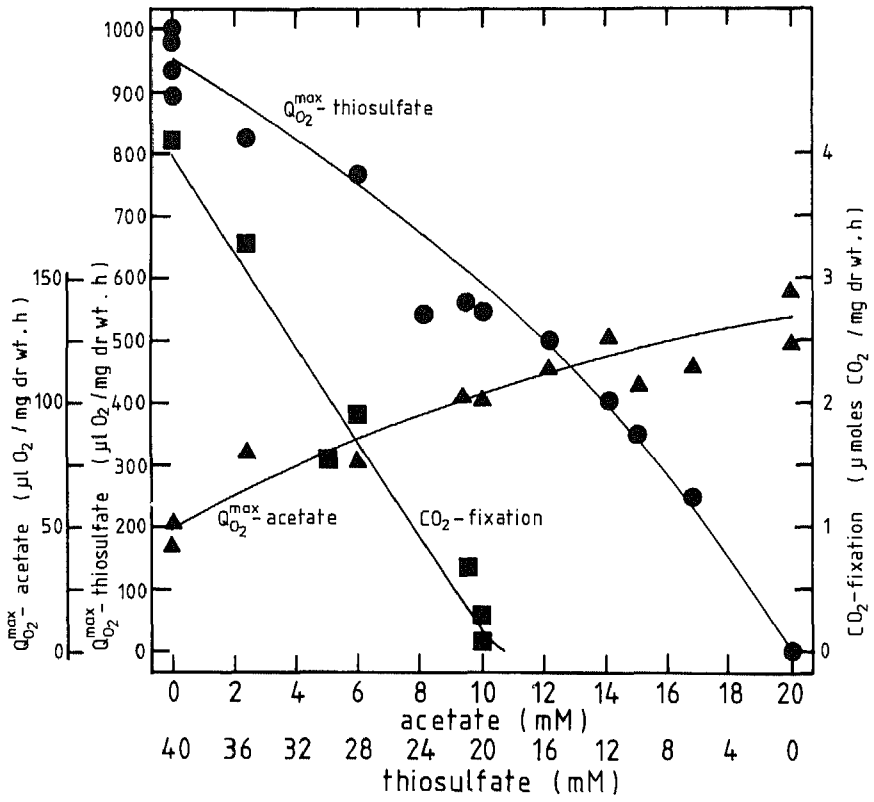

Fig. 2 – Carbon dioxide fixing potential and maximum substrate oxidation potentials of whole cells of *Thiobacillus* A2 as a function of different acetate and thiosulphate concentrations in the chemostat feed. Data obtained at a steady state with a dilution rate of 0.05 h^{-1}. $Q_{O_2}^{max}$ thiosulphate (●—●), $Q_{O_2}^{max}$ acetate (▲—▲), carbon dioxide fixation potential (■—■).

