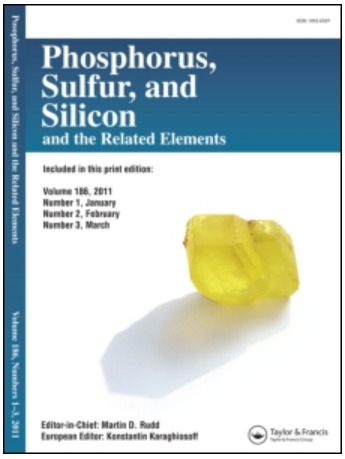
This article was downloaded by:
On: *30 January 2011*
Access details: *Access Details: Free Access*
Publisher *Taylor & Francis*
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Phosphorus, Sulfur, and Silicon and the Related Elements

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713618290

Microbial Metabolism of Inorganic Sulfur Compounds

Hans G. Trüper[a]
[a] Institut für Mikrobiologie der Rheinischen Friedrich Wilhelms-Universität, Bonn, Fed. Rep. of Germany

To cite this Article Trüper, Hans G.(1985) 'Microbial Metabolism of Inorganic Sulfur Compounds', Phosphorus, Sulfur, and Silicon and the Related Elements, 24: 1, 165 — 198
To link to this Article: DOI: 10.1080/03086648508073401
URL: http://dx.doi.org/10.1080/03086648508073401

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.informaworld.com/terms-and-conditions-of-access.pdf

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

Phosphorus and Sulfur, 1985, Vol. 24, pp. 165-198
0308-664X/85/2401-0165/$30.00/0
© 1985 Gordon and Breach, Science Publishers, Inc.
and OPA Ltd. Printed in the United Kingdom

MICROBIAL METABOLISM OF INORGANIC SULFUR COMPOUNDS

HANS G. TRÜPER
Institut für Mikrobiologie der Rheinischen Friedrich Wilhelms-Universität, Meckenheimer Allee 168
D-5300 Bonn, Fed. Rep. of Germany

Abstract Inorganic sulfur compounds are used by microorganisms (bacteria, fungi, algae) and plants for assimilation, i.e. biosynthesis of sulfur-containing cell constituents.
Quantitatively, within the biogeochemical cycle of sulfur the utilization of inorganic sulfur compounds in bacterial energy metabolism, i.e. dissimilatory sulfur utilization, is of far higher importance. Reduced sulfur compounds serve as electron donors for photosynthesis and respiration, whereas inorganic sulfur compounds of oxidation levels above sulfide serve as electron donors in anaerobic respiration as well as in fermentation. In still other bacteria reduced sulfur compounds act as protective agents against hydrogen peroxide.

Downloaded At: 08:34 30 January 2011

A. INTRODUCTION

Most inorganic sulfur compounds occurring in nature are available for microbial metabolism. An important prerequisite for their utilization is that they should be present dissolved in water, such as the anions sulfide, polysulfides, thiosulfate, polythionates, sulfite and sulfate or at least suspended or submerged in water such as elemental sulfur or heavy metal sulfides (e.g. pyrite).

Sulfur at all oxidation levels is utilized by microbes - especially by bacteria - for quite different purposes. Like all other living beings microorganisms contain sulfur in their amino acids, coenzymes and other structural elements. For biosynthesis of these components inorganic sulfur compounds are taken up and assimilated.

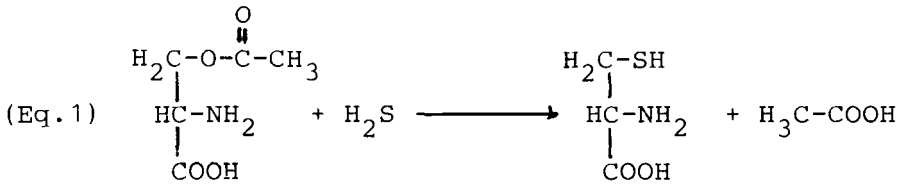
While assimilation thus serves the biosynthesis of sulfur containing cell constituents, dissimilatory pathways are part of the energy metabolism in several groups of bacteria. Inorganic sulfur compounds at oxidation levels below that of sulfate may serve as oxidizable electron donors in photosynthesis and oxygen-dependent as well as nitrate-dependent respiration. On the other hand, inorganic sulfur compounds at oxidation levels above that of sulfide may serve as reducible electron acceptors in anaerobic respiration and fermentative metabolism.
A third aspect in microbial sulfur metabolism is that of protection against hydrogen peroxide.
In the following, these three aspects, assimilation, dissimilation and protective mechanisms shall be discussed in detail.

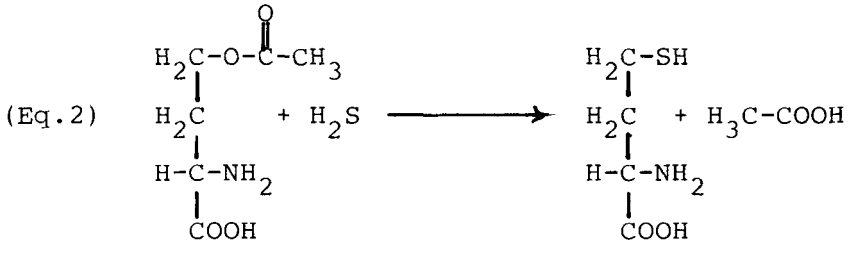
Downloaded At: 08:34 30 January 2011

B. INORGANIC SULFUR COMPOUNDS FOR ASSIMILATION

Uptake of any compound into a living organism and its anabolic use is called "assimilation". The assimilated sulfur bound in the biosphere of the Earth (including dead organic matter) following a recent estimate (1) amounts to 5.6×10^9 metric tons.
At the oxidation level of sulfate (+ 6), sulfur is a component of structural molecules such as sulfatides, sulfated polysaccharides (in algal cell walls) or sulfolipids.
Reduced sulfur (- 2) is required by living organisms for the synthesis of the sulfur-containing amino acids cysteine, methionine and of coenzymes and cofactors like biotin, thiamin, coenzyme A, lipoic acid, glutathion, coenzyme M, thioredoxin, ferredoxin and other iron-sulfur proteins, as well as β-lactam antibiotics and other substances.

The most common way to bind reduced sulfur to the carbon sceleton is the biosynthesis of cysteine (or homocysteine) catalyzed by O-acetylserine sulfhydrylase (Eq. 1) or O-acetylhomoserine sulfhydrylase (Eq. 2).





Homocysteine is a direct precursor of methionine. O-acetylserine sulfhydrylase has been found in most bacteria studied so far (2,3,4). In yeast, high activities of O-acetylhomoserine sulfhydrylase have been observed pointing towards physiological importance of direct biosynthesis of homocysteine from sulfide (5).

In anoxic natural environments usually sulfide is abundant and several large groups of strictly anaerobic bacteria depend on sulfide as their sulfur source for amino acid synthesis, e.g., the methanogenic bacteria, the phototrophic green bacteria and several of the phototrophic purple bacteria (6). Other anaerobes such as the propionibacteria (7), several clostridia, and most phototrophic

Downloaded At: 08:34 30 January 2011

purple bacteria (Chromatiaceae, Ectothiorhodospiraceae and Rhodospirillaceae) utilize sulfide for this purpose in their natural environment (6).

Also elemental sulfur can be utilized as an assimilatory sulfur source by many anaerobic bacteria. Before uptake into the cells it is usually reduced to sulfide by mechanisms not yet studied in detail (7,8,9).

