The Role of Specialists and Generalists in Microbial Population Interactions

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In many environments highly specialized bacteria co-exist with generalists, i.e. bacteria able to metabolize a large diversity of substrates. In order to understand the mechanisms of interaction between these types of bacteria, model experiments in continuous culture have been carried out with two typical specialists and one generalist. It could be shown that the generalist can compete successfully with specialists for growth limiting substrates when mixtures of substrates are available. Under these conditions the generalist can utilize these mixtures simultaneously. Another advantage of generalists might lie in their capability to continue to grow when the supply of different substrates alternate. In that case specialists would alternatively grow and starve. Model-competition experiments indicate that, in general, the success of specialists was favoured by increased length of growth and starvation periods.

The breakdown of organic and inorganic compounds in nature is carried out by an enormous diversity of bacteria which are adapted to a variety of physical and chemical environmental parameters. In a given environment, many microorganisms may coexist which often have completely different metabolic capabilities, but sometimes also have overlapping properties. Typically, one can find highly specialized organisms, able to metabolize only one or a few compounds, coexisting with very versatile organisms, termed generalists. The latter are able to metabolize a great diversity of organic compounds.

From the ecological point of view one may ask how to explain this coexistence of microorganisms and how their respective physiological properties may give them an advantage or disadvantage under a given growth condition. Similarly, from the point of view of management of sewage treatment plants, one may ask how the

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regime of a plant may select certain metabolic types and how this may have a bearing on properties of the plant such as susceptibility to pulse charges and loading capacity.

In order to understand the complicated reactions occurring between specialists and generalists within a complex mixture of other organisms, a thorough insight into their basic metabolic properties, that is their physiological behavior, is needed. Unfortunately the physiology of most microorganisms is impossible to study in the very complicated mixtures in which they naturally occur. Therefore, as an initial approach, it has been necessary to study specialists and generalists in pure cultures, and in artificial composite mixtures of organisms in order to obtain insight into their ecological niches.

When grown on their specific substrate in the laboratory, the specialists are characterized by very high specific growth rates ($\mu_{\text{max}}$), whereas the generalists turn out to possess relatively low maximum specific growth rates on the substrates they can utilize. Even at very low concentrations of a substrate, as they generally occur in the environment, the versatile organisms grow relatively slowly.

Figure 1 shows a generalized picture of the relationship between the growth limiting substrate and the specific growth rate of a specialist and a generalist.

It may be asked whether the generalists might be able to grow faster than specialists in mixtures of substrates. However, under such conditions the generalists often show sequential substrate utilization, known as diauxie. A well-known example is the growth of the bacterium Escherichia coli on mixtures of glucose and lactose. First the glucose is utilized, and only when this compound is completely metabolized, the enzymes needed for lactose utilization will be induced, allowing growth on lactose. Thus at high concentrations of mixtures of substrates, simultaneous utilization in this case is not possible.