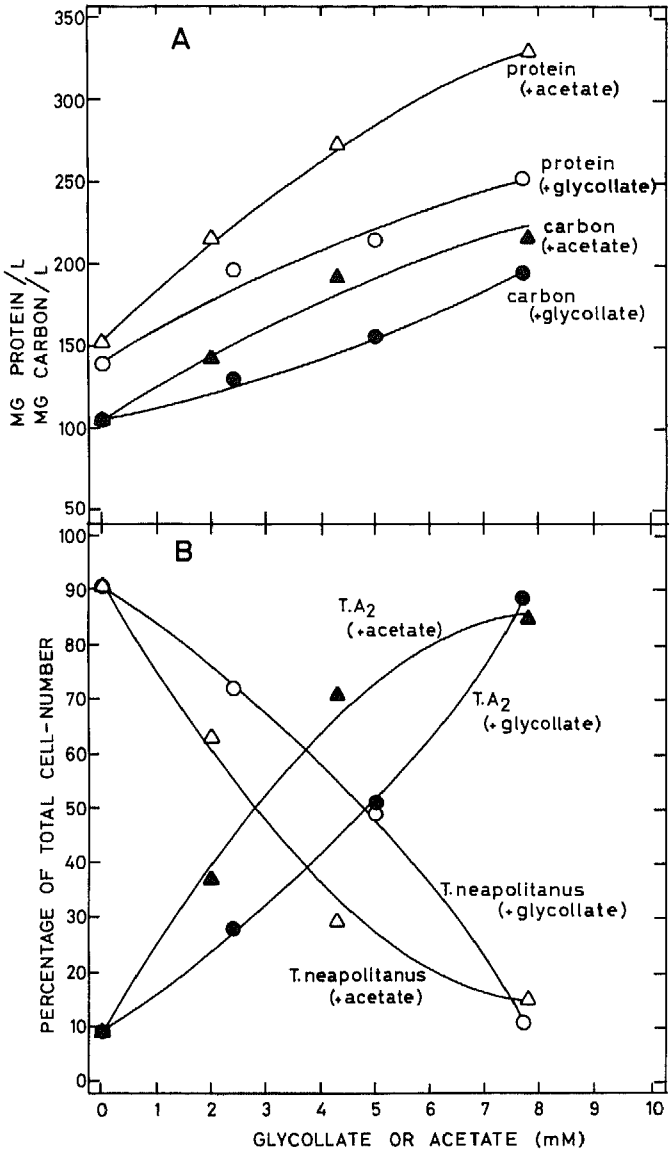

Fig. 3 A+B – Effect of different levels of organic substrate on the competition between *Thiobacillus* A2 and *T. neapolitanus* for thiosulphate in a chemostat run at a dilution rate of 0.07 h^{-1}. The inflowing medium contained thiosulphate (40 mM) with either acetate or glycollate at concentrations ranging from 0–7 mM. A: Protein or carbon in culture with thiosulphate + acetate (△—△, ▲—▲) or glycollate (○—○, ●—●). B: The percentage *Thiobacillus* A2 in the mixture on thiosulphate + acetate (▲—▲) or glycollate (●—●) and that of *T. neapolitanus* with thiosulphate + acetate (△—△) or glycollate (○—○).

In further experiments it was shown that the versatile species became dominant, in cultures containing all three organisms, when the concentrations of acetate and thiosulphate in the mixture were of the same magnitude. This clearly demonstrates the importance of the mixtrophic way of life.

Gottschal & Thingstad (1982) have developed a mathematical model which could be used to predict the outcome of competition experiments between versatile and specialist species at a steady state in a chemostat. The model was based on the assumption that both the specific growth rate and the specific substrate consumption rate are exclusively dependent on the substrate concentration in the chemostat.

The classic Monod equation to describe the dependence of the specific growth rate may be developed to show the growth of autotrophic or heterotrophic organisms in a chemostat as follows:

$$\mu_{tA}(s_t) = \frac{\mu_{tA}^{max} \cdot s_t}{K_{tA} + s_t} \tag{1}$$

and

$$\mu_{aH}(s_a) = \frac{\mu_{aH}^{max} \cdot s_a}{K_{aH} + s_a} \tag{2}$$

However, the situation for the mixotroph is more complicated since it can grow on either, or both, of the substrates. μ_M (s_a, s_t) is not merely the sum of equations (1) and (2) since the presence of a second substrate decreases the substrate-oxidizing capacity required for growth on the first substrate (Fig. 2) (Gottschal & Kuenen, 1980a). However, at lower substrate concentrations when the relationship between μ and s is nearly linear, and the rate of substrate supply is probably the only rate-limiting step, it seems reasonable to suppose that the specific growth rate (μ_M (s_a, s_t)) is the sum of the two separate specific growth rates, $\mu_M(s_a)$ and $\mu_M(s_t)$. Equations (1) and (2) may then be combined to give:

$$\mu_M\,(s_a, s_t) = \frac{\mu_{aM}^{max} \cdot s_a}{K_{aM} + s_a} + \frac{\mu_{tM}^{max} \cdot s_t}{K_{tM} + s_t} \tag{3}$$

or, in simple terms:

$$\mu_M\,(\text{total}) = \mu_M\,(\text{acetate}) + \mu_M\,(\text{thiosulphate}).$$

This is only dependent on the steady state $\bar{s}$ (acetate) or $\bar{s}$ (thiosulphate).

Using this simple model, predictions for the outcome of competition in two-membered cultures were made. The experimental values in Fig. 4 (shown as dots and circles) show a linear relationship as predicted by the model. This implies that the steady state concentration of *Thiobacillus* A2 in the mixture is directly proportional to the acetate concentration in the inflowing medium. The case was analogous for the values from the culture containing the specialist heterotroph and the versatile bacterium.