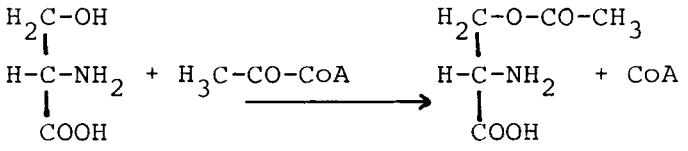
Chambers and Trudinger (3) found a reaction in Pseudomonas aeruginosa and several other bacterial species by which S-sulfocysteine was formed from thiosulfate and O-acetylserine. The enzyme, S-sulfocysteine synthase, was partly purified. A survey in a number of phototrophic bacteria showed that this activity occurred in all purple sulfur bacteria (Chromatiaceae and Ectothiorhodospiraceae) studied, as well as in (only) three species of the "non-sulfur" purple bacteria (Rhodospirillaceae) (4,1o) belonging now to the genus Rhodocyclus (11). Comparative studies of O-acetylserine sulfhydrylase and S-sulfocysteine synthase activities in Rhodocyclus tenuis (formerly Rhodospirillum) and Chromatium vinosum showed that both bacteria possessed two proteins with O-acetylserine sulfhydrylase activity, of which one possessed additional S-sulfocysteine synthase activity (1o,12).

Certainly the possession of the latter activity allows assimilation of thiosulfate. Cysteine may be formed from S-sulfocysteine by reductive splitting as reported from fungi (13,14) or, perhaps, by hydrolysis.

Cysteine and homocysteine biosynthesis from the O-acetyl carbon precursor requires biosynthesis of that precursor. This is accomplished by the enzyme serine (or homoserine) transacetylase (2) (Eq.3):

Downloaded At: 08:34 30 January 2011

(Eq.3)



Also this enzyme is regularly found in a wide variety of microbes and plants (cf.15).

Aerobic environments and certain anaerobic (preferably freshwater) environments do not contain sufficient amounts of inorganic sulfur compounds other than sulfate. Consequently plants, fungi and most bacteria possess the ability of assimilatory sulfate reduction. Sulfate is the thermodynamically most stable form of sulfur under most terrestrial conditions. Direct reduction to sulfite at pH 7 is not possible due to the oxidation reduction potential of the sulfate/sulfite couple (-516 mV)(15). Therefore an activation by adenylation is necessary to overcome this thermodynamical barrier. Two activated forms of sulfate exist: 5'-adenylylsulfate (APS) and 3'-phospho-5'-adenylylsulfate (PAPS), cf. Fig. 1.

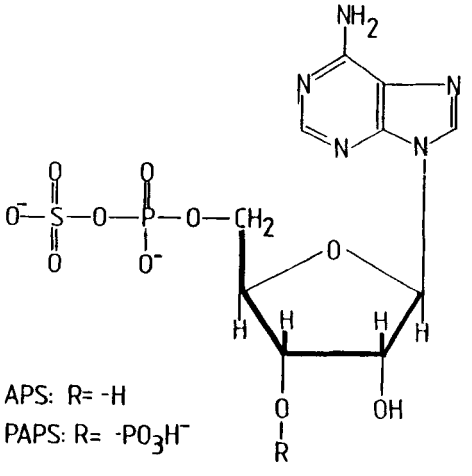


Fig.1: Chemical structure of adenylylsulfate and 3'-phosphoadenylylsulfate.

Downloaded At: 08:34 30 January 2011

The pathways leading to APS and PAPS were established by De Meio, Lipmann, Bandurski and their colleagues, whose work has been extensively reviewed elsewhere (e.g., 15,16,17).
The activation reactions are:

(Eq.4) $ATP + SO_4^{2-} \rightleftharpoons APS + PP_i$

(Eq.5) $ATP + APS \rightleftharpoons PAPS + ADP$

As the equilibrium for ATP-sulfurylase (Eq.4) is in favor of ATP synthesis, the reaction must be pulled towards APS synthesis. This, in addition to APS kinase (Eq.5) is accomplished by inorganic pyrophosphatase (Eq.6).

(Eq.6) $PP_i + H_2O \rightleftharpoons 2\ P_i + H^+$

ATP-sulfurylases have been purified from many bacteria, fungi, plants, and animal tissues. APS-kinase was partly purified from yeast (18) and fully purified only from the green alga Chlamydomonas reinhardii (19).
In many organisms, PAPS is the substrate for sulfotransferases leading to the formation of sulfate esters, sulfolipids etc. Not all microorganisms, however, possess such compounds, and therefore may not need PAPS formation.
As far as assimilatory sulfate reduction is concerned there is a surprising dichotomy in the pathways occurring in microorganisms. While in higher plants and eukaryotic algae, as well as in part of the prokaryotes APS reduction occurs (15), in fungi and an other part of the prokaryotes (including the enterobacteria) PAPS is the substrate entering the reductive pathway. This dichotomy

Downloaded At: 08:34 30 January 2011

even occurs within the cyanobacteria (2o) and within the anoxygenic phototrophic bacteria (21-23). A general scheme of assimilatory reduction of APS and PAPS is given in Fig. 2.

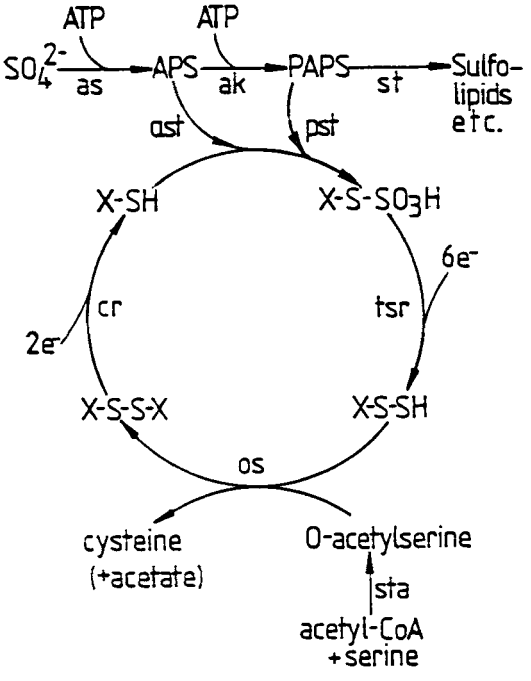


Fig.2: Simplified scheme of assimilatory sulfate reduction. ak, APS kinase; as, ATP sulfurylase; ast, APS sulfotransferase; cr, reductase of the oxidized carrier; os, O-acetylserine sulfhydrylase; pst, PAPS sulfotransferase, st, sulfotransferases; sta, serine transacetylase; tsr, thiosulfonate reductase.

The sulfo group of APS or PAPS is transferred upon a sulfhydryl-containing carrier/protein (X) and is reduced carrier-bound to a sulfydryl group itself. It then reacts with O-acetylserine catalyzed by O-acetylserine sulfhydrylase to form cysteine (plus acetate). The oxidized carrier (X-S-S-X) is then reduced again. Different results and opinions exist about the nature of the carrier as well as about the reductases ("thiosulfonate" reductase and the reductase of the oxidized carrier) and their electron donors involved (15). As the turnover rates of assimilatory sulfate reduction, i.e. the actual amounts of the assimilatory enzymes, are extremely low,

Downloaded At: 08:34 30 January 2011

the study of single enzyme steps poses difficult problems.
In the pathways shown in Fig.2 no free sulfur intermediates occur. Since a long time, however, sulfite reductases reducing free sulfite to sulfide have been known in organisms with an assimilatory system for sulfate.

Such enzymes have been carefully studied by Siegel and his colleagues (cf. 17) who elucidated the complex nature and siroheme structure of this 6-electron transfer enzyme. As this enzyme activity copurifies with that of "thiosulfonate reductase" (24,25) they may be properties of a single protein (26). This question needs to be clarified.

