Coexistence of Organisms Competing for the Same Substrate: An Example Among the Purple Sulfur Bacteria[1]

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Abstract

The purpose of this study was to find a possible explanation for the coexistence of large and small purple sulfur bacteria in natural habitats. Experiments were carried out with *Chromatium vinosum* SMG 185 and *Chromatium weissei* SMG 171, grown in both batch and continuous cultures. The data may be summarized as follows: (a) In continuous light, with sulfide as growth rate-limiting substrate, the specific growth rate of *Chr. vinosum* exceeds that of *Chr. weissei* regardless of the sulfide concentration employed. Consequently, *Chr. weissei* is unable to compete successfully and is washed out in continuous cultures. (b) With intermittant light-dark illumination, the organisms showed balanced coexistence when grown in continuous cultures. The "steady-state" abundance of *Chr. vinosum* was found to be positively related to the length of the light period, and that of *Chr. weissei* to the length of the dark period. (c) Sulfide added during darkness is rapidly oxidized on subsequent illumination, resulting in the intracellular storage of reserve substances, which are later utilized for growth. The rate of sulfide oxidation/mg cell N/hr was found to be over twice as high in *Chr. weissei* as in *Chr. vinosum*. The observed coexistence may be explained as follows. In the light, with both strains growing, most of the sulfide will be oxidized by *Chr. vinosum* [see (a)]. In the dark, sulfide accumulates. On illumination, the greater part of the accumulated sulfide will be oxidized by *Chr. weissei* [see (c)]. A changed light-dark regimen should then have the effect as observed [see (b)]. These observations suggest that intermittant illumination may, at least in part, explain the observed coexistence of both types of purple sulfur bacteria in nature.

Introduction

Purple sulfur bacteria (Chromatiaceae or Thiorhodaceae) thrive in the anaerobic parts of a variety of aquatic habitats, provided illumination occurs [8, 13]. In such habitats they often occur in such abundance that colored water is observed macroscopically [9]. Often hydrogen sulfide, generated by *Desulfovibrio*, is used as electron donor, as shown by the presence of sulfur

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globules inside the cells. Such natural enrichments have been observed for several species, including both the small strains like *Chr. vinosum* or *Thiopedia* [7, 20] and the larger organisms like *Chr. okenii* or *Chr. weissei* [15]. However, when such samples are taken to the lab, the large organism initially found to be the dominant species is rapidly overgrown by small species.

The aim of this study was to find a possible explanation for the relative abundance of the large type photosynthetic bacteria in such natural habitats. Likely, the inability of the large species to compete successfully with the small species in laboratory cultures is a matter of culture conditions. In this respect, Pfennig's [10-12] observations are highly suggestive. He recommended a number of culture conditions for the selective enrichment of the large purple bacteria, such as:

1. a temperature of 15°-20°C rather than 30°C
2. a slightly acidic pH (6.5-6.8)
3. the addition of vitamin B_{12}
4. a low light intensity (10-30 fc)
5. intermittant illumination, like daylight
6. reduced sulfide concentrations

In preliminary experiments, we found the pH and the temperature to be of minor importance. The availability of B_{12} seems to be irrelevant for this study, since we are dealing with habitats supporting growth of the large species and thus may be assumed to contain B_{12}.

For these and other reasons, it was decided to study the influence of the light conditions and the substrate concentration in more detail.

The experiments were carried out with two *Chromatium* species, grown in both batch and continuous cultures, either as pure cultures or as mixed populations.

Materials and Methods

Organisms. Experiments were carried out with *Chromatium vinosum* strain SMG 185 and *Chromatium weissei* strain SMG 171, both generously provided by Norbert Pfennig. In the text the strain numbers are omitted.

Media and Growth Conditions. The organisms were grown in media as described before [4, 5], supplemented with vitamin B_{12} (20 µg/liter), and yeast extract (20 mg/liter). These additions are essential only for growing *Chr. weissei*, but were added to all media for comparison. As a rule, sulfide was used as electron donor and CO_2 as carbon source. The pH of the media was adjusted to 7.0, and the incubation temperature was 25°C. The cultures were illuminated either continuously or intermittantly, employing a light intensity of 100-200 fc incandescent light.

The experiments were performed both in batch cultures and in continuous cultures. In the experiments on the determination of the specific sulfide oxidation rate (q), double-strength culture solutions were employed.