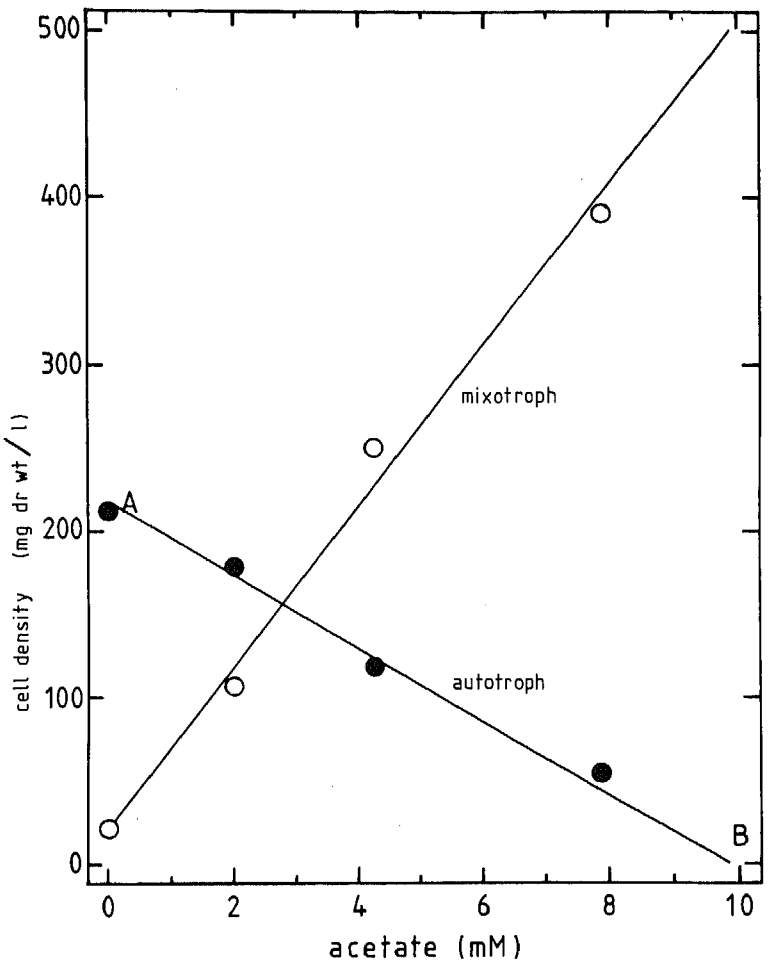

Fig. 4 – Cell density of the mixotroph, *Thiobacillus* A2, (o—o) and the autotroph *T. neapolitanus* (•—•) in a mixed chemostat culture after reaching steady state at a dilution rate of 0.075 h^{-1}. Growth was simultaneously limited by thiosulphate (40 mM) and increasing amounts of acetate (0–10 mM) in the feed. "A" represents the cell density of the autotroph when the acetate level is 0 and "B" is the acetate concentration when the density of the autotroph becomes 0.

It should be noted especially that the presence of *T. neapolitanus* forces the versatile *Thiobacillus* A2 to display a metabolic state different from that observed in pure culture at the same thiosulphate to acetate ratio. This is easily understood if it is realized that for as long as *T. neapolitanus* is present, *Thiobacillus* A2 receives considerably less thiosulphate than it would in a pure culture under similar conditions. By referring to Fig. 4, it may be seen that as

long as *T. neapolitanus* is able to maintain itself in the chemostat, the steady state concentrations of thiosulphate must be equal to that in a pure culture of *T. neapolitanus* since it can grow at the required rate ($\mu = D$). This is true even when *T. neapolitanus* represents as little as 0.5% of the population at steady state. This means that, in the mixed culure, the *Thiobacillus* A2 cells are exposed to the same thiosulphate concentration irrespective of the acetate/thiosulphate ratio. Now, as pointed out before, the growth rate of *Thiobacillus* A2 will be:

$$\mu_M \text{ (total)} = D = \mu_M \text{ (acetate)} + \mu_M \text{ (thiosulphate)}.$$

