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Lithotrophic growth of *Sulfurospirillum deleyianum* with sulfide as electron donor coupled to respiratory reduction of nitrate to ammonia

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Abstract *Sulfurospirillum deleyianum* grew in batch culture under anoxic conditions with sulfide (up to 5 mM) as electron donor, nitrate as electron acceptor, and acetate as carbon source. Nitrate was reduced to ammonia via nitrite, a quantitatively liberated intermediate. Four moles of sulfide were oxidized to elemental sulfur per mole nitrate converted to ammonia. The molar growth yield per mole sulfide consumed, Y_m, was 1.5 ± 0.2 g mol^{-1} for the reduction of nitrate to ammonia. By this type of metabolism, *S. deleyianum* connected the biogeochemical cycles of sulfur and nitrogen. The sulfur reductase activity in *S. deleyianum* was inducible, as the activity depended on the presence of sulfide or elemental sulfur during cultivation with nitrate or fumarate as electron acceptor. Hydrogenase activity was always high, indicating that the enzyme is constitutively expressed. The ammonia-forming nitrite reductase was an inducible enzyme, expressed when cells were cultivated with nitrate, nitrite, or elemental sulfur, but repressed after cultivation with fumarate.

Key words Sulfide oxidation · Nitrate ammonification · Sulfur respiration · Sulfur reductase · *Sulfurospirillum deleyianum*

Abbreviations $<S^0>$ Biogenic elemental sulfur · S_R^8 Crystalline elemental sulfur

Dedicated to Prof. Norbert Pfennig on the occasion of his 70th birthday

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Introduction

Recently, the facultatively sulfur-reducing bacterium "Spirillum" 5175 isolated by Wolfe and Pfennig (1977) was described as the type strain of the new genus and species *Sulfurospirillum deleyianum* (Schumacher et al. 1992). Growth of this versatile bacterium occurs with hydrogen or formate as electron donor, acetate as carbon source, and one of the following electron acceptors: dioxygen (2%); nitrate and nitrite reduced to ammonia; elemental sulfur, thiosulfate and sulfite reduced to sulfide; dimethyl sulfoxide reduced to dimethyl sulfide; fumarate, malate, and aspartate reduced to succinate. Energy metabolism with formate as electron donor coupled to respiratory nitrate ammonification has been described for *S. deleyianum* (Schumacher and Kroneck 1992). Growth coupled to respiratory reduction of elemental sulfur to sulfide has been shown for *S. deleyianum*; furthermore, the production of elemental sulfur from sulfide under anoxic conditions with malate or fumarate as electron acceptor has also been reported for this bacterium (Wolfe and Pfennig 1977). Growth with sulfide as electron donor and fumarate as electron acceptor has been demonstrated for *Wolinella succinogenes* (Macy et al. 1986). Thermodynamically, the anaerobic oxidation of sulfide ($E_o'(S^o/H_2S)$ = –245 mV) coupled to the reduction of nitrate to nitrite (E_o' = +433 mV) and nitrite to ammonia (E_o' = +340 mV) may also support growth according to a respiratory mode of energy transduction. The first indication of this pathway came from the finding that resting cells of *Desulfovibrio desulfuricans* CSN catalyze the oxidation of sulfide to sulfate coupled to the reduction of nitrate to ammonia (Dannenberg et al. 1992). However, growth of a bacterium with sulfide and nitrate (reduced to ammonia) as energy source has not yet been demonstrated.

Here, evidence is provided that *S. deleyianum* is able to grow by the anaerobic oxidation of sulfide to elemental sulfur coupled to the reduction of nitrate to ammonia. In addition, *S. deleyianum* was cultivated under various conditions in order to study the expression of sulfur reductase.

Materials and methods

Media and growth conditions

Sulfurospirillum deleyianum (DSM 6946^T), taken from our stock culture collection, was cultivated in batch cultures (10-l flasks) as described previously (Zöphel et al. 1988). Cells were cultivated with 20 mM fumarate as sole energy and carbon source, or with 20–40 mM formate as electron donor, 10 mM acetate as carbon source, and nitrate (10 mM), fumarate (20 mM), crystalline elemental sulfur, or polysulfide sulfur as electron acceptor. Cultivation with H_2S gas as electron donor and 10 mM fumarate as electron acceptor was performed by continuous addition (0.12 l h^{-1}) of H_2S with a peristaltic pump as described previously (Macy et al. 1986). Cultures were incubated with stirring at 28°C under an atmosphere of N_2/CO_2 (90/10, v/v). The pH was discontinuously controlled at 7.2 with 2 M solutions of Na_2CO_3 or H_2SO_4. With H_2 as electron donor, the headspace of the culture was flushed (0.2 l h^{-1}) with H_2/CO_2 (90/10, v/v) during cultivation. Crude extracts from these cells were prepared as described previously (Zöphel et al. 1991).