Estimation of Growth Constants. Little is known about the functional relationship between an inhibitory substrate (e.g., sulfide) and the specific growth rate of organisms utilizing that substrate (e.g., *Chromatium*). To enable calculations, the function as proposed by Haldane and used by several others was used.

With the symbols as suggested by Andrews [1], this inhibition function may be described as

$$\mu = \hat{\mu} \frac{s}{(K_s + s)\left(1 + \frac{s}{K_i}\right)} \tag{1}$$

where μ = specific growth rate, hr^{-1}
$\hat{\mu}$ = theoretical maximum specific growth rate in the absence of inhibition, hr^{-1}
s = sulfide concentration, mmol/liter
K_s = saturation constant, equal to the lowest sulfide concentration at which $\mu = \frac{1}{2}\hat{\mu}$, mmol/liter
K_i = inhibition constant, equal to the highest sulfide concentration at which $\mu = \frac{1}{2}\hat{\mu}$, mmol/liter

With very high values of K_i, indicating that the substrate is not inhibitory, Eq. (1) becomes similar to the Monod relationship, and $\hat{\mu}$ equals μ_{max}. The maximum attainable growth rate in the presence of inhibition is described as $\hat{\mu}_{max}$ for which the critical dilution rate (D_c) may serve as an approximate value.

Estimation of $\hat{\mu}$ and K_s. Continuous cultures were run with sulfide as growth rate-limiting substrate [5]. At various dilution rates, the steady-state sulfide concentration was measured. K_s and $\hat{\mu}$ were then estimated graphically from a double reciprocal plot (Burk-Lineweaver) of D vs. s [4].

Estimation of K_i. Batch cultures with increasing sulfide concentration (1-8 mM) were inoculated. Growth was estimated from O.D. readings [2]. The specific growth rate was estimated in the early log phase and plotted versus the initial sulfide concentration. This is not a very accurate approach. However, the magnitude of K_i has only little influence on μ at very low sulfide concentrations.

Estimation of the Specific Sulfide Oxidation Rate q. Organisms were grown in 5-liter batch cultures with automatic pH control by repeated feeding with sulfide. After all sulfide had been oxidized to sulfate (no more storage of sulfur), light was omitted for a 1- to 3-hr period during which sulfide was added to a final concentration of about 0.5 mmol/liter. The data on sulfide and cell nitrogen obtained on subsequent illumination were used to calculate q in mmol sulfide oxidized/mg cell N/hr.

Competition Experiments. Experiments with the two organisms growing in one solution were performed in 20-liter flow-controlled continuous cultures at various light-dark regimen. Samples were collected at regular time intervals and fixed immediately for several analyses. Optical density and pH (pH-stat) were recorded automatically.

Analyses. Sulfide was determined after Trüper and Schlegel [18] and intracellular elemental sulfur and bacteriochlorophyll after Van Gemerden [2]. Cell nitrogen concentra-

tions were determined with a Technicon Autoanalyzer with continuous digestor. In the competition experiments the contribution of the two strains to the total biomass (biovolume) was determined as follows. Samples were fixed, diluted as required with dust-free saline, and counted at small threshold intervals employing a Coulter-counter model Fn with a 50-μm aperture tube. Calibration was done with mono-sized particles. Counts were corrected for coincidence and substrated to yield a size-frequency diagram. The numbers in each category were multiplied with the specific volumes and added to yield the total biovolume. Because of the great difference in size between the two strains (mean cell volume of *Chr. weissei* is five times that of *Chr. vinosum*), virtually no overlap occurs, and the contribution of the two strains could nicely be calculated.

Results

Experiments with Pure Cultures

Influence of Sulfide Concentrations. In most cases, it is hard to determine on what substrate organisms are growing in their natural habitats. However, dealing with purple sulfur bacteria, this is not so difficult. The presence of intracellular sulfur globules observed in *Chromatium* cells in natural samples demonstrates beyond any doubt that sulfide has been oxidized. Although the simultaneous utilization of other electron donors is likely, sulfide may be regarded an electron donor of importance.

Therefore, the relation between the specific growth rate and the sulfide concentrations was determined for both *Chr. vinosum* and *Chr. weissei*. This was done by estimating the essential parameters from batch and continuous cultures. The values obtained for $\hat{\mu}$, K_s, and K_i (Table 1) were then substituted in Eq. (1).