Since $\bar{s}$ (thiosulphate) is constant, μ_M (thiosulphate) is also constant and since D is unchanged, μ_M (acetate) is constant as well. This can only hold true if $\bar{s}$ (acetate) is also unvarying. Thus at steady state, *Thiobacillus* A2 is exposed to constant concentrations of both substrates, irrespective of their ratio in the medium feed. The physiological condition of *Thiobacillus* A2 must therefore remain unaltered. Since, as stated before, *Thiobacillus* A2 receives less thiosulphate in mixed culture than it does as a monoculture, it is forced to maintain a more heterotrophic metabolism than it would in pure culture. In fact, it seems reasonable to suppose that *Thiobacillus* A2 will be in a metabolic state equivalent to the point where *T. neapolitanus* is about to be excluded from the culture. In short, in mixed culture, *Thiobacillus* A2 will not display the range of adaptation evident in pure culture (Fig. 2).

Another interesting point which arose from the mathematical modelling of Gottschal and Thingstad, among others (Frederickson, 1977, Taylor & Williams, 1974, Yoon *et al.*, 1977, de Freitas & Frederickson, 1978), is that the yield of organisms on the respective substrates is an important parameter in the outcome of the competition. This is in contrast with the results obtained for competition for a single substrate where success is dependent only on μ_{max} and K_s, and is essentially independent of the yield. The effect of yield during competition for mixed substrates can easily be rationalized. A higher yield on acetate would allow *Thiobacillus* A2 to increase its population density very rapidly with increasing acetate levels, thus allowing it to claim all of the thiosulphate at a lower acetate: thiosulphate ratio than if its yield were lower.

The general validity of the outcome of the experimental and theoretical model systems was further demonstrated by various enrichment experiments in continuous culture. Using different ratios of growth limiting acetate and thiosulphate, mixed cultures were produced in which the dominant organism was, with one exception, a facultative chemolithoautotroph. In the fifth culture, the dominant species was a chemolithotrophic heterotroph (Table 3). These experiments confirm the ecological advantage of the mixotrophic way of life. Analogous results have been found for a versatile and a specialist *Clostridium* species (Laanbroek *et al.*, 1979), and also for methylotrophs in preliminary studies by Harder (quoted in Kuenen & Gottschal, 1982). From the model studies on the competition of sulphur-oxidizing bacteria, a generalization concerning their

Table 3

Results of enrichment cultures (I–VII) after 15–20 volume changes in the chemostat under dual limitation by thiosulphate and acetate at a dilution rate of 0.05 h^{-1}

(I–V were enrichments from freshwater samples; VI and VII had been inoculated with marine mud. Concentration of thiosulphate (T) and acetate (A) in the inflowing medium are given in millimoles per litre. After Gottschal & Kuene (1980b).)

Culture	Substrate concentration	Dominant physiological type	Percentage of total number
Freshwater			
I	30T + 5A	facultative chemolithoautotroph	82
II	10T + 15A	facultative chemolithoautotroph	75
III	30T + 5A	facultative chemolithoautotroph	85
IV	20T + 10A	facultative chemolithoautotroph	50
V	10T + 15A	chemolithotrophic heterotroph	86
Marine			
VI	30T + 5A	obligate chemolithoautotroph + heterotroph	67 + 37
VII	10T + 15A	obligate chemolithoautotroph + heterotroph	81 + 19

possible role in the turnover of natural sulphur compounds can be made. Specialist sulphur oxidizers may become dominant in an environment where the relative turnover rate of sulphur compounds and organic substrates is high, whereas versatile species would dominate when both the substrate turnover rates were similar. At a low turnover rate of inorganic and organic substrates, carbon dioxide fixation would no longer be advantageous and chemolithoheterotrophic sulphur oxidizers would come to the fore (See case V, Table 3).

