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Geomicrobiology of Acid Mine Drainage in the weathering zone of pyrite-bearing schists in the Rudawy Janowickie Mountains (Poland)

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We report geomicrobiological analysis of acid water reservoirs and Acid Mine Drainage (AMD) developed in the weathering zone of pyrite-bearing schists near the closed-down pyrite mine in Wieściszowice (southwestern Poland). The analysis focused on two reservoirs characterized by different physical and chemical properties (pH, redox potential, content of sulphates and heavy metals), emphasizing geomicrobiological relationships taking place in this AMD setting and describing the microbiological processes that significantly influence biogeochemical cycles of sulphur and iron in the water reservoirs analysed. The reservoir water also harbours numerous large, organized microbial structures in the form of streamers, studied in detail here using optical and electron microscopy and by microbiological cultivation and molecular methods. The Wieściszowice mine slime streamers are characterized by the co-occurrence of typical chemolithoautotrophic microorganisms oxidizing sulphur and iron together with sulphate-reducing bacteria. These structures probably depend on the occurrence of iron(II) in the surrounding environment.

Key words: Acid Mine Drainage, microbial communities, pyrite, weathering zone.

INTRODUCTION

Weathering zones of sulphide ores containing pyrite are places where chemical processes linked with the oxidation of sulphides of heavy metals are often accelerated by biotic processes, in which microorganisms play the main role. In this context, many geochemical and geomicrobiological studies are focused on Acid Mine Drainage and sulphur cycle microorganisms, such as chemolithoautotrophic sulphur bacteria and sulphate-reducing bacteria (Postgate, 1984; Gibson, 1990; Fauque et al., 1991; Hao et al., 1996; Nordstrom et al., 1999; Ehrlich, 2001; Hallberg, 2010). Many microorganisms oxidizing sulphide and sulphur also oxidize some metals, especially iron, thus favouring rapid development of the weathering zone, a phenomenon that is difficult to attribute merely to chemical processes. One result of the microbial activity is the development of strongly acid conditions in the weathering zone of sulphidic ore deposits, resulting in the formation of acid mine drainage (Fortin et al., 1996; Johnson, 1998; Ehrlich, 2001; Baker and Banfield, 2003).

The weathering zone of the pyrite-bearing schists studied is located within the closed-down pyrite mine in Wieściszowice in Rudawy Janowickie, Western Sudetes (Fig. 1). Laminated chlorite-sericite schists mineralized with pyrite, forming a belt that is approximately 200 m wide and 4 km long, were subject to exploitation till 1925. The schists contain quartz, muscovite, chlorite, plagioclases, epidote and calcite. Pyrite mineralization impregnates the rock; its average content reaches approximately 10%. The weathering zone developed in the mine workings yields a diverse paragenesis of secondary sulphate minerals (Table 1), such as gypsum, copiapite, pickeringite, fibroferrite, slavikite, melanterite, epsomite and schwertmannite (Balcerzak et al., 1992; Parafiniuk, 1996; Parafiniuk and Siuda, 2006).

Weathering processes of the pyrite schists exposed in the mine workings has resulted in a local hydrochemical anomaly characterized by high concentrations of sulphates and metal

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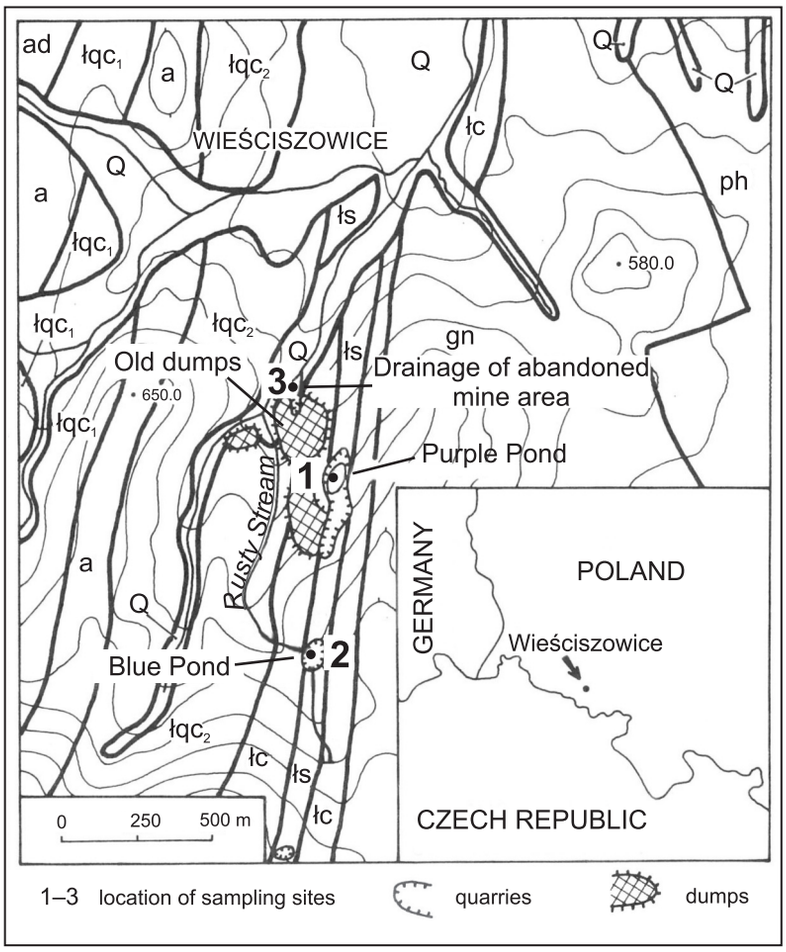

Fig. 1. Geological sketch of the environs of Wieściszowice (after Parafiniuk, 1996, modified)

a – amphibolites, ad – diopside amphibolites, gn – gneisses, łc – chlorite schists, łs – pyrite-bearing sericite-chlorite schists, $łqc_1$ – massive quartz-chlorite schists, $łqc_2$ – fibrous quartz-chlorite schists, ph – phyllites, Q – Quaternary deposits

ions, especially those of Fe, Al, and Mg in ground and surface water. Water draining from the soluble weathering products fills the lowermost parts of the mine workings, forming small reservoirs, characterized in part by low pH and high mineralization typical of AMD. The two largest, the Purple Pond and Blue Pond, differ in colour and chemical composition of the water. The Blue Pond (150 m long, 35 m wide and about 10 m deep) fills a small, upper working of the mine situated at 650 m a.s.l. It is the deeper of the two and its water has a light bluish colour. The Purple Pond (max. 430 m long, 150 m wide and 2.5 m deep) is located deeper in the mine, in the largest mine working at a level of approximately 555 m a.s.l. and has dark brown colour and high mineralization. The Blue Pond is an overflow reservoir, whereas the Purple Pond does not have surface runoff and is drained through a series of fractures and underground mine workings by a small stream located below dumps of reworked pyrite-bearing schists (Fig. 2). The water level in both lakes is variable and depends on the meteorological conditions.

