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Continuous chemotrophic growth and respiration of Chromatiaceae species at low oxygen concentrations

Jörg Overmann* and Norbert Pfennig

Fakultät für Biologie, Universität Konstanz, Postfach 5560, W-7750 Konstanz, Federal Republic of Germany

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Abstract. Endogenous and maximum respiration rates of nine purple sulfur bacterial strains were determined. Endogenous rates were below 10 nmol O_2 · (mg protein · min)$^{-1}$ for sulfur-free cells and 15–35 nmol O_2 · (mg protein · min)$^{-1}$ for cells containing intracellular sulfur globules. With sulfide as electron-donating substrate respiration rates were considerably higher than with thiosulfate. Maximum respiration rates of *Thiocystis violacea* 2711 and *Thiorhodovibrio winogradskyi* SSP1 (254.8 and 264.2 nmol O_2 · (mg protein · min)$^{-1}$, respectively) are similar to those of aerobic bacteria. Biphasic respiration curves were obtained for sulfur-free cells of *Thiocystis violacea* 2711 and *Chromatium vinosum* 2811. In *Thiocystis violacea* the rapid and incomplete oxidation of thiosulfate was five times faster than the oxidation of stored sulfur. A high affinity of the respiratory system for oxygen (K_m = 0.3 0.9 µM O_2, V_{max} = 260 nmol O_2 · (mg protein · min)$^{-1}$ with sulfide as substrate, K_m = 0.6 2.4 µM O_2, V_{max} = 14 40 nmol O_2 · (mg protein · min)$^{-1}$ with thiosulfate as substrate), for sulfide (K_m = 0.47 µM, V_{max} = 650 nmol H_2S · (mg protein × min)$^{-1}$, and for thiosulfate (K_m = 5–6 µM, V_{max} = 24–72 nmol $S_2O_3^{2-}$ · (mg protein · min)$^{-1}$ was obtained for different strains. Respiration of *Thiocystis violacea* was inhibited by very low concentrations of NaCN (K_i = 1.7 µM) while CO concentrations of up to 300 µM were not inhibitory. The capacity for chemotrophic growth of six species was studied in continuous culture at oxygen concentrations of 11 to 67 µM. *Thiocystis violacea* 2711, *Amoebobacter roseus* 6611, *Thiocapsa roseopersicina* 6311 and *Thiorhodovibrio winogradskyi* SSP1 were able to grow chemotrophically with thiosulfate/acetate or sulfide/acetate. *Chromatium vinosum* 2811 and *Amoebobacter purpureus* ML1 failed to grow under these conditions. During shift from phototrophic to chemotrophic conditions intracellular sulfur and carbohydrate accumulated transiently inside the cells. During chemotrophic growth bacteriochlorophyll *a* was below the detection limit.

Key words: Chromatiaceae – Continuous culture – Respiration – Chemotrophic growth – Oxygen

The impact of molecular oxygen on the growth of purple sulfur bacteria (Chromatiaceae) has been investigated for a considerable period of time. Batch culture experiments of Pfennig (1971), Bogorov (1974), Gorlenko (1974), Kondratieva et al. (1976) and Kämpf and Pfennig (1980, 1986) revealed the capacity of *Thiocapsa roseopersicina*, *Amoebobacter roseus*, *Thiocystis violacea*, *Chromatium vinosum*, *C. minus*, *C. violascens* and *C. gracile* to grow in the dark by oxidizing reduced inorganic sulfur compounds with oxygen. In contrast, green sulfur bacteria did not grow chemotrophically (Kämpf and Pfennig 1980).

The growth experiments cited were performed either in batch or in fed-batch culture (Kämpf and Pfennig 1986). Under these conditions chemotrophically growing cells still contained photosynthetic pigments (Gorlenko 1974, Kondratieva et al. 1976, Kämpf and Pfennig 1986). Furthermore, growth of some strains under dark conditions continued for a limited period of time but ceased after repeated transfer into fresh culture medium (Kämpf and Pfennig 1986). Therefore long-term continuous culture experiments are necessary to prove unequivocally the capacity for chemotrophic growth of Chromatiaceae with molecular oxygen.

Up to now, infinite chemotrophic growth in continuous culture and the absence of photosynthetic pigments was demonstrated exclusively for *Thiocapsa roseopersicina* strain M1 by de Wit and van Gemerden (1987, 1990a, b). The aim of the present study was to assess the capacity of six other Chromatiaceae species for continuous chemotrophic growth and to determine their respiratory activity under various conditions.

* *Present address and address for correspondence:* J. Overmann, Department of Microbiology, University of British Columbia, # 300-6174 University Boulevard, Vancouver, B.C., Canada V6T 1Z3

Materials and methods

Source of strains

Amoebobacter purpureus strain ML1 and *Thiorhodovibrio winogradskyi* strain SSP1 (Overmann et al. 1991, 1992) were isolated from the chemocline and littoral sediment, respectively, of meromictic Mahoney Lake (British Columbia, Canada). All other strains were obtained from our culture collection at the University of Konstanz. For comparison, *Thiocapsa roseopersicina* strain M1 was kindly provided by Dr. H. van Gemerden, University of Groningen, The Netherlands.

Respiration rates

For initial measurements of respiration rates strains were grown in batch culture in completely filled 250 ml screw cap bottles at 150 μmol $m^{-2} s^{-1}$ of tungsten light (phototrophic conditions, growth medium see below) or under chemotrophic conditions in the dark on a rotary shaker in one liter Erlenmeyer flasks containing 200 ml phosphate buffered growth medium (Kämpf and Pfennig 1980) under an atmosphere of 3% O_2 and 1% CO_2 in N_2.