One should realize that in many natural and seminatural environments concentrations of substrates are generally low, usually below the $m$ and even often below the $m$ range. Under such conditions, the growth rate of microorganisms will be limited by the concentration of their substrates, and mixed substrate utilization might be possible. In the laboratory, growth under dual substrate limitation can be conveniently created in a flow-controlled chemostat, or continuous culture. In the growth medium supplied to the culture, all ingredients necessary for growth are in excess except for the two substrates in question. It has been shown for $E. coli$ by Silver and Mateles (1) that under such conditions simultaneous utilization of glucose and lactose was possible. Analogous results have been obtained for other bacteria, such as Pseudomonas xanthobacter growing on mixtures of formate and acetate (2) and Thiobacillus strain A2 growing on thiosulfate and acetate (3). Our own work on the generalist Thiobacillus A2 may serve as
Figure 1. The relationship between the concentration ($s$) of the growth-limiting substrate and the specific growth rate ($\mu$) of a typical specialist and a typical generalist bacterium growing on the same substrate.
an example. T. A2 is a very versatile organism able to grow on at least 25-30 different organic substrates, and, in addition, also capable of autotrophic growth on inorganic reduced sulfur compounds. (See Table I). Under the latter conditions it oxidizes, for example, thiosulfate or sulfide as a source of energy while using carbon dioxide as the only carbon source for growth. When grown in batch culture in the presence of high concentrations of acetate and thiosulfate T. A2 clearly shows a biphasic utilization of the two compounds (Figure 2). However when grown in the chemostat under growth limitation by this mixture, simultaneous utilization of acetate and thiosulfate is possible irrespective of the available ratio of thiosulfate and acetate (Figure 3). Under such conditions, acetate and thiosulfate concentrations in the culture are below the detection level. The metabolic machinery of T. A2 adapts to the required turnover rates of the respective substrates. For example, the ability to oxidize thiosulfate is present at maximum capacity when only thiosulfate is supplied to the culture, whereas no thiosulfate respiration capacity is present when only acetate is available. Interestingly, the ability of the cells to assimilate CO₂ for cell carbon is efficiently adapted to the available organic carbon in the culture. This implies that when the ratio of thiosulfate to acetate is relatively high, acetate is used primarily as a carbon source to "save" energy for CO₂ fixation. Further work in our laboratory by others (4 and 5) has shown that simultaneous utilization of mixtures of other substrates is equally possible.

The aim of our research was then focussed on the question of whether this generalist would be able to compete successfully for growth-limiting substrates with specialists during mixotrophic growth under nutrient limitation. For our purpose we used the three model organisms shown in Table II. These included a typical generalist, namely the versatile *Thiobacillus* A2, and two specialists. The first specialist was the chemolithoautotroph, *Thiobacillus neapolitanus*, which can grow very fast in minerals-thiosulfate medium using CO₂ as its carbon source, and the second was a chemoorganoheterotroph *Spirillum* G7, which can grow very rapidly on minerals-acetate medium using acetate as carbon and energy source. (See also Table I).

A series of experiments was carried out in continuous culture to study the competition between sets of two and three organisms. In the first experiment, *Thiobacillus* A2 and *Thiobacillus neapolitanus* were each grown separately in a thiosulfate-limited chemostat. Once steady states had been established at a fixed dilution rate, the cultures were mixed one to one (v/v) and the change in the percentages of the two organisms was followed until no further change could be observed for one or two volume changes. Figure 4 shows that in minerals-thiosulfate medium, *Thiobacillus* A2 was out-competed by the specialist. T. A2 was not completely eliminated since it has been shown that the specialist excretes glycolate (6) which can be consumed by T. A2.
Table I

Types of metabolism of "model" bacteria used for the study of competition between "specialists" and "generalists".

GENERALIST:
Facultative chemolitho(auto)troph ("mixotroph") (Thiobacillus A2)

\[ S_2O_3^{\text{-}} (\text{or } S^{\text{2-}}) \xrightarrow{O_2} SO_4^{\text{-}} \]

Energy source: and/or
(many) organic compound(s) \( \xrightarrow{O_2} \) CO\(_2\) cellmaterial

Carbon source: and/or
(many) organic compound(s) \( \xrightarrow{} \) cellmaterial

SPECIALIST I
Obligate chemolitho(auto)troph (Thiobacillus neapolitanus)

\[ S_2O_3^{\text{-}} (\text{or } S^{\text{2-}}) \xrightarrow{O_2} SO_4^{\text{-}} \]

Energy source: \( \xrightarrow{} \) cellmaterial

Carbon source: CO\(_2\) \( \xrightarrow{} \) cellmaterial

SPECIALIST II
Chemo(organoheterotroph (Spirillum G7)

Energy source: (few) organic compounds \( \xrightarrow{O_2} \) CO\(_2\)

