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Geomicrobiological Aspects of the Oxidation of Reduced Sulfur Compounds by Photosynthesizing Bacteria

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Abstract

The activity of photosynthesizing sulfur bacteria in a continuous culture was studied. The bacteria were isolated from the natural environment with the use of the Winogradski column. Isolated bacteria were cultured in synthetic medium and in the effluent from the column containig HS-. Sulphides, the main product of reduction of sulfates in phosphogypsum, were used by green sulfur bacteria in the photosynthetic column. Almost 70% reduction of the concentration of sulfides was observed. After the experiment, diffractometric methods where employed to analyze the sediment from the column.

Key words: *Chlorobium sp*, photosynthesizing sulfur bacteria, sulfate reducing bacteria, sulfidogenesis, sulfur cycle

Introduction

In nature, the photosynthesizing sulfur bacteria play a very important role in the sulfur cycle, especially in those water environments in which reduced inorganic sulfur compounds are present as the result of the biological reduction of sulfates. Sulfide is one of the main substrates which can be used by many autotrophic microorganisms such as colorless sulfur bacteria such as the genus *Thiobacillus* and photosynthesizing sulfur bacteria which can conduct the light – dependent process – photosynthesis. The second group is widely distributed in the natural environment and is represented by numerous genera such as *Chlorobium* and *Chromatium*. However, this group is not homogeneous. It can be divided into four subgroups. Green sulfur bacteria (*Chlorobiales*), conducting photosynthesis which are strictly (obligatory) autotrophic and anaerobic microorganisms (Overmann and Tuschak 1997; Alexander *et al.*, 2002). These bacteria can fix carbon dioxide by reversed TCA cycle and can use sulfides as a donor of electrons (Eraso and Kaplan, 2001). In the European climate, they grow in narrow zones in fresh water ecosystems (stratified lakes) with no oxygen, but there is enough light for bacterial photosynthesis. These bacteria can store sulfur as a reserve material outside the cell. Such species as: *Chlorobium limicola*, *C. thiosulfatophilum*. Purple sulfur bacteria (*Chromatiales*) belong here – an enormous group including microorganisms which can also fix carbon dioxide and use sulfides as an electron donor. Unlike in the previous group, in this case sulfur is stored inside the cell (exception: subfamily – *Ectothiorhodospiraceae*). These bacteria also occur in stratified lakes (Gemerden, 1986; Eraso and Kaplan, 2001). Purple nonsulfur bacteria – a very interesting group including microorganisms capable of photosynthesis, but often only using it as an energy source. The source of carbon is an external organic compound, therefore these bacteria are photoheterotrophs. This process is also present in the fourth group – green nonsulfur bacteria, with such species as *Chloroflexus aurantiacus* (Eraso and Kaplan, 2001).

In the natural environment bacterial photosynthesis is inseparably connected with sulfidogenesis, a process driven by sulfate reducing bacteria (SRB).

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These bacteria can use sulfate as an final acceptor of hydrogen in the respiratory chain, simultaneously utilizing organic compounds. Organic substances, which can be used by SRB, include: organic acids, alcohols, even some aromatic compounds (Fauque *et al.*, 1991; Hansen 1994; Colleran *et al.*, 1995; Hao *et al.*, 1996). Since the biological reduction of sulfate is strictly anaerobic, this process occurs first of all in sediments of lakes and seas. If more sulfates are dissolved in the water, the development of SRB may lead to generating a significant amount of sulfides and hydrogen sulfide. On the other hand, sulfides are a good substrate for green and purple sulfur bacteria, growing also in anaerobic conditions (Overmann and Gemerden, 2000; Overmann, 2001). The effect of growth is the production of sulfate or, depending on conditions, accumulation of sulfur and other mineral compounds in the environment. From the geological point of view, the origin of deposits sulfur is probably connected with reactions depending on SRB and sulfur bacteria. It is generally accepted that bacteria of various kinds play a significant role in geochemical cycles of C, N, S, P and in other geologic processes, like mineral diagenesis and dissolution. It seems certain that since the early Precambrian the activity of microbes has had an impact on the evolution of the Earth's surface. Microbial activity has also had an impact on the composition of the atmosphere (Ehrlich, 1998; Fortin and Langley, 2005).

