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Growth Yields of Green Sulfur Bacteria in Mixed Cultures with Sulfur and Sulfate Reducing Bacteria

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Abstract. 1. Dry weight yields from mixed cultures of *Prosthecochloris aestuarii* or *Chlorobium limicola* with the sulfur reducing *Desulfuromonas acetoxidans* were determined on different growth limiting amounts of acetate, ethanol or propanol. The obtained yields agreed well with values predicted from stoichiometric calculations. 2. From mixed cultures of two *Chlorobium limicola* strains with *Desulfovibrio desulfuricans* or *D. gigas* on ethanol as the growth limiting substrate, dry weight yields were obtained as calculated for the complete utilization of the ethanol by the mixed cultures. 3. Dry weight yield determinations for two pure cultures of *Chlorobium limicola* with different growth limiting amounts of sulfide in the absence and presence of excess acetate confirmed that acetate is incorporated by *Chlorobium* in a fixed proportion to sulfide; compared to the yield in the absence of acetate the yield is increased two to threefold in the presence of acetate. 4. The lowest possible sulfide concentrations necessary for optimal growth of mixed cultures of either *Prosthecochloris* or *Chlorobium* with *Desulfuromonas* on acetate were 7–8 mg H_2S per liter of medium. 5. Doubling times at the growth rate limiting light intensities of 5, 10, 20, 50, 100 and 200 lux were determined under optimal growth conditions for the following phototrophic bacteria: *Prosthecochloris aestuarii*, *Chlorobium phaeovibrioides*, *Chromatium vinosum* and *Rhodopseudomonas capsulata*. Reasonably good growth was still obtained with *Prosthecochloris* at 10 and 5 lux light intensity at which no growth of the purple bacteria could be observed.

Key words: Green sulfur bacteria – *Chlorobium limicola* – *Prosthecochloris aestuarii* – *Desulfuromonas acetoxidans* – *Desulfovibrio* species – Purple bacteria – Syntrophic mixed cultures – Growth yields – Light limitation – Acetate – Ethanol – Propanol.

Recently we described the anaerobic bacterium *Desulfuromonas acetoxidans* that reduces elemental sulfur to hydrogen sulfide by the oxidation of acetate to CO_2, acetate being the electron donor and carbon source (Pfennig and Biebl, 1976). It was shown that in the presence of acetate, *Desulfuromonas* strains form robustly growing syntrophic cultures when mixed with pure cultures of phototrophic green sulfur bacteria. For both types of bacteria, the mixed culture provides particular good growth conditions as there is mutual use of the metabolic product of one bacterium by the other. Although the tolerance for sulfide is remarkably high (30 mmol H_2S/l), pure cultures of *Desulfuromonas* growing on acetate and sulfur are inhibited by sulfide before significant cell densities are reached. Pure cultures of phototrophic green sulfur bacteria, on the other hand, can be grown to high cell densities. As they tolerate only low concentrations of sulfide, it has to be supplied repeatedly during growth. Moreover, many strains – particularly from marine habitats – are unable to oxidize most of the excreted elemental sulfur further to sulfate. As a result, only low cell yields are obtained in such cultures. The sulfur precipitates in the form of globules when its solubility in the culture medium is exceeded.

When a pure culture of *Desulfuromonas* and of a green sulfur bacterium are cultivated together with acetate in the light, the growth patterns are different. The green sulfur bacterium oxidizes sulfide to a dissolved, readily metabolizable form of sulfur which is immediately reduced to sulfide by the acetate oxidizing *Desulfuromonas*. In such a mixed culture neither globules of elemental sulfur appear, nor does sulfide accumulate. The culture grows at the expense of the organic substrate which is degraded by *Desulfuromonas* and thus made available for the green bacterium.

Up to now sulfate reducing bacteria were considered to be the main producers of the sulfide necessary

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for the phototrophic sulfur bacteria. Mixed cultures of *Desulfovibrio* and *Chlorobium* were studied by Butlin and Postgate (1954) in order to understand the sulfur deposits in a sulfate rich desert salt lake. Matheron and Baulaigue (1976) showed that mixed cultures of *Chlorobium*, *Desulfovibrio* and *Escherichia coli* can be grown in the light over many transfers as stable mixed populations with glucose as the sole substrate. The syntrophic relations between *Desulfovibrio* and *Chromatium* were studied in detail by van Gemerden (1967) with respect to the natural sulfur cycle.