Respiration rates were determined in washed (50 mM potassium phosphate buffer, pH 7.4) cell suspensions employing a Clark type oxygen electrode (Model 53, Yellow Springs Instruments, Ohio, USA) in a thermostatted 10-ml cuvette. The protein concentration of cell suspensions ranged between 100 and 300 μg · ml $^{-1}$. For calibration, aliquots of oxygen saturated buffer were injected into the assay and respiration rates calculated from the decrease of oxygen concentration with time. Substrates (sulfide and thiosulfate) were dissolved in degassed distilled water and stored under a nitrogen atmosphere.

Continuous chemotrophic growth

The growth medium contained per liter of distilled water: $KH_2PO_4 \times H_2O$, 0.25 g; NH_4Cl, 0.34 g; KCl, 0.34 g; $MgSO_4 \times 7\ H_2O$, 0.5 g; $CaCl_2 \cdot 2\ H_2O$, 0.25 g; $NaHCO_3$, 1.50 g; $Na_2S_2O_3 \times 5\ H_2O$, 0.99 g; Na-acetate, 0.33 g; vitamin B_{12}, 0.02 mg; trace element solution SL 12 (Overmann et al. 1992), 1 ml. To media for strains M1, 5813, ML1, SSP1 requiring elevated salt concentrations, NaCl (20.0 g · l^{-1}) and $MgCl_2 \cdot 6\ H_2O$ (2.8 g · l^{-1}) were added.

Experiments were performed in a modified laboratory scale fermentor Biostat V (B. Braun, Melsungen, FRG). Incubation temperature was controlled with a Braun Thermomant thermostat. Dissolved oxygen was measured with an autoclavable polarographic oxygen electrode (Pt-Ag/AgCl electrode IL, Ingold, Urdorf, Switzerland). The combined pH electrode (No. 60216100, Metrohm AG, Herisau, Switzerland) was inserted in the autoclaved fermentor vessel after 1 min of sterilization in boiling 0.5 M HCl. To ensure anoxic conditions during anaerobic phototrophic growth, all connections except the pump hose (Tygon) were made of Isoversinic tube.

Defined gas mixtures of N_2, CO_2 and − under conditions enabling chemotrophic growth − pressurized air were adjusted with needle valves (Hoke, Cresskil, New Jersey, USA) and introduced directly into the culture. A pH of 7.6 ± 0.1 (with thiosulfate as substrate) and 7.2 ± 0.1 (with sulfide) was maintained by adjusting the CO_2 flow manually with a needle valve at the beginning of each experiment. Regulation of the oxygen partial pressure in the chemostat was accomplished by a proportional integral regulator (B. Braun) and a three way magnetic valve (Huba Control, Würenlos, Switzerland) which added alternatively pressurized air or N_2 to the main gas stream. Thus, the gas flow through the culture was maintained at a constant rate. The range of oxygen concentrations applied was 11–67 μM.

During phototrophic growth the culture was illuminated continuously from opposite sides with 3000 μmol $m^{-2} s^{-1}$ of reflector tungsten lamps.

The oxygen tolerance of various Chromatiaceae strains increased in growth medium supplemented with acetate as organic carbon source (Kämpf and Pfennig 1980). In preliminary experiments with acetate-free thiosulfate medium and in the absence of additional reducing compounds *Chromatium vinosum* 2811 failed to grow even under anoxic conditions. Therefore the medium was routinely supplemented with sodium acetate. In contrast to purple nonsulfur bacteria, Chromatiaceae species proved unable to use acetate as electron-donating substrate for respiration (Göttert, personal communication).

For growth of *Thiorhodovibrio winogradskyi* strain SSP1 which does not oxidize thiosulfate, sterile Na_2S-solution was pumped into the culture from an additional reservoir (Beeftink and van Gemerden 1979) to yield a final concentration of 1 mM. In these experiments the second reservoir contained all other nutrients in elevated concentrations according to the mixing ratio of both reservoir solutions.

Analytical procedures

Optical density of cell suspensions was measured at 650 nm in a Bausch and Lomb Spectronic 70 photometer (Rochester, NJ, USA). Protein was measured after Hartree (1972) and carbohydrate after Herbert et al. (1971). Dry cell mass was determined after filtration of cell suspensions on predried glass fiber filters GF 92 (Schleicher & Schuell, Dassel, FRG) and drying for 24 h at 105 °C.

Bacteriochlorophyll *a* was extracted overnight at 4 °C with acetone (99.5%); a specific extinction coefficient of 92.3 ml × (mg · cm)$^{-1}$ (Steenbergen and Korthals 1982) was employed. The detection limit was 4 μg · l^{-1}.

Sulfide was measured colorimetrically by the methylene blue method (Cline 1969), thiosulfate and tetrathionate after Kelly et al. (1969) and zero valence sulfur ("S^0") after Taylor et al. (1989). For determination of sulfate, an ion chromatographic system (Sykam S 3110, Garching, FRG, equipped with a conductivity detector S 1000 and a Shimadzu, Kyoto, Japan, C-R3A integrator) was employed. Acetate was measured by gas chromatography (Schink and Pfennig 1982).

Results and discussion

Specific rates and stoichiometry of respiration

Maximum respiration rates at a non-limiting concentration of oxygen (20 μM) and thiosulfate (50 μM) or sulfide (20 μM) (compare Fig. 2 and Table 3) did not differ significantly for phototrophically and chemotrophically grown cells. Even after identical incubation conditions, the mean standard deviation of maximum respiration rates between parallels was 50% (n = 5–8). With sulfide, respiration rates were considerably higher than with thiosulfate (Table 1). Very low maximum respiration rates were found for *Amoebobacter purpureus* ML1 while *Thiorhodovibrio winogradskyi* SSP1 showed the highest rates. Endogenous respiration rates (i.e. in the absence of sulfide or thiosulfate) of cells free of intracellular sulfur globules generally were below 10 nmol O_2 · (mg protein · min)$^{-1}$. Cells containing sulfur globules exhibited endogenous rates of 15–35 nmol O_2 · (mg protein · min)$^{-1}$ (Table 1).