We have recently been offered the opportunity to study a continuous flow system on the "grand scale". This is allowing us to test our hypothesis under much more "natural" and complex conditions than is possible in the laboratory. A denitrifying reactor for the anaerobic oxidation of sulphide has been running on a pilot plant scale at a local factory in Delft as part of a complete effluent treatment system (patent number EP 0051888A1) (see Fig. 5). The industrial effluent is first passed through a Hubert reactor where most of the organic materials are converted into methane. As the original waste has a high sulphate

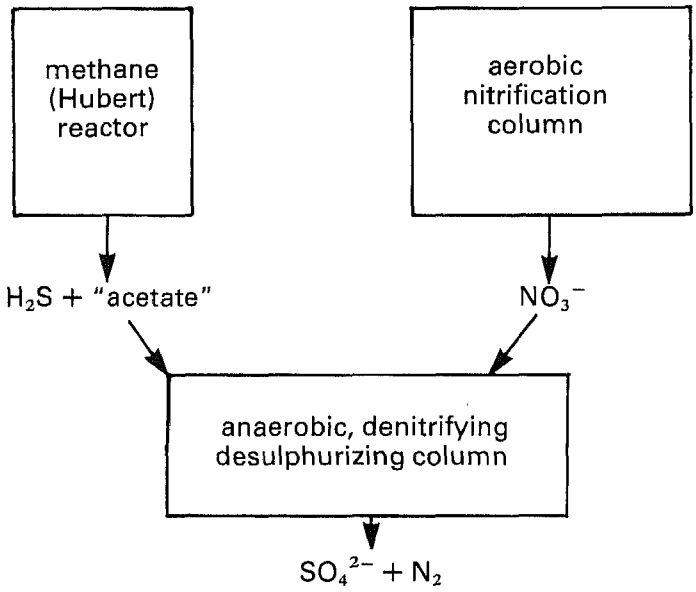

Fig. 5 – Schematic representation of an anaerobic, denitrifying, sulphide-oxidizing system linked to a methane reactor and an aerobic nitrification system.

content, the effluent from the methane reactor unavoidably contains a considerable amount of sulphide. Furthermore, the Hubert reactor effluent inevitably contains a certain amount of small organic molecules, such as acetate, which have not been consumed in the methane reactor. Subsequently, the effluent passes into the denitrification column which contains a fluidized bed of sand particles that provide anchorage for the bacterial culture. It is hoped, eventually, to provide nitrate for the column by feeding part of the desulphurized effluent through an aerobic nitrifying column, and then recirculating the nitrate produced into the anaerobic column. The denitrifying stage of the system provides an unrivalled opportunity for the study of sulphur metabolism at very low redox potentials, and under much more "natural" conditions than could be provided by a laboratory chemostat.

Our hypothesis predicted that the conditions existing within the column (i.e. with sulphide and low concentrations of acetate available as substrates) should give a selective advantage to those organisms able to grow mixotrophically rather than to chemolithotrophic or heterotrophic specialists.

A typical isolate from the column should therefore display a metabolic profile which has not previously been reported in the literature, and which would include the following features:

1. An ability to grow anaerobically at the expense of nitrate as the electron acceptor.

2. The ability to grow aerobically. Some of the liquids flowing into the column are not made anaerobic before addition.
3. An ability to metabolize reduced sulphur compounds both aerobically and anaerobically.
4. The ability to utilize some of those organic substances likely to be present (e.g. acetate).
5. The ability to grow mixotrophically at a rate which would allow survival under the conditions existing in the column.

We have been able to confirm these expectations by isolating from the column an organism which, fulfilling all these predictions, is therefore:

1. a facultative anaerobe
2. a mixotroph both aerobically and anaerobically.
3. a facultative autotroph under both sets of conditions.

The new isolate has been given the name of *Thiosphaera pantotropha* (Robertson & Kuenen, 1982. Robertson & Kuenen, submitted). Although this isolate has been selected for detailed study, it is not the only species in the column to display these features, and it is clear that a whole group of similar organisms exists.

Preliminary results also indicate that in this system obligate autotrophs may be absent, or only present in very small numbers, since we have not yet managed to isolate any from the bacterial population. This view is also supported by respirometric experiments. If the acetate and thiosulphate (or sulphide) were being used by separate (specialist) organisms, one would expect that the denitrification rate of samples supplied with a mixture of the two substrates would be the sum of the rates for each substrate. This was not the case.