Lithotrophic growth with sulfide and nitrate as energy source

Sulfide oxidation coupled to the reduction of nitrate by *S. deleyianum* was investigated in 11.5-l batch culture experiments in bicarbonate-buffered freshwater medium, with the initial concentrations of 5 mM sulfide, 1.2 mM nitrate, 10 mM acetate as carbon source, 2 mM NH_4Cl as nitrogen source, and 0.3 mM L-cysteine as sulfur source. The stock solution containing 0.5 M Na_2S was autoclaved and cooled under N_2. The pH of the culture was initially adjusted to 7.2. The stoppered culture flask was filled, leaving a head space of approximately 0.2 l. Cultures were incubated at 28°C under an atmosphere of N_2/CO_2 (90/10, v/v) with stirring. Samples were withdrawn from the culture with a gas-tight syringe through a butyl rubber stopper, and sulfide was determined immediately. Growth was followed by determination of protein. For this, the bacteria were removed from samples by centrifugation (5 min, 12,000 × *g*, 4°C). The supernatant was stored in liquid nitrogen until analysis of nitrate, nitrite, and ammonia. The dried sediment was suspended in water for protein determination. This method was also used to follow growth with formate (7.5 mM) as electron donor, which was added to the sulfide/nitrate culture after oxidation of sulfide as electron donor ceased.

Cell density

During cultivation of *S. deleyianum* with formate as electron donor in the presence of fumarate or nitrate as electron acceptor, growth was followed by measuring the OD_{578} in 1.0-cm cuvettes and by determination of protein concentration. The protein concentration of growing cultures increased linearly (317 ± 3 mg protein l^{-1} OD_{578}^{-1}) in the early- and late-exponential growth phase and was calibrated against the bacterial dry weight (475 ± 15 mg dry cells l^{-1} OD_{578}^{-1}; Schumacher and Kroneck 1992).

Enzyme activities

Sulfur reductase (EC 1.97.1.3) activity was determined manometrically under an atmosphere of dihydrogen with a Warburg apparatus according to Fauque et al. (1979) using a hydrophilic sulfur suspension as the electron acceptor (Zöphel et al. 1988). Hydrogen sulfide evolved during the reduction was trapped by a filter paper soaked with 0.2 ml 6% NaOH in the center of the Warburg flask. Hydrogenase (EC 1.18.99.1) activity was also determined manometrically (Umbreit et al. 1972) with 10 mM methylene blue as electron acceptor. Cytochrome *c* nitrite reductase activity was determined as formation of ammonia from nitrite with reduced methyl viologen as electron donor, as described previously (Schumacher et al. 1994). All activities were measured at 37°C.

Sulfur compounds

Crystalline sulfur (Fluka, Neu-Ulm, Germany) was powdered before use in growth experiments. Polysulfide sulfur (Gebhardt et al. 1985) and hydrophilic sulfur suspension (Janek 1933) were prepared as described previously.

Analytical methods

Sulfide (Cline 1969), formate (Lang and Lang 1972), nitrite, and ammonia (Boltz and Taras 1978) were determined as described previously. Nitrate was determined by HPLC using an LCA A03 column (4.6 × 125 mm, Sykam, Gilching, Germany). Protein was determined with bicinchoninic acid (Smith et al. 1985) using bovine serum albumin as standard.

A gray sulfur compound was obtained from a culture of *S. deleyianum* cultivated with sulfide as electron donor. The compound was separated from cells by washing five times in 0.5 M sucrose, 50 mM K-phosphate buffer, pH 7.5 (1,000 × *g*, 5 min, 4°C). Thereafter, the solid was carefully washed in H_2O (10,000 × *g*, 10 min, 4°C) to remove the sucrose prior to element analysis. The sulfur compound was dried at 100°C and dissolved in carbon disulfide. The suspension was filtered to separate the CS_2-insoluble compounds.