This study has been undertaken to determine the possibilities of generating sulfur and other mineral substances resulting from the presence of photosynthesizing sulfur bacteria. The obtained date could contribute to our knowledge about interactions between bacteria involved in the sulfur cycle in respect of both geomicrobiological point of view and the possibility of utilizing these bacteria in the treatment of sulfate – rich wastewater.

Experimental

Materials and Methods

Isolation and cultivation of microorganisms. Photosynthetic sulfur bacteria were isolated from Lake Piaseczno near Łęczna (Pojezierze Łęczyńsko-Włodawskie, Poland). Two samples, consisting of water and sediment (about 1:1), were taken near the shore of the lake. This material was used as an inoculum for the Winogradski Column, which is a method of enrichment cultivation. The material from the lake, water supplemented with NaCl (1%) and phosphogypsum (0.6 g) was put into the column (V = 900 ml). The culture was maintained in the light (about 500 lux) at 28–30°C. After two weeks, the obtained culture of green sulfur bacteria was used in the main experiment in bed reactor with glass beads, called a photosynthetic column (V = 1020 ml). The medium fed into the bed reactor with a peristaltic pump was the modified Pfennig's solution and contained: KH_2PO_4 – 0.3 g, KCl – 0.34 g, NH_4Cl – 0.34 g, $CaCl_2$ – 0.11 g, $MgSO_4$ – 0.5 g, $NaHCO_3$ – 15 ml of 10% solution, $Na_2S_2O_3$ – 0.5 g, $Na_2S \times 9H_2O$, CH_3COONa – 0.12 g, thioglycolate – 0.1 g, yeast extract – 0.5 g, distilled water – 1l, pH – 6.7.

Dilution time (D) is a parameter characterizing velocity of medium flow in column in time. This parameter is expressed by the equation:

$$D = \frac{f}{V}$$

Where: f – velocity of flow [ml/24 h]; V – volume [ml].

After 100 days the medium was changed to the effluent from the column, in which the phosphogypsum with distillery decoctions was utilized by sulfate reducing bacteria (sulfidogenesis). This solution was contaminated by SRB and other heterotrophic bacteria; therefore prior to use, the solution was filtered but not under strictly sterile conditions. This experiment was carried out in the same conditions as in the Winogradski Column.

Chemical analysis. The samples were taken from influent and effluent of photosynthetic column every 3–4 days to monitor the changes of the sulfide concentration. The sulfide concentration was determined by iodometric method. Sulfates concentration analyzed using barium chloride and spectroscopic measurement (SPECTRONIC 20 GENESYS 20™). At the end of the experiment, two samples of the materials from on the surface of column was made using a DRON-2 X-ray diffractometer.

Results and Discussion

Oxidation of sulfides was studied during 128 days of the process. Figure 1 shows the effectiveness of oxidation of sulfides depending on dilution time (D), where the medium was the Pfennig solution (to the 98th day of the experiment). During the first 26 days the concentration of sulfides in the effluent was stable despite an increase in dilution time. Over the next 10 days the effectiveness decreased. On the 30th day of the experiment, the medium was enriched in sulfide (200 mg/l = 68 mg/l/24 h). However, the effectiveness did not change rapidly, which could indicate good conditions favoring the growth of green bacteria in the column.

This assay made it possible to establish the dilution time at 0.27/24 h for the next part of the experiment, in which the medium was effluent from column