As cells grown under anoxic conditions in the light respired reduced sulfur compounds at high specific rates the respiratory enzyme system must be constitutive in Chromatiaceae. In several *Thiocapsa* strains oxidation of thiosulfate and sulfite is mediated by the same enzyme

Table 1. Specific respiration rates (RR) of various Chromatiaceae species[a]

Strain	Substrate	maxmimum RR nmol O_2 · (mg protein · min)$^{-1}$	Stoichiometry µM Substrate : µM O_2	endogenous RR nmol O_2 · (mg protein · min)$^{-1}$	
				$-S^0$	$+S^0$
Chromatium vinosum D	$S_2O_3^{2-}$	10.0	2:1	4.2	24.2
	H_2S	177.3	3.3:1	8.4	n.d.
Chromatium vinosum 2811	$S_2O_3^{2-}$	16.7	1.7:1	2.0	n.d.
	H_2S	52.9	n.d.	11.9	n.d.
Thiocystis violacea 2711	$S_2O_3^{2-}$	33.5	1.8:1	2.2	28.9
	H_2S	254.8	n.d.	13.0	n.d.
Thiocapsa roseopersicina 6311	$S_2O_3^{2-}$	69.9	1.9:1	5.9	29.3
Thiocapsa roseopersicina M1	$S_2O_3^{2-}$	80.9	1.8:1	5.0	15.4
Amoebobacter roseus 6611	$S_2O_3^{2-}$	65.5	1.8:1	4.8	21.7
Amoebobacter pendens 5813	$S_2O_3^{2-}$	111.8	1.8:1	7.6	34.9
Amoebobacter purpureus ML1	H_2S	24.5	n.d.	9.8	5.7
Thiorhodovibrio winogradskyi SSP1	H_2S	264.2	2.5:1	5.9	n.d.

[a] Maximum respiration rates corrected for endogenous rates (without exogenous substrate). Endogenous rates for cells without ($-S^0$) and with ($+S^0$) microscopically visible sulfur globules. As no significant differences were detected between respiration rates of phototrophically or chemotrophically grown cells, the values were combined. Mean values of 5–8 determinations. n.d. = not determined

system during phototrophic as well as during chemotrophic growth (Trüper 1984; Dahl and Trüper 1989). In phototrophic organisms the cytochrome bc_1-complex is often involved in photosynthetic as well as respiratory electron transport (Scherer 1990). The high specific respiration rates of phototrophically grown cells have to be attributed to the presence of these components of the respiratory chain and a high activity of a terminal oxidase.

With NaCN an inhibitor constant of $K_i = 1.7$ µM was determined for respiration of chemotrophically grown cells of *Thiocystis violacea* 2711. In contrast, no inhibition of respiration could be detected with CO at a concentration of 300 µM.

Purple nonsulfur bacteria contain two different types of terminal oxidases. Electron transport occurs from ubiquinone via the cytochrome bc_1-complex to cytochrome c_2 oxidase (cyt b_{380}) or directly from ubiquinone to the alternative oxidase (cyt b_{250}) (Venturoli et al. 1987; Richaud et al. 1986; Zannoni and Fuller 1988). While cytochrome c_2 oxidase is inhibited by very low cyanide concentrations (inhibitor constant $K_i = 1$–5 µM), the alternative oxidase requires much higher concentrations ($K_i = 500$ µM KCN). Only the alternative oxidase is inhibited by CO ($K_i = 770$ µM, o-type cytochrome). According to the low inhibition constant of cyanide ($K_i = 1$–7 µM) and the insensitivity against CO in our experiments, *Thiocystis violacea* 2711 cells possess cytochrome c_2 oxidase as the terminal electron acceptor.

The two steps of thiosulfate oxidation with molecular oxygen can be described by:

$$2\,S_2O_3^{2-} + O_2 \rightarrow 2\,S^0 + 2\,SO_4^{2-} \quad (1)$$

$$2\,S^0 + 2\,H_2O + 3\,O_2 \rightarrow 2\,SO_4^{2-} + 4\,H^+ \quad (2)$$

$$2\,S_2O_3^{2-} + 4\,O_2 + 2\,H_2O \rightarrow 4\,SO_4^{2-} + 4\,H^+ \quad (3)$$

where reaction (1) includes the activity of thiosulfate reductase, rhodanese and sulfite oxidase. Incomplete oxidation of thiosulfate in reaction (1) requires 2 mol of thiosulfate per 1 mol O_2, while during complete oxidation (reaction 3) the ratio $S_2O_3^{2-} : O_2$ is 1 : 2. The same holds true for oxidation of H_2S. The respective ratio found in the experiments was 1.7 : 1 to 2.0 : 1 with thiosulfate and 2.5 : 1 to 3.3 : 1 with sulfide as electron-donating substrate (Table 1). This indicates incomplete oxidation of reduced sulfur substrates during the short-time incubations.