Results

Energy metabolism during sulfide oxidation with nitrate as electron acceptor

In a batch culture experiment (Fig. 1), *S. deleyianum* grew at pH 7.2 with sulfide as electron donor, nitrate as electron acceptor, and acetate as carbon source. During growth, the formation of elemental sulfur (<S^0>) in the form of globules was observed under the microscope; <S^0> was not further oxidized in the presence of excess nitrate or nitrite. The catabolic reactions are consistent with the stoichiometry of the following equations:

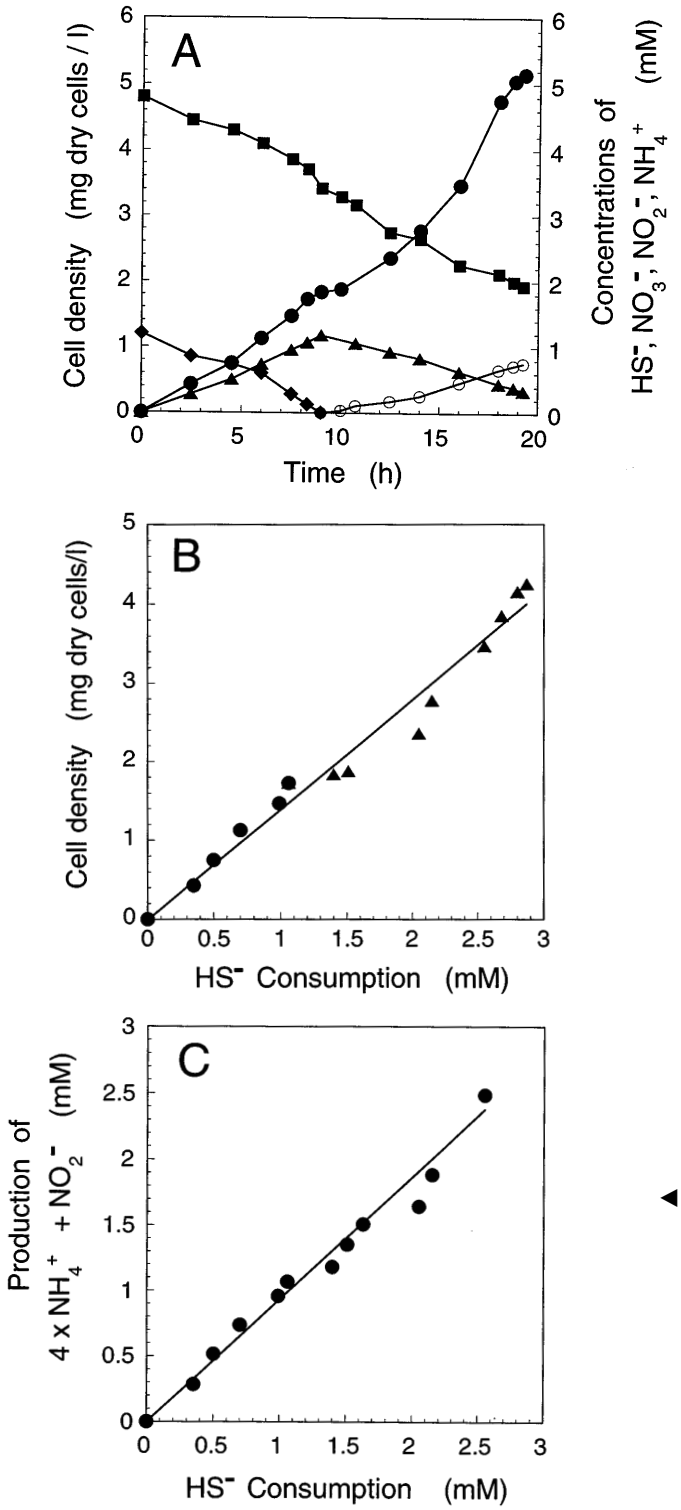
$$H_2S + NO_3^- \rightarrow NO_2^- + <S^0> + H_2O \quad (1)$$