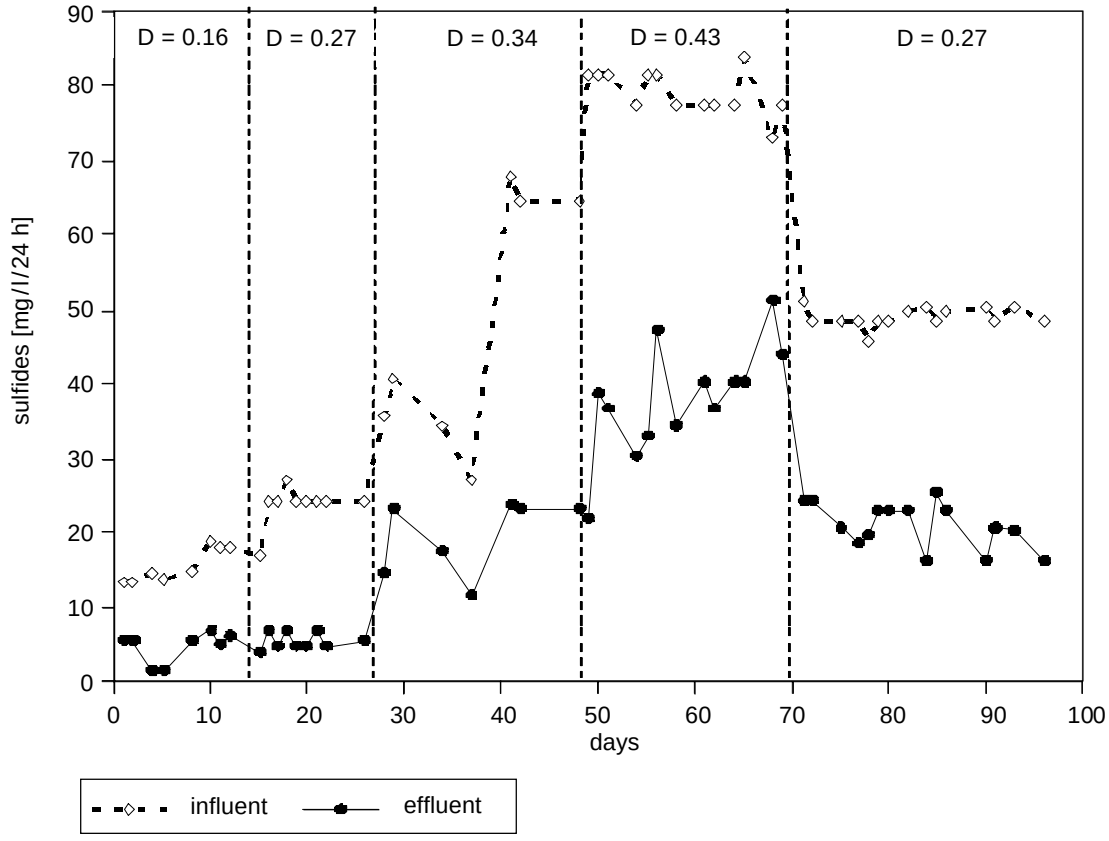

Fig. 1. Changes of sulfides in the influent and effluent of the photosynthetic column in modified Pfennig's medium