In some experiments phototrophically grown cells of *Thiocystis violacea* 2711 free of intracellular sulfur globules exhibited a biphasic respiration curve (Fig. 1). Following the addition of thiosulfate, the specific respiration rate was 44.8 nmol O_2 · (mg protein · min)$^{-1}$ and decreased abruptly to 16.9 nmol O_2 · (mg protein × min)$^{-1}$. The endogenous rate was very low in this case (2.4 nmol O_2 · (mg protein · min)$^{-1}$). Similarly, a biphasic curve of oxygen consumption was found for *Chromatium vinosum* 2811. The molar ratio between thiosulfate added and oxygen consumed was 1.8 : 1 at the end of the first phase of high respiration rate and

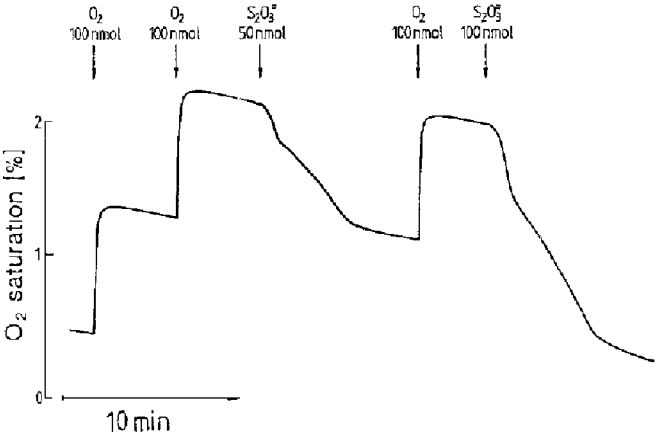

Fig. 1. Biphasic curve of oxygen depletion during respiration of *Thiocystis violacea* 2711 cells. T = 30 °C. Cells free of intracellular sulfur droplets, grown under phototrophic conditions. *Arrows* indicate addition of oxygen and thiosulfate

0.6 : 1 at the end of the second phase. These ratios are in good agreement with the expected values for incomplete and complete oxidation.

If reaction (2) would not start before complete exhaustion of thiosulfate (reaction 1), the stoichiometry values at the end of reaction (1) should vary statistically around a mean value of $S_2O_3^{2-} : O_2 = 2 : 1$. However, the values measured (1.7 : 1 to 2.0 : 1) were systematically lower indicating oxidation of intracellular sulfur concomitant to thiosulfate oxidation. Similarly, a slow oxidation of intracellular sulfur in parallel with sulfide oxidation was demonstrated during phototrophic growth of *Chromatium okenii* (Trüper 1964).

Conversion of oxygen consumption rates during biphasic respiration of *Thiocystis violacea* yielded a rate of 55.8 nmol $S_2O_3^{2-}$ · (mg protein · min)$^{-1}$, Eq. (1) for the first phase and a rate of 11.3 nmol S^0 · (mg protein × min)$^{-1}$ for the second phase. In this calculation the concomitant oxidation of the intracellular sulfur during reaction (1) was considered. The rate of incomplete oxidation of thiosulfate thus was 4.9 times higher than the oxidation rate of intracellular sulfur. For *Chromatium vinosum* D a very similar ratio of 4.6 was reported for biphasic sulfate production with oxygen by Smith and Lascelles (1966).

Depending on the endogenous respiration rate the stoichiometry of thiosulfate oxidation in *Thiocystis violacea* 2711 varied considerably (Table 2). Biphasic respiration curves were not obtained at high endogenous rates. The low respiration rate during the second phase of biphasic respiration (Fig. 1) is similar to the "endogenous" rates measured with *Thiocystis violacea* in other experiments and with various Chromatiaceae species (Table 1). Obviously the stoichiometries in Table 1 resulted from the fact that the very low respiration rates with intracellular sulfur are often indistinguishable from endogenous rates. Accordingly, cells apparently free of microscopically visible sulfur globules exhibited endogenous rates higher than 2.4 nmol O_2 · (mg protein · min)$^{-1}$ suggesting the presence of small amounts of stored sulfur inside the cells.

Determination of the K_m values of respiration for thiosulfate, sulfide and oxygen revealed a high affinity for O_2 (K_m = 0.3 0.9 µM O_2, V_{max} = 260 nmol O_2 · (mg protein · min)$^{-1}$ with sulfide as substrate, K_m = 0.6 2.4 µM O_2, V_{max} = 14–40 nmol O_2 · (mg protein · min)$^{-1}$ with thiosulfate as substrate) (Fig. 2, Table 3) for all Chromatiaceae species studied. The affinity for sulfide of *Thiorhodovibrio winogradskyi* SSP1 was very high (K_m = 0.47 µM, V_{max} = 650 nmol H_2S · (mg protein · min)$^{-1}$ · K_m values of *Chromatium vinosum* 2811 and *Thiocystis violacea* 2711 for thiosulfate were between 5 and 6 µM (V_{max} = 24–72 nmol $S_2O_3^{2-}$ · (mg protein × min)$^{-1}$. Following the method of Eisenthal and Cornish-Bowden (1974), *C. vinosum* 2811 was grown phototrophically in a series of chemostat experiments and a value of K_S = 7.4 µM thiosulfate was calculated which is comparable to the K_m of respiration.