$$3H_2S + NO_2^- + 2H^+ \rightarrow NH_4^+ + 3<S^0> + 2H_2O \quad (2)$$

The growth curve (Fig. 1A) exhibited two phases separated by an intermediate lag phase. Growth during the first phase ($\mu = 0.074 \pm 0.002$ h^{-1}) at the expense of nitrate led to quantitative accumulation of nitrite (Eq. 1) at a rate of 0.10 ± 0.01 mmol NO_2^- h^{-1}. At maximum nitrite concentration, the ratio of $nitrate_{consumed}/sulfide_{consumed}$ was 0.98, in accordance with Eq. 1. Nitrite reduction started only after nitrate was exhausted from the medium. During the second phase ($\mu = 0.063 \pm 0.005$ h^{-1}), energy gain was only sustained by reduction of nitrite to ammonia (Eq. 2) at a rate of 0.07 ± 0.01 mmol NO_2^- h^{-1}. Cell formation was a linear function of the amount of sulfide consumed, and linear regression was carried out with the data points of the two phases. Within the experimental error, the molar growth yield, $Y_m = 1.5 \pm 0.2$ g (mol sulfide)$^{-1}$ (slope of line, Fig. 1B), appeared to be identical for growth with the reduction of nitrate to nitrite (Eq. 1) and with the re-

duction of nitrite to ammonia (Eq. 2). The recovery of reducing equivalents as the measured amount of nitrite and ammonia was a linear function of sulfide consumption [slope of line in Fig. 1C, 0.94 ± 0.05 mol 2[H] (mol sulfide)$^{-1}$].

When *S. deleyianum* was cultivated with 5 mM nitrate and 4 mM sulfide, nitrite accumulated and ammonia was not formed. The ratio $sulfide_{consumed}/nitrite_{formed}$ was 1.03, in accordance with Eq. 1. Biomass formation depended on the presence of sulfide plus nitrate or nitrite, in addition to acetate as carbon source. Sulfide oxidation was also dependent on the presence of cells and an electron acceptor. In control experiments with cell-free medium containing 4 mM sulfide plus 5 mM nitrate or 5 mM nitrite, oxidation of sulfide did not occur within 3 days. Growth occurred only in the presence of up to 5 mM sulfide; higher concentrations of sulfide were toxic to *S. deleyianum.* The elemental sulfur as a product of the sulfide/nitrate culture was also formed after cultivation of *S. deleyianum* with sulfide as electron donor and fumarate as electron acceptor. After centrifugation of the culture suspension, a gray sediment was separated from the cells that consisted mainly (97%) of elemental sulfur according to C,H,N,S analysis (Table 1). The CS_2-insoluble part (3%) was mainly carbon (50%), with small amounts of nitrogen, hydrogen, and sulfur, with a melting point above 300°C. The biogenic gray sulfur formed in the sulfide/nitrate culture served as electron acceptor for anaerobic growth of *S. deleyianum* with 7.5 mM formate as electron donor, which was added to the culture after oxidation of sulfide ceased. Biomass formation, measured as protein increase, was accompanied by the production of sulfide and the consumption of formate (data not shown).



Expression of enzymes

Sulfur reductase activity was measured with crude extracts of *S. deleyianum* cultivated under various conditions (Table 2). Highest sulfur reductase activities were measured after cultivation with crystalline sulfur [1.1 µmol H_2 min^{-1} (mg protein)$^{-1}$, given as 100%] or polysulfide sulfur (30–50%) as electron acceptor during growth with H_2 or formate as electron donor. Cultivation of *S. deleyianum* with H_2S gas or sulfide as electron donor and fumarate as electron acceptor resulted in a high cell yield with only low (5%) sulfur reductase activity. Cells cultivated with L-cysteine as reductant and sulfur source, formate as electron donor, and fumarate or nitrate as electron acceptor, did not show significant sulfur reductase activity (Table 2). When L-cysteine was replaced by sulfide, sulfur reductase activity was present in *S. deleyianum.* The hydrogenase activity was always induced [13–18 µmol H_2

◀ **Fig. 1A–C** Growth of *Sulfurospirillum deleyianum* in minimal medium with sulfide (oxidized to elemental sulfur), nitrate (reduced to ammonia via nitrite), acetate, and L-cysteine. **A** Cell density (*filled circles*) and concentration of sulfide (*filled squares*), nitrate (*filled diamonds*), nitrite (*filled triangles*), and ammonia (*open circles*) as a function of growth time. **B** Cell density as a function of sulfide consumption during the reduction of nitrate to nitrite (*filled circles*) and nitrite to ammonia (*filled triangles*); the line [slope = 1.5 ± 0.2 g (mol sulfide)$^{-1}$] was obtained by linear regression. **C** Reducing equivalents resulting from oxidation of sulfide recovered in nitrite and free ammonia (*filled circles*) as a function of sulfide consumption; the line [slope = 0.94 ± 0.05 mol 2[H] (mol sulfide)$^{-1}$] was obtained by linear regression