C. INORGANIC SULFUR COMPOUNDS AS ELECTRON DONORS FOR RESPIRATION AND PHOTOSYNTHESIS

As long as an inorganic sulfur compound can be oxidized to sulfate, i.e., is at a lower oxidation level, it is suited as an electron donor for respiration in many chemolithoautotrophic bacteria, as well as for anoxygenic photosynthesis in phototrophic bacteria (Table 1). Not all of these bacteria, however, have the same utilization pattern for such "reduced sulfur compounds".
In respiring and photosynthesizing living organisms biochemical energy is generated by electron flow from an electron donor via several intermediate "electron carriers" towards an electron acceptor. These electron carriers are located in an orderly manner at and within membranes. The electron transport across these membranes gives rise to a membrane potential difference and a proton-motive force, which in turn produce energy for growth, transport, mobility etc. (27). Thus an organism

Downloaded At: 08:34 30 January 2011

needs suitable electron donors as well as acceptors.

1. INORGANIC SULFUR COMPOUNDS AS ELECTRON DONORS FOR RESPIRATION

As in most chemolithoautotrophic bacteria the oxidation reduction potential of the inorganic electron donor is insufficient, i.e., too positive to reduce the pyridine nucleotides (NAD, NADP) required for CO_2 fixation by the Calvin cycle, these organisms rely on reverse electron flow in their respiratory electron transport chain. **Textbooks** usually depict this situation in a way that the ATP formed during electron flow from e.g., thiosulfate via cytochrome c to cytochrome oxidase drives a reverse electron flow from cytochrome c via cytochrome b/ubiquinone to NAD.

Wheelis (28) has recently shown by a thermodynamic analysis on the basis of the Mitchell hypothesis (27) that in most chemolithoautotrophic bacteria, including the sulfur oxidizing bacteria, respiration can generate a proton-motive force easily sufficient to drive both ATP synthesis and reverse electron transport directly.

There exist many bacteria that live by an anaerobic metabolism based on electron flow from organic carbon compounds as donors to nitrate as acceptor. This process is called dissimilatory nitrate reduction (or denitrification, or nitrate respiration). This property is never obligatory but facultative.

Only very few denitrifying bacterial species exist that replace organic matter by reduced sulfur compounds as electron donors and that gain their carbon by CO_2 fixation, i.e. Thiobacillus denitrificans and Thiomicrospira denitrificans (29). These bacteria are anaerobic chemolithoautotrophs and fix CO_2 via the Calvin cycle.

Downloaded At: 08:34 30 January 2011

They are the only metabolic type besides phototrophs that can oxidize reduced sulfur compounds in the absence of oxygen. They are widely distributed in nature, but they rarely form conspicuous mass developments. The sulfur metabolism of Thiobacillus denitrificans has been studied in detail (3o-32) and, as molecular oxygen is not involved in it, has been found rather similar to that of phototrophic sulfur bacteria (33, and cf. below):
The enzymes APS reductase and siroheme sulfite reductase are present, further a thiosulfate-splitting enzyme that can be measured by its rhodanese (thiosulfate sulfur transferase) activity. The organism is able to store a labile form of sulfur (probably polysulfides) inside the cell (3o), not, however, in form of "sulfur globules" as they occur in some phototrophic bacteria. This sulfur is formed during thiosulfate splitting or during sulfide oxidation by cytochrome c_{552} (3o,34). It is oxidized to sulfite by a reverse siroheme sulfite reductase (3o,31). Fig. 3 gives an overall picture of sulfur metabolism in Thiobacillus denitrificans.

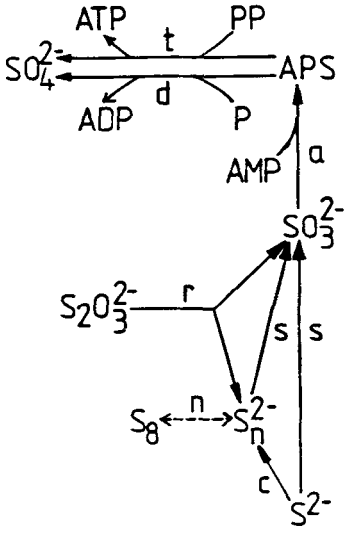


Fig.3: Dissimilatory sulfur metabolism in Thiobacillus denitrificans. a, APS reductase; c, cytochrome c 552; d, ADP sulfurylase; n, nonenzymatic step (?); r, thiosulfate sulfur transferase (rhodanese); s, reverse siroheme sulfite reductase; t, ATP sulfurylase.

Downloaded At: 08:34 30 January 2011

Under aerobic conditions there exists a wide variety of microorganisms able to grow while oxidizing reduced sulfur compounds. Their metabolic types include obligate chemolithoautotrophs (Thiobacillus and Thiomicrospira species), facultative chemolithoautotrophs (Thiobacillus species, Sulfolobus species, Thermotrix thiopara, Paracoccus denitrificans), chemolithoheterotrophs (Thiobacillus perometabolis, Pseudomonas species) chemoorganoheterotrophs (Pseudomonas species, Beggiatoa species, the fungus Aureobasidium pullulans, many bacteria including actinomycetes)(35-37). Also many morphologically wellknown bacteria considered as chemolithoautotrophs - although so far not really well studied with respect to their metabolism - are involved in the oxidation of sulfur compounds: Beggiatoa species, Thiothrix, Thiospira, Thermothrix, Thioploca, Thiovulum, Macromonas, Achromatium (synonym: Thiophysa), Thiobacterium (35,38,39). Some phototrophic sulfur bacteria perform an aerobic dark metabolism oxidizing reduced sulfur compounds. This property is, however, limited to certain species of the family Chromatiaceae, e.g. Chromatium vinosum, Thiocapsa roseopersicina and Thiocystis violacea (4o).

Of all aerobic groups mentioned above certainly the Thiobacillus species are best studied with respect to physiology, ecology and even economy. The sulfur metabolism of thiobacilli has recently been thoroughly reviewed by Kelly (41), who emphasized that the occurence of polythionates during aerobic sulfide/sulfur oxidation by thiobacilli is merely a byproduct due to non-biological reactions of sulfur compounds with oxygen and with each other. In principle, Kelly expressed the expert opinion that the "central pathway" of inorganic sulfur oxidation seems to operate with single-sulfur units with larger

Downloaded At: 08:34 30 January 2011

molecules of sulfur being peripheral to the main energy-yielding process. This means that in principle sulfur metabolism of aerobic thiobacilli is similar to that in denitrifying thiobacilli.

For aerobic sulfur oxidations free oxygen is of threefold importance: First, it is involved directly in respiratory electron transport as final electron acceptor; second, it is competing with the organism for reduced sulfur compounds in oxidizing these chemically; and third, it has been proven that oxygen is directly involved in the enzymatic oxidation of elemental sulfur in Thiobacillus thiooxidans (42) by a sulfur oxygenase.

Thiobacilli are responsible for naturally occurring and industrial ore leaching processes, rock weathering, concrete and building material deterioration. They are widely distributed in nature, especially in sulfur springs, in deep-sea hydrothermal vents, open sulfur ore deposits, acid mine waters, sulfur stockpiles, marine muds etc.. Thiobacilli-like bacteria have recently been found as endosymbionts in the tissues of invertebrate animals from sulfide-rich habitats, such as the large tubeworm Riftia pachyptila from pacific hydrothermal vents and certain other worms and molluscs (43,44).

The genera Beggiatoa, Thiothrix, Thioploca, Thiospira, Thiovulum, Macromonas, Achromatium and Thiobacterium, although of high environmental importance in marine, estuarine and sulfur spring environments (3,38,39,45,46), are less well studied than the thiobacilli, especially with respect to metabolic properties and their enzymatic pathways. The reasons herefore are that they require extremely delicate balancing of environmental parameters (such as O_2 and H_2S concentrations, pH, etc.) and therefore cannot be handled by conventional microbiological

Downloaded At: 08:34 30 January 2011

enrichment and pure culture techniques (39). As in the recent literature pure cultures of Beggiatoa, Thiothrix and Thiospira have been mentioned, an increase of information may be expected in the near future.