The relation between the specific growth rate and the sulfide concentration is shown in Fig. 1. Both strains are inhibited by sulfide, as has been found for other Chromatiaceae [4, 5]. The inhibition, however, is much more serious for *Chr. weissei* than it is for *Chr. vinosum*. Together with the low maximum growth rate attainable by *Chr. weissei* ($\hat{\mu}_{max} = 0.04\ hr^{-1}$) and the relatively poor affinity for sulfide (K_s = 0.010 mmol sulfide/liter) observed for the same organism, this results in a specific growth rate of *Chr. weissei* which is lower than that of *Chr. vinosum*, regardless of the sulfide concentration employed.

Effect of Dark Periods. In the dark, sulfide is not oxidized by photosynthetic bacteria. This holds for both strains, suggesting that dark periods would have no effect other than slowing down the overall growth rate when judged over a longer period of time.

In previous experiments [3], strongly reduced specific growth rates were found on illumination of a culture previously kept in the dark for several hours. Nevertheless, sulfide was rapidly oxidized, resulting in the synthesis of polyglucose and the formation of elemental sulfur, both accumulated intracellularly.

Table 1
Strain Parameters[a]

Organism	(1) $\hat{\mu}$ (hr^{-1})	(2) $\hat{\mu}_{max}$ (hr^{-1})	(3) K_s (mmol sulfide/liter)	(4) K_i (mmol sulfide/liter)	(5) q (mmol sulfide/mg cell N/hr)
(A) *Chr. vinosum* SMG 185	0.130	0.117	0.007	2.5	0.0185
(B) *Chr. weissei* SMG 171	0.050	0.040	0.010	0.7	0.0415

[a] Key to numbers in parentheses:

(1) and (3): From Burk-Lineweaver plots of steady-state data.
(2): From Eq. (1), in practice equal to D_c.
(4): From batch cultures with increasing initial sulfide concentrations.
(5): From short-term batch culture experiments.

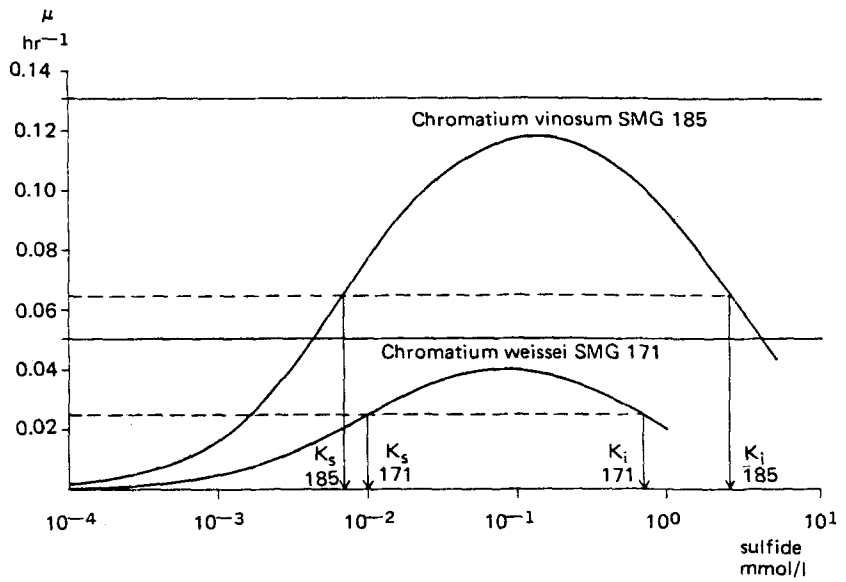

Fig. 1. Relation between specific growth rate and sulfide concentration for *Chr. vinosum* and *Chr. weissei*. Slopes calculated by substituting the experimental data on $\hat{\mu}$, K_S, and K_i in Eq. (1), for various values of s. Full horizontal lines represent $\hat{\mu}$, broken lines represent $\frac{1}{2}\hat{\mu}$.

Comparable experiments were carried out with the two strains studied. Typical examples are shown in Figs. 2 and 3.

On illumination, growth was not observable, but sulfide was rapidly oxidized. Both strains synthesize polyglucose under these conditions. One difference between the two strains was the amount of sulfur stored. In *Chr. vinosum* cultures 54-57% of the added sulfide was recovered as sulfur, whereas in *Chr. weissei* cultures the corresponding figure was 85-89%. Another striking difference was found for the specific rate of sulfide oxidation q, expressed as mmol sulfide oxidized/mg cell N/hr. The value of q was more than twice as high for *Chr. weissei* as for *Chr. vinosum* (Table 1). This suggests that in a mixed culture of both organisms a proportionally high amount of any sulfide accumulated will be oxidized by *Chr. weissei*.