The geochemical characteristics of the reservoirs are apparent, but prior to this study were not subject to limnological and geomicrobiological studies. Large microbial aggregates growing in the small AMD-type stream draining water from the mine and the Purple Pond, likewise have not previously been studied microbiologically. These take the form of slime streamers, often larger than 20 cm: biofilms consisting of extensive deposits of extracellular substances in which microorganisms are embedded. Poorly crystalline iron oxyhydroxides are precipitated on the surface of the slime streamers. The presence of these structures is restricted to the upper part of the runoff. Such structures have not been noted in reservoirs filling the bottom of the workings.

Our investigations focused on the geomicrobiological characteristics of the water reservoirs and the AMD in Wieściszowice, and determining the influence of microbial communities on the biogeochemical cycles of sulphur and iron in the weathering zone of the pyrite-bearing schists.

MATERIAL AND METHODS

Water samples for chemical analysis were collected five times from each reservoir at a distance of approximately 1 m from the reservoir margin. In the field, the samples were filtered through a cellulose filter (millipore) at 0.45 μm mesh size and transported airtight to the laboratory in order to determine the

Table 1

Sulphate minerals occurring in the weathering zone of pyrite-bearing schists at Wieściszowice

Mineral	Chemical formula	Solubility in water	Colour	Occurrence
Fibroferrite	$Fe(OH)(SO_4) \cdot 5H_2O$	good	greyish	common
Magnesocopiapite	$MgFeFe_4^{3+}(OH)_2(SO_4)_6 \cdot 20H_2O$	very good	yellow	common
Slavikite	$NaMg_2\,Fe_5^{3+}(OH)_6(SO_4)_7 \cdot 33H_2O$	good	green	rather common
Pickeringite	$MgAl_2(SO_4)_4 \cdot 22H_2O$	very good	white	rather common
Melanterite	$FeSO_4 \cdot 7H_2O$	very good	whitish	rare
Gypsum	$CaSO_4 \cdot 2H_2O$	poor	white	common
Alunogen	$Al_2(SO_4)_3 \cdot 18H_2O$	very good	white	rare
Epsomite	$MgSO_4 \cdot 7H_2O$	very good	white	rare
Schwertmannite	$Fe_8O_8(OH)_{8-2x}(SO_4)_x \cdot nH_2O$ $1 \le x < 1.75$	very poor	brown	rather common

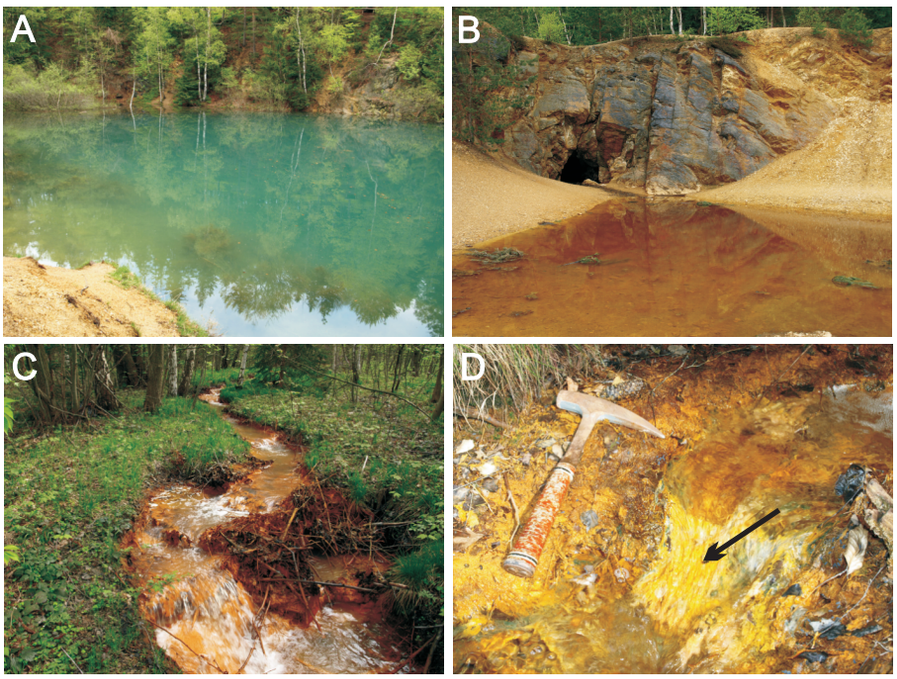

Fig. 2. Study area

A – Blue Pond; **B** – Purple Pond (height and width of outcrop – 7 m and 20 m respectively); **C** – stream from waste dump of reworked schists; **D** – slime streamers in the upper reach of the stream flowing from the waste dump (hammer is 35 cm long)

chemical composition. Fe^{2+} ions were detected in filtered field samples, acidified with 1 M HCl. For microbiological analysis, five samples each were collected from the water and the bottom deposit from both water reservoirs and introduced into sterile 100 ml containers. The samples of water were collected directly from the reservoir surface. The thickness of the samples of sediment was about 15 cm. In addition, samples from microbial mats and from microbial communities occurring abundantly in AMD in streams draining the main mine working were collected.

The pH of the water was measured in the field with a combination electrode, and the redox potential of the water was measured using a carbon electrode.

MICROBIOLOGICAL DETERMINATIONS

The abundance of bacteria capable of thiosulphate oxidation was determined by plating samples on thiosulphate agar composed of: $(NH_4)_2SO_4$ – 0.12 g, K_2HPO_4 – 4.00 g, KH_2PO_4 – 4.00 g, $MgSO_4 \cdot 7H_2O$ – 0.10 g, $CaCl_2$ – 0.10 g, $FeCl_3 \cdot 6H_2O$ – 0.03 g, $MnSO_4 \cdot H_2O$ – 0.02 g, $Na_2S_2O_3 \cdot 5H_2O$ – 10.00 g, purified agar (Merck) 20 g, and distilled water 1000 ml (pH 6.7). The abundance of hyphal fungi capable of thiosulphate oxidation was determined on the same medium, but to which penicillin and streptomycin had been added. All determinations were made in triplicate by plating samples serially diluted in sterile 0.9% NaCl solution.

Thiosulphate-oxidizing ability was studied in triplicate in enrichment cultures in the following medium: $(NH_4)2SO_4$ – 0.12 g, K_2HPO_4 – 4.00 g, KH_2PO_4 – 4.00 g, $MgSO_4 \cdot 7H_2O$ – 0.10 g, $CaCl_2$ – 0.10 g, $FeCl_3 \cdot 6H_2O$ – 0.03 g, $MnSO_4 \cdot H_2O$ – 0.02 g, $Na_2S_2O_3 \cdot 5H_2O$ – 10.00 g, and distilled water 1000 ml (pH 6.7). Elemental sulphur-oxidizing ability was studied in triplicate enrichment cultures in the following medium: NH_4Cl – 0.12 g, KH_2PO_4 – 3.00 g, $MgCl_2 \cdot 6H_2O$ – 0.10 g, $CaCl_2 \cdot 2H_2O$ – 0.14 g, powdered sulphur 10 g, and distilled water 1000 ml (pH 4.2). Enrichment cultures from reservoir water or AMD were initiated with 5 ml of respective inoculum. Enrichment cultures from bottom reservoir deposits or slime streamers were initiated with 1 g of respective inoculum. Each sample of the material studied was introduced to 50 ml of medium and incubated at 25°C in a mechanical shaker. Incubation continued for two weeks, during which the concentration of sulphate ions was tested. Controls were set up like the corresponding enrichments, except that the respective inocula had been pretreated with 5% formaldehyde.