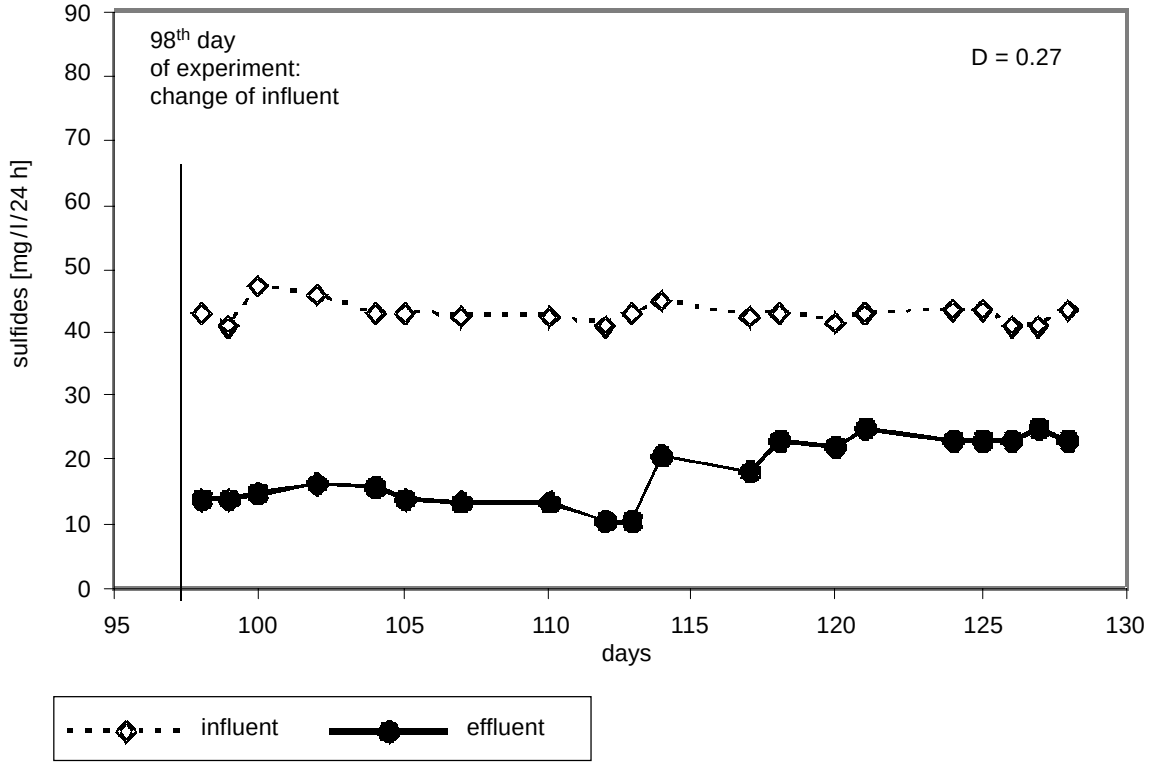

Fig. 2. Changes of sulfides in the photosynthetic column after the change of medium to effluent from the column with SRB

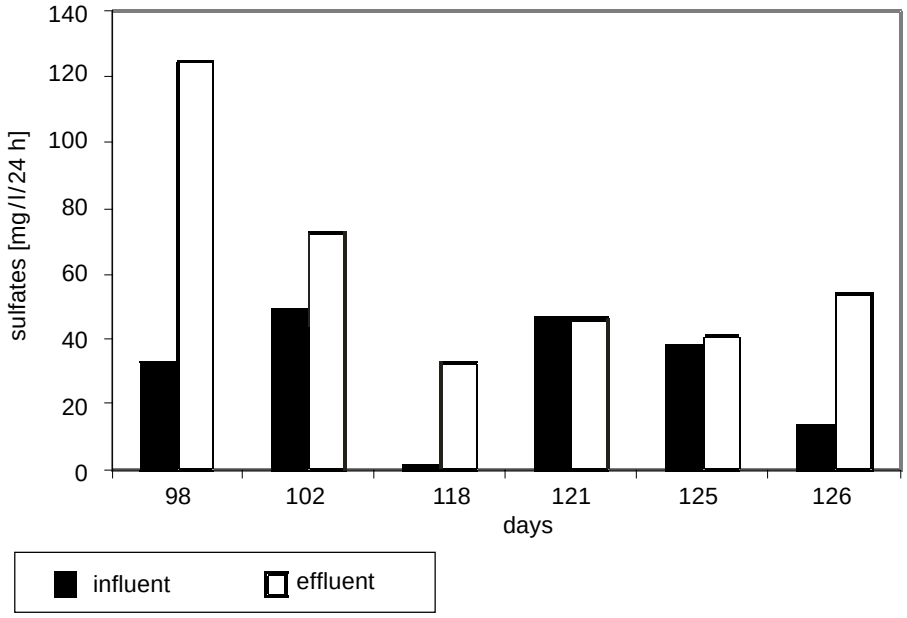

Fig. 3. Change of sulfates in the influent and effluent of the photosynthetic column between 98th and 126th day of the experiment.

with SRB (98th–128th day). The result is shown in Figure 2 and 3. Figure 2 shows changes of sulfide concentration during 30 days of the experiment. During the first 15 days of the second stage (98th–114th day), the concentration of sulfides was stable. After 114th day effectiveness of oxidation decreased (to 50%). This could be caused by proliferation of sulfate reducing bacteria (SRB), since the reactor contained organic compounds coming from the medium as well as those resulting from bacterial photosynthesis, the development of heterotrophic SRB was possible. Figure 3 shows concentration of sulfate on selected days. On the 98th day an increased amount of sulfate in the effluent was observed, which included sulfate from oxidizing of sulfide and thiosulfate from the previous medium before its change. Thiosulfate may be used by some green sulfur bacteria (for instance *Chlorobium thiosulfatophilum*) and sulfate could be generated as an effect of this process. During the next days sulfates in the effluent came only from the oxidizing of sulfides and from the influent.