Experiments with Mixed Cultures of Chr. vinosum *and* Chr. weissei

As one would expect from Fig. 1, the continuous cultivation of both strains in one culture with sulfide as growth rate-limiting factor and illuminated continuously invariably resulted in complete wash-out of *Chr. weissei*.

As mentioned previously, it is unlikely that sulfide acts as the sole electron donor in natural habitats. Organic substrates may be utilized as well. At

the moment, no data are available on the relation of specific growth rate of purple sulfur bacteria and the concentration of organic substrates. However, some mixed continuous culture experiments were done employing a reservoir solution containing both sulfide and acetate. Acetate is known to be utilized in the presence of both CO_2 and H_2S [14, 17]. In case *Chr. weissei* showed a higher affinity for acetate than *Chr. vinosum*, one would expect coexistence of

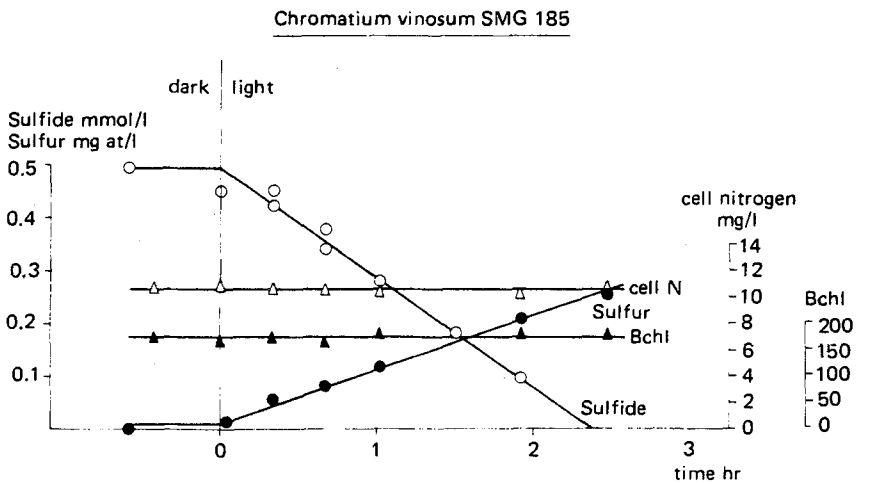

Fig. 2. Oxidation of sulfide observed in a pure culture (batch) of *Chr. vinosum*. Cells were grown until intracellular sulfur was depleted, thereafter sulfide was added in the dark. The data were used to calculate the value of q mentioned in Table 1.

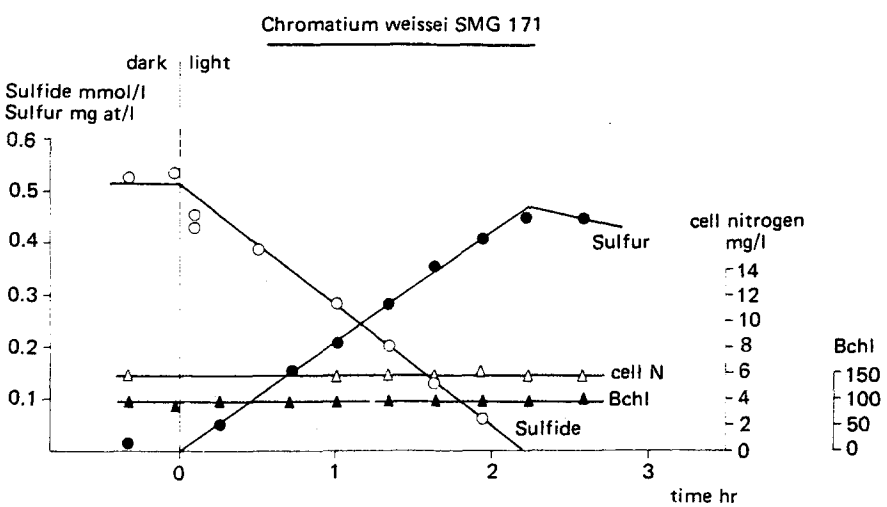

Fig. 3. Oxidation of sulfide observed in a pure culture (batch) of *Chr. weissei*. Procedures similar to those described in legend of Fig. 2.

the two organisms under such conditions. This idea had to be abandoned, since *Chr. weissei* was washed out completely. Organic substrates other than acetate will not necessarily show the same effect. In similar experiments inoculated with *Chr. vinosum* only, the addition of acetate raised the maximum growth rate attainable from 0.117 to 0.129 hr^{-1}.