The present paper reports on growth yields of mixed pure cultures of green sulfur bacteria and sulfur or sulfate reducing bacteria using organic substrates. The results are compared with yields of pure cultures of *Chlorobium* grown in the absence and presence of acetate. Finally, the unique capacity of green sulfur bacteria to grow at extremely low light intensities is demonstrated by comparing the growth rates of purple bacteria and of mixed cultures of green bacteria.

MATERIALS AND METHODS

Sources of Organisms. The following pure cultures of phototrophic and sulfur reducing bacteria were taken from our collection: *Chlorobium limicola* strain 9330 (freshwater) and 3331 (marine), *Prosthecochloris aestuarii* strain 5030 (marine), *Chlorobium phaeovibrioides* strain 2631 (marine), *Chromatium vinosum* strain D, *Rhodopseudomonas capsulata* strain Kb1, *Desulfuromonas acetoxidans* strain 5071 (marine) and strain 11070 (marine). The sulfate reducing bacteria *Desulfovibrio desulfuricans* Essex 6 (= DSM 692) and *D. gigas* DSM 496 were obtained from the German Collection of Microorganisms, DSM, in Göttingen.

Media and Growth Conditions. Composition and preparation of the basal medium for *Desulfuromonas* and green sulfur bacteria were described by Pfennig and Biebl (1976); the NH_4Cl-content was raised to 0.5 g per liter. For cultures of *Desulfovibrio* and *Chlorobium* strain 9330 the following medium was used (components in g/l): KH_2PO_4, 1.0; NH_4Cl, 0.5; $MgSO_4 \cdot 7\,H_2O$, 0.4; $CaCl_2 \cdot 2\,H_2O$, 0.1; $NaHCO_3$, 2.0; $Na_2S \cdot 9\,H_2O$, 0.3 or as indicated; trace element solution SL8, 1 ml per liter; pH was adjusted to 6.8. Trace element solution SL8 is a modification of SL4 (Pfennig and Lippert, 1966) and has the following composition (values in mmol per liter distilled water): EDTA-$Na_2 \cdot 2\,H_2O$, 14; $FeCl_2 \cdot 4\,H_2O$, 7.5; $ZnCl_2$, 0.5; $MnCl_2 \cdot 4\,H_2O$, 0.5; H_3BO_3, 1.0; $CoCl_2 \cdot 6\,H_2O$, 0.8; $CuCl_2 \cdot 2\,H_2O$, 0.1; $NiCl_2 \cdot 6\,H_2O$, 0.1; $Na_2MoO_4 \cdot 2\,H_2O$, 0.15; pH 6.5. Media for *Chlorobium* strains contained vitamin B_{12} (20 µg/l). Screw-capped bottles or tubes filled completely with medium were used as culture vessels. The organic substrates were added from sterile stock solutions prior to inoculation. Cultures were incubated at 28° C and, if not otherwise indicated, at a light intensity of about 500 lux.

The minimum amount of sulfide necessary to start a culture was determined in a medium reduced with 0.05% of thioglycollate. Growth experiments under low light intensity were done in 22 ml screw-cap tubes, the absorbancy of which was directly measured in a Bausch and Lomb Spectronic 88 photometer at 650 nm. A 25 watt tungsten light bulb served as the light source in a long, black walled cabinet. A light intensity of 5 lux was obtained in 2.5 m distance from the light source. Light intensities were measured with a Metrawatt Luxmeter, type Metrux K. Incubation temperature was 28° C. *Chromatium vinosum* was grown at pH 7.3 in the mineral medium used for *Chlorobium* strain 9330 supplemented with 0.2% D,L-malate and 0.1% ascorbate as a reductant. *Rhodopseudomonas capsulata* strain Kb1 was grown in a mineral medium for purple nonsulfur bacteria (Pfennig, 1969) at pH 6.8 in the presence of 0.1% yeast extract and 0.2% succinate.