Table 2. Stoichiometry of thiosulfate oxidized to oxygen reduced at different endogenous respiration rates of *Thiocystis violacea* 2711[a]

Endogenous respiration rate nmol O_2 · (mg protein · min)$^{-1}$	µM $S_2O_3^{2-}$: µM O_2
2.4 (p)	0.6 : 1
3.8 (c)	0.7 : 1
4.0 (p)	1.2 : 1
8.5 (c)	2.4 : 1
47.7 (p)	2.0 : 1

[a] At endogenous rates of ≥ 4.0 nmol O_2 · (mg protein · min)$^{-1}$ no biphasic respiration curve was observed. p = phototrophically, c = chemotrophically grown cells

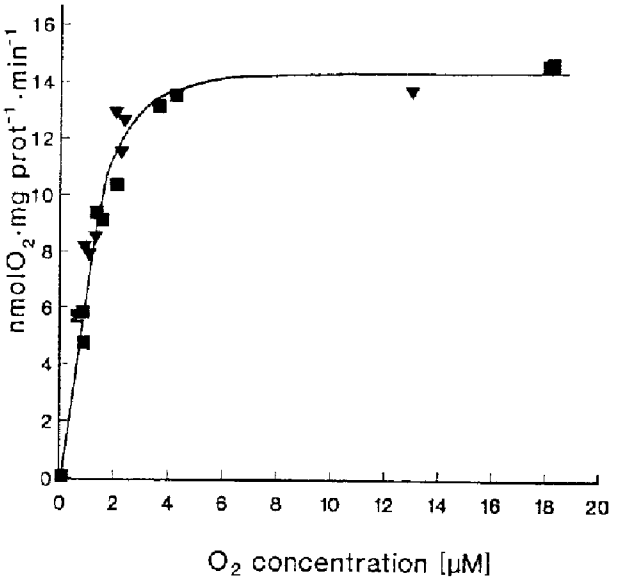

Fig. 2. Dependence of the specific respiration rate of *Chromatium vinosum* 2811 on oxygen concentration. Rates were determined first at stepwise increased *(rectangles)*, than at stepwise decreased *(triangles)* concentrations of oxygen

Table 3. K_m values of O_2 and electron-donating substrates for three different Chromatiaceae species

Strain	Growth conditions	$K_m(O_2)$ [µM]	$K_m(S_2O_3/H_2S)$ [µM]
C. vinosum 2811	phototrophic	1.1 ± 0.4	4.8 ± 2.5 (S_2O_3)
	chemotrophic[a]	1.2 ± 0.4	n.d.
T. violacea 2711	phototrophic	2.4 ± 0.4	6.2 ± 1.6 (S_2O_3)
	chemotrophic	0.6 ± 0.1	n.d.
T. winogradskyi SSP1	phototrophic	0.9 ± 0.1	0.5 ± 0.1 (H_2S)
	chemotrophic	0.3 ± 0.1	n.d.

[a] Cells harvested 4 days after shift to chemotrophic conditions. *C. vinosum* did not grow continuously under these conditions (see text). n.d. = not determined

Continuous chemotrophic growth

With the exception of *Chromatium vinosum* D and *Thiocapsa roseopersicina* M1 which were investigated earlier (Kämpf and Pfennig 1986; de Wit and van Gemerden 1987, 1990a, b) and *Amoebobacter pendens* 5813, all species listed in Table 1 were examined for their capacity to grow chemotrophically with oxygen.

Pressurized air was added in small pulses to a phototrophically grown culture of *Thiocystis violacea* 2711 after reaching the steady state. The oxygen concentration in the culture medium was 11.1 μM (± 0.6 μM) and the dilution rate 0.67 day^{-1}. Within ten days the *Thiocystis* cell suspension in the chemostat lost its purple-violet color completely. Concomitantly, bacteriochlorophyll *a* declined below the detection limit following the washout