In continuous cultures, illuminated continuously, the sulfide concentration at low dilution rates is beyond the limits of detection [4]. Conceivably, under such artificial conditions a high specific sulfide oxidation rate is not advantageous as competition is controlled by the affinity for sulfide.

Therefore the competition experiments in continuous cultures were repeated employing fluctuating sulfide concentration. This was performed both practically and naturally by the continuous addition of the sulfide-containing reservoir solution in combination with intermittant illumination.

At first, an illumination regimen of 6 hr light-6 hr dark was selected. Assuming the length of one cycle to be 1, such a regimen is indicated as $l = 0.5$. The data obtained were interesting (Fig. 4).

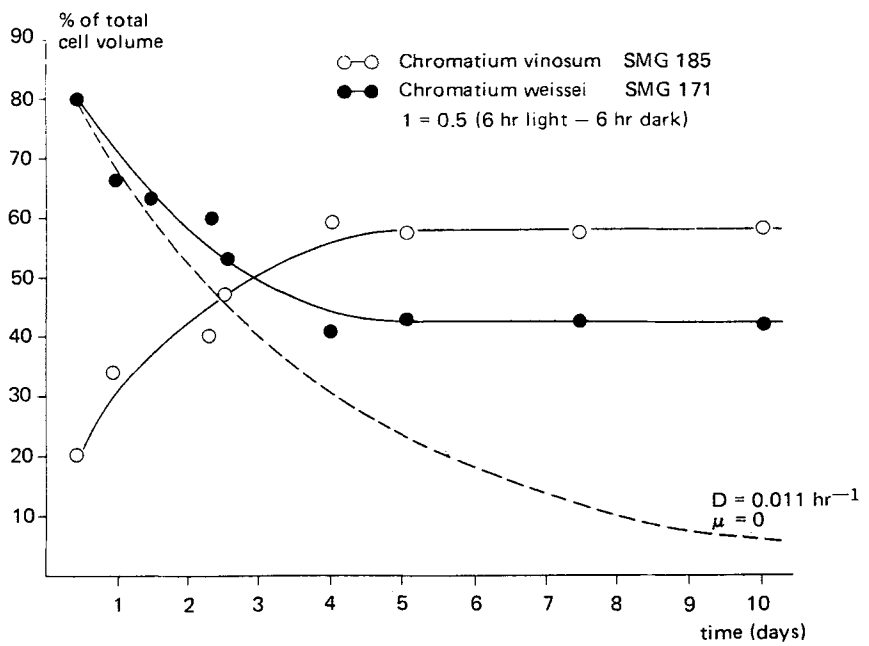

Fig. 4. Time course of the relative abundance of *Chr. vinosum* and *Chr. weissei* in a mixed continuous culture ($D = 0.011$ hr^{-1}) illuminated intermittantly ($l = 0.5$). Dotted line represents wash-out rate of *Chr. weissei* if μ were zero.

A high proportion of the inoculum biomass was *Chr. weissei*. Gradually the contribution of *Chr. vinosum* to the total biomass (biovolume) increased. However, unlike the phenomena observed in continuously illuminated mixed cultures, *Chr. weissei* was able to sustain. The final result was a coexistence of both strains lasting for several days.

Figure 5 is part of the recording sheet of a similar experiment showing optical density, pH, and sulfide concentration as measured during a 24-hr period of established coexistence. The increase in O.D. right after illumination is due to the formation of intracellular sulfur [2]. The gradual decrease in O.D. thereafter is caused by the oxidation of sulfur to sulfate. As a result of the low dilution rate (D = 0.011 hr^{-1}), the decrease in O.D. in the dark period is very small. By comparing the O.D. at, e.g., the end of the light periods, the culture may be regarded to represent a "steady state."

In a parallel experiment, once balanced coexistence was established, the illumination regimen was changed from 6 hr light-6 hr dark (l = 0.5) to continuous light (l = 1). Consistent with the previous data, an increasing population of *Chr. vinosum* was observed, eventually resulting in complete washout of *Chr. weissei*. Likely, *Chr. weissei* predominantly depends on the amount of sulfide accumulated in the dark, whereas *Chr. vinosum* is oxidizing a high proportion of the sulfide during periods of low concentration, as observed at daytime.