Yields. Growth yields were determined as dry weight. The cell material of a 100 ml bottle was harvested by centrifugation and dried in beakers of aluminium foil. The yield of a control without organic carbon substrate was subtracted to account for growth with the sulfide initially present in the medium. Sulfide was determined according to Pachmayr using the version of Trüper and Schlegel (1964).

RESULTS AND DISCUSSION

Carbon Sources for Syntrophic Mixed Cultures with Desulfuromonas

Pure cultures of *Desulfuromonas acetoxidans* in addition to acetate, use ethanol and propanol as electron donors and carbon sources; butanol is an electron donor substrate, if a carbon source like succinate is present in the medium (Pfennig and Biebl, 1976). After a lag period of 10–12 days, cultures also develop with pyruvate in the presence of sulfur (unpublished result).

In mixed cultures with *Chlorobium* or *Prosthecochloris* acetate, ethanol or propanol were used by both *Desulfuromonas* strains tested (11070 and 5071). Small amounts of these substrates (less than 0.05%) were sufficient to produce deeply green cultures sometimes of viscous consistency. No growth was obtained with butanol, irrespective of succinate being added or not; pyruvate also failed to support syntrophic growth. If both butanol and pyruvate were combined, satisfactory development could be observed. However, the latter combination of substrates was not studied quantitatively in the present paper.

Growth Yields of Mixed Cultures with Desulfuromonas on Acetate, Ethanol and Propanol

Two different mixed cultures, *Prosthecochloris aestuarii* strain 5030 plus *Desulfuromonas acetoxidans* strain 5071 and *Chlorobium limicola* strain 3331 plus *Desulfuromonas acetoxidans* strain 11070, were used to measure dry weight yields on acetate, ethanol and propanol. The results in Table 1 show that the yields per mol of substrate were almost constant over the range of concentrations studied. The average yields on acetate were 47 g dry weight/mol for the *Prosthecochloris* culture and 41 g dry weight/mol for the *Chlorobium* culture. The corresponding values for ethanol were 63 g and 58 g dry weight/mol. Propanol was entirely consumed only by the mixed culture containing *Desulfuromonas* strain 5071 with a yield of 92 g

Table 1. Dry weight yields from mixed cultures of two different green sulfur bacteria with different *Desulfuromonas* strains grown on acetate, ethanol and propanol

Substrate	mmol	*Chlorobium limicola* strain 3331 plus *Desulfuromonas* strain 11070		*Prosthecochloris aestuarii* strain 5030 plus *Desulfuromonas* strain 5071	
		mg Cell material	g Cell material per mol	mg Cell material	g Cell material per mol
Acetate	0.1	4.3	43	5.1	51
	0.2	8.4	42	9.1	46
	0.3	11.8	39	14.0	47
	0.4	15.4	38.5	17.7	44
Ethanol	0.1	5.3	53	6.4	64
	0.2	11.0	55	12.9	64
	0.3	17.8	59	18.4	61
	0.4	25.5	64	–	–
Propanol	0.1	4.8	–	9.0	90
	0.2	5.8	–	19.4	97
	0.3	6.6	–	26.4	88
	0.4	4.3	–	36.3	91

dry weight/mol; in the culture with *Desulfuromonas* strain 11070 growth ceased for unknown reasons at an early stage when about 6 mg dry weight per 100 ml of medium were formed.

The experimental results can be evaluated on the basis of the following theoretical considerations. In a phototrophically growing mixed culture the organic carbon substrates can entirely be converted to cell material. When the formula for cell composition of van Dijken and Harder (1975) is used, the amount of cell material formed by *Prosthecochloris* or *Chlorobium* is given by the following equation:

$$17\ H_2S + 8\ CO_2 \xrightarrow{\text{light}} 2\ (C_4H_7O_3) + 17\ S + 10\ H_2O.$$

For each mol of sulfide oxidized to elemental sulfur, 12.12 g dry weight can be formed. When acetate is the substrate and the formation of cell material is not considered, *Desulfuromonas* can theoretically form 4 mol of H_2S for each mol of acetate oxidized:

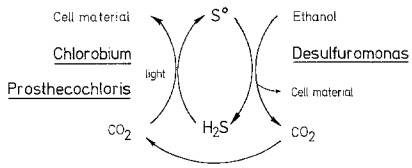
$$CH_3COOH + 4\ S + 2\ H_2O \rightarrow 2\ CO_2 + 4\ H_2S.$$

In mixed cultures, therefore, a growth yield of $4 \times 12.12 = 48.5$ g dry weight per mol of acetate should be obtained. Practically, about 3.65 instead of 4 mol of H_2S are formed, as about 8.7% acetate are consumed in the formation of *Desulfuromonas* cell material (Pfennig and Biebl, 1976). By calculating with 4 mol of sulfide, the amount of cell material assessed too much for *Chlorobium*, corresponds for stoichiometric reasons exactly to the amount of cell material which is in fact formed by *Desulfuromonas* from acetate directly. No error is, therefore, introduced when the yields of the mixed cultures are calculated as described. According to the equations for ethanol and propanol

$$CH_3CH_2OH + 6\ S + 3\ H_2O \rightarrow 2\ CO_2 + 6\ H_2S$$

$$CH_3CH_2CH_2OH + 9\ S + 5\ H_2O \rightarrow 3\ CO_2 + 9\ H_2S$$

1 mol of ethanol yields 6 mol of H_2S and, therefore, can give rise to 72.7 g cell material, and 1 mol of propanol yields 9 mol of H_2S allowing 109.1 g cell material to be formed by the mixed culture. Comparison of the calculated values with the experimentally obtained dry weight yields (Table 1) reveals a reasonably good agreement.



Scheme 1

Different from ethanol and propanol, acetate can be incorporated by green sulfur bacteria in the light in a certain proportion to H_2S and CO_2 (Sadler and Stanier, 1960). It is, therefore, unknown how much of the acetate is directly used by *Chlorobium* or *Prosthecochloris* and how much is metabolized by *Desulfuromonas*. The following stoichiometric calculations show that it is actually irrelevant for the final growth yield of mixed cultures how much of the acetate is consumed by the green sulfur bacteria, since the remaining part is made available by *Desulfuromonas* in the form of reducing equivalents in H_2S.

1. Maximum hypothetical yield on acetate assuming direct assimilation:

$$17\ CH_3COOH \xrightarrow{light} 8\ (C_4H_7O_3) + 2\ CO_2 + 6\ H_2O$$
(*Chlorobium* or *Desulfuromonas*)

2. Yield on acetate via the sulfur cycle:

$$17\ CH_3COOH + 68\ S + 34\ H_2O \rightarrow 34\ CO_2 + 68\ H_2S$$
(*Desulfuromonas*)

$$68\ H_2S + 32\ CO_2 \xrightarrow{light} 8\ (C_4H_7O_3) + 68\ S + 40\ H_2O$$
(*Chlorobium*)

Sum: $17\ CH_3COOH \xrightarrow{light} 8\ (C_4H_7O_3) + 2\ CO_2 + 6\ H_2O$ (Mixed culture).

The quantitative results of our mixed culture experiments fully confirm these considerations.

Growth Yields of Mixed Cultures with Desulfovibrio Species on Ethanol

As *Desulfovibrio* strains are unable to oxidize acetate, ethanol was used as the organic electron donor substrate for the mixed cultures of sulfate reducing bacteria with green sulfur bacteria. Using the freshwater strain 9330 of *Chlorobium limicola* and either *Desulfovibrio desulfuricans* or *D. gigas*, maximum dry weight yields of 72 and 68 g cell material per mol of ethanol were determined (Table 2). This result is surprising, as *Desulfovibrio* species form only 1 mol sulfide from 2 mol of ethanol and excrete acetate as an endproduct:

$$2\ CH_3CH_2OH + SO_4^{2-} \longrightarrow 2\ CH_3COO^- + H_2S + 2\ H_2O.$$

As the obtained yields are close to the theoretical values for complete assimilation of ethanol (see preceeding section), nearly all acetate formed must have been consumed by the *Chlorobium* strain 9330. Sadler and Stanier (1960) determined a two times higher cell yield with acetate than without. In a *Chlorobium*–*Desulfovibrio* mixed culture the dry weight yield would be 24 g per mol of ethanol if acetate would not be used; actually, the dry weight yield was nearly three times higher (Table 2). This result is understood when it is taken into account that in mixed cultures with sulfate reducing bacteria, yields approaching the theoretical value are obtained only when the green sulfur bacteria completely oxidize sulfide–via sulfur–to sulfate, and not only to elemental sulfur as they do in mixed cultures with *Desulfuromonas*. In case of complete oxidation, *Chlorobium* obtains per mol of H_2S not only two but rather 8 electrons which can all be used for the incorporation of acetate.

Table 2. Dry weight yields from mixed cultures of *Chlorobium limicola* strain 9330 with two *Desulfovibrio* strains grown on ethanol

mmol Ethanol	*Chlorobium limicola* strain 9330 together with			
	D. desulfuricans strain Essex 6		*D. gigas* strain DSM 496	
	mg Cell material	g Cell material per mol	mg Cell material	g Cell material per mol
0.05	3.6	72	3.4	68
0.1	6.7	67	6.4	64
0.15	10.0	67	9.6	64
0.2	12.7	64	12.7	64

The stoichiometry of the processes in mixed cultures with sulfate-reducing bacteria can be described by the following equations:

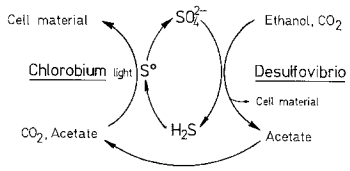
1. *Desulfovibrio*:

$$34\ CH_3CH_2OH + 17\ SO_4^{2-} \longrightarrow 34\ CH_3COO^- + 17\ H_2S + 34\ H_2O.$$

2. *Chlorobium*:

$$17\ H_2S + 34\ CH_3COO^- + 28\ CO_2 + 16\ H_2O \xrightarrow{light} 24\ (C_4H_7O_3) + 17\ SO_4^{2-}.$$

Thus, per mol of ethanol consumed 72.7 g cell material can be obtained which is the same yield as in the case of the *Desulfuromonas* mixed culture on ethanol.



Scheme 2

Growth Yields of Chlorobium Pure Cultures with and without Acetate

The mixed culture studies with green sulfur bacteria and sulfur or sulfate-reducing bacteria on acetate and ethanol as organic substrates called for quantitative determinations of the extent to which acetate increased the yields of pure cultures of *Chlorobium* in the presence of growth limiting amounts of sulfide. The results presented in Table 3 show that for comparable amounts of sulfide *Chlorobium limicola* strain 9330 formed about three times more cell material in the presence of acetate than without. The salt-requiring *Chlorobium limicola* strain 3331 also used in *Desulfuromonas* mixed cultures was somewhat less efficient on acetate. Although our strains incorporated

Table 3. Dry weight yields from pure cultures of two different *Chlorobium limicola* strains grown in the absence and presence of acetate (excess) with growth-limiting amounts of sulfide

Strain	Acetate added mmol	Sulfide added mmol	Cell material		Increase of yield by acetate
			mg	g per mol of sulfide	
9330	–	0.053	1.9	36	–
	–	0.126	4.9	39	–
	–	0.199	6.5	33	–
	1.67	0.056	5.4	96	2.7×
	1.67	0.126	12.4	99	2.5×
	1.67	0.195	21.2	109	3.3×
3331	–	0.057	2.5	43	–
	–	0.123	5.4	44	–
	–	0.195	8.3	43	–
	1.67	0.056	4.9	87	2.0×
	1.67	0.126	13.6	108	2.4×
	1.67	0.202	21.7	107	2.5×

acetate slightly better than Sadler and Stanier's strain, the experiments confirm their results, that incorporation of acetate is strictly dependent on the initial sulfide concentration. Pure cultures of *Chlorobium* with acetate and a sulfide concentration as low as used in the mixed culture experiments would cease to grow at rather low cell densities.

The *Chlorobium* pure culture experiments suggest that in acetate limited mixed cultures up to two thirds of the acetate may be directly assimilated by *Chlorobium* so that only the remaining part is oxidized by *Desulfuromonas*.