On the basis of these data, the balance of sulfur in column during 102th–128th day of experiment was predicted, this being shown in Fig. 4. The total amount of sulfur in the influent was 200mg/l, but the amount of sulfur in the effluent was 130 mg/l. The difference (70 mg/l) probably precipitated as elemental sulfur on the surface of the column, which was confirmed by diffractometric studies. The result of diffractometric studies of precipitates is presented in Fig. 5. This analysis correlated with chemical data and confirmed the precipitation and cumulation of sulfur in residue. Besides the sulfur, apatite and calcite were present in precipitate. The formation of calcite ($CaCO_3$)

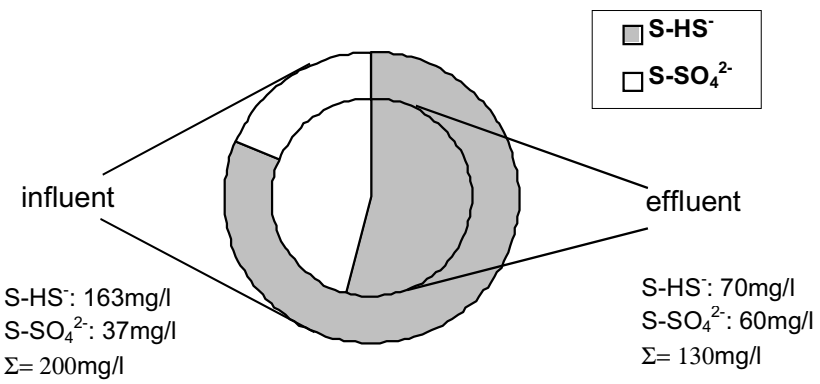

Fig. 4. Balance of the sulfur in the photosynthetic column made on the basis of the experimental data (102th – 128th day of experiment).