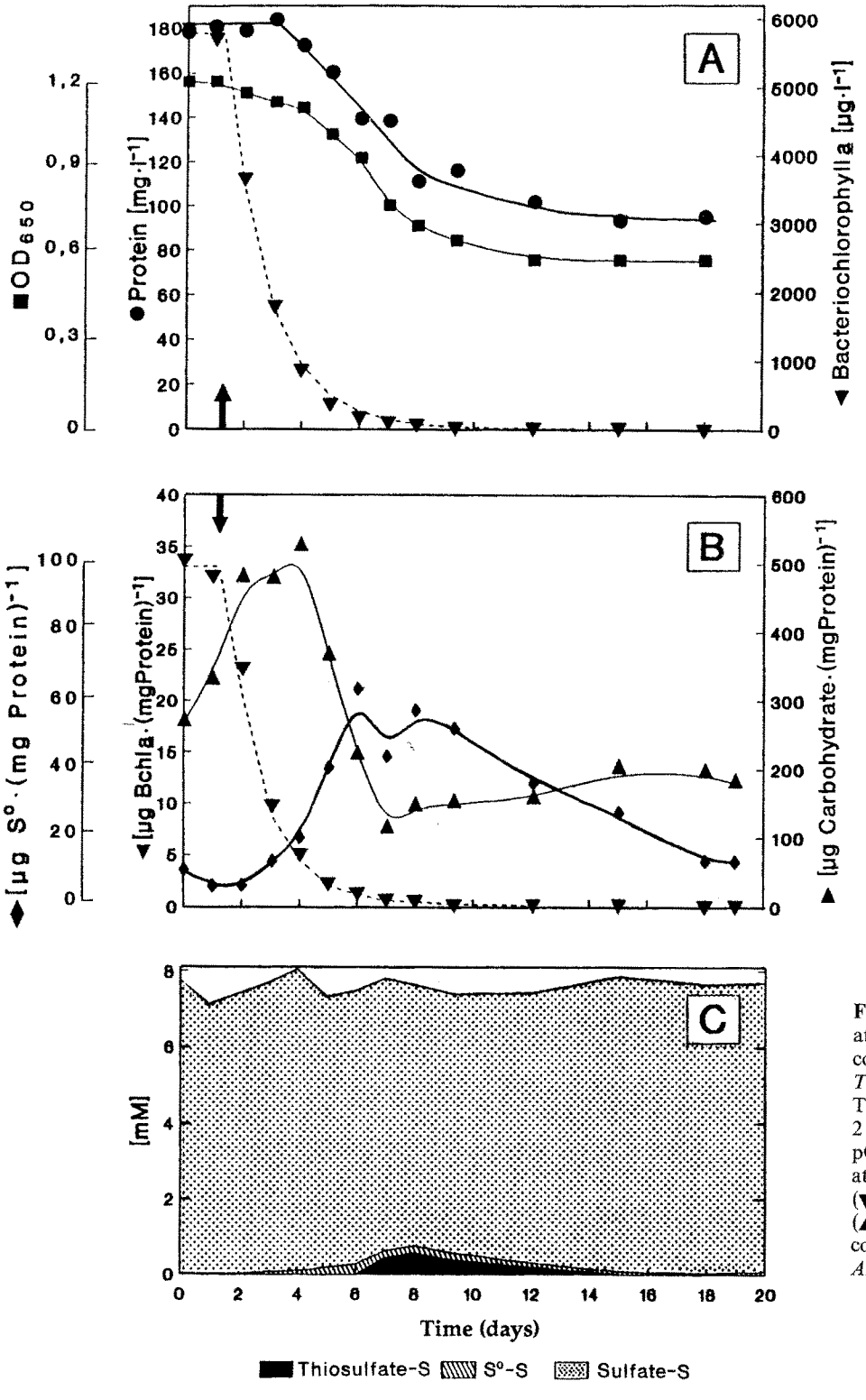

Fig. 3. Time course of biomass parameters at phototrophic and chemotrophic conditions in a continuous culture of *Thiocystis violacea* 2711. D = 0.67 day^{-1}, T = 25 °C, pH = 7.6 ± 0.1, illumination 2 × 3000 μmol m^{-2} s^{-1} (tungsten light), pO_2 = 1% (11 μM). (■) optical density at 650 nm, (●) protein concentration, (▼) bacteriochlorophyll concentration, (▲) intracellular carbohydrate concentration, (◆) intracellular sulfur. *Arrow* indicates start of oxygen supply

curve (Fig. 3) while the protein concentration decreased from a mean value of 181 mg · l^{-1} during phototrophic growth to 93 mg · l^{-1}. The decrease of bacteriochlorophyll *a* indicates repression of its synthesis by oxygen but no active breakdown. As revealed by electron microscopy, the decrease of bacteriochlorophyll a was paralleled by a decrease of the amount of pigment-bearing intracytoplasmic membrane vesicles.

Cells grew chemotrophically under light conditions as well as in the dark.

During phototrophic growth and assuming a mean biomass composition of $\langle C_4H_8O_2N\rangle$ (Harder and van Dijken 1976), a biomass of 384 mg (dry cell mass) · l^{-1} can be expected from 4 mM $S_2O_3^{2-}$ and 4 mM acetate according to the following equation

$$17\,S_2O_3^{2-} + 17\,CH_3COO^- + 23\,H_2O + 30\,CO_2 + 16\,NH_4^+ \rightarrow 16\langle C_4H_8O_2N\rangle + 34\,SO_4^{2-} + 33\,H^+ . \quad (4)$$

A value of 359 mg (dry cell mass) · l^{-1}, thus corresponding to 93% of the theoretical value was obtained experimentally.

After the shift to conditions enabling chemotrophic growth, a dry cell mass concentration of 186 mg · l^{-1} was found. If 4 mM acetate is converted completely into bacterial biomass according to

$$16\,CH_3COO^- + 8\,NH_4^+ + S_2O_3^{2-} + 6\,H^+ \rightarrow 8\langle C_4H_8O_2N\rangle + 11\,H_2O + 2\,SO_4^{2-} , \quad (5)$$

a dry cell mass concentration of 204 mg · l^{-1} can be expected theoretically. The actual yield was 91% of this value. In the present experiment the cell yield was considerably lower than in chemotrophically growing batch cultures of *Thiocystis violacea* at similar substrate concentrations (Kämpf and Pfennig 1980). Of the total 4 mM thiosulfate added, only 0.25 mM or 6.3% must have been oxidized during acetate assimilation. Thus it appears that most of the thiosulfate was respired for energy conservation by the *Thiocystis* cells in this experiment. From the dilution rate D (0.67 day^{-1}), the protein concentration $c_{protein}$ (93 mg · l^{-1}) and the thiosulfate concentration in the reservior c_{thio} (4 mM, minus 0.25 mM for reduction of acetate = 3.75 mM), a respiration rate (RR) of

$$RR = D \cdot c_{thio} \cdot 2/c_{protein} = 37.5\ \text{nmol}\ \ O_2 \cdot (\text{mg protein} \cdot \text{min})^{-1}$$

was calculated which corresponds well with the maximum respiration rate measured in batch cultures (mean value: 33.5 nmol O_2 · (mg protein · min)$^{-1}$, Table 1). Obviously, *Thiocystis* cells growing continuously under chemotrophic conditions respired at maximum rate.

During the shift to chemotrophic growth the intracellular sulfur content increased transiently (Fig. 3B) although thiosulfate was the growth-limiting substrate. Before and after this phase, the sulfur content of the culture was low (15–40 µM) and thiosulfate was oxidized almost completely. The mean recovery of sulfur atoms derived from thiosulfate in this experiment was 95% (Fig. 3C). Similarly to *Thiocystis violacea*, intracellular sulfur concentrations in other species tested increased during the transition to chemotrophic growth. The same phenomenon was observed for *Thiocapsa roseopersicina* M1 (de Wit and van Gemerden 1987).