Carbon source: (few) organic compounds \( \xrightarrow{} \) cellmaterial
Figure 2. Maximum substrate oxidation capacities ($Q_{O2}^{max}$) and substrate concentration during growth of T. A2 on a mixture of acetate and thiosulfate in batch culture. The inoculum consisted of cells from an acetate-grown culture. Key: ●, $Q_{O2}^{max}$-thiosulfate; ▲, $Q_{O2}^{max}$-acetate; ○, thiosulfate concentration; △, acetate concentration. Reproduced, with permission, from Ref. 3. Copyright 1980, Springer-Verlag.
Figure 3. Maximum substrate oxidation potentials and carbon dioxide fixation potential of whole cells of T. A2 as a function of different acetate and thiosulfate concentrations in the reservoir medium of the chemostat cultures. Key: •, $Q_{O_2}^{max}$-thiosulfate; ▲, $Q_{O_2}^{max}$-acetate; ■, CO$_2$-fixation potential. Reproduced, with permission, from Ref. 3. Copyright 1980, Springer-Verlag.

Data were obtained with cells from thiosulfate- and/or acetate-limited chemostat cultures in steady state at a dilution rate of 0.25 h$^{-1}$. Acetate and thiosulfate concentrations in the cultures were below the detection level. Cellulose carbon in the cultures ranged from 110 mg C/L (40 mM thiosulfate) to 200 mg C/L (20 mM acetate).
When acetate was supplied to the inflowing medium, the number of T. A2 cells increased since T. neapolitanus cannot utilize acetate. Figure 5 shows that with increasing acetate concentration the percentage of T. A2 gradually increased and coexistence of the two organisms became possible. Above a concentration of about 10 mM acetate, the specialist was eliminated from the culture. The explanation for this lies in the ability of T. A2 to utilize acetate and thiosulfate simultaneously under these growth conditions. With increasing acetate concentration, the absolute concentration of the T. A2 cells increased and allowed these organisms to claim an increasingly larger share of thiosulfate. Eventually, the ratio of acetate to thiosulfate became such that T. A2 was able to reduce the thiosulfate concentration to below the level at which T. neapolitanus could maintain the required growth rate, resulting in the wash out of the specialist. Very similar results were obtained with mixtures of thiosulfate and glycolate (4) or thiosulfate and glucose (3).

Competition experiments between the specialist Spirillum G7 and T. A2 gave analogous results. In acetate medium Spirillum G7 out-competed T. A2 completely. Thiobacillus A2 out-competed the specialist when more than 10 mM thiosulfate was present in the acetate-minerals medium. Obviously, under "natural" conditions in the environment, or in sewage treatment plants, the generalist would have to compete with both specialists at the same time. Therefore, three-membered cultures were also studied. The results, as summarized in Figure 6, show that coexistence of the three organisms was possible over a large range of concentration ratios with T. A2 dominating the culture. The coexistence of three organisms does not agree with theoretical predictions which will be discussed below. In any case, the outcome of these experiments clearly indicated that when mixed substrates are supplied, generalists have metabolic advantages which allow them to claim a "niche", that is, a right of existence in the natural environment. The validity of this generalization was confirmed by the results of enrichment cultures carried out in the chemostat inoculated with natural samples. Table III shows the outcome of such enrichment cultures performed with different mixtures of thiosulfate and acetate. In all cases when fresh water inocula were used, a dominant culture of a generalist Thiobacillus was obtained. Interestingly, in 4 out of 5 cases the generalist was a facultative chemolithotroph able to grow autotrophically. However, in one case where relatively high amounts of acetate were presented to the culture, a bacterium able to obtain energy from thiosulfate but not able to fix CO₂ came to the fore. This is not surprising, since at this ratio CO₂ fixation is not required (compare Figure 3 for T. A2).

As mentioned before, a somewhat puzzling result of the competition experiments with the three-membered culture was that coexistence of three organisms seemed possible. In several theore-
Table II

Maximum specific growth rates $\mu_{\text{max}}$ (h$^{-1}$) of the specialized, obligately chemolitho(auto)trophic *Thiobacillus neapolitanus*, the versatile, facultatively chemolithoautrophic *Thiobacillus A2* (a generalist), and a specialized heterotrophic *Spirillum G7*, during growth in minerals medium supplemented with thiosulfate (t), acetate (a) or mixtures of both substrates (t + a). *T. neapolitanus* and *Spirillum G7* cannot grow on acetate or thiosulfate respectively (10).