Therefore, one would expect some kind of relation with the light regimen. To verify this point, competition experiments were also run with an illumination regimen of 18 hr light-6 hr dark (l = 0.75) and a regimen of 4 hr light-8 hr dark (l = 0.33). The data are summarized in Table 2.

The "steady-state" data found with continuous illumination (l = 1.0) were discussed previously. Unexpectedly, *Chr. weissei* was unable to sustain at an 18 hr light-6 hr dark regimen (l = 0.75; see Discussion). A light regimen of 4 hr

Fig. 5. Part of recording sheet showing time course of pH, O.D., and sulfide (estimated manually) during established coexistence of *Chr. vinosum* and *Chr. weissei* in a comparable experiment as shown in Fig. 4 (l = 0.5).

light-8 hr dark ($l = 0.33$) indeed resulted in a steady state with a high population of *Chr. weissei*.

Discussion

It was found that *Chr. vinosum* grows faster than *Chr. weissei* irrespective of the sulfide concentration. It was also observed that *Chr. weissei* in the presence of higher sulfide concentrations managed to oxidize sulfide faster than *Chr. vinosum*. The data suggest that *Chr. weissei* is able to sustain in mixed cultures by its high specific sulfide oxidation rate (q), whereas *Chr. vinosum* does so by its high specific growth rate (μ). The two strains selected were thought to be representative. Data collected in the meanwhile do suggest that the phenomena observed may indeed be of more general importance.

Although intermittant illumination results in constant cell densities when judged over a longer period of time, it should be realized that we are dealing with transient states. The development of true steady states is prevented under such conditions. Therefore, it may not be correct to explain the processes during the light periods by data obtained during steady states. Experiments will be carried out to verify this point. In the meanwhile, it is assumed that growth in the light periods is controlled by the cell parameters shown in Table 1. The coexistence of the two strains may then be mathematically described as follows.

Table 2
Relative Abundance of Chr. vinosum *and* Chr. weissei *during "Steady-State" Coexistence in Relation to the Light Regimen*

Illumination regimen			Relative abundance in % biovolume	
	l	Organism	Inoculum	During "steady state"
Continuous light	1	*Chr. vinosum*	10	100
		Chr. weissei	90	0
18 hr light 6 hr dark	0.75	*Chr. vinosum*	30	100
		Chr. weissei	70	0
6 hr light 6 hr dark	0.5	*Chr. vinosum*	20	63
		Chr. weissei	80	37
4 hr light 8 hr dark	0.33	*Chr. vinosum*	60	30
		Chr. weissei	40	70

In continuous light, with both strains growing, sulfide added to the mixed culture will be oxidized by both organisms. The rate of oxidation for either one of the strains will be proportional to its cell density (x) and its specific growth rate (μ). As long as both organisms consume proportionally as much substrate, as their cell densities contribute to the total cell density, the ratio between the two organisms will not change. Dealing with the organisms A (*Chr. vinosum*) and B (*Chr. weissei*), having identical yields/mmol sulfide, the proportion of organism A would be

$$\frac{x_A}{x_A + x_B} = \frac{\mu_A x_A}{\mu_A x_A + \mu_B x_B} \tag{2}$$

When $\mu_A > \mu_B$, organism A will take over. In continuous culture, eventually organism B will be washed out completely ($\mu_B < D$).

In the dark, any sulfide introduced into the mixed culture will accumulate. In the following light period, the sulfide will be oxidized rapidly by both organisms. In this case, the overall rate of oxidation will be proportional to the specific sulfide oxidation rate (q) and the cell density (x). If sulfide were added to the culture in the dark only, an unchanged ratio between x_A and x_B may be expected when

$$\frac{x_A}{x_A + x_B} = \frac{q_A x_A}{q_A x_A + q_B x_B} \tag{3}$$

The oxidation of sulfide eventually results in growth. Therefore, still assuming a sulfide input in the dark only, a constant ratio x_A/x_B may be expected only if q_A and q_B were equal.