In chemotrophically grown cells of *Thiocapsa roseopersicina*, the activity of enzymes involved in thiosulfate oxidation (thiosulfate reductase, rhodanese, adenosin phosphosulfate reductase, ADP sulfurylase, ATP sulfurylase and sulfite oxidoreductase) is increased as compared to phototrophically grown cells (Dahl and Trüper 1989). As in all experiments intracellular sulfur accumulated transiently during the shift to chemotrophic growth, de novo synthesis and/or activation of the sulfur-oxidizing enzyme must be considerably slower compared to the other enzymes. Several findings in the literature support this conclusion. Redox calculations of de Wit and van Gemerden (1987) demonstrated that the oxidation rate of intracellular sulfur decreases during the shift of *Thiocapsa roseopersicina* M1 cells to chemotrophic growth. Hurlbert (1967) observed a decreased conversion of intracellular sulfur to sulfate by *Chromatium vinosum* in the presence of oxygen while the oxidation of the thiosulfate sulfane-sulfur to intracellular sulfur remained at a constant rate. The oxidation of sulfur appears to be the rate-limiting step during oxidation of polysulfides (Visscher et al. 1990). During chemolithoautotrophic growth, cells of *Thiocapsa roseopersicina* BBS strongly accumulated sulfur droplets which in this case even inhibited further growth (Kondratieva et al. 1975).

Amoebobacter roseus 6611 and *Thiocapsa roseopersicina* 6311 were able to grow continuously under chemotrophic conditions (Figs. 4, 5). In order to shorten the time required per experiment the dilution rate was increased. *Thiocapsa roseopersicina* reached a biomass yield (163 mg protein · l^{-1}) comparable to *Thiocystis violacea* (181 mg protein · l^{-1}). For *Amoebobacter roseus* the yield was considerably lower (Fig. 4). This may be attributed to the higher dilution rate of D = 0.9 day^{-1} which is closer to the maximum growth rate of chemotrophically growing cultures of *Thiocapsa roseopersicina* M1 (de Wit and van Gemerden 1987). At high dilution rates, substrates are not utilized completely and the growth yield is decreased (Herbert et al. 1956). Possibly the same holds true for the slightly decreased yield of *Thiocapsa roseopersicina* 6311 mentioned above (D = 0.7 day^{-1} in this experiment).

Cultivation of *Thiorhodovibrio winogradskyi* SSP1 proved to be very difficult under conditions enabling chemotrophic growth. As this strain is unable to utilize thiosulfate, sulfide was added. For growth of strain SSP1 the sulfide concentration had to be decreased to 1 mM and the oxygen partial pressure to 1% (11 µM). Nevertheless, the yield measured for phototrophically grown cells amounted to only 23% of the theoretical growth yield (24.3 versus 103.3 mg protein · l^{-1}) indicating inhibition of cell growth of this strain. The dilution rate chosen in this experiment (D = 0.6 day^{-1}) was considerably lower than the maximum growth rate measured with sulfide and acetate as substrates in batch culture (2.0 day^{-1}, Overmann et al. 1992). The protein concentration did not decrease concomitantly to the bacteriochlorophyll *a* concentration at the end of the exper-

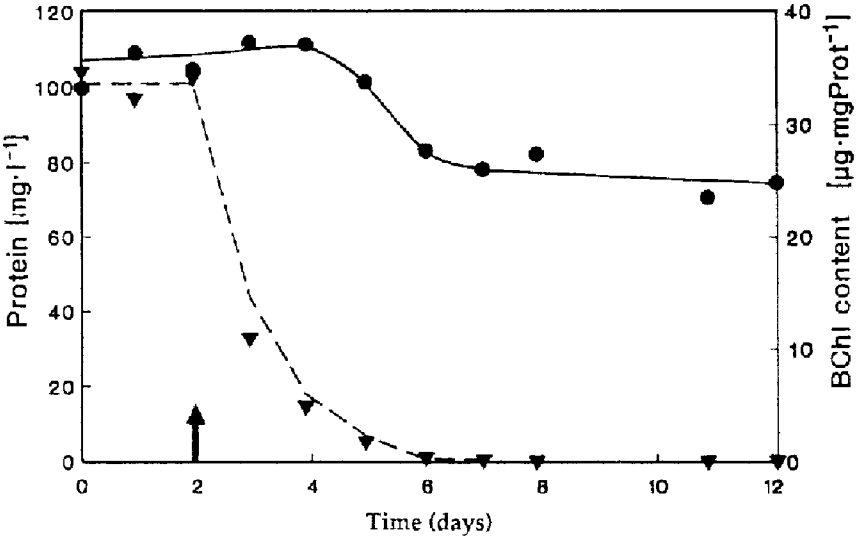

Fig. 4. Protein concentration (●) and specific bacteriochlorophyll content (▼) during phototrophic and chemotrophic growth of a continuous culture of *Amoebobacter roseus* 6611. D = 0.9 day^{-1}, T = 30 °C, pO_2 = 6%. *Arrow* indicates start of oxygen supply