Most of the previous sections have involved selection in a steady-state type of environment. Although such environments do occur in nature, fluctuations in the substrate supply or other conditions are more common. It was assumed that, in an environment undergoing rapid or irregular fluctuations, the ecological advantage would be possessed by the species which can adapt with the conditions, and which can "scavenge" a discontinuous supply of very low concentrations of both inorganic and organic substrates, rather than being dependent on any one. In other words, the advantage should again be with the versatile species. With this in mind, the growth of *Thiobacillus* A2 was studied under an alternating supply of growth-limiting thiosulphate and acetate (Gottschal, Nanninga & Kuenen, 1981). It could be shown that the bacterium grew uninterruptedly, provided that the intervals of alternation were 4 hours or less. An example is shown in Fig. 6. Under such conditions, *Thiobacillus* A2 slowly repressed its thiosulphate-oxidizing and carbon dioxide-fixing potentials during the acetate period; the reverse being true during thiosulphate supply. At the end of a 4-hour

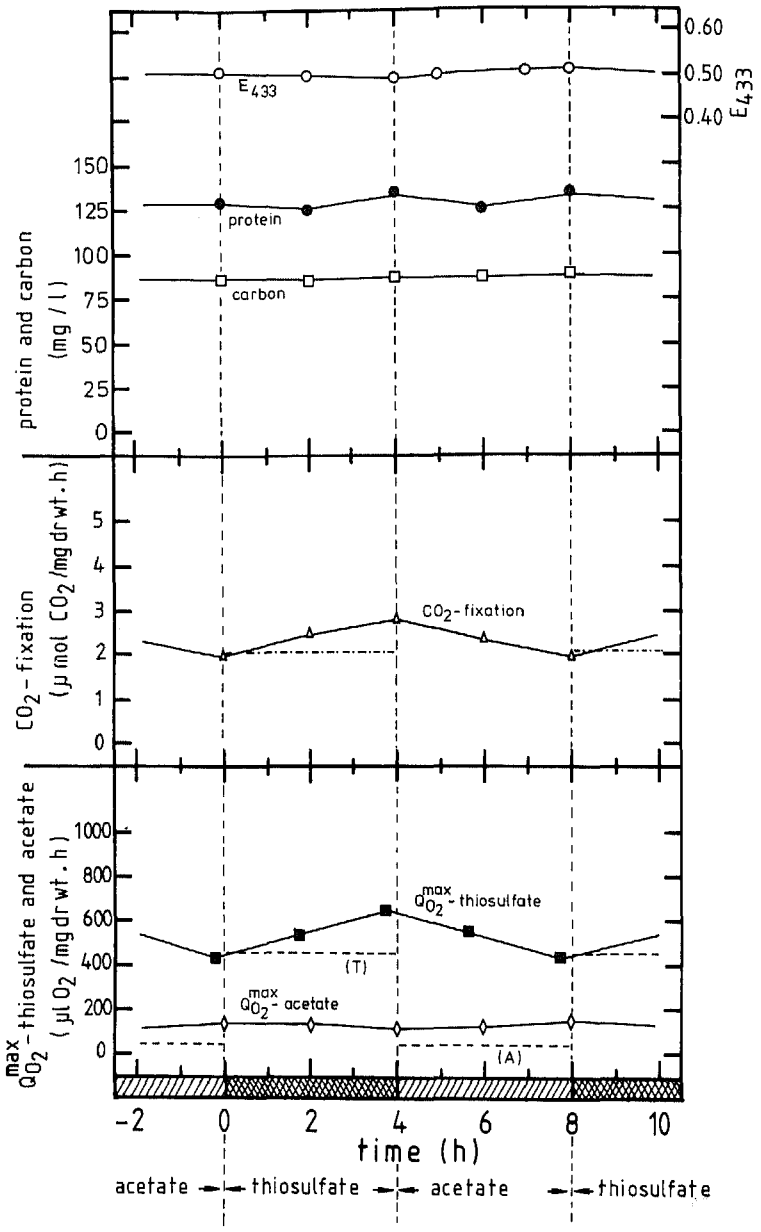

Fig. 6 – *Thiobacillus* A2 grown under 4-hour supplies of growth limiting acetate and thiosulphate in continuous culture (D = 0.05 h^{-1}). Optical density (○—○), protein (●—●), carbon (□—□), maximum CO_2 fixation capacity (△—△), maximal thiosulphate oxidizing capacity (■—■), maximal acetate oxidizing capacity (◇—◇).

supply of acetate, the thiosulphate oxidizing potential was just sufficient to allow thiosulphate oxidation at the onset of the following thiosulphate period. However, longer intervals between medium changes meant that *Thiobacillus* A2 was forced to re-induce its thiosulphate utilizing system and thiosulphate accumulated transiently in the culture.