Similarly, Fe(II)-oxidizing ability was determined in Silverman medium (Silverman and Lundgren, 1959) composed of: $(NH_4)_2SO_4$ – 3.0 g, KCl – 0.1 g, K_2HPO_4 – 0.5 g, $MgSO_4 \cdot 7H_2O$ – 0.5 g, $Ca(NO_3)_2$ – 0.01 g, $FeSO_4 \cdot 7H_2O$ – 44 g, H_2SO_4 (0.5 M) 10 ml, and distilled water 1000 ml. The medium was sterilized by filtration. During incubation, measurements of Fe^{2+} ion concentrations were made and compared to readings of corresponding controls.

The selective medium Easicult S (Orion Diagnostica, Finland, Cat. No. 67687) was applied to reservoir water samples, reservoir bottom samples, and AMD to confirm the presence of sulphate reducing bacteria (SRB). Samples (approximately 100 μl) were inoculated aseptically by means of a sterile pipette into separate test tubes containing the test medium, which were then stoppered securely and incubated at 25°C for 7 days. Sulphate-reduction was indicated by blackening of a portion of the test medium.

MICROSCOPY

Microscopy was carried out with a scanning electron microscope outfitted with EDX (*JEOL JSM-6380LA*) and with an epifluorescence microscope (*NIKON Eclipse E600W*). For electron microscopy, the preparations were coated with carbon,

whereas for fluorescence microscopy the samples were stained with acridine orange. The structure of microbial communities (streamers) from the AMD was tested in cross-sections of the structures studied using purpose-modified histological techniques as follows. A sample of the streamer (0.5 × 0.5 cm) was fixed in the 4% formaldehyde in phosphate buffer (0.1M, pH 7) for 12 h and was dehydrated in a graded ethanol series (1 h in 50, 70, 80, 90 and 100% ethanol). Next, the sample was cleared by immersing for 2 h in xylene and was transferred to a mixture consisting of equal parts of paraffin wax and xylene. After 2 h, the sample was transferred to pure paraffin wax and placed in an oven (60°C) for 5 h. The sample was embedded in paraffin and sectioned using a microtome, and the sections were transferred to xylene and hydrated gently in serially diluted ethanol (100–50%). Sections were stained with an aqueous mixture of methylene blue and eosine (0.5%) for 2 minutes. The stained sections were dehydrated and mounted in DPX synthetic balsam.

CHEMICAL DETERMINATIONS

Sulphates in the cultures were measured using the turbidimetric method after reaction with barium chloride according to the method described by Kolmert et al. (2000); metal concentrations and total iron in the water samples from the reservoirs were determined using the ICP-OES method on an *Optima 5300DV* spectrometer. Fe^{2+} concentration in water samples was determined in a *Thermo Scientific* spectrophotometer after reaction with phenanthroline (Harvey et al., 1955).

MOLECULAR ANALYSIS OF MICROORGANISMS AND DNA SEQUENCING

Bacterial genomic DNA was extracted using a Genomic Mini isolation kit (A&A Biotechnology) according to the manufacturer's instructions. The purity and concentration of DNA preparation were determined spectrophotometrically at 260 nm, and the DNA was used as a template for Polymerase Chain Reaction (PCR). Approximately 100 ng of DNA was used as the template for PCR amplification of nearly full-length bacterial 16S rRNA gene fragments using the universal primers 27F (5'AGAGTTTGATCCTGGCTCAG3') and 1492R (5'GGTTACCTTGTTACGACTT3') to amplify a 1540-bp segment from the 16S rRNA gene. AmpliTaq polymerase (Invitrogen) or MARATHON polymerase (A&A Biotechnology) was employed in PCR reaction. The reactions were performed using a *PTC-200* thermal cycler (MJ Research, Inc., USA) under optimized conditions: 95°C for 5 min; 20 cycles of 95°C for 30 s, 53°C for 30 s, 72°C for 90 s; followed by 15 cycles of 95°C for 30 s, 46°C for 30 s, 72°C for 1.5 min; and a final extension at 72°C for 10 min. Amplification products were purified using a *NucleoSpin® Extract II kit* (Macherey-Nagel) and analysed by electrophoresis in 1% (wt/vol $^{-1}$) agarose gel in a 1 x TBE running buffer containing ethidium bromide (0.5 μg ml^{-1}) at 4.8 V cm^{-1} for 1 h. A 100-bp DNA ladder (Invitrogen) was used as a size marker. Gels were photographed using a Syngene gel documentation system. The PCR products were directly sequenced on an *ABI3730 DNA Analyzer* (Applied Biosystems) using the primers 27F, 1492R, F357 (5'GCCTACGGAGGCAGCAG3'), 519R (5'ATTACCGCGGCTGCTGG3') and 926R (5'CCGTCAATTCCTTTGAGTTT3'), corresponding to the conserved regions of the 16S rRNA gene sequence. DNA sequences were assembled using the *Linux* programs phred/phrap/consed and checked manually. The 16S rDNA sequences obtained were then compared with those in the National Centre for Biotechnology Information (NCBI) database using the *Blast 2.0* program. A multiple alignment with isolated sequences retrieved from the NCBI Reference mRNA and Microbes Assembled Genomes databases was generated using the program *Muscle* and edited manually using *BioEdit* (http://www.mbio.ncsu.edu/BioEdit/BioEdit.html).

RESULTS

HYDROCHEMISTRY OF THE STUDIED WATER RESERVOIRS

The chemical composition of water collected from surface layer in the reservoirs studied differed significantly. Samples from the Blue Pond showed slightly acid pH of approximately 5.5–6.0, and redox potential within 238–315 mV (Table 2). Sulphate concentration did not exceed 100 mg l^{-1}, whereas the heavy metal concentration (Co, Ni, Cu) was at the level of 0.01 mg l^{-1}. Much higher values of the chemical elements analysed were noted in samples from the Purple Pond. High concentrations of SO_4^{2-} ions (about 2000 mg l^{-1}) and of heavy metals were observed. Water from this reservoir was also characterized by much lower pH values, which were in the range of 2.5–3.0. The redox potential of water from this reservoir was within 430–550 mV. The content of iron in water samples from the two reservoirs was intriguing.