Table 1 Element analysis of the globular gray sulfur compound isolated from a sulfide/nitrate culture of *Sulfurospirillum deleyianum*

Element	Gray sulfur compound	
	CS_2-soluble part (%)	CS_2-insoluble part (%)
Carbon	1.65	47.30
Hydrogen	0.36	6.96
Nitrogen	–	10.06
Sulfur	96.94	3.79

min^{-1} (mg protein)$^{-1}$] in *S. deleyianum* cultivated under the various conditions. The ammonia-forming nitrite reductase activity was induced after cultivation with sulfide or formate as electron donor and with nitrate, nitrite, or crystalline sulfur as electron acceptor, but it was repressed after cultivation with fumarate as electron acceptor (Table 2).

Discussion

Energy metabolism of nitrate ammonification by oxidation of sulfide

The sulfur-reducing bacterium *S. deleyianum* was shown to grow by the oxidation of sulfide to elemental sulfur coupled to the reduction of nitrate to ammonia in a minimal medium. To our knowledge, this is the first demonstration of bacterial growth with sulfide plus nitrate or nitrite (reduced to ammonia) as energy source. This kind of energy-conserving metabolism of this organism connects the biogeochemical cycles of nitrogen and sulfur. Resting cells of *Desulfovibrio desulfuricans* CSN catalyzed the oxidation of sulfide to sulfate linked to the reduction of nitrate to ammonia (Dannenberg et al. 1992). However, growth of sulfate-reducing bacteria according to this pathway has not yet been demonstrated. It has been reported that sulfide even inhibited growth with nitrate as electron acceptor in sulfate-reducing bacteria, such as *Desulfovibrio desulfuricans* (Dalsgaard and Bak 1994). Anaerobic oxidation of sulfide coupled to the reduction of nitrate to dinitrogen has been demonstrated for denitrifying bacteria of the genus *Thiobacillus* (Aminuddin and Nicholas 1973).

Cell formation was a linear function of sulfide consumption with nitrate or with nitrite as electron acceptor, indicating that *S. deleyianum* performs electron transport phosphorylation with nitrate and nitrite as acceptor. A fermentative generation of ATP was excluded under these conditions because substrate-level phosphorylation did not result from these substrates in *S. deleyianum*. Within the experimental error (±15%), the molar growth yield with sulfide as electron donor and with nitrate or nitrite as electron acceptor appeared to be identical. However, with formate as electron donor, a significant difference in growth yield (≈20%) was reported earlier for growth of *S. deleyianum* with nitrate reduced to nitrite and nitrite reduced to ammonia (Schumacher and Kroneck 1992). A difference in molar growth yield of *S. deleyianum* for the reduction of nitrate and nitrite with sulfide cannot be excluded because of the experimental error. The molar growth yield for the reduction of nitrate to nitrite is higher than the reduction of nitrite to ammonia when *Campylobacter sputorum* biovar *bubulus* and *Desulfovibrio desulfuricans* (Essex 6) are cultivated with dihydrogen as electron donor (De Vries et al. 1980; Seitz and Cypionka 1986). The reducing equivalents resulting from oxidation

Table 2 Cell yield, doubling time, and specific activities of sulfur reductase and nitrite reductase of *Sulfurospirillum deleyianum* cultivated with various electron donors, electron acceptors, carbon sources, and reductants. Sulfur reductase activity of crude extract was determined manometrically in an atmosphere of dihydrogen. The activity with hydrophilic sulfur, 1.1 μmol H_2 min^{-1} (mg protein)$^{-1}$, was set to 100%; hydrogenase was always induced [13–18 μmol H_2 min^{-1} (mg protein)$^{-1}$]. Nitrite reductase activity of crude extract was determined as the formation of ammonia from nitrite; the activity of cells cultivated with nitrite, 11 μmol NO_2^- min^{-1} (mg protein)$^{-1}$, was set to 100% (*n.d.* not determined)