2. INORGANIC SULFUR COMPOUNDS AS ELECTRON DONORS FOR PHOTOSYNTHESIS

Phototrophic bacteria occur in a wide variety of anoxic environments such as stratified lakes, coastal lagoons, fjords, estuaries, intertidal flats, salt marshes, salt lakes, soda lakes, waste ponds, sewage lagoons. In nature, mass developments of phototrophic bacteria are obvious by their red, purple and green colors that are due to their light harvesting and reaction center pigments, carotenoids and bacteriochlorophylls. Although they are able to utilize a limited number of organic compounds they are as important primary producers, i.e., CO_2-fixing organisms in light anoxic as algae are in oxic environments.

The use of reduced sulfur compounds as electron donors for anoxygenic photosynthesis has been found in all groups of phototrophic eubacteria (47-49), i.e. in "purple bacteria" (families Rhodospirillaceae, Chromatiaceae, Ectothiorhodospiraceae), in "green bacteria" (families Chlorobiaceae, Chloroflexaceae) and in cyanobacteria (blue-green algae). In the Chlorobiaceae and Chromotiaceae all species oxidize sulfide and elemental sulfur, some also thiosulfate and sulfite. In the Rhodospirillaceae and the cyanobacteria not all species are able to utilize reduced sulfur compounds during photosynthesis.

In bacterial photosynthesis, ATP is generated by photophosphorylation during a cyclic electron flow, i.e. the light-excited reaction center bacteriochlorophyll emits

Downloaded At: 08:34 30 January 2011

Downloaded At: 08:34 30 January 2011

Table 1: Metabolic microbial types using inorganic sulfur compounds as electron donors (49)

Metabolic type	Mechanisms	Microorganisms
Chemotrophic Sulfur Oxidation -anaerobic-	El.-Don.: Sulfide, Sulfur, Thiosulfate, (Sulfite) El.-Acc.: Nitrate Product: Sulfate Carbon source: CO_2	Thiobacillus denitrificans Thiomicrospira denitrificans
Chemotrophic Sulfur Oxidation -aerobic-	El.-Don.: Sulfide, Sulfur, Thiosulfate, (Sulfite) El.-Acc.: O_2 Product: Sulfate Carbon source: CO_2 or organic compounds	Thiobacillus Thiomicrospira Sulfolobus Thermothrix Paracoccus Pseudomonas Beggiatoa Thiothrix Thiospira

Downloaded At: 08:34 30 January 2011

(Table 1 continued):

Metabolic type	Mechanisms	Microorganisms
		Thioploca Macromonas Achromatium Thiobacterium Chromatiaceae (dark, some species) Many heterotrophs
Phototrophic Sulfur Oxidation -anaerobic-	El.-Don.: Sulfide Sulfur Thiosulfate (Sulfite) Photosynthesis (CO_2 Fixation) Product: Sulfate Carbon source: CO_2 and/or organic compounds	Chlorobiaceae Chromatiaceae Ectothiorhodospiraceae Rhodospirillaceae (some) Chloroflexaceae Cyanobacteria (some)

an electron which reduces a "primary acceptor". From there, the electron flows back to the bacteriochlorophyll via a number of electron carriers including ubiquinones (or menaquinones) and cytochromes. This electron transport is arranged in membranes and leads to the generation of ATP following Mitchell's chemiosmotic hypothesis (27).

The hydrogen carrier required for CO_2 fixation, NAD(P), cannot be reduced directly by reduced sulfur compounds because its redox potential is -32o mV.

In the Chlorobiaceae the photosynthetic primary electron acceptor is a membrane-bound ferredoxin with a redox potential (E_o') of -54o mV (5o) thus sufficiently electronegative to reduce NAD(P). Indeed this reduction occurs via ferredoxin. The consequence is that electrons are taken out of the cyclic electron flow at a highly negative level. The resulting lack of electrons is balanced by an electron inflow to the reaction center from reduced sulfur compounds via cytochromes c. In the Rhodospirillaceae and perhaps the Chromatiaceae the redox potential of the primary acceptor is above that of $NADH_2$ (5o, 51). In these organisms the reduction of NAD occurs via an ATP-dependent reverse electron flow from reduced sulfur compounds via cytochromes and other electron carriers.

Photosynthetic carbon dioxide fixation and sulfide (or thiosulfate) oxidation are stoichiometrically linked by van Niel's (52) overall equations (Eq. 7-12) ($\langle CH_2O \rangle$ stands for organic matter):

$$\text{(Eq.7)} \qquad CO_2 + 2\ H_2S \xrightarrow{\text{light}} \langle CH_2O \rangle + H_2O + 2\ S^o$$

$$\text{(Eq.8)} \qquad 3\ CO_2 + 2\ S^o + 5\ H_2O \xrightarrow{\text{light}} 3\langle CH_2O \rangle + 2\ H_2SO_4$$

Downloaded At: 08:34 30 January 2011

(Eq.9) $2\ CO_2 + H_2S + 2\ H_2O \xrightarrow{light} 2\langle CH_2O\rangle + H_2SO_4$

(Eq.10) $CO_2 + 2\ Na_2S_2O_3 + H_2O \xrightarrow{light} \langle CH_2O\rangle + 2\ S^o + 2\ Na_2SO_4$

(Eq.11) $3\ CO_2 + 2\ S^o + 5\ H_2O \xrightarrow{light} 3\langle CH_2O\rangle + 2\ H_2SO_4$

(Eq.12) $2\ CO_2 + Na_2S_2O_3 + 3\ H_2O \xrightarrow{light} 2\langle CH_2O\rangle + H_2SO_4 + Na_2SO_4$

A great deal of information about the action of reduced sulfur compounds as photosynthetic electron donors has been derived from experiments with pure cultures, i.e. cell suspensions (or "whole cells") in contrast to work with cell extracts or purified enzymes.

Most species of phototrophic bacteria depend on or are at least capable of utilizing reduced sulfur compounds as electron donors. All organisms with this capacity utilize sulfide. Elemental sulfur is readily utilized by all Chromatiaceae, Ectothiorhodospiraceae, and Chlorobiaceae, whereas Rhodospirillaceae usually cannot oxidize it. The utilization of thiosulfate is more common in Chromatiaceae and Rhodospirillaceae than in Chlorobiaceae, and only very few species oxidize sulfite or tetrathionate (47).

During oxidation of sulfide, sulfur appears in the form of globules inside (Chromatiaceae) or outside the bacterial cells.

An interesting phenomenon is seen in the alkaliphilic Ectothiorhodospira species: The alkaline pH of their media allows the detection of polysulfides as intermediates of

Downloaded At: 08:34 30 January 2011

sulfide oxidation. The average chain lenghth of these has been found to be 4(53), i.e., that chain lenghths between 1 and 7 may occur. We suppose that the elemental sulfur originating from sulfide is immediately reacting with surplus sulfide (at alkaline pH values) thus forming polysulfide. As soon as the sulfide is exhausted the bacteria start to oxidize the polysulfides and immediately elemental sulfur globules appear outside the cells (53,54). Although the pH inside Chromatiaceae and the optimal pH for the Chlorobiacease and the optimal pH for the Chlorobiaceae is rather close to 7.o, i.e., polysulfides are much less stable under these conditions, the formation of intra- and extracellular "sulfur globules" may follow the same scheme in a different time relationship.

Evidence is accumulating that these "sulfur globules" are not elemental sulfur but rather large globular agglomerates of long chain polysulfides. Besides by their globular non-crystaline shape this is supported by activation energy measurements during cyanolysis (9,53,54) a lack of sulfur ring molecules (R. Steudel, pers. comm.), hydrophilic properties (53,9) and density measurements (55).