Table 2

The chemistry of water from the Blue Pond, Purple Pond and drainage of the abandoned mine area

	Blue Pond	Purple Pond	Drainage
n	5	4	3
pH	5.0–6.0	2.5–3.0	2.5–3.0
Eh [mV]	238–315	456–590	460–575
macroelements [mg l^{-1}]			
Na	1.35–2.92	4.69–5.27	5.6–6.52
Mg	3.21–7.10	88.1–140	171–173
Al	0.79–2.12	48.1–73.8	65.7–94
Ca	13.5–16.7	146–209	173–326
Fe^{2+}	n.d.	35–61	54
Fe_{tot}	0.01–0.10	113–131	157–236
SiO_2	7.50–18.3	46.4–50.9	63.3–73.3
SO_4^{2-}	71–96	2010–2440	2389–2630
microelements [μg l^{-1}]			
Li	7–9	40–50	60
K	100–1380	250–290	600–670
Mn	70–160	850–1870	2700–3590
Co	10	160–240	280–400
Ni	10	110–180	190–270
Cu	<1–5	1110–1280	1470–2450
Zn	<1	120–310	390–550
Sr	20	100–170	270–390
Pb	3–5	5–8	8
PO_4^{3-}	<3	<3	<3 – 200

n – count of samples; n.d. – not determined

In the Blue Pond the concentration of total iron did not exceed 0.1 mg l^{-1}, whereas in the Purple Pond it lay between 114–132 mg l^{-1}. Moreover, in samples from the Purple Pond the presence of ferrous ions at a level of 45 mg l^{-1} was noted, which was about 37% of the total iron in the solution (averagely 124 mg l^{-1}). This is a significant result, particularly in the context of the presence of iron(II)-oxidizing bacteria at a very low pH. Very similar concentrations of the chemical solutes studied were obtained from the AMD stream flowing out below the Purple Pond. In general, the water from the Purple Pond was homogeneous and the redox potential was similar throughout the entire column of water. However, the Blue Pond seems to be vertically zoned as regards the depth and concentration of H_2S. The redox potential decreased with depth from a value of ca. 260 mV to ca. –50 mV near the bottom. The content of S^{2-} in a sample of water collected with sediment (at a distance of approximately 1 m from the reservoir margin) was about 1.5 mg l^{-1}.

ABUNDANCE AND ACTIVITY OF MICROORGANISMS IN ENRICHED CULTURES

Analysis of the abundance of bacteria oxidizing thiosulphate determined on thiosulphate agar showed significant differences between the water reservoirs studied (Fig. 3). In samples from the Blue Pond the abundance in the bottom deposit was at the level of 10^5 cfu $g_{d.m.}^{-1}$ (colony-forming units/g dry mass), whereas in the deposit from the Purple Pond this abundance was smaller, on average 10^2–10^3 cfu $g_{d.m.}^{-1}$. Similarly, water samples from the Blue Pond displayed much higher abundances of the bacteria than similar water samples from the Purple Pond. Analysis also revealed a considerable content of hyphal fungi growing on the thiosulphate agar. Detection of their abundance in agar, with addition of antibiotics inhibiting bacterial growth, indicated that in both reservoirs the fungi growing on thiosulphate agar were equally abundant (103 $g_{d.m.}^{-1}$) and their occurrence was essentially restricted to the bottom deposit.

The potential thiosulphate- and elemental sulphur-oxidizing ability as well as the Fe(II)-oxidizing ability of enrichment cultures prepared with water- and bottom deposit-samples was quantified (Figs. 4 and 5). The initial pH of the medium for observing thiosulphate-oxidizing activity was 6.7 and for observing elemental-sulphur oxidizing activity was 4.2. The amount of sulphate production after 10 days of incubation of the various culture enrichments in the thiosulphate medium was a measure of the potential thiosulphate-oxidizing activity contributed by the respective enrichment inocula. It correlated with the bacterial abundance in the respective inocula enumerated on thiosulphate agar. In enrichment cultures inoculated with samples of deposits from the Blue Pond an increase of SO_4^{2-} concentration by almost 4000 mg l^{-1} was observed after 10 days. In the case of cultures from the Purple Pond, such concentrations were noted only in one enriched culture with a sample of the bottom deposit. The opposite trend was observed in enrichment

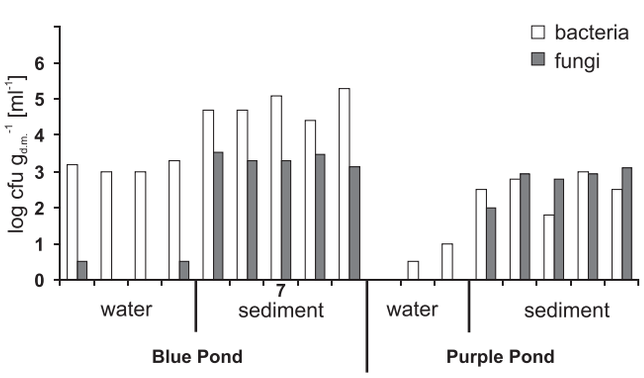

Fig. 3. An abundance of bacteria and hyphal fungi in samples of water and sediment from Blue and Purple ponds, determined on thiosulphate agar

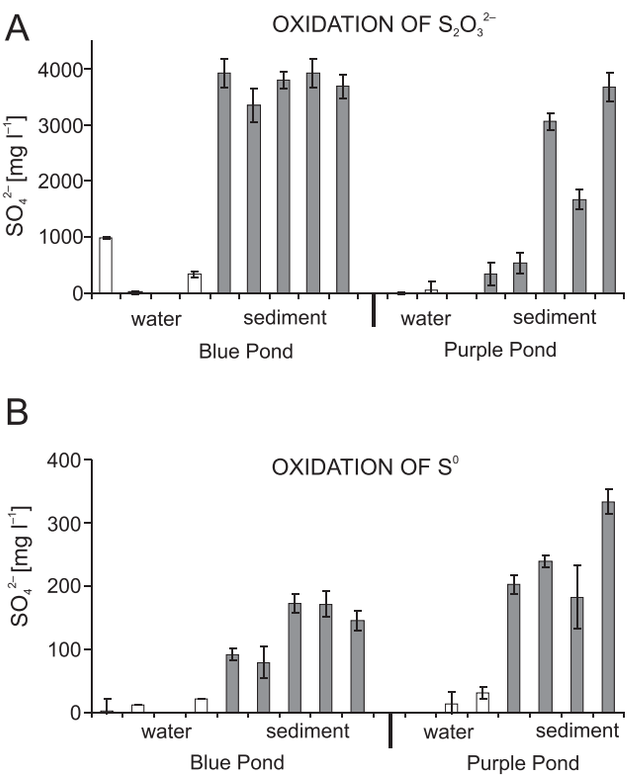

Fig. 4. Increase of SO_4^{2-} ion concentration in enriched cultures after 10 days of incubation on medium with thiosulphate (oxidation of $S_2O_3^{2-}$ ions) – A and on medium with elemental sulphur (oxidation of S^0) – B

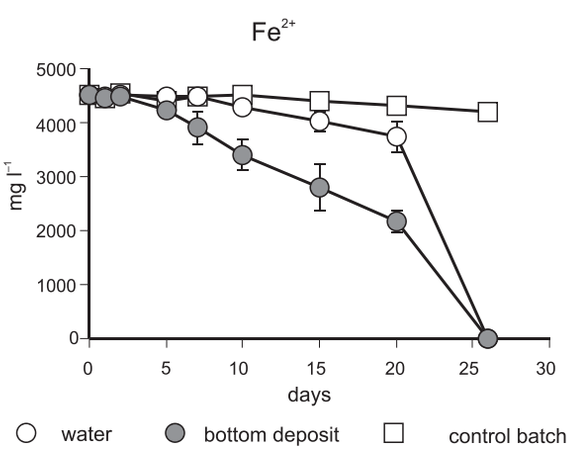

Fig. 5. Changes of Fe^{2+} concentration in cultures inoculated with material from the Purple Pond

Standard deviation is shown

cultures prepared in an elemental-sulphur medium. Here, the SO_4^{2-} ion concentrations determined were higher in enrichment cultures inoculated with bottom deposit from the Purple Pond.