<table>
<thead>
<tr>
<th></th>
<th>t</th>
<th>a</th>
<th>t + a</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>T. neapolitanus</em></td>
<td>0.35</td>
<td>-</td>
<td>0.35</td>
</tr>
<tr>
<td><em>Thiobacillus A2</em></td>
<td>0.10</td>
<td>0.22</td>
<td>0.22</td>
</tr>
<tr>
<td><em>Spirillum G7</em></td>
<td>-</td>
<td>0.43</td>
<td>0.43</td>
</tr>
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</table>

Figure 4. Competition in continuous culture between *T. neapolitanus* (the specialist) and *T. A2* (the generalist) for thiosulfate as the only growth-limiting substrate. Key: ●, relative cell number of *T. A2*; ○, *T. neapolitanus*. Reproduced, with permission, from Ref. 4. Copyright 1979, Springer-Verlag.

The chemostat was run at a dilution rate of 0.05 h$^{-1}$ with a 40 mM thiosulfate concentration in the reservoir medium. Organisms had been pregrown separately in continuous culture at $D = 0.03$ h$^{-1}$ and at zero time mixed in a 1:1 ratio.
Figure 5A. Effect of different concentrations of organic substrate on the outcome of the competition between T. A2 and T. neapolitanus for thiosulfate. Protein concentration and organic content in the cultures are shown, limited by thiosulfate plus acetate or glycollate. Reproduced, with permission, from Ref. 4. Copyright 1979, Springer-Verlag.

The chemostat was run at a dilution rate of 0.07 h⁻¹. The inflowing medium contained thiosulfate (40 mM) together with either acetate or glycollate at concentrations ranging from 0-7 mM. Relative cell numbers, protein content, and organic cell carbon in the culture were determined after steady states had been established.
Figure 5B. Effect of different concentrations of organic substrate on the outcome of the competition between T. A2 and T. neapolitanus for thiosulfate. Conditions as in Figure 2A. The percentage of T. A2 cells and of T. neapolitanus cells in cultures are shown, with thiosulfate plus acetate or glycollate in the reservoir medium. Reproduced, with permission, from Ref. 4. Copyright 1979, Springer-Verlag.
Figure 6. Results of competition between the generalist T. A2, and two specialists, T. neapolitanus and Spirillum G7 for thiosulfate and acetate as growth-limiting substrates in the chemostat at a dilution rate of 0.07 h⁻¹. Concentrations in the inflowing medium ranged from 0–20 mM for acetate and from 0–40 mM for thiosulfate. After a steady state had been established relative cell numbers were determined. Solid line, experimental data; dashed line, outcome of the competition as predicted from mathematical modeling.
Table III

Results of enrichment cultures (I - V) from fresh water samples after 15-20 volume changes in the chemostat under dual substrate limitation of thiosulfate (t) and acetate (a) at a dilution rate of 0.05 h⁻¹, using different mixtures of t + a. Adapted from Gottschal and Kuenen, ([1]).

<table>
<thead>
<tr>
<th>Number</th>
<th>Sample</th>
<th>Substrate concentration in medium (mM)</th>
<th>% of total</th>
<th>Dominant population</th>
<th>Metabolic type</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>canal</td>
<td>30 t + 5 a</td>
<td>82</td>
<td></td>
<td>facultative chemolithotroph</td>
</tr>
<tr>
<td>II</td>
<td>canal</td>
<td>10 t + 15 a</td>
<td>75</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>III</td>
<td>canal</td>
<td>30 t + 5 a</td>
<td>85</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>IV</td>
<td>ditch</td>
<td>20 t + 10 a</td>
<td>50</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>V</td>
<td>ditch</td>
<td>10 t + 15 a</td>
<td>86</td>
<td></td>
<td>chemolithoheterotroph</td>
</tr>
</tbody>
</table>
tical treatments of the growth of microbial populations on mixtures of substrates it has been predicted that the maximum number of organisms which can coexist can never be more than the number of growth-limiting substrates supplied to the culture. This holds if no interaction other than mere competition takes place in the culture. Based on previous publications of other workers Gottschal and Thingstad (7) have developed a mathematical model for growth of the three organisms in the particular case.