From the growth yields obtained in the absence of acetate, under photolithoautotrophic conditions, it can be seen (Table 3) that both *Chlorobium* strains must have oxidized most of the elemental sulfur which they released into the medium as an intermediate oxidation product of sulfide. According to the equation

$$17\,H_2S + 32\,CO_2 + 28\,H_2O \xrightarrow{\text{light}} 8\,(C_4H_7O_3) + 17\,H_2SO_4$$

per mol of sulfide 48.5 g dry cell material could at most be expected when all elemental sulfur was oxidized to sulfate. The best experimental values obtained with strain 3331, 44 g and 43 g dry weight per mol of H_2S, are reasonably close to the theoretical maximum.

Minimum Sulfide Concentrations for Mixed Cultures with Desulfuromonas

Most green sulfur bacteria photooxidize sulfide to elemental sulfur which is released to the medium and oxidized further to sulfate when sulfide is limiting. In an initial sulfur-free, acetate containing mixed culture of *Chlorobium limicola* and *Desulfuromonas acetoxidans*, the latter bacterium depends in its activity to oxide acetate and to form sulfide on the elemental sulfur which is formed by *Chlorobium* in the light. In order to initiate growth of the mixed culture an initial amount of sulfide has to be added. The question, how large the minimum amount of sulfide must be to allow growth to high cell density, is of great interest, as it represents the quantity of sulfur which is oxidized and reduced and thus serves as the electron transfer catalyst on which growth is dependent. Every molecule of sulfur which is oxidized to sulfate by *Chlorobium* is lost for the interspecies electron transfer as *Desulfuromonas* cannot reduce sulfate.

In thioglycolate reduced media, full development of *Chlorobium* strain 3331 plus *Desulfuromonas* strain 11070 and *Prosthecochloris* strain 5030 plus *Desulfuromonas* strain 5071 was obtained, when 7–8 mg H_2S were added per liter of medium; below 4 mg H_2S per liter no growth occurred.

In the preceeding section we showed that at most two thirds of the dry weight yield of pure cultures of green sulfur bacteria may be formed by the incorporation of acetate. The minimum amount of sulfide required for growth in the presence of acetate can, therefore, be calculated by the following equations:

$$17\,CH_3COOH \xrightarrow{\text{light}} 8\,(C_4H_7O_3) + 2\,CO_2 + 6\,H_2O$$

$$34\,H_2S + 16\,CO_2 \xrightarrow{\text{light}} 4\,(C_4H_7O_3) + 34\,S + 20\,H_2O$$

Sum: $$34\,H_2S + 14\,CO_2 + 17\,CH_3COOH \xrightarrow{\text{light}} 12\,(C_4H_7O_3) + 34\,S + 26\,H_2O.$$

Accordingly, in the presence of acetate a pure culture of a green sulfur bacterium may at most form 36.35 g cell material per mol of H_2S; this corresponds to 8.55 mg cell material with 8 mg H_2S. With the same 8 mg H_2S initially present, a mixed culture with *Desulfuromonas* forms about 485 mg cell material per liter in the presence of 10 mmol of acetate. This means that the initially added 8 mg H_2S must have been oxidized to sulfur by *Chlorobium* and reduced back to H_2S by *Desulfuromonas* at least 57 times.

As the green sulfur bacterium is potentially capable of oxidizing the elemental sulfur further to sulfate – in which case the sulfur would be lost for the interspecies sulfur cycle – it has to be assumed that the *Desulfuromonas* cells have a very high affinity to the excreted elemental sulfur for which they compete successfully with the green sulfur bacteria.

Our experiments also show that there can hardly be any storage of elemental sulfur inside the *Chlorobium* cells, since storage would render the sulfur in-

accessible for *Desulfuromonas* and certainly result in the excretion of sulfate, as it is true for the sulfur storing purple sulfur bacteria. Our attempts to establish syntrophic mixed cultures of *Desulfuromonas* with *Chromatium vinosum* and *Thiocapsa roseopersicina* failed. This confirms that in cultures of these bacteria no elemental sulfur is being released into the medium.