Electron donor	Electron acceptor	Carbon source	Reductant[a]	Cell yield[b] (g l^{-1})	Doubling time (h)	Sulfur reductase (%)	Nitrite reductase (%)
Formate	Crystalline sulfur	Acetate	Na_2S	0.1	170	100	90
Formate	Polysulfide sulfur	Acetate	Na_2S	0.3	30	30	n.d.
H_2	Polysulfide sulfur	Acetate	Na_2S	0.2	35	50	n.d.
H_2	Polysulfide sulfur	Pyruvate	Na_2S	0.5	15	10	n.d.
H_2S (aq)	Fumarate	Acetate	Na_2S	0.6	8	5	< 5
Formate	Fumarate	Acetate	Na_2S	1.3	5	11	< 5
Formate	Fumarate	Acetate	L-Cysteine	1.3	5	< 1	< 5
Formate	$NO_3^-\rightarrow NH_4^+$	Acetate	Na_2S	0.6	8	5	100
Formate	$NO_3^-\rightarrow NH_4^+$	Acetate	L-Cysteine	0.6	8	< 1	100
Formate	$NO_3^-\rightarrow NO_2^-$	Acetate	L-Cysteine	0.6	8	< 1	100
Formate	$NO_2^-\rightarrow NH_4^+$	Acetate	L-Cysteine	0.4	9	< 1	100
Sulfide	$NO_3^-\rightarrow NH_4^+$	Acetate	L-Cysteine	n.d.	11	< 1	100

[a] Na_2S (1.4 mM), L-Cysteine (0.3 mM)
[b] Wet weight of sedimented cells after removal of gray sulfur

of sulfide to elemental sulfur were recovered (94 ± 5%) in nitrite and free ammonia; the residual reducing equivalents are expected to be conserved in the biomass produced. It was shown that nitrite and ammonia are the only products of catabolic nitrate reduction.

Growth of *S. deleyianum* with sulfide and nitrate was biphasic with an intermediate lag phase at the maximum concentration of nitrite. Such a biphasic growth has also been observed during growth of *S. deleyianum* with formate and nitrate as energy source (Schumacher and Kroneck 1992). A competition between nitrate and nitrite for a pool of reduced quinones, supplied by formate via formate dehydrogenase, has been shown for resting cells of *Escherichia coli*; nitrite is not reduced until nitrate is exhausted (Abou-Jaoudé et al. 1979). Note that menaquinone-6, shown to function as electron carrier to the cytochrome *c* nitrite reductase in *Wolinella succinogenes* (Bokranz et al. 1983), is the major quinone (90%) in *S. deleyianum* (Collins and Widdel 1986). At present, it cannot be decided whether biphasic growth of *S. deleyianum* with nitrate as electron acceptor is the result of regulation on the level of enzyme activity or of enzyme expression.

Expression of enzymes involved in catabolic reactions

Previous data suggest that sulfur reductase is a constitutive enzyme in *S. deleyianum* because its activity is present in cells cultivated with various electron acceptors, such as fumarate, nitrate, or crystalline sulfur (Zöphel et al. 1988). However, the sulfur reductase activity is absent when sulfide is replaced by L-cysteine as reductant and sulfur source for cultivation of *S. deleyianum* with fumarate or nitrate. As a consequence, sulfur reductase in *S. deleyianum* is not constitutively expressed, but appears to be inducible by sulfide or elemental sulfur. This conclusion is in accordance with the recent isolation of a sulfur-regulated gene (Laudenbach et al. 1991). Sulfide dehydrogenase (oxidizing sulfide to elemental sulfur) and sulfur reductase (reducing sulfur to elemental sulfide) seemed to be two independently expressed enzyme activities in *S. deleyianum*. Otherwise, cells cultivated with sulfide as electron donor and fumarate or nitrate as electron acceptor would be expected to have sulfur reductase activity as high as the cells cultivated with crystalline sulfur or polysulfide sulfur. The periplasmic sulfide dehydrogenase has also been shown to differ from the sulfur reductase in the physiologically related bacterium, *Wolinella succinogenes* (Kreis-Kleinschmidt et al. 1995). However, sulfide dehydrogenase is identical to the sulfur reductase in the hyperthermophile *Pyrococcus furiosus* (Ma and Adams 1994).