When the competition experiments were repeated using the 4-hour alternating supplies of substrates, *Thiobacillus* A2 proved to be unable to maintain itself in a three-membered culture. The explanation for this unexpected result lies in the adaptability of *Thiobacillus* A2 and the rigidity of the two specialists. For example, *T. neapolitanus* maintained a very high thiosulphate oxidizing capacity during the acetate supply period, which would amount to a starvation period for this organism. On the appearance of thiosulphate in the culture, it was able to oxidize the thiosulphate at a much higher rate than the versatile species. *Thiobacillus* A2 was therefore forced to be "a very good chemolithotroph" during the thiosulphate period and a "very good heterotroph" during the acetate period. It was not able to accomplish this, since its "reactivity", that is, its ability to respond with full potential to a sudden change in the environment, was lower than those of the specialists.

A summary of the typical properties of versatile and specialist species as exemplified by *T. neapolitanus* and *Thiobacillus* A2 is given in Table 4.

In order to establish whether specialist species would dominate in continuous enrichment cultures made under fluctuating conditions, experiments were made using alternating thiosulphate and acetate supplies. The dominant organism isolated was called *Thiobacillus* S. This species was found to be a facultative autotroph, able to grow on acetate, glycollate, succinate, lactate and thiosulphate (Spijkerman & Kuenen, unpublished results). Moreover, although its capacity for thiosulphate utilization dropped during culture in the absence of thiosulphate, it was never completely lost. This appears to be an important difference from *Thiobacillus* A2. Other differences are shown in Table 5. Rather than the rapid switches of metabolism shown by *Thiobacillus* A2, *Thiobacillus* S was found to have developed a different survival strategy. During the periods of heterotrophic growth, it stored significant quantities of poly-β-hydroxybutyrate which were then utilized as a supplementary carbon source during the initial stages of growth on thiosulphate when its carbon dioxide fixing capacity was low (Fig. 7).

Thus, by showing a less dramatic response than *Thiobacillus* A2, *Thiobacillus* S is able to retain enough "reactivity" to compete successfully with specialist organisms present in the enrichment inoculum.

In conclusion, our experiments have shown a number of the ecological principles concerning co-existence and competition. It has been shown that a mixotrophic type of metabolism may be both a blessing and a curse, since the advantages must be paid for by a loss of reactivity. The results of the enrichment cultures supplied with mixtures of acetate and sulphide, together with the

Table 4

Differences between the specialist model organism, *T. neapolitanus,* and the versatile model organism, *Thiobacillus* A2. Table adapted from Beudeker *et al.,* (1982)

Data collected from Gottschal *et al.* (1979, 1981a, 1981b), Beudeker *et al.* (1982), Smith & Hoare (1977), Matin (1978).

Specialist *T. neapolitanus*	Versatile *Thiobacillus* 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.
2. High specific growth rate on single substrate (μ_{max} on thiosulphate = 0.35 h^{-1}).	Low specific growth rate on single substrate (μ_{max} on thiosulphate = 0.10 h^{-1}). Relatively high specific growth rate on mixed substrate (μ_{max} on thiosulphate plus acetate = 0.22 h^{-1}).
3. High affinity for reduced sulphur compounds.	Relatively low affinity for reduced sulphur compounds.
4. High overcapacity of respiratory capacity during substrate-limited growth.	Low overcapacity of respiratory capacity during substrate-limited growth.
5. Low "flexibility" with respect to energy generation and organic carbon assimilation; "constitutive" enzymes.	High "flexibility"; "inducible" enzymes for energy generation and carbon assimilation.
6. High "reactivity" towards few substrates.	Low "reactivity" towards many substrates.
7. Metabolic "lesions".	Many pathways often overlapping.
8. Low endogenous respiration.	High endogenous respiration in autotrophically and heterotrophically grown cells.
9. Very resistant to starvation.	Less resistant to starvation.
10. Ecological niche: in environments with continuous or fluctuating supply of reduced sulphur compounds and a low turnover of organic compounds.	Ecological niche: in environments with simultaneous presence of both inorganic and organic substrates ("mixotrophic" conditions).