During growth of Chromatiaceae on thiosulfate "sulfur globules" appear inside the cells. They are entirely derived from the sulfane group of thiosulfate (56,57). Of the green thiosulfate-using bacteria only Chlorobium vibrioforme forma thiosulfatophilum forms extracellular sulfur globules from thiosulfate (58).

Rhodopseudomonas sulfoviridis oxidizes sulfide via intermediate intracellular sulfur which, however, does not appear as microscopically visible globules inside the cells, although it has been chemically identified. It probably represents long chain polysulfides.

Only in Rhodobacter sulfidophilus sulfite is excreted in

Downloaded At: 08:34 30 January 2011

measurable amounts as an intermediate in sulfide and thiosulfate oxidation (59). Rhodopseudomonas marina produces thiosulfate from sulfide (6o). Thiosulfate formation from sulfide was found to occur in Chlorobium limicola f. thiosulfatophilum alongside with the formation of elemental sulfur (61). It was proven that in this species neither thiosulfate is an intermediate of elemental sulfur oxidation, nor is elemental sulfur an intermediate of thiosulfate oxidation (61). Chlorobium vibrioforme f. thiosulfatophilum also forms thiosulfate plus elemental sulfur from sulfide but the formation of sulfur continues at the expense of thiosulfate after sulfide is exhausted (58).

In batch cultures of Rhodomicrobium vannielii (family Rhodospirillaceae) Hansen detected simultaneous thiosulfate and elemental sulfur formation during growth on sulfide (62). He proved, however, by chemostat cultures, that this was due to a formation of tetrathionate which chemically reacted with the residual sulfide forming thiosulfate plus sulfur.

This chemical reaction mechanism does not account for the occurence of thiosulfate plus sulfur in cultures of Chlorobium limicola f. thiosulfatophilum, because there polythionates could not be detected (61).

Tetrathionate is formed from thiosulfate by Chromatium vinosum at pH values below 7.0 (63), by Rhodopseudomonas palustris (when concentrations of thiosulfate above 1o mM are present) (64), and by Rhodopila globiformis (always) (65).

In Chromatiaceae and most other phototrophic bacteria, sulfate is the end product of sulfur oxidation, although the oxidation of extracellular sulfur by Chlorobiaceae and Ectothiorhodospiraceae is often incomplete.

Downloaded At: 08:34 30 January 2011

Rhodospirillum rubrum, Rhodobacter capsulatus, and Rb. sphaeroides cannot further oxidize elemental sulfur formed from sulfide (47).

The present state of knowledge on the enzymology of photolithotrophic sulfur metabolism in phototrophic bacteria is summarized in Fig. 4.

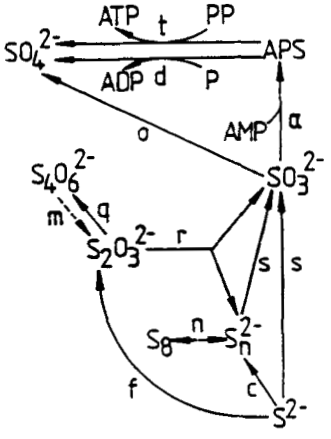


Fig.4: Pathways of light/anaerobic dissimilatory sulfur metabolism in phototrophic bacteria. a, APS reductase; c, cytochrome c; d, ADP sulfurylase; f, flavocytochrome c; m, chemical reduction of tetrathionate by H_2S; n, non-enzymatic step (?); o, sulfite: acceptor oxidoreductase; q, thiosulfate: acceptor oxidoreductase (tetrationase); r, thiosulfate sulfur transferase (rhodanese); s, reverse siroheme sulfite reductase; t, ATP sulfurylase.

The enzymatic steps in Fig. 4 are not common to all phototrophic bacteria but depict the different pathways for which evidence exists in different bacterial species.

Figure 4 shows that all sulfur compounds oxidized to sulfate have to pass through sulfite.

The scheme allows to differentiate three metabolic sections with a group of enzymes each:

a: oxidation of sulfide and elemental sulfur

b: utilization of thiosulfate

c: oxidation of sulfite

Downloaded At: 08:34 30 January 2011

Sulfide may be utilized by 3 different enzymes leading to polysulfides, thiosulfate, or sulfite, respectively. The enzymatic step from sulfide to polysulfides and sulfur clearly involves cytochromes of the c type (66, 53, 9) as biocatalysts and electron acceptors.

The formation of thiosulfate from sulfide is catalyzed by flavocytochrome c as it occurs in Chromatium and Chlorobium species (67).

A "reverse" siroheme-containing sulfite reductase is responsible for sulfite formation from sulfide directly as well as from polysulfides and/or elemental sulfur. This enzyme has been purified from Chromatium vinosum and characterized (33).

Thiosulfate undergoes splitting to elemental sulfur and sulfite catalyzed by an enzyme that may be measured by its rhodanese activity. There is some evidence from work with Thiobacillus denitrificans and comparison with Chromatium vinosum, that this enzyme may be identical with thiosulfate reductase (31,61).

Chlorobium limicola forma thiosulfatophilum (68), Chromatium vinosum (63), Rhodopseudomonas palustris (69), and Rhodopila globiformis (65) contain thiosulfate: acceptor oxidoreductase, earlier called tetrathionase, forming tetrathionate from thiosulfate.

An enzyme oxidizing or splitting tetrathionate has not been found in phototrophic bacteria, so far. As mentioned above, however, under natural environmental conditions a chemical reduction of tetrathionate by sulfide may readily occur.

The enzyme APS reductase, an essential enzyme in dissimilatory sulfate-reducing bacteria (see below) and two Thiobacillus species was found to occur also in

Downloaded At: 08:34 30 January 2011

Chromatiaceae and Chlorobiaceae (7o,71). The enzyme was purified from Thiocapsa roseopersicina (72) and Chlorobium limicola f. thiosulfatophilum (73). In Chromatium vinosum (74) and Chr. warmingii (W. Leyendecker, pers. comm.) it is firmly membrane-bound and cannot be solubilized. Species of Ectothiorhodospira and the Rhodospirillaceae do not possess APS reductase (47, 48). Also Chromatium purpuratum and Chr. gracile lack this enzyme (48 and H.M. Ulbricht, pers. comm.).

The intermediary formation of APS demands an additional enzyme to split off the sulfate moiety. This step allows conservation of the phosphate binding energy contained in APS.

The enzyme ADP sulfurylase replaces the sulfate moiety of APS by inorganic phosphate thus producing adenosine-diphosphate (Eq. 13).

(Eq. 13) $\quad APS + P_i \longrightarrow ADP + SO_4^{2-}$

ADP then can be disproportionated by the enzyme adenylate kinase with the result of 1 ATP + 1 AMP per 2 ADP.

This pathway has been found in Chlorobium vibrioforme f. thiosulfatophilum (48, U. Bias, pers. comm., 75) and six species of the Chromatiaceae (48, H.M. Ulbricht, pers. comm.) not, however, in Rhodospirillaceae.