Ecological significance of sulfurogenic nitrate ammonification

Growth of *Wolinella succinogenes* with sulfide as electron donor (converted to elemental sulfur) and fumarate as electron acceptor has been demonstrated (Macy et al. 1986). The formation of elemental sulfur from sulfide by *S. deleyianum* under anoxic conditions in the presence of malate or fumarate has been reported previously (Wolfe and Pfennig 1977); in addition we were able to cultivate *S. deleyianum* with sulfide or H_2S gas as electron donor and fumarate as electron acceptor. In contrast to nitrate and nitrite, malate and fumarate are usually not present in large quantities in anaerobic environments except in specific locations. However, in the presence of nitrate, biologically mediated sulfur production from sulfide coupled to nitrate ammonification may represent an ecologically significant process in dark anoxic habitats with a low redox potential. Nitrate ammonification has to be considered as a potential sink of sulfide, in addition to chemical oxidation or precipitation as iron sulfide. The reduction of nitrate to ammonia or to dinitrogen is regulated by the bacterial community that is selected under the given environmental conditions. Nitrate-ammonifying bacteria exhibit a higher affinity for nitrate ($K_m \approx 0.05$ μM, Dalsgaard and Bak 1994) than denitrifying bacteria ($K_m \approx 1.8$–13.7 μM, Murray et al. 1989). It has also been demonstrated for estuarine sediments that at low nitrate concentration, ammonification is the favored process (King and Nedwell 1985, 1987). There is evidence that nitrate-ammonifying sulfate-reducing bacteria compete successfully with denitrifying or nitrate-fermenting bacteria for nitrate and nitrite (Seitz and Cypionka 1986). Thus, at low nitrate concentration, bacterial sulfide oxidation may favor the formation of ammonia rather than of dinitrogen.

The biogenic sulfur formed during oxidation of sulfide served as electron acceptor for growth of *S. deleyianum* with formate as electron donor; sulfur respiration has been demonstrated for this bacterial strain with dihydrogen as electron donor (Wolfe and Pfennig 1977). Sulfur respiration and the sulfurogenic oxidation of sulfide define suitable environmental niches for bacteria such as *S. deleyianum*. Syntrophic interactions of *S. deleyianum* with other microorganisms may contribute to sulfur diagenesis. The potential significance of interspecies sulfur transfer has even been demonstrated for *S. deleyianum* by a successful syntrophic cultivation of this bacterium with *Chlorobium* (Wolfe and Pfennig 1977).

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References

Abou-Jaoudé A, Chippaux M, Pascal MC (1979) Formate-nitrite reduction in *Escherichia coli* K12. 1. Physiological study of the system. Eur J Biochem 95:309–314

Aminuddin M, Nicholas DJD (1973) Sulphide oxidation linked to the reduction of nitrate and nitrite in *Thiobacillus denitrificans*. Biochim Biophys Acta 325:81–93

Bokranz MJ, Katz J, Schröder I, Roberton AM, Kröger A (1983) Energy metabolism and biosynthesis of *Vibrio succinogenes* growing with nitrate or nitrite as terminal electron acceptor. Arch Microbiol 135:36–41

Boltz DF, Taras MJ (1978) Nitrogen. In: Boltz DF, Howell JA (eds) Colorimetric determination of nonmetals. Chemical analysis, vol 8. Wiley, New York, pp 197–251

Cline JD (1969) Spectrophotometric determination of hydrogen sulfide in natural waters. Limnol Oceanogr 14:454–458

Collins MD, Widdel F (1986) Respiratory quinones of sulphate-reducing and sulphur-reducing bacteria: a systematic investigation. System Appl Microbiol 8:8–18

Dalsgaard T, Bak F (1994) Nitrate reduction in a sulfate-reducing bacterium, *Desulfovibrio desulfuricans*, isolated from rice paddy soil: sulfide inhibition, kinetics, and regulation. Appl Environ Microbiol 60:291–297

Dannenberg S, Kroder M, Dilling W, Cypionka H (1992) Oxidation of H_2, organic compounds and inorganic sulfur compounds coupled to reduction of O_2 or nitrate by sulfate-reducing bacteria. Arch Microbiol 158:93–99

De Vries W, Niekus HGD, Boellaard M, Stouthamer AH (1980) Growth yields and energy generation by *Campylobacter sputorum* subspecies *bubulus* during growth in continuous culture with different hydrogen acceptors. Arch Microbiol 124:221–227