Different from the purple sulfur bacteria, the green sulfur bacteria lack an intracytoplasmic membrane system; all components of their photosynthetic electron transport system are located in the cytoplasmic membrane (Olson et al., 1976). This structural characteristic is in agreement with the physiological property that the first oxidation product of sulfide, elemental sulfur, is directly released into the culture medium.

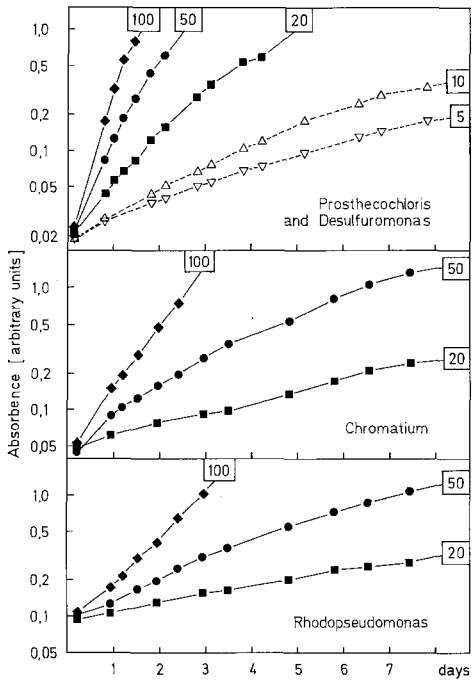


Fig. 1. Photoorganotrophic growth of different cultures of a green sulfur bacterium (upper part: *Prosthecochloris aestuarii* strain 5030 plus *Desulfuromonas acetoxidans* strain 5071) and two purple bacteria (central part: *Chromatium vinosum* strain D; lower part: *Rhodopseudomonas capsulata* strain Kb1). At the end of each growth curve the corresponding light intensity is indicated in lux. Each set of cultures received optimal carbon sources, the *Prosthecochloris* mixed culture acetate, *Chromatium* malate and as a reductant ascorbate, and *Rhodopseudomonas* succinate plus yeast extract. Optical densities were followed with a Spectronic 88 photometer at 650 nm

Doubling Times of Green Sulfur Bacteria and Purple Bacteria at Limiting Light Intensities

In the presence of sufficient amounts of acetate or ethanol, mixed cultures of green sulfur bacteria with *Desulfuromonas* strains reach unusually high cell densities with a high degree of self-shading. It was to be expected, therefore, that green sulfur bacteria are capable to grow at lower light intensities than other phototrophic bacteria. The use of mixed cultures instead of pure cultures of green bacteria is of advantage when growth is followed by turbidity measurements as no highly refractile globules of elemental sulfur appear and no addition of sulfide is required during growth.

In Figure 1 growth of the mixed culture *Prosthecochloris* strain 5030 plus *Desulfuromonas* strain 5071 on acetate is compared with photoorganotrophic growth of the two representative purple bacteria *Chromatium vinosum* strain D and *Rhodopseudomonas capsulata* strain Kb1 at light intensities between 5 and 100 lux. Both cultures of purple bacteria reached the lower limit of growth at 20 lux; a slow increase in turbidity was still observed and corresponded to doubling times of 85 and 90 h (Table 4). In contrast to this, the *Prosthecochloris* mixed culture showed a doubling time of 16 h at 20 lux; even at 5 lux it did grow reasonably well with a doubling time of 48 h.

A mixed culture of the bacteriochlorophyll e containing, brown coloured *Chlorobium phaeovibrioides* strain 2631 with *Desulfuromonas* strain 11070 exhibited doubling times between those of *Prosthecochloris* and the purple bacteria (Table 4). Apparently, the brown coloured *Chlorobium* species with their extended ab-

Table 4. Doubling times of different phototrophic bacteria at different growth rate limiting light intensities

Light intensity	*Prosthecochloris* strain 5030 (mixed culture on acetate)	*Chlorobium phaeovibrioides* strain 2631 (mixed culture on acetate)	*Chromatium vinosum* strain D	*Rhodopseudomonas capsulata* strain Kb1
Lux	h	h	h	h
5	48	118	no growth	no growth
10	36	81	no growth	no growth
20	16	43	85	90
50	9	22	30	40
100	6	12	14	19
200	n.d.[a]	n.d.[a]	11	9

[a] n.d. = not determined

sorption range due to additional carotenoids, have no selective advantage in a nonselective radiation environment.