Another pathway to conserve the energy of APS is the replacement of its sulfate moiety by inorganic pyrophosphate, catalyzed by the action of ATP sulfurylase. Inorganic pyrophosphate is a common product of biosynthetic reactions, especially of protein biosynthesis, and therefore readily available during the exponential growth phase of cells. ATP sulfurylase has been shown to catalyze this reaction in Chlorobium limicola f. thiosulfato-

Downloaded At: 08:34 30 January 2011

Downloaded At: 08:34 30 January 2011

Table 2: Metabolic microbial types using inorganic sulfur compounds as electron acceptors (49)

Metabolic type	Mechanisms	Microorganisms
Dissimilatory Sulfate Reduction -anaerobic-	El.-Acc.: Sulfate Thiosulfate Sulfite El.-Don.: Organic compounds or H_2 Product: H_2S Carbon source: organic compounds or CO_2	Desulfovibrio Desulfotomaculum Desulfomonas Desulfobacter Desulfobulbus Desulfococcus Desulfosarcina Desulfonema Thermodesulfobacterium
Dissimilatory Sulfur Reduction -anaerobic-	El.-Acc.: Sulfur El.-Don.: Organic compounds or H_2 Product: H_2S Carbon source: organic compounds or CO_2	Desulfuromonas Desulfovibrio (some) Campylobacter (some) Wolinella Beggiatoa Thermoproteus Thermodiscus

Downloaded At: 08:34 30 January 2011

(Table 2 continued)

Metabolic type	Mechanisms	Microorganisms
		Pyrodictium Thermococcus Thermofilum Desulfurococcus many methanogenic bacteria
Fermentation during maintenance in the dark -anaerobic-	El.-Acc.: Sulfur El.-Don.: Carbo-hydrates Product: H_2S	Chromatiaceae Ectothiorhodospiraceae Chlorobiaceae

philum (U. Bias, pers. comm.).
An enzyme bypassing the formation of APS in phototrophic bacteria is sulfite: acceptor oxidoreductase (47,48) (Eq. 14):

(Eq. 14) $SO_3^{2-} + H_2O \longrightarrow SO_4^{2-} + 2\ e^- + 2\ H^+$

This enzyme occurs in most Chromatiaceae in addition to the APS pathway, in a few cases instead of the latter (H.M. Ulbricht, pers. comm.); it was also found in Rhodobacter sulfidophilus (59).

D. INORGANIC SULFUR COMPOUNDS AS ELECTRON ACCEPTORS IN ANAEROBIC RESPIRATION AND FERMENTATION

Theoretically all reducible inorganic sulfur compounds may serve as electron acceptors. A list of respective microorganisms is presented in Table 2.

Downloaded At: 08:34 30 January 2011

1. DISSIMILATORY SULFATE REDUCTION

The most abundant sulfur compound in nature is sulfate, and the most common process of anaerobic respiration in this context is dissimilatory sulfate reduction.
This process is carried out by strictly anaerobic bacteria in anoxic environments where sulfate is available. The majority of bacterial species involved in dissimilatory sulfate reduction are chemoorganoheterotrophic, i.e., they rely upon organic carbon compounds as electron donors and carbon source. They are, together with methanogenic bacteria, the most important final consumers of organic compounds in the anaerobic food chain in nature, predominantly in the aquatic environment (36,45). The main carbon sources of sulfate-reducing bacteria are

fatty acids with up to 18 carbon atoms. Also ethanol and benzoate are utilized. Some of the sulfate-reducing bacteria are chemolithoautotrophic, i.e. able to grow with sulfate, molecular hydrogen and carbon dioxide (76). The ability of these bacteria to use H_2 as an electron donor leads to cathodic depolarization on the surface of submerged iron and steel structures such as drilling rigs, pipelines etc., thus having corrosive effects of enormous economic consequences.

The bacterial species involved in dissimilatory sulfate reduction exhibit a wide variety of morphological forms (76,77). The most abundant numbers of sulfate reducing bacteria are found in the anoxic sediments of the oceans, in estuarine intertidal mud flats ("Watten"), in salt marshes, fresh water sediments, meromictic lakes and lagoons as well as in sewage. Their presence is usually indicated by strong H_2S smell and black mud due to FeS precipitation. The pathway of sulfur metabolism in dissimilatory sulfate-reducing bacteria consists of the enzymes adenosine triphosphate sulfurylase (Eq.4), APS reductase (Eq.15) and siroheme sulfite reductase (Eq.16) catalyzing one activation and two reductive steps, respectively.

Downloaded At: 08:34 30 January 2011

(Eq.15) $$APS + 2\ e^- \longrightarrow AMP + SO_3^{2-}$$

(Eq.16) $$SO_3^{2-} + 6\ e^- + 8\ H^+ \longrightarrow H_2S + 3\ H_2O$$

In Desulfovibrio species ATP sulfurylase is pulled towards APS formation by inorganic pyrophosphatase, whereas in Desulfotomaculum the binding energy of pyrophosphate is conserved by transfer upon acetate to form acetyl phosphate (78).

Most sulfate reducing bacteria can also reduce sulfite and thiosulfate. For the latter they possess an active thiosulfate reductase (78). There exist detailed recent reviews on the biochemistry of sulfur metabolism in dissimilatory sulfate-reducing bacteria (15,78).

2. DISSIMILATORY SULFUR REDUCTION

Bacteria demanding elemental sulfur as an electron acceptor during oxidation of organic carbon compounds (ethanol, acetate) were first discovered by Pfennig and Biebl (79) and named Desulfuromonas. Dissimilatory sulfur reduction is not restricted to Desulfuromonas. Also several Desulfovibrio species (80), Wolinella succinogenes and Campylobacter strains are able to grow with elemental sulfur as the sole electron acceptor (81,82).

The discovery of a deep phylogenetic gap between the "archaebacteria" and the "eubacteria" by Woese and Fox (83), the latter comprising almost all hitherto known bacteria, the former including mainly groups living in extreme environments (extremely halophilic bacteria, methanogenic bacteria, acidothermophilic bacteria) has led to an enforced search for "new" archaebacteria. The groups of Zillig and Stetter have found a number of anaerobic acidothermophilic archaebacteria depending on sulfur reduction in sulfataras: Thermoproteus tenax (84), Desulfurococcus mucosus and D. mobilis (85), Thermodiscus maritimus (86), Thermofilum pendens (87), Thermococcus celer (88). The optimal growth temperatures of these species range between 85° and 90°C, the optimal pH values between 5.2 and 6.8 (86). Further, Pyrodictium occultum with an optimal growth temperature of 105°C was described by Stetter (89,86). The recently detected ability of methanogenic bacteria to reduce

Downloaded At: 08:34 30 January 2011

elemental sulfur (8) may turn out as an important electron sink during their metabolism as well as a mechanism of self-maintaining their anaerobic environments.

3. SULFUR FERMENTATION

Under anaerobic conditions in the dark phototrophic sulfur bacteria (Chromatiaceae, Ectothiorhodospiraceae, Chlorobiaceae) perform a fermentative metabolism during which elemental sulfur acts as an electron sink for electrons from carbohydrate fermentation. Under these conditions - and darkness probably occupies more of their life than light - they slowly produce H_2S. This aspect of sulfur metabolism has been carefully studied quantitatively in Chromatium vinosum (90) but enzymatic studies are still lacking.

This process is certainly not an anaerobic respiration but clearly a fermentation.

The flavocytochrome c-552 of Chr. vinosum has been reported to possess elemental sulfur reductase activity (91), forming sulfide. The flavocytochrome of Chlorobium vibrioforme f. thiosulfatophilum, however, does not possess this activity (58).

Also the sulfide oxidizing bacterium Beggiatoa (see above) has been reported to reduce elemental sulfur under anaerobic conditions (92).

One should expect that many more bacteria are able to make use of elemental sulfur (and perhaps thiosulfate and sulfite) as an electron sink under strictly anaerobic conditions - as long as such compounds are available.

The anaerobic fermenting bacterium Clostridium pasteurianum possesses a sulfite reductase, reducing sulfite to sulfide, that is considered a dissimilatory enzyme (93). This organism is not capable, however, of dissimilatory sulfate reduction.