A decrease in iron(II) concentration was noted in enrichment cultures prepared in Silverman medium inoculated with water or bottom samples from the Purple Pond (Fig. 5). Iron oxidation resulted in the change of the solution colour to yellow-brown and precipitation of brown mineral phases. After 26 days, the decrease in iron concentration reached 100% compared to the control, whereas no iron(II) oxidation was observed in Silverman medium-enrichment cultures inoculated with water- or bottom-samples from the Blue Pond.

ANALYSIS OF MICROBIAL COMMUNITY (SLIME STREAMERS) FROM THE AMD

In the small AMD stream draining water from the main mine working, natural, grey-white and light brown, compact, mucilaginous streamers of microbial growth up to 20 cm long were present in dense formations on deposit fragments and at the margin of the upper reach of the stream (Fig. 2D). Microscopic analysis (Figs. 6 and 7) showed the presence of microorganisms in these structures with sizes typical of bacteria (2–5 μm), variable morphologically, secreting large quantities of slime that formed the mucilaginous structure of the streamer. Other from bacterial cells, dense groups of green cells randomly distributed on the streamer surface were also observed. Analysis using an electron microscope with an energy-dispersive X-ray spectroscopy (EDX) detector showed the presence of oxygen and iron on the surface of the fragments studied, as well as areas highly enriched in sulphur. Cross-sections of the streamer examined showed a fragmented layered structure on which poorly crystalline mineral material was locally present. Fluorescence microscopy confirmed the presence of variable morphotypes of bacteria in the streamer, as well as clusters of microbial cells showing autofluorescence, probably belonging to cyanobacteria.

In order to determine the ability of the microbial streamer studied to oxidize elemental sulphur and iron, an experiment was conducted under laboratory conditions, in which fragments

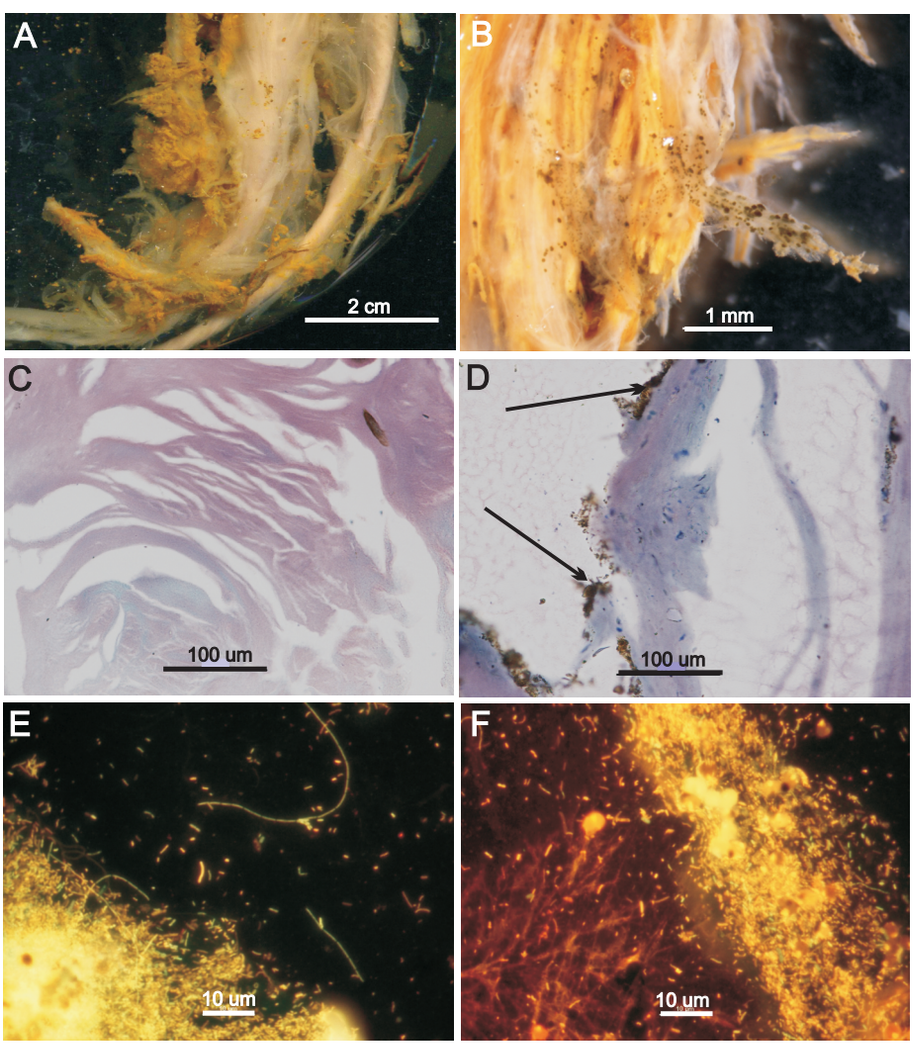

Fig. 6. Microbial community (slime streamers) from the stream flowing from the schist waste dump

A – general morphology of a streamer; **B** – streamer fragments viewed with a stereoscopic microscope; small clusters of photosynthesizing microorganisms are visible; **C**, **D** – cross-section of a streamer; poorly crystallized iron oxyhydroxides on surface of the layered structure are marked; **E**, **F** – streamer fragments viewed with fluorescence microscopy