The model is based on simple Monod kinetics for growth. The specific growth rate of the specialist autotroph (A) on thiosulfate (t) is

\[ \mu_{tA} (s_t) = \frac{\mu_{\text{max}}}{K_{tA} + s_t} \cdot s_t \]

in which \( s_t \) is the concentration of the thiosulfate and \( K_{tA} \) the substrate saturation constant of the autotroph for thiosulfate.

Similarly, for growth of the heterotroph (H) on acetate (a) one obtains

\[ \mu_{aH} (s_a) = \frac{\mu_{\text{max}}}{K_{aH} + s_a} \cdot s_a \]

The growth rate for the mixotrophic generalist (M) has been taken as the sum of the two separate growth rates:

\[ \mu_M (s_a, s_t) = \frac{\mu_{\text{max}}}{K_{aM} + s_a} \cdot s_a + \frac{\mu_{\text{max}}}{K_{tM} + s_t} \cdot s_t \]

As pointed out by Gottschal and Thingstad (7), this is an approximation which can only be valid at low growth rates where the relationship between \( \mu \) and \( s \) is nearly linear. This model does not take into account that the generalist adapts its substrate oxidizing capacity to the ratio between the two substrates available in the mixture. This would probably lead to an over-estimation of \( \mu_{\text{max}}^M \) and \( \mu_{\text{max}}^A \). It could be shown, however, that in a two-membered culture the decrease of \( \mu_{\text{max}}^A \) would have a constant value since the physiological state of the generalist (M) would be constant.

Although the model allowed accurate prediction of the competition between two organisms (Figure 7 a,b), it could indeed not account for the anomaly observed in the experiments using three organisms. The dotted lines of Figure 6 describe the predicted steady state values. The parameters used in this prediction had been derived from the outcome of the results obtained from the two-membered cultures.

Several possibilities explaining this anomaly have been con-
Figure 7A. Cell density of the generalist (mixotroph T. A2, O) and the autotroph (specialist T. neapolitanus, ●) in mixed chemostat culture at steady state, growing at a dilution rate of 0.075 h⁻¹. Growth was simultaneously limited by thiosulfate (reservoir medium concentration, $S_{1}^{0} = 40$ mM) and by increasing concentrations of acetate in the reservoir medium ($S_{2}^{0} = 0$–10 mM).
Figure 7B. Cell density of the mixotroph (○) and the heterotroph (specialist Spirillum G7, ●) in mixed chemostat-culture at steady state, growing at a dilution rate of 0.075 h⁻¹. Growth was simultaneously limited by acetate (Sₐ = 20 mM) and by increasing concentrations of thiosulfate in the inflowing medium (0-10 mM).
sidered. First of all, the assumption that no interactions other than competition for the substrates occur may be incorrect. Although this possibility cannot be ruled out, it is unlikely since the spent medium from each of the pure cultures did not influence the growth of the other(s). T. neapolitanus did however excrete glycollate, which can be utilized by T. A2 and by Spirillum G7. However, the effect of glycollate should be greatest when the concentration of T. neapolitanus is highest, and this is apparently not the case.

A more complicated explanation may lie in the fact, indicated above, that in the theoretical treatment, it has been assumed that the growth of T. A2 is the sum of the separate specific growth rates on the individual substrates. As long as only two organisms are present in the culture, T. A2 may indeed be in a constant metabolic state. However, the physiological state of T. A2 in the presence of T. neapolitanus is very different from that of T. A2 in the presence of Spirillum G7. At the very moment that T. neapolitanus is removed from the culture the metabolic state of T. A2 will change to that found in pure culture during mixed substrate consumption (Figure 3). As a result, the assumed $K_s$ and $u_{max}$ values for T. A2 will alter and in fact it can be shown that this will make T. A2 less competitive. As pointed out by Gottschal and Thingstad (7), it may therefore be that the rate at which the shift in population occurs in the three-membered culture is much smaller than that in the two-membered cultures. Mathematical simulation experiments show that if a shift in parameters happened, it might very well take 100 or more volume changes before a real steady state would be attained. If this were the case, changes might have taken place so slowly that they were not noticed in the actual experiments, even though, in one experiment, a measurement of ratios was made after 30 volume changes.