Table 5

Differences shown by *Thiobacillus* A2 and *Thiobacillus* S in their reactions to variations in the ratio of acetate and thiosulphate in the medium, demonstrating the less dramatic variations in the metabolism of *Thiobacillus* S (Spijkerman & Kuenen, unpublished results; Gottschal & Kuenen, 1980a)

Organism	Substrate		$Q_{O_2}^{max}$		CO_2 fixation
	Acetate mM	Thio. mM	Acetate (μl O_2/mg protein/h)	Thio	by whole cells nmol CO_2/min/mg protein)
Thiobacillus S	10	0	196	45	0.45
	10	10	85	257	–
	4	32	157	1291	19.5
Thiobacillus A2	10	0	300	0	0
	10	10	230	100	0
	4	32	100	1700	80

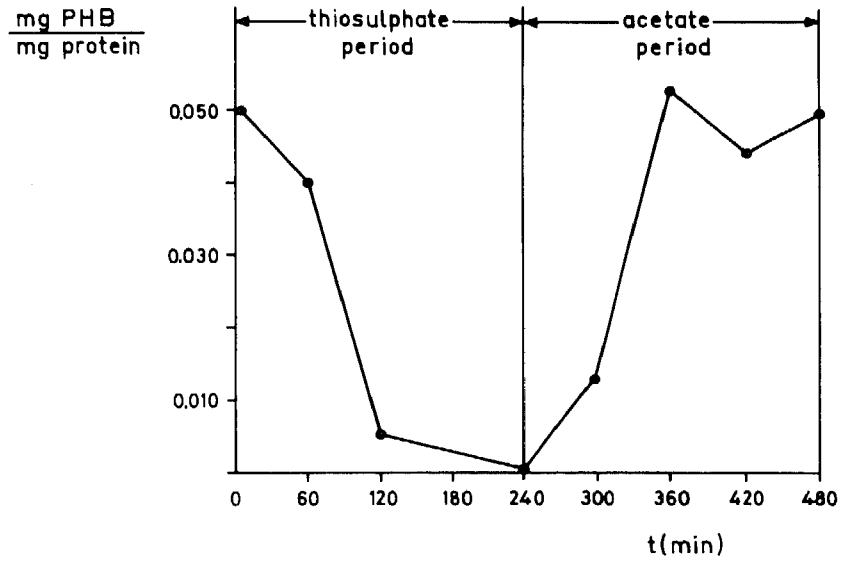

Fig. 7 – Fluctuation of the poly-β-hydroxybutyrate content of *Thiobacillus* S cells during alternating 4 hour periods of thiosulphate and acetate in a chemostat. ($D = 0.05\ h^{-1}$). (Spijkerman & Kuenen, unpublished results.)

observations on the denitrifying column, show that the principles found for mixed-substrate monocultures can be applied to much more complex systems. The outcome of the enrichments run with alternating supplies of acetate and thiosulphate clearly demonstrate that the conclusions drawn from "model" experiments cannot always be applied generally and should therefore be treated with caution.

The mathematical modelling has provided us with more insight into the exact nature of competition for mixed substrates. The concept that the physiological state of an organism competing with others for mixed substrates at a fixed dilution rate must be constant, and thus be independent of the ratio of the substrates in the feed, is of particular interest.

It is hoped that the study of many more composite mixed cultures, and of additional continuous enrichments with different mixtures of substrate, will extend our understanding of the inter-relationships between bacteria in simple and complex biological systems.

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SYMBOLS USED

D = dilution rate

$\mu_j (s_1, s_2)$ = specific growth rate of population j as a function of s_1 and s_2

μ_{max} = maximum specific growth rate

$\bar{s}$ = specific substrate concentration in steady state

s_i = concentration of substrate i in the culture

K_{ij} = concentration of substrate i which permits half-maximum specific growth rate of population j

Y_{ij} = cell yield of population j on substrate i

A = autotroph

H = heterotroph

M = mixotroph

a = acetate

t = thiosulphate