Downloaded At: 08:34 30 January 2011

E. "DETOXIFYING" SULFUR OXIDATION

Recently, Dubinina and Grabovich (94) succeeded in culturing the colorless sulfur bacterium Thiospira winogradskii, that turned out to be a heterotrophic, aerobic bacterium. The authors found that the deposition of intracellular sulfur globules in this species is due to a non-specific reaction between sulfide and hydrogen peroxide. When thiosulfate was added to Thiospira cultures, it was oxidized to tetrathionate by H_2O_2. When organic nutrients were added, e.g., succinate the cells started to form H_2O_2. Both, sulfide and thiosulfate oxidation was blocked by the addition of the enzyme catalase It therefore appears that in catalase-lacking bacteria reduced sulfur compounds may have a detoxifying importance in destroying toxic H_2O_2.

The occurence of intracellular sulfur globules in the nonsulfur bacterium Sphaerotilus natans (95) that lives in polluted waters, may be due to a lack of catalase. In this respect it is interesting that addition of catalase has been found to improve the culture conditions for the large filamentous colorless sulfur bacterium Beggiatoa (96).

A symbiotic relationship in the rizosphere of rice plants involving Beggiatoa is most probably responsible for detoxifying that rizosphere of H_2S (97): The plant is protected by Beggiatoa oxidizing H_2S to intracellular sulfur globules and a plant root catalase in turn protects Beggiatoa from autointoxication by decomposing its self-produced H_2O_2 (97, 98).

The soil amoeba Acanthamoeba castellani appears to possess two mechanisms for oxidative detoxification of H_2S (99) neither of which has been fully elucidated.

The detoxifying importance of microbial sulfide oxidation

Downloaded At: 08:34 30 January 2011

in nature is neither assessed quantitatively nor is it fully understood. The detoxifying effect may be obviously self protective for single microbial species as well as a mutual symbiotic one for large habitats such as rice plantations, salt marshes and other estuarine habitats.

REFERENCES

(1) P.A. Trudinger, Phil.Trans.R.Soc.Lond.B. 298, 563-581 (1982).
(2) N.M. Kredich and G.M. Tomkins, J. biol. Chem. 241, 4955-4965 (1966).
(3) L.A. Chambers and P.A. Trudinger, Arch. Mikrobiol. 77, 165-184 (1971).
(4) G. Hensel and H.G. Trüper, Arch. Microbiol. 1o9, 1o1-1o3 (1976).
(5) H. Cherest, F. Eichler and H. de Robichon-Szulmajster, J. Bacteriol. 97, 328-336 (1969).
(6) H.G. Trüper, in The Photosynthetic Bacteria, edited by R.K. and W.R. Sistrom (Plenum Publishing Corporation, New York, 1978), Chap. 35, pp. 677-69o.
(7) L.I. Vorobeva and I.A. Charakhchyan, Mikrobiologiya 52, 875-879 (1983).
(8) K.O. Stetter and G. Gaag, Nature (Lond.), 3o5, 3o9-311 (1983).
(9) J. Then and H.G. Trüper, Arch. Microbiol. 139, 295-298, 1984.
(1o) G. Hensel and H.G. Trüper, Arch. Microbiol. 134, 227-232 (1983).
(11) J.F. Imhoff, H.G. Trüper and N. Pfennig, Internat. J. System. Bacteriol. 34, 34o-343 (1984).
(12) G. Hensel and H.G. Trüper, Arch. Microbiol. 13o, 228-233 (1981).
(13) T. Nakamura and R. Sato, Seikagaku 37, 1-13 (1965).
(14) T.S. Moodin and I.H. Segel, Biochim. Biophys. Acta 167, 78-88 (1968).
(15) P.A. Trudinger and R.E. Loughlin in Amino Acid Metabolism and Sulphur Metabolism (Vol.19A of Comprehensive Biochemistry, edited by M. Florkin and E.H. Stotz), edited by A. Neuberger and L.L.M. van Deenen (Elsevier, Amsterdam, 1981), Chap. 3, pp. 165-256.
(16) H.D. Peck, jr., in The Enzymes (3rd. ed. Vol. 1o), edited by P.D. Boyer (Academic Press, New York,

Downloaded At: 08:34 30 January 2011

1974) Chap. 21, pp. 651-669.
(17) L.M. Siegel in Metabolism of Sulfur Compounds (Metabolic Pathways, 3rd. ed., Vol.7), edited by D.M. Greenberg (Academic Press, New York, 1975) Chap.7, pp. 217-286.
(18) P.W. Robbins and F. Lipmann, J.Biol.Chem. 233, 685-69o (1958).
(19) H.G. Jender and J.D. Schwenn, Arch. Microbiol. 138, 9-14 (1984).
(2o) A. Schmidt, FEMS Microbiol. Lett. 1, 137-14o (1977).
(21) A. Schmidt and H.G. Trüper, Experientia 33, 1oo8-1oo9 (1977).
(22) J.F. Imhoff, Arch. Microbiol. 132, 197-2o3 (1982).
(23) H.G. Trüper in Handbook of Biosolar Resources (Vol.1, Part 1) edited by A. Mitsui and C.C. Black, jr. (CRC Press, Boca Raton, 1982) pp. 283-287.
(24) M.L.S. Tsang and J.A. Schiff, Plant Physiol. 56, 36 (1975).
(25) W. Dott, Doctoral Thesis, Univ. of Bonn, Germany 1977, 245 pp.
(26) A. Trebst in Handbook of Biosolar Resources (Vol 1, Part 1) edited by A. Mitsui and C.C. Black, jr. (CRC Press, Boca Raton, 1982) pp. 289-298.
(27) P. Mitchell, Chemiosmotic Coupling in Oxidative and Photosynthetic Phosphorylation (Glynn Research, Bodmin, 1966), 192 pp.
(28) M. Wheelis, Arch. Microbiol. 138, 166-169 (1984).
(29) J.G. Kuenen and O.H. Tuovinen in The Prokaryotes edited by M.P. Starr, H. Stolp, H.G. Trüper, A. Balows and H.G. Schlegel, Springer-Verlag, Berlin/ Heidelberg/New York, 1981), Chap. 81, pp. 1o23-1o36.
(3o) M. Schedel and H.G. Trüper, Arch. Microbiol. 124, 2o5-21o (198o).
(31) M. Schedel and H.G. Trüper, Biochim. Biophys. Acta, 568, 454-467 (1979).
(32) T.J. Bowen, F.C. Happold and B.F. Taylor, Biochim. Biophys. Acta 118, 566-576 (1966).
(33) M. Schedel, M. Vanselow and H.G. Trüper, Arch. Microbiol. 121, 29-36 (1979).
(34) J.P. Aubert, J. Millet and G. Milhaud, Annal.Inst. Pasteur 96, 559-576 (1959).
(35) J.G. Kuenen and R.F. Beudeker, Phil. Trans. R. Soc. Lond. B, 298, 473-497 (1982).
(36) N. Pfennig and F. Widdel, Phil.Trans.R.Soc.Lond.B., 298, 433-441 (1982).
(37) K. Killham, N.D. Lindley and M. Wainwright, Appl. Envir. Microbiol. 42, 629-631 (1981).
(38) W. Wiessner in The Prokaryotes edited by M.P. Starr, H. Stolp, H.G. Trüper, A. Balows and H.G. Schlegel