In spite of the discrepancy between the theoretical and practical models, it should be stressed that both models clearly indicate the ecological advantages of a generalist type of physiology.

Recent work by Slanbroek, Smit, Klein-Nulend and Veldkamp (8) shows that this phenomenon may also explain the coexistence of versatile and specialist Clostridium species. Furthermore, results obtained by Harder and co-workers (W. Harder, University of Groningen, unpublished) indicated that the same principle may apply to generalists and specialists among the methanol utilizing bacteria.

Mixotrophic growth of the generalists is apparently only one possible advantage of a versatile metabolism. Another possible benefit might lie in the capability of versatile organisms to grow continuously with alternating supplies of two substrates. This has also been studied in detail for the same set of three organisms shown in Table II. (9). Figure 8 gives an example of one series of experiments designed to show that the generalist can grow uninterrupted during an alternating supply of 4h chiosulfate,
Figure 8. T. A2 grown under alternate limitation of acetate and thiosulfate in continuous culture at a dilution rate of 0.05 h⁻¹. Thiosulfate (40 mM) or acetate (10 mM) was supplied to the culture, each for 4 h. The dashed lines indicate the minimum activities needed for uninterrupted growth. Reproduced, with permission, from Ref. 9. Copyright 1981, Society for General Microbiology.
4h acetate, in a chemostat run at a dilution rate of 0.05 h⁻¹. Under these conditions, the overcapacity of T. A2 to oxidize either of the substrates was marginal. During the acetate period, the ability to oxidize thiosulfate was repressed more than the acetate oxidation potential was during the thiosulfate period. When the acetate period was extended relative to the thiosulfate period, T. A2 no longer maintained a sufficiently high thiosulfate oxidation potential to permit continued growth at the same rate. As a result, population densities started to fluctuate and thiosulfate transiently accumulated.

Interestingly, the two specialist organisms grew well in pure culture on their specialist substrate under a regime of 4h substrate (thiosulfate or acetate) and 4h starvation. Of course, during the starvation period a proportion of the specialists was washed out of the chemostat, but the remaining organisms retained a very high overcapacity to oxidize the substrate, permitting them to grow instantaneously at a high rate after the starvation period.

When a competition experiment was carried out between T. A2 and T. neapolitanus under alternating substrate conditions, the two organisms appeared to coexist in equal numbers. This suggested that T. neapolitanus would utilize practically all of the thiosulfate, whereas T. A2 was growing on acetate only.

The explanation of this result lies in the versatility of T. A2. During the acetate period T. A2 reduced its thiosulfate oxidizing capacity whilst T. neapolitanus was washed out but retained its high thiosulfate oxidation potential. As a result, at the beginning of the thiosulfate period, the T. neapolitanus population could oxidize the thiosulfate at a much higher rate than T. A2. Direct measurement of the substrate levels in the chemostat showed that under these conditions T. neapolitanus could maintain the concentration of the substrate 10 fold lower than T. A2 was able to (Figure 9). Thus, during the thiosulfate period in the mixed culture T. neapolitanus could grow much faster than T. A2. Furthermore, the very low concentration of thiosulfate imposed by T. neapolitanus obviously led to a further reduction of the thiosulfate oxidizing potential of T. A2. In other words, the generalist was forced by the specialist to grow as a heterotroph on acetate only.

T. A2 is, however, not a specialist heterotroph and the addition of the specialist acetate-utilizing Spirillum G7 to the two-membered culture led to wash out of T. A2, resulting in a coexisting population of the two specialists (Figure 10a).