In natural habitats, the production of sulfide is not affected by light-dark changes. The same holds for the experiments in the present study, where sulfide was added continuously. Under such conditions the dark events may compensate the light events. Consequently, under such conditions a constant ratio between the cell densities of the organisms A and B may be expected when

$$\frac{x_A}{x_A + x_B} = \left(\frac{\mu_A x_A}{\mu_A x_A + \mu_B x_B}\right) l + \left(\frac{q_A x_A}{q_A x_A + q_B x_B}\right) d \tag{4}$$

when l and d represent the quantity of sulfide added during the light and the dark period, respectively. For practical reasons we assumed $l + d$ to be 1. With a constant rate of sulfide production or input, the quantities added during light and dark periods are proportional to the length of the light and dark period, respectively.

The ratio x_A/x_B is not affected by dilution. This does not imply that the dilution rate (D) is of no importance. In the light period, except for the very first half-hour (Fig. 5), only insignificant amounts of sulfide are washed out. This is not so during the dark period. However, in the present study we employed a reservoir concentration S_R of 2 mM sulfide and a dilution rate D of 0.011 hr^{-1}. The theoretical value of s at the end of a 6-hr dark period (t) according to $s = S_R\ (1 - e^{-Dt})$ is 0.130 mM sulfide. Ignoring any wash-out of sulfide [as done in Eq. (4)] the sulfide concentration after 6 hr dark should be 0.132 mM ($D \times S_R \times t$). The actual concentration was 0.13 mM.

It was found that *Chr. weissei* was unable to maintain itself with a regimen of 18 hr light-6 hr dark, despite its advantage of having a superior q. It is therefore of interest to estimate the critical length of the dark period and the light period. These limits can be calculated by realizing that coexistence means that the value of x_A/x_B is between 0 and ∞. In case $x_A/x_B = 0$, organism B will take over; with increasing values for x_A/x_B the proportion of x_A increases, being 100% when $x_A/x_B = \infty$. Solving Eq. (4) for x_A/x_B yields

$$\frac{x_A}{x_B} = -\frac{(\mu_A q_B - \mu_B q_A)\, l + (q_A - q_B)\, \mu_B}{(\mu_A q_B - \mu_B q_A)\, l + (q_A - q_B)\, \mu_A} \tag{5}$$

The limits of coexistence thus are

$$\frac{x_A}{x_B} = 0, \quad \text{if } l = \frac{-q_A + q_B}{\frac{\mu_A}{\mu_B}\, q_B - q_A} \tag{6}$$

and

$$\frac{x_A}{x_B} = \infty, \quad \text{if } l = \frac{(-q_A + q_B)\, \frac{\mu_A}{\mu_B}}{\frac{\mu_A}{\mu_B}\, q_B - q_A} \tag{7}$$

From the parameters mentioned, q_A and q_B have been determined experimentally. Knowing the values of $\hat{\mu}$, K_s, and K_i for both organisms, μ_A and μ_B could be calculated if the sulfide concentration in the light period were determinable. However, the sulfide concentration falls apparently to zero after the sulfide accumulated in the dark period has been oxidized. This advantageously eliminates the necessity of knowing μ_A and μ_B separately. At such low concentrations the relation between μ and s is almost linear for both organisms. Therefore μ_A/μ_B may be calculated at any very low value of s (e.g., 10^{-3} or 10^{-4} mM sulfide). According to Table 1, μ_A/μ_B will be 3.6 up to $s = 10^{-3}$ mM. Solving Eqs. (6) and (7) then yields possible coexistence of the two organisms at any value of l be-

tween 0.176 and 0.633, corresponding to a light period between 4.22 and 15.19 hr/24-hr period.

Figure 6 shows the theoretical magnitude of x_A and x_B in relation to the length of the light period calculated according to Eq. (4). The actual data sofar obtained (Table 2, "steady state" values also plotted in Fig. 6) are in reasonable agreement with the theoretical data. This holds as well for the unexpected observation that *Chr. weissei* (organism B) was unable to sustain at a regimen of 18 hr light-6 hr dark.

At first sight one should expect to end up with a pure culture of *Chr. weissei* by employing a light regimen of, e.g., 4 hr light-20 hr dark ($l < 0.176$). However, in a continuous flow system the misleading result will be a pure culture of *Chr. vinosum*, provided care is taken to lower the dilution rate to values inacceptable for practical reasons ($D < 0.006$ hr^{-1}).