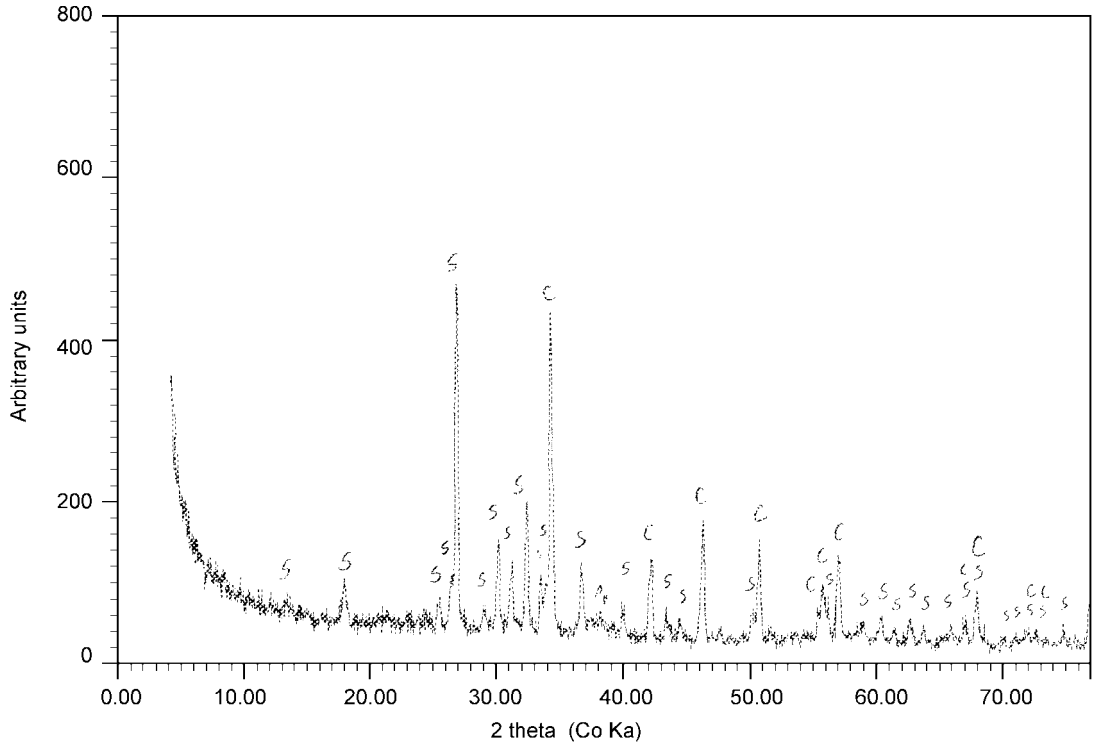

Fig. 5. Diffractometric analysis of the residue from the photosynthetic column after the end of the experiment. The symbols indicate: S – sulfur, C – calcite, Ap – apatite.

was probably due to the growth and activity of SRB, as has been reported previously (Kowalski *et al.*, 2003; Wolicka, 2006).

Conclusions. The obtained results indicate that sulfur bacteria can oxidize sulfides and store the sulfur in the environment which was confirmed by diffractometric studies. Calcite was also present in the precipitate, probably as an effect of the activity of SRB. In the photosynthetic column photosynthesizing sulfur bacteria and probably sulfate reducing bacteria were present. This experiment also seems to reveal something that could be conceived as the sulfur cycle in artificial conditions. The photosynthesizing sulfur bacteria grew in the presence of organic compounds, which is important because theoretically these conditions are not favorable for the growth of these autotrophic bacteria.

It seems that the application of the column and the method of continuous cultivation are appropriate to research correlations between anaerobic sulfur bacteria and their activity.

Literature

Alexander B., J.H.Andersen, R.P.Cox and J.F. Imhooff. 2002. Phylogeny of green sulfur bacteria on the basis of gene sequences of 16S rRNA and of the Fenna-Matthews-Olson protein. *Arch. Microbiol.* 178: 131–140

Colleran E., S. Finnegan and P.Lens.1995. Anaerobic treatment of sulphate-containing waste streams. *Antonie van Leeuwenhoek* 67: 29–46

Eraso J., M. and S. Kaplan. 2001. *Photoautotrophy. Encyclopedia of Life Sciences*, Nature Publishing Group.

Erlich H.L. 1998. Geomicrobiology: its significance for geology. *Earth-Science Reviews* 45: 45–60

Fauque G., J. Legall and L. L. Barton. 1991. *Sulfate-reducing and Sulfur-reducing Bacteria. Variations in Autotrophic Life*. (Eds) Shively J.M. and Barton L.L. Academic Press Ltd.

Fortin D. and S. Langley. 2005. Formation and occurrence of biogenic iron-rich minerals. *Earth-Science Reviews* 72: 1–19

van Gemerden H. 1986. Production of elemental sulfur by green and purple sulfur bacteria. *Arch. Microbiol.* 146: 52–56.

Hansen T. A. 1994. Metabolism of sulfate-reducing procaryotes. *Antonie van Leewenhoek* 66: 165–185.

Hao O. J., J. M. Chen, L. Huang and R. L. Buglass. 1996. Sulfate-reducing bacteria. *Crit. Rev. Environ. Sci. Technol.* 26: 155–187.

Kowalski W., W. Hołub, D. Wolicka, M. Przytocka-Jusiak and M. Błaszczyk. 2003. Sulphur balance in anaerobic cultures of microorganisms in medium with phosphogypsum and sodium lactate. *Arch. Mineral.* 54: 33–40.

Overmann J. 2001. Diversity and ecology of phototrophic sulfur bacteria. *Microbiology Today* 28: 116–118.

Overmann J. and H. van Gemerden. 2000. Microbial interactions involving sulfur bacteria: implications for the ecology and evolution of bacterial communities. *FEMS Microbiol. Rev.* 24: 591–599.

Overmann J. and C. Tuschak. 1997. Phylogeny and molecular fingerprinting of green sulfur bacteria. *Arch. Microbiol.* 167: 302–309.

Wolicka D. 2006. Biotransformation of phosphogypsum in cultures of bacteria selected from petroleum [In:] *Proceedings of the International Conference Protection and Restoration of the Environment VIII pp .217–218, Chania – Crete, Greece, 3–7 June.*