Further experiments showed that an alternating supply of two different mixtures of thiosulfate and acetate led to coexistence of all three organisms (Figure 10 b,c). Obviously, under an alternating supply of specialist substrates, generalists similar to T. A2 clearly are at a disadvantage. However, it could be shown that nature harbours organisms akin to T. A2 which are better
Figure 9. The accumulation of sulfide under alternate supply of 4 h acetate, 4 h sulfide to a continuous culture of T. A2. O, sulfide concentration in the culture at pH 8.0; and ♦, at pH 7.5. Competition experiments all had been carried out at pH 7.5. Sulfide is a substrate which, in these experiments, is entirely equivalent to thiosulfate, but has the advantage of being detectable down to a concentration of less than 1 μM. In cultures of T. neapolitanus grown at pH 7.5 the sulfide concentration never increased to above 4 μM. Reproduced, with permission, from Ref. 12. Copyright 1982, H. Veenman en Zonen BV.
Figure 10. Competition in continuous culture between T. A2, T. neapolitanus and Spirillum G7 for thiosulfate and acetate as growth-limiting substrates. The dilution rate was 0.05 h⁻¹ with intermittent feeding of two media containing thiosulfate, or acetate, or both. These media were supplied alternately to the culture, each during 4 h. Key: •, T. A2; ■, Spirillum G7; and ○, T. neapolitanus as percentage of the total cell number present in the culture. A: One medium contained 10 mM acetate, the other 40 mM thiosulfate. B: One medium contained 2.2 mM acetate plus 34.4 mM thiosulfate, the other 10 mM thiosulfate plus 6.4 mM acetate. C: One medium contained 5.7 mM acetate plus 31.0 mM thiosulfate, the other 6 mM thiosulfate plus 11.7 mM acetate. Reproduced, with permission, from Ref. 9. Copyright 1981, Society for General Microbiology.
adapted to the regime described in Figure 10a. When a continuous culture under this regime was inoculated with fresh water mud, a dominant population of a spirillum-shaped facultative chemolitho- trophe developed (9). The success of this organism was probably due to its ability to "store" acetate as a polymer (poly-ß-hydroxy- butyrate) in the cells. This polymer was used during the thiosul- fate period as a source of carbon (J.G. Kuenen and A. Spijkerman, unpublished results). In this way, the thiosulfate utilizing spirillum could save energy for CO₂ fixation during the thiosulfate period.

The experiments described in this paper clearly show the possible advantages and disadvantages of the specialist versus generalist strategies for survival. Our present hypothesis is that specialist organisms will be successful under conditions where the turnover rate of their specialist substrate is high relative to that of other substrates. On the other hand, versatile organisms might have an advantage when the relative turnover rates of several of their growth substrates are in the same order of magnitude. Obviously when the supplies of different substrates strongly fluctuate or even alternate, the outcome of competition between specialists and generalists for growth-limiting substrates will be determined by the length of the supply periods. As yet it has not been possible to confirm these predictions with direct measurements of generalists and specialists in natural mixed cultures. At present we are developing methods for the measurement of the contributions of the different population in suitable systems, namely sewage treatment plants, which receive supplies of, for example, sulfide and organic substrates.

A better understanding of the selection of different populations in the mixed cultures of sewage treatment plants may facilitate control and management of these plants. An example may help to illustrate this point. The presence of specialist populations will improve the "resilience" of the plant to strong fluctuations, since the organisms usually possess a high overcapa- city. In contrast if, during supply of sulfide and organic com- pounds, chemolithothrophic heterotrophs are selected, the population will become extremely sensitive to strong fluctuations of sulfide, not only because their maximum respiratory capacity is low, but also because such organisms tend to produce toxic pro- ducts inhibitory for their own metabolism of sulfide. In such a case, a sudden pulse of sulfide would lead to accumulation of sulfide, subsequent formation of toxic products, and further inhibi- tion of the sulfide oxidation, leading to even further accumu- lation of this compound. With prior knowledge of this selection, one might be able to absorb this peak loading, thus avoiding unsatisfactory performance of the sewage treatment plant.
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Literature Cited


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