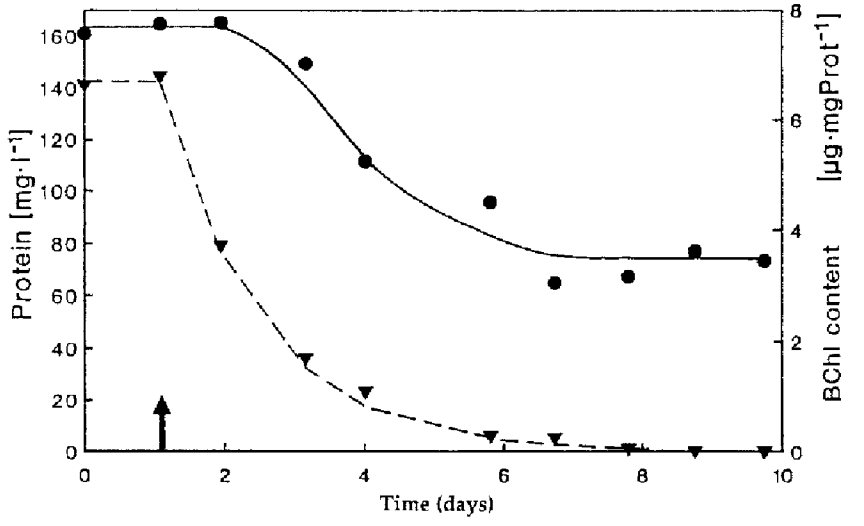

Fig. 5. Continuous culture of *Thiocapsa roseopersicina* 6311 under phototrophic and chemotrophic conditions. D = 0.7 day^{-1}, T = 30 °C, pO_2 = 1%. *Symbols* as in Fig. 4

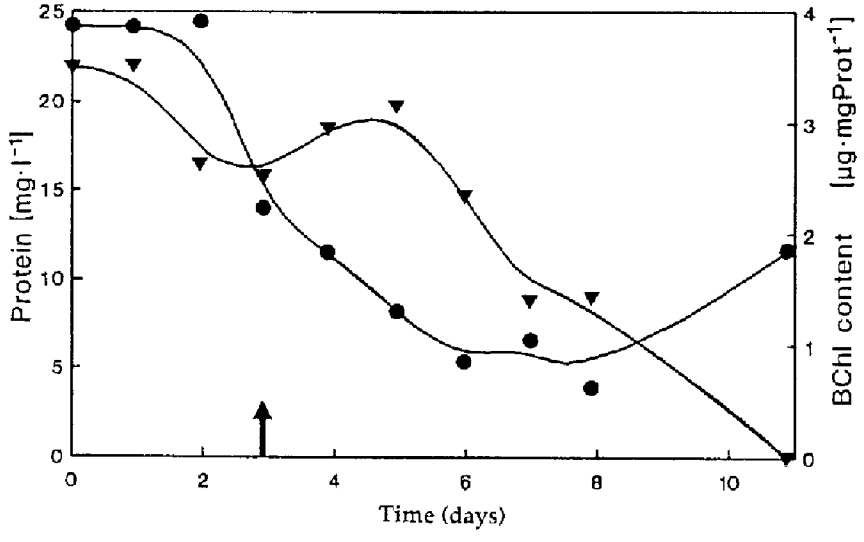

Fig. 6. Continuous culture of *Thiorhodovibrio winogradskyi* SSP1 under phototrophic and chemotrophic conditions. D = 0.6 day^{-1}, T = 30 °C, pO_2 = 1%. *Symbols* as in Fig. 4

iment (Fig. 6). *Thiorhodovibrio winogradskyi* SSP1 thus appears to be capable of chemotrophic growth.

The capacity for chemotrophic growth of *Chromatium vinosum* 2811 was studied in the same way at different oxygen concentrations and light intensities. After the switch to microoxic conditions (pO_2 = 1%) the intracellular bacteriochlorophyll content decreased and the carbohydrate content increased, but the sulfur concentration in the culture remained low. During the four days following the shift to microoxic conditions, the growth yield remained high at 91% of the theoretical value. Eventually cells formed clumps and adhered to the wall of the culture vessel. After six days, formation of large amounts of foam indicated considerably lysis of *Chromatium* cells. Similar results were obtained at low incubation light intensity (500 instead of 3000 μmol m^{-2} s^{-1}) and at different oxygen concentrations (11 and 56 μM). Obviously *Chromatium vinosum* 2811 is unable to grow chemotrophically under these conditions.

Amoebobacter purpureus ML1 was washed out of the chemostat after shift from anoxic to microoxic conditions (D = 0.5 day^{-1}). This result corresponds well to the poor growth of this strain which was obtained in uniformly inoculated deep agar tubes incubated under oxic conditions in the dark (method of Kämpf and Pfennig 1980).

Conclusions

The present study shows that the capacity for continuous chemotrophic growth is not confined to the strain *Thiocapsa roseopersicina* M1 but is more widespread among the Chromatiaceae. A correlation exists between this capacity and high respiration rates.

The maximum respiration rates observed with the strains *Thiocystis violacea* 2711 and *Thiorhodovibrio winogradskyi* SSP1 are similar to those of aerobic bacteria (Cypionka and Meyer 1982). Chromatiaceae contain a terminal oxidase with a very high oxygen affinity (K_m = 0.34–2.4 μM). So far, only the very similar K_m value for *Thiocapsa roseopersicina* M1 0.64–1.42 μM; de Wit and van Gemerden 1987) has been reported. The oxygen affinity of Chromatiaceae is fully comparable to that of aerobic bacteria (Cypionka et al. 1985). Chromatiaceae thus are able to take advantage of the very low oxygen concentrations which are present in boundary layers like the chemocline of meromictic lakes or at the top of sediment layers. Interestingly, most species which were able to grow continuously under microoxic conditions are often found in the redoxcline of laminated limnic and marine sediment systems (van Gemerden et al. 1989; Pfennig 1989).

The diurnal variations of light intensity and sulfide concentration in natural habitats are considerably faster than the minimum time of five days required for the transition to chemotrophic growth in the present experiments. A loss of photosynthetic pigments and complete change of chemotrophic growth may rarely occur under natural conditions. More likely, the physiological flexibility as now demonstrated for several Chromatiaceae species provides a selective advantage during short period of simultaneous presence of reduced sulfur compounds and oxygen in the absence of light.

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