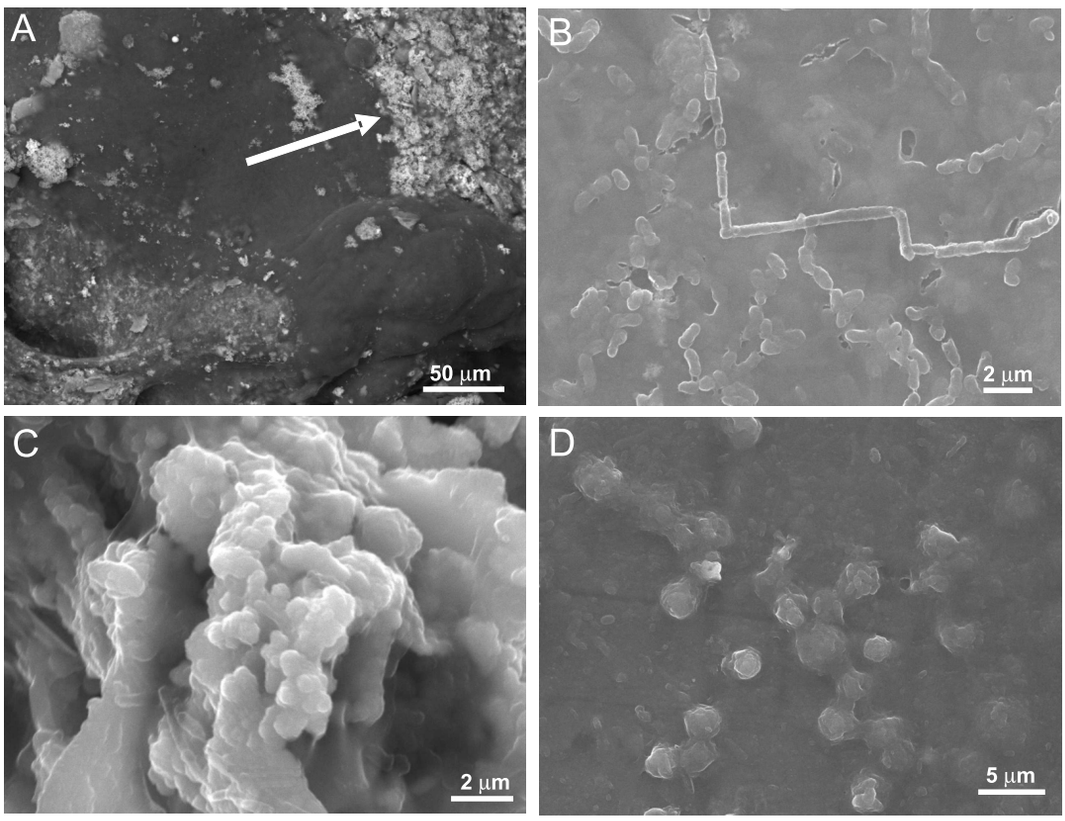

Fig. 7. Microbial community (slime streamers) from the stream flowing from the schist waste dump (SEM)

A – streamer surface in slight magnification, poorly crystallized iron oxyhydroxides covering the surface are marked; **B**, **C** – streamer surface; **D** – microorganism cells on the streamer surface visible as green concentrations in light microscopy

of slime streamers were inoculated into the microbiological media described above. Plots in Figure 8 show changes in sulphate and iron(II) concentration compared to the control batch. These experiments showed that the microbes in the streamer inoculum were able to oxidize elemental sulfur and iron under acid conditions. Easicult S tests indicated the presence of microorganisms capable of sulphate reduction.

Identification of microorganisms comprising the slime streamers studied and microbial communities within the bottom deposits in both water reservoirs required isolation of DNA and sequencing a fragment of gene 16S rRNA; the results were compared with genome fragments of known microorganism strains in existing databases (Tables 3 and 4). Analysis showed that the sequenced fragments of gene 16S rRNA indicate significant similarity to strains that were either autotrophs or heterotrophs. Material from slime streamers showed the presence of strains similar to bacteria typical of the AMD environment, such as *Acidithiobacillus ferrooxidans*, but also typically saprophytic bacteria such as *Bacillus subtilis or B. amyloliquefaciens*. It is interesting that the presence of SRB e.g., *Desulfococcus oleovorans* and *Desulfotalea psychrophila* was also detected. In turn, the SRB were present in significant numbers in the bottom deposits of both the Purple and the Blue ponds.

We also isolated and characterized 16S rRNA genes from streamer fragments that harbored photosynthetic microbes. The analysis (Table 3) showed the presence of cyanobacteria e.g., representing the genera *Arthrospira*, *Acaryochloris*, *Cyanobium*, *Cyanothece* and *Synechococcus*.

DISCUSSION

The Purple Pond was characterized by significantly higher mineralization and higher concentration of heavy metals compared to the Blue Pond. This might seem mainly the effect of differences in hydrology, though it is also possible that the hydrogen sulphide in the Blue Pond can affect the concentration of metals, which can be precipitated as sulphides and retained in the sediments. The Blue Pond is recharged and drained by water from the nearby Rusty Stream. The Purple Pond does not have a surface runoff, but to a minor extent its drainage is possible through the basement and the underground mine workings. Water of the Purple Pond collects much more acid products of weathering of the pyrite-bearing schists than the Blue Pond, which fills with water in contact with poorly exposed schists in the smaller mine working. Both these factors cause significant differences in water chemistry (Balcerzak et al., 1992; Parafiniuk, 1996). During weathering, the pyrite-bearing schists exposed in the mine supplies considerable amounts of sulphates, iron, magnesium, aluminium, calcium and other prod-

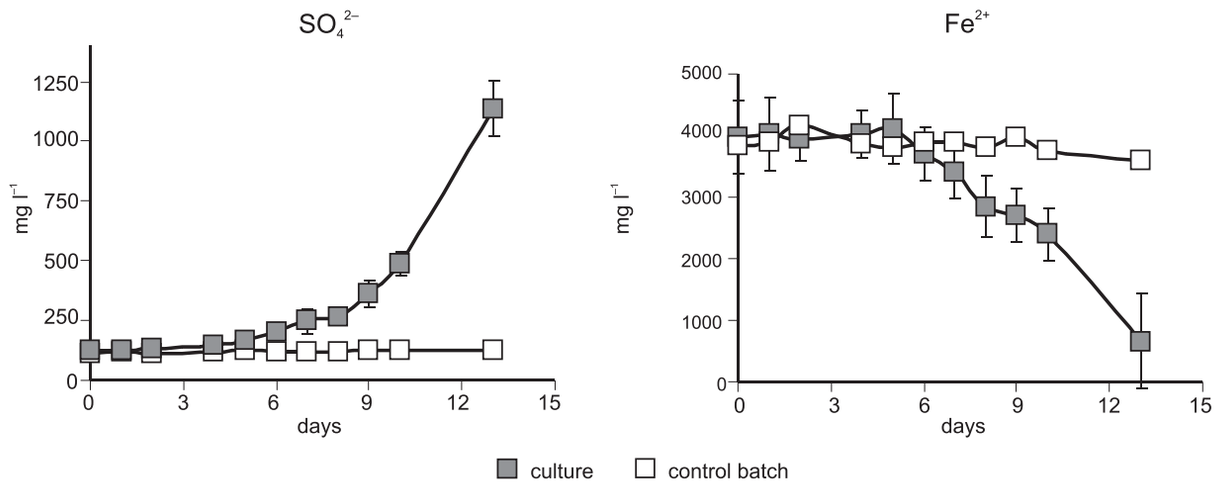

Fig. 8. Changes of SO_4^{2-} and Fe^{2+} concentrations in cultures with material from slime streamers from the acid runoff stream from the schist waste dump

Standard deviation is shown

Table 3

Genetic analysis (16S rRNA) of the bacterial community from slime streamers isolated from drainage of the mine nearby the Purple Pond