Fauque G, Herve D, LeGall J (1979) Structure-function relationships in hemoproteins: the role of cytochrome c_3 in the reduction of colloidal sulfur by sulfate-reducing bacteria. Arch Microbiol 121:261–264

Gebhardt NA, Thauer RK, Linder D, Kaulfers PM, Pfennig N (1985) Mechanism of acetate oxidation to CO_2 with elemental sulfur in *Desulfuromonas acetoxidans*. Arch Microbiol 141: 392–398

Janek A (1933) Ein neues Verfahren zur Herstellung von Schwefelsolen. Kolloid Z 64:31–32

King D, Nedwell DB (1985) The influence of nitrate concentration upon the end product of nitrate dissimilation by bacteria in anaerobic salt marsh sediment. FEMS Microbiol Ecol 31:23–28

King D, Nedwell DB (1987) The adaptation of nitrate-reducing bacterial communities in estuarine sediments in response to overlying nitrate load. FEMS Microbiol Ecol 45:15–20

Kreis-Kleinschmidt V, Fahrenholz F, Kojro E, Kröger A (1995) Periplasmic sulphide dehydrogenase (Sud) from *Wolinella succinogenes*: isolation, nucleotide sequence of the *sud* gene and its expression in *Escherichia coli*. Eur J Biochem 227:137–142

Lang E, Lang H (1972) Spezifische Farbreaktion zum direkten Nachweis der Ameisensäure. Z Anal Chem 260:8–10

Laudenbach DE, Ehrhardt D, Green L, Grossman A (1991) Isolation of a sulfur-regulated gene encoding a periplasmically localized protein with sequence similarity to rhodanese. J Bacteriol 173:2751–2760

Ma K, Adams MWW (1994) Sulfide dehydrogenase from the hyperthermophilic archaeon *Pyrococcus furiosus*: a new multifunctional enzyme involved in the reduction of elemental sulfur. J Bacteriol 176:6509–6517

Macy JM, Schröder I, Thauer RK, Kröger A (1986) Growth of *Wolinella succinogenes* on H_2S plus fumarate and on formate plus sulfur as energy sources. Arch Microbiol 144:147–150

Murray RE, Parsons LL, Smith MS (1989) Kinetics of nitrate utilization by mixed populations of denitrifying bacteria. Appl Environ Microbiol 55:717–721

Schumacher W, Kroneck PMH (1992) Anaerobic energy metabolism of the sulfur-reducing bacterium "Spirillum" 5175 during dissimilatory nitrate reduction to ammonia. Arch Microbiol 157:464–470

Schumacher W, Kroneck PMH, Pfennig N (1992) Comparative systematic study on "Spirillum" 5175, *Campylobacter* and *Wolinella* species. Description of "Spirillum" 5175 as *Sulfurospirillum deleyianum* gen. nov., spec. nov. Arch Microbiol 158:287–293

Schumacher W, Hole U, Kroneck PMH (1994) Ammonia-forming cytochrome *c* nitrite reductase from *Sulfurospirillum deleyianum* is a tetraheme protein: new aspects of the molecular composition and spectroscopic properties. Biochem Biophys Res Commun 205:911–916

Seitz HJ, Cypionka H (1986) Chemolithotrophic growth of *Desulfovibrio desulfuricans* with hydrogen coupled to ammonification of nitrate or nitrite. Arch Microbiol 146:63–67

Smith PK, Krohn RI, Hermanson GT, Mallia AK, Gartner FH, Provenzano MD, Fujimoto EK, Goeke NM, Olson BJ, Klenk DC (1985) Measurement of protein using bicinchoninic acid. Anal Biochem 150:76–85

Umbreit WW, Burris RH, Stauffer JF (1972) Manometric and biochemical techniques. Burgess, Minneapolis

Wolfe RS, Pfennig N (1977) Reduction of sulfur by spirillum 5175 and syntrophism with *Chlorobium*. Appl Environ Microbiol 33:427–433

Zöphel A, Kennedy MC, Beinert H, Kroneck PMH (1988) Investigations on microbial sulfur respiration. 1. Activation and reduction of elemental sulfur in several strains of eubacteria. Arch Microbiol 150:72–77

Zöphel A, Kennedy MC, Beinert H, Kroneck PMH (1991) Investigations on microbial sulfur respiration. 2. Isolation, purification, and characterization of cellular components from Spirillum 5175. Eur J Biochem 195:849–856