One might say that organisms having high q values are more adapted to the occasional presence of high sulfide concentrations. For organisms with lower

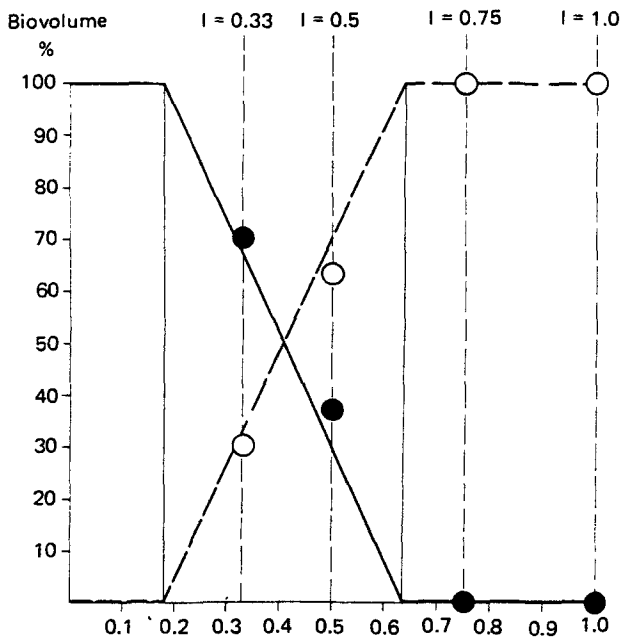

Fig. 6. Relative "steady-state" abundance of *Chr. vinosum* and *Chr. weissei* in relation to the light regimen (l). Lines represent the theoretical abundance, calculated according to Eqs. (4), (6), and (7), employing the cell parameters shown in Table 1. Broken line, *Chr. vinosum*; solid line, *Chr. weissei*; circles represent the actual data observed; Open circles, *Chr. vinosum*; Closed circles, *Chr. weissei*.

q values, but with a high affinity for sulfide (like *Chr. vinosum*), the continuous presence of very low sulfide concentrations would then be more profitable. Experiments are in progress to determine the rate-limiting step for both organisms.

One should keep in mind, however, that *Chr. weissei*, as all large *Chromatium* species [14, 18, 21], is unable to carry out assimilatory sulfate reduction. Growth of these organisms thus depends on the presence of sulfide in their habitats.

Mostly, it is extremely difficult to indicate precisely the critical factors controlling the events in natural habitats. Also, it is highly unlikely that the events are controlled by the same factors all the time.

In the present study some conditions (e.g., temperature and light intensity) employed led to growth stimulation of *Chr. vinosum* rather than *Chr. weissei* (Table 3). Nevertheless, *Chr. weissei* managed to cope with the situation provided the proper illumination regimen was selected. Therefore, in natural habitats the marked effect of the length of day and night may be reinforced by other factors such as light intensity. Experiments are in progress to investigate the effect of a more natural increase in light intensity than employed in this study.

Sulfide is oxidized to sulfur and eventually to sulfate. In the oxidation of sulfur to sulfate, three times as much reducing power is released as in the oxidation of sulfide to sulfur. Nevertheless, only the rate of oxidation of sulfide is of importance in competition among these organisms. This is due to the fact that

Table 3
Comparison of Culture Conditions

	Large species, e.g., *Chr. weissei*[a]	Conditions employed in present study	Small species, e.g., *Chr. vinosum*[a]
Temperature	15°-20°C	25°C	25°-30°C
pH	6.5-6.8	7.0	6.6-7.2
Light intensity	10-30 fc	100-200 fc	70-200 fc
Illumination regimen[b]	0.67	0.19-0.68	1.00

[a] Conditions recommended by Pfennig [12] for selective enrichment of organisms indicated.
[b] Figures indicate length of light period assuming sum of one light period and one dark period to be 1.

the storage polymer produced, as well as the sulfur formed, are stored intracellularly. Therefore, a high sulfide oxidation rate is a powerful weapon for those organisms unable to compete successfully by means of high specific growth rates. It is known, however, that the storage of sulfur cannot continue infinitely. One-third of the dry weight is found to be the maximal storage capacity for both small and large strains [2, 18, 22]. Thus, for cells already maximally loaded with sulfur a high q value is of no advantage. But then, under such conditions of excessive availability of electron donors, competition is marginal. It would be of interest to know the mechanism by which those apparently "careless" organisms, viz., *Chlorobium* or *Ectothiorhodospira*, not accumulating sulfur intracellularly [19], and even some times excreting vast amounts of intermediates [16], do manage to sustain themselves. Thus, organisms such as those recently isolated by Hansen [6] may prove important.

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