Strain	Similarity [%]	Site of isolation
Acidaminococcus sp. D21 cont 1.71	90	bacteria from slime streamers (drainage of mine)
Acidithiobacillus ferrooxidans ATCC 23270	86	
Acidithiobacillus ferrooxidans ATCC 53993	85	
Acidothermus cellulolyticus 11B	87	
Acinetobacter baumanii AB0057	82	
Aeromonas salmonicida	72	
Bacillus amyloliquefaciens	95	
Bacillus coagulans 36D1 ctg473	94	
Bacillus subtilis SMY	96	
Desulfococcus oleovorans Hxd3	89	
Desulfotalea psychrophila LSv54	91	
Pseudomonas syringae 1448A	88	
Staphylococcus saprophyticus ATCC 15305	98	
Streptococcus thermophilus CNRZ1066	93	
Arthrospira platensis str. Paraca	100	cyanobacteria from slime streamers (drainage of mine)
Arthrospira sp. PCC 8005	99	
Arthrospira maxima CS-328	99	
cyanobacterium UCYN-A	98	
Acaryochloris marina MBIC11017	97	
Cyanobium sp. PCC 7001	96	
Cyanothece sp. CCY0110	96	
Cyanothece sp. PCC 8801	96	
Cyanothece sp. PCC 8802	96	
Thermosynechococcus elongatus BP-1	95	
Trichodesmium erythraeum IMS101	94	
Nostoc azollae 0708	93	
Anabaena variabilis ATCC 29413	92	
Synechococcus sp. CC9902	91	

ucts of erosion that have a fundamental influence on the water chemistry in these reservoirs. Strong acidification by products of pyrite oxidation results in the formation of unstable mineral phases of chlorite and sericite schists, particularly chlorite, whose decomposition is the main source of magnesium, aluminium and calcite supplying calcium to the reservoirs studied. A characteristic feature is the very low concentration of alkali resulting from the resistance of sericite to decomposition in an acid environment (Parafiniuk, 1996).

Acid water reservoirs such as those occurring near Wieściszowice are also known from many weathering zones around other sulphide ore deposits. With regard to chemical composition, they are similar to other widely discussed occurrences of AMD (Murad et al., 1994; Webster et al., 1994; Leduc et al., 2002; Joeckel et al., 2005). Limnological, microbiological and hydrogeochemical studies of such reservoirs, formed in pyrite mines near Rio Tinto in the "Spanish Pyrite Belt", have been published by Sánchez-España et al. (2008), González-Toril et al. (2011) and Sánchez-Andrea et al. (2011). Water from these reservoirs is characterized by physical and chemical properties similar to those found in the workings of the Wieściszowice mine. Chemical characteristics mentioned by Sánchez-España et al. (2008) vary from neutral, with relatively low metal content, e.g., the Los Frailes reservoir (pH = 7.2, 0.07 mg Fe l^{-1}) to extremely acid such as the Corta Atalaya with pH = 1.2, iron

Table 4

Genetic analysis (16S rRNA) of the sediment samples from the Blue and Purple ponds

Strain	Similarity [%]	Site of isolation
Desulfatibacillum alkenivorans AK-01	90	sediment from the Blue Pond
Desulfovibrio salexigens DSM 2638 ctg4	95	
Geobacillus kaustophilus HTA426	92	
Geobacillus thermodenitrificans NG80-2	85	
Marinobacter aquaeolei VT8	95	
Methanococcoides burtonii DSM 6242	90	
Shewanella baltica OS185	99	
Shewanella putrefaciens 200 ctg6	95	
Anaeromyxobacter dehalogenans 2CP-C	88	sediment from the Purple Pond
Coprothermobacter proteolyticus DSM 5265	68	
Desulfatibacillum alkenivorans AK-01	91	
Desulfococcus oleovorans Hxd3	93	
Desulfohalobium retbaense DSM 5692	86	
Desulfohalobium retbaense DSM 5692	85	
Desulfomicrobium baculatum DSM 4028	87	
Desulfotomaculum reducens MI-1	96	
Desulfuromonas acetoxidans DSM 684 ctg	97	
Dialister invisus DSM 15470	80	
Thermoanaerobacter pseudethanolicus ATCC 332	90	
Thermus thermophilus HB27	71	
Thioalkalivibrio sp. HL-EbGR7	95	
Thioalkalivibrio sp. K90mix ctg2	95	

content (36.7 g l^{-1}) and copper content (1.3 g l^{-1}). Most of these basins are, however, typical acid reservoirs with pH within 2.2–3.6 (Sánchez-España et al., 2008), for which interesting microbiological investigations were also presented. These may allow for conclusions on the key role of bacteria oxidizing Fe^{2+} in the iron cycle in the environments described and the role of bacteria reducing iron, whose activity may be the source of Fe^{2+} ions.

Microbiological analysis of the Wieściszowice reservoirs indicates significant differences between these basins, reflected in the comparison of bacterial abundance determined on thiosulphate agar and in the results from culture enrichments. The most important cause of these differences is probably the chemical composition of water from the respective reservoirs. In the Blue Pond the pH is only slightly acid and there is high production of hydrogen sulphide that may be oxidized by sulphur bacteria, which do not necessarily belong to acidophilic species. Such bacteria, e.g. *Thiobacillus*, very often have the ability to oxidize both hydrogen sulphide and thiosulphate (Holt et al., 2000). Due to instability at pH below 4 (Pronk, 1990), thiosulphate is available to sulphur bacteria only at neutral or slightly acid pH. Thiosulphate oxidation is a property not only of sulphur bacteria; it is, however, a feature often occurring in chemolithoautotrophic microorganisms capable of growth on thiosulphate as the sole energy source (Kappler et al., 2001). All samples of deposits from the Blue Pond in enriched cultures resulted in considerable increase of sulphate concentration from thiosulphate oxidation by contrast to cultures with elemental sulphur under acid conditions. The opposite relationship was noted in the case of the Purple Pond, from which samples in cultures enriched with sulphur yielded much higher concentrations of sulphates in comparison to samples from the Blue Pond. This may indicate the presence of microorganisms capable of elemental sulphur oxidation and preferring an acid environment. Although molecular analysis (16S rRNA) did not indicate the presence of bacteria similar to *Acidithiobacillus* sp. in the Purple Pond, their presence was noted in samples of streamers growing in the stream from the waste dump with a chemical composition similar to that from the Purple Pond. These streamers are an interesting example of a microbial assemblage composed of many different microorganisms. The bacterial communities in the streamers vary in composition with respect to organisms capable of oxidizing elemental sulphur and iron, and also with respect to SRB and cyanobacteria. A crucial role in the streamers is assumed to be played by microorganisms capable of iron oxidation because these streamers are present only in the uppermost stretch of the stream flowing from the waste dump, where a significantly high concentration of iron(II) is still noticeable. Further downstream, Fe^{2+} concentration decreases and considerable amounts of precipitating mineral phases with iron(III) appear. Similar structures in an AMD environment were studied by Bond et al. (2000), who noted the presence of microorganisms classified into *Acidithiobacillus*, *Leptospirillum* and *Sulfobacillus*. The occurrence of SRB in the streamers was an interesting observation. Their presence was supported both by molecular analysis and in cultivation tests. Such microorganisms were noted in both water reservoirs studied. Most known SRB are heterotrophic species, strictly anaerobic, preferring neutral or slightly alkaline environments, growing in the presence of sulphate ions (Gibson, 1990; Rampinelli et al., 2008). Such conditions occur in the Blue Pond, which is shown by the presence of hydrogen sulphide in this reservoir. The second reservoir, the Purple Pond, does not seem as favourable to these bacteria, although all genetic analyses of samples collected from this setting indicate the presence of SRB. It seems that some species belonging to this group of microorganisms are not sensitive to acid conditions (Praharaj and Fortin, 2004; Rampinelli et al., 2008). It seems that low pH value is not necessarily a limiting factor for SRB activity. It is possible that the quantity and quality of organic carbon can strongly affect the SRB metabolism (Koschorreck, 2008). Moreover, some analyses indicate the ability of SRB to grow under microaerophilic conditions, particularly in bacterial communities (Baumgartner et al., 2006).