Downloaded At: 08:34 30 January 2011

(Springer Verlag, Berlin/Heidelberg/New York, 1981) Chap. 22, pp. 380-389.
(39) J.W.M. la Riviere and K. Schmidt in The Prokaryotes edited by M.P. Starr, H. Stolp, H.G. Trüper, A. Balows and H.G. Schlegel (Springer Verlag, Berlin/Heidelberg/New York, 1981) Chap. 82, pp. 1037-1048.
(40) C. Kämpf and N. Pfennig, Arch. Microbiol. 127, 125-137 (1980).
(41) D.P. Kelly, Phil.Trans.R.Soc.Lond.B. 298, 499-528 (1982).
(42) I. Suzuki, Biochim.Biophys.Acta 110, 97-101 (1965).
(43) A.J. Southward, E.C. Southward, P.R. Dando, G.H. Rau, H. Felbeck and H. Flügel, Nature (Lond.) 293, 616-620 (1981).
(44) H. Felbeck, J.J. Childress and G.N. Somero, Nature (Lond.) 293, 291-293 (1981).
(45) B.B. Jørgensen, Phil.Trans.R.Soc.Lond.B. 298, 543-561 (1982).
(46) H. Troelsen and B.B. Jørgensen, Estuar.Coastal Shelf Sci. 15, 255-266 (1982).
(47) H.G. Trüper in Biology of Inorganic Nitrogen and Sulfur edited by H. Bothe and A. Trebst (Springer-Verlag, Berlin/Heidelberg/New York, 1981) pp. 199-211.
(48) H.G. Trüper and U.Fischer, Phil.Trans.R.Soc.Lond.B 298, 529-542 (1982).
(49) H.G. Trüper in Sulfur, its Significance for Chemistry, for the Geo-, Bio- and Cosmosphere and Technology edited by A. Müller and B. Krebs (Studies in Inorganic Chemistry, Vol.5) (Elsevier, Amsterdam, 1984) pp. 367-382.
(50) D.B. Knaff in The Photosynthetic Bacteria edited by R.K. Clayton and W.R. Sistrom (Plenum, New York, 1978) Chap. 32, pp. 629-640.
(51) R. van Grondelle, L.N.M. Duysens, J.A. van der Wel and H.N. van der Wal, Biochim. Biophys. Acta 461, 188-201 (1977).
(52) C.B. van Niel, Arch. Mikrobiol. 3, 1-112 (1931).
(53) J. Then and H.G. Trüper, Arch. Microbiol. 135, 254-258 (1983).
(54) J. Then, Doctoral Thesis, Univ. Bonn, Germany, 1984, 133 pp.
(55) R. Guerrero, J. Mas and C. Pedros-Alio, Arch. Microbiol.137, 350-356 (1984).
(56) A.J. Smith, Biochem. J. 94, 27 p (1965).
(57) H.G. Trüper and N. Pfennig, Antonie van Leeuwenhoek J. Microbiol. Serol. 32, 261-271 (1966).
(58) M. Steinmetz and U. Fischer, Arch. Microbiol. 131, 19-26 (1982).
(59) O. Neutzling, C. Pfleiderer and H.G. Trüper, J. Gen. Microbiol. (in Press) (1984).
(60) J.F. Imhoff, System. Appl. Microbiol. 4, 512-521 (1983).

Downloaded At: 08:34 30 January 2011

(61) M. Schedel, Doctoral Thesis, Univ. Bonn, Germany, 1977; 15o pp.
(62) T.A. Hansen, Doctoral Thesis, Univ. Groningen, Netherlands, 1974; 69 pp.
(63) A.J. Smith, J. Gen. Microbiol. 42, 371-38o (1966).
(64) N.A. Rodova and L.V. Pedan, Mikrobiologiya 49, 221-226 (198o).
(65) J. Then and H.G. Trüper, Arch. Microbiol. 13o, 143-146 (1981).
(66) U. Fischer and H.G. Trüper, FEMS Microbiol. Lett. 1, 87-9o (1977).
(67) U. Fischer, Doctoral Thesis, Univ. Bonn, Germany, 1977, 16o pp.
(68) A. Kusai and T. Yamanaka, Biochim. Biophys. Acta 325, 3o4-314 (1973).
(69) K. Knobloch, J.H. Eley and M.I.H. Aleem, Arch. Microbiol. 8o, 97-114 (1971).
(7o) H.H. Thiele, Antonie van Leeuwenhoek J. Microbiol. Serol. 34, 35o-356 (1968).
(71) H.G. Trüper and H.D. Peck, jr. Arch. Mikrobiol. 73, 125-142 (197o).
(72) H.G. Trüper and L.A. Rogers, J. Bacteriol. 1o8, 1112-1121 (1971).
(73) J. Kirchhoff and H.G. Trüper, Arch. Microbiol. 1oo, 115-12o (1974).
(74) J.D. Schwenn and M. Biere, FEMS Microbiol. Lett. 6, 19-22 (1979).
(75) S. Khanna and D.J.D. Nicholas, J. Gen. Microbiol. 129, 1365-137o (1983).
(76) F. Widdel, Doctoral Thesis, Univ. Göttingen, Germany, 198o, 443 pp.
(77) N. Pfennig, F. Widdel and H.G. Trüper in The Prokaryotes, edited by M.P. Starr, H. Stolp, H.G. Trüper, A. Balows and H.G. Schlegel (Springer-Verlag, Berlin/Heidelberg/(New York,1981) Chap. 74, pp. 926-94o.
(78) H.D.Peck, jr., and J. LeGall. Phil. Trans. R. Soc. Lond. B., 298, 443-466 (1982).
(79) N. Pfennig and H. Biebl, Arch. Microbiol. 11o, 3-12 (1976).
(8o) H. Biebl and N. Pfennig, Arch. Microbiol. 112, 115-117 (1977).
(81) R.S. Wolfe and N. Pfennig, Appl. Envir. Microbiol. 33, 427-433 (1977).
(82) H.J. Laanbroek, L.J. Stal and H. Veldkamp, Arch. Microbiol. 119, 99-1o2 (1978).
(83) C.R. Woese and G.E. Fox, Proc. Natl. Acad. Sci. (Wash.) 74, 5o88-5o9o (1977).

Downloaded At: 08:34 30 January 2011

(84) W. Zillig, K.O. Stetter, W. Schäfer, D. Janekovic, S. Wunderl, I. Holz and P. Palm, Zbl. Bakt. Hyg. I. Abt. Orig. C, 2, 2o5-227 (1981).
(85) W. Zillig, K.O. Stetter, D. Prangishvilli, W. Schäfer, S. Wunderl, D. Janekovic, I. Holz and H. Palm, Zbl. Bakt. Hyg. I. Abt. Orig. C. 3, 3o4-317 (1982).
(86) F. Fischer, W. Zillig, K.O. Stetter and G. Schreiber, Nature (Lond.), 3o1, 511-513 (1983).
(87) W. Zillig, A. Gierl, G. Schreiber, S. Wunderl, D. Janekovic, K.O. Stetter and H.P. Klenk, System. Appl. Microbiol. 4, 79-87 (1983).
(88) W. Zillig, I. Holz, D. Janekovic, W. Schäfer and W.D. Reiter, System. Appl. Microbiol. 4, 88-94 (1983).
(89) K.O. Stetter, Nature (Lond.) 3oo, 258-26o (1982).
(9o) H. van Gemerden, Arch. Mikrobiol. 64, 118-124 (1968).
(91) Y. Fukumori and T. Yamanaka, J. Biochem. (Tokyo), 85, 14o5-1414 (1979).
(92) D.C. Nelson and R.W. Castenholz, J. Bacteriol. 147, 14o-154 (1981).
(93) G. Harrison, C. Curle and E.J. Laishley, Arch. Microbiol. 138, 72-78 (1984).
(94) G.A. Dubinina and M.Yu. Grabovich, Mikrobiologiya 52, 5-12 (1983).
(95) V.B.D. Skerman, G. Dementjeva and B.J. Carey, J. Bacteriol. 73, 5o4-512 (1957).
(96) S.D. Burton and R.Y. Morita, J. Bacteriol. 88, 1755-1761 (1964).
(97) M.M. Joshi and J.P. Hollis, Science 195, 179-18o (1977).
(98) A.D. Heritage and R.C. Foster, Microb. Ecol. 1o, 115-121 (1984).
(99) D. Lloyd, B. Kristensen and H. Degu, J. Gen. Microbiol. 126, 167-17o (1981).

Downloaded At: 08:34 30 January 2011