These measurements confirm earlier observations that green sulfur bacteria are capable to grow at lower light intensities than any other phototrophic organisms.

CONCLUSIONS

In comparison to the purple sulfur bacteria, the green sulfur bacteria are physiologically much more restricted. All species are dependent on reduced sulfur compounds and incorporate organic substrates, often solely acetate, only in a fixed proportion to the inorganic electron donor sulfide or thiosulfate. Also, green sulfur bacteria are unable to store the first oxidation product of H_2S, elemental sulfur, inside the cells for periods of H_2S depletion; the sulfur is released into the surrounding medium and may be lost as a potential electron donor. In contrast to most purple bacteria, all green sulfur bacteria are nonmotile and cannot photo- and chemotactically adjust to optimal growth conditions. In spite of these disadvantages in comparison to the purple sulfur bacteria, the green sulfur bacteria are common in most anaerobic, light exposed aquatic environments.

The experimental results obtained in the present study contribute to our understanding of the reasons for the successful competition of the green sulfur bacteria with purple sulfur bacteria in anaerobic sulfide containing habitats.

The most efficient utilization of low light intensities by the green sulfur bacteria has to be considered first. As we have shown, these bacteria are able to grow reasonably well at light intensities which are too small to allow multiplication of any other phototrophic organisms. This special capacity has its structural basis in the unique *Chlorobium* vesicles which are attached to the inner side of the cytoplasmic membrane (Cohen-Bazire et al., 1964; Cruden and Stanier, 1970). The vesicles are highly enriched with photochemically inactive bacteriochlorophyll *c*, *d* or *e*; they serve a light harvesting function with an energy transfer that is directed back to the cytoplasmic membrane. The latter carries the photosynthetic electron transport system with reaction center bacteriochlorophyll *a*, cytochromes and carotenoids (Olson et al., 1976). The strong light absorption by the cells of green sulfur bacteria is apparent in the light microscope: individual cells with a diameter of only 0.4 to 1.0 µm appear bright yellowish green, while cells of comparable width from purple bacteria appear colourless. Their efficient light harvesting system allows the green sulfur bacteria to occupy ecological niches in natural environments which do not provide sufficient light for the growth of purple sulfur bacteria. Also, the light harvesting bacteriochlorophylls *c*, *d* and *e* absorb radiation in the range between 700 and 760 nm which is not absorbed by any other phototrophic organisms (Pfennig, 1967; Gloe et al., 1975).

In good agreement to this, the green sulfur bacteria are regularly found below the layers of all other phototrophic organisms. This is not only true for pools and stratified lakes (Gorlenko and Kusnezow, 1972; Oláh et al., 1973; Caldwell and Tiedje, 1975; Cohen, 1975) but also for the layers of phototrophic organisms in marine muddy or sandy sediments (Fenchel, 1969; Jørgensen and Fenchel, 1974).

The anaerobic ecological niches with the lowest light intensities are often also those with the highest concentrations of sulfide and breakdown products of the anaerobic decomposition of organic substances by chemoorganotrophic bacteria. Under these conditions, the green sulfur bacteria may take advantage of their pronounced capacity for syntrophism with sulfur and sulfate reducing bacteria. Due to the metabolic activity of these chemoorganotrophs, the green sulfur bacteria obtain most directly the necessary sulfide for growth from the anaerobic oxidation of simple organic compounds which they cannot utilize directly as electron donors and carbon sources. On the other hand, by their release of elemental sulfur and sulfate, the green sulfur bacteria regenerate the necessary electron acceptors for the more or less complete anaerobic oxidation of organic compounds which otherwise could not be metabolized by these chemoorganotrophs.

Thus, by way of syntrophy the green sulfur bacteria attain the ecophysiological level of the purple sulfur bacteria with respect to both the use of simple organic substances as well as the economic utilization of excreted elemental sulfur.

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