It seems thus that the group may play a significant role in acid environments such as the AMD in Wieściszowice, particularly considering the fact that such waters are usually enriched in sulphates due to oxidation of sulphide minerals.

SUMMARY AND CONCLUSIONS

The occurrence of microbial communities capable of oxidizing inorganic compounds of sulphur and iron within acid water reservoirs is linked to the presence of mineral compounds acting as the source of energy in these waters. The presence of typical microorganisms capable of full oxidation of thiosulphate to sulphates is demonstrated, as well as the pres-

ence of SRB, which is significant because of the acid reaction of these reservoirs and their high redox potential. In the Blue Pond, water pH was determined to be in the range of 5–6, which favours the growth of SRB as well as sulphur microorganisms preferring a slightly acid environment, whereas the very low water pH and high redox potential in the Purple Pond is presumably not favourable for the development of SRB. However, this group of bacteria was found both in culture tests and by molecular analysis.

Differences in the water chemistry between the reservoirs studied are reflected in the potential activity of the isolated microbial communities capable of thiosulphate oxidation. Culture experiments showed that the microorganism assemblage isolated from the Purple Pond was characterized by lower potential ability to oxidize thiosulphate to sulphates, a process that is conducted by sulphur microorganisms preferring less acid settings.

The presence of organized microbial structures in the AMD outflow from the mine is a notable feature. The presence of these slime streamers was restricted to the uppermost stretch of the stream; likewise they did not occur in the acid Purple Pond, despite the fact that water samples from the stream and reservoir had an almost identical chemical composition. Therefore, it can be concluded that an important factor influencing the development of slime streamers is good water aeration, attainable in a small flowing stream, but an aspect limiting their growth is the availability of iron(II). With decrease of iron(II) concentration downstream, slime streamers disappeared completely. The structures described occur as layered biofilm and it is possible that they are a complex system of microorganisms characterized by differences in physiology. Culture tests and molecular studies showed the presence of microorganisms oxidizing iron within the slime streamers and it seems that the biotic process of iron(II) to iron(III) oxidation successfully competes with its abiotic oxidation in flowing water conditions, resulting in the development of numerous microbial structures, possibly physiologically also utilizing this process. The analyses allow tentative determination of microbiological processes linked to the sulphur and iron cycle. Figure 9 shows a scheme of processes that may have crucial meaning for the shaping of the biogeochemical cycle of sulphur and iron in two chemically different water reservoirs in the weathering zone of the pyrite-bearing schists at Wieściszowice. In the Blue Pond the dominating processes in the sulphur cycle are the microbiological reduction of sulphates in the bottom deposits and oxidation of hydrogen sulphide by microorganisms preferring slightly acid and neutral conditions. In the Purple Pond, a process dominating among the obligatory or facultative chemolithotrophic microflora is most probably iron oxidation. This can be shawn by the abundance of weathering sulphate minerals containing Fe^{3+}, such as copiapite, fibroferrite or slavikite, whereas ferrous sulphates occur only sporadically.

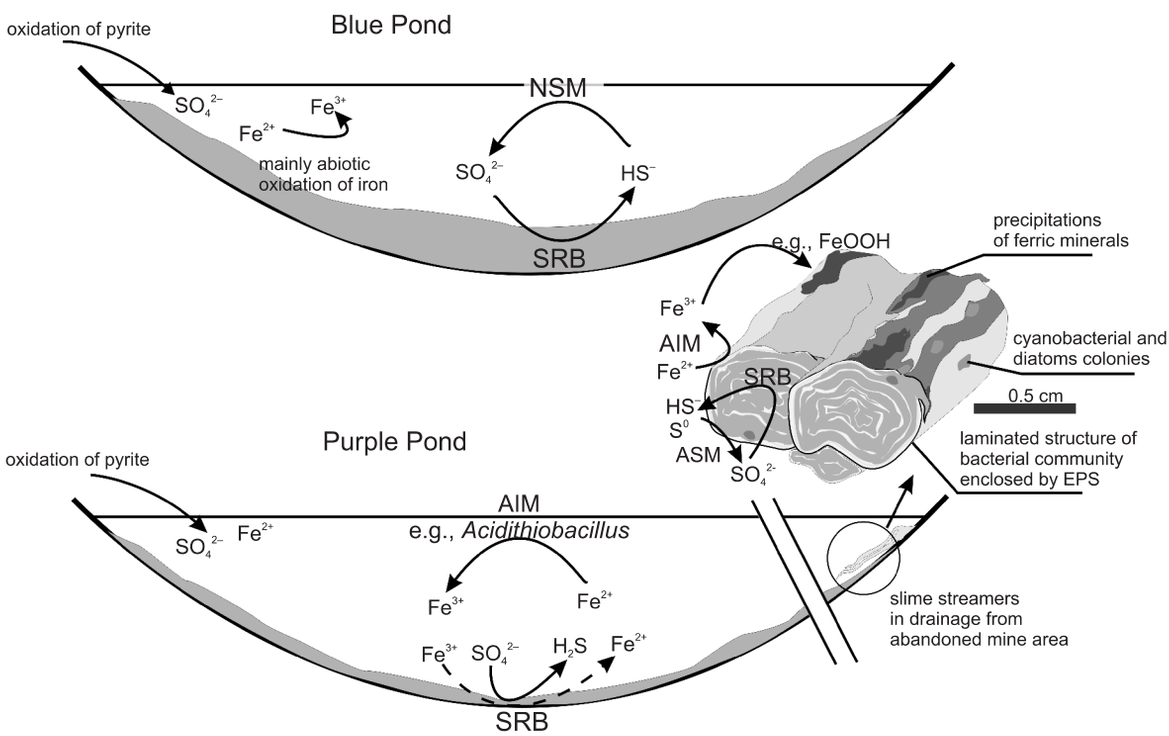

Fig. 9. The potentially most important processes in the water reservoirs studied having influence on the shape of the biogeochemical cycle of sulphur and iron

AIM – acidophilic iron-oxidizing microorganisms, ASM – acidophilic sulphur microorganisms, EPS – Extracellular polymeric substances, NSM – neutrophilic sulphur microorganisms, SRB – sulphate-reducing bacteria

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