BIOLOGICAL TREATMENT OF OVERBURDEN FROM LIGNITE OPENCAST MINING IN ORDER TO AVOID SEEPAGE OF ACID MINE WATER

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ABSTRACT

The formation of acid mine water (AMD) in pyrite containing materials like coal, lignite, overburden material from lignite mining or sulfidic ores is a bacterially catalyzed oxidation process, which is initiated when the material attains contact with air and water.

The formation of sulfate and the mobilization of heavy metals can be reversed by bacterially catalyzed sulfate reduction which produces H_2S at the expense of organic substrates being oxidized in the process.

Laboratory experiments, as well as an outdoor pilot scale study, have shown that biological treatment of pyrite-containing overburden is a suitable method for avoiding the seepage of AMD: Seepage water from biologically treated overburden had a pH of 5.2, a redox potential of + 120 mV and a sulfate concentration of approximately 7 g/L, while in the untreated control the corresponding values were 1.5, + 630 mV and about 60 g/L, respectively.

The success of the process and the necessity of pH-increasing pre-treatment depend mainly on the degree of pre-oxidation of the overburden being treated and on the organic substrates added. Suitable substrates are those which provide alkalinity together with good degradability e.g. sewage sludge.

Treatment is accompanied by changes in the structure of the microbial community in the overburden. These changes were monitored using classical microbiological and molecular biological methods.

INTRODUCTION

In 1996, 187 million tons of lignite were won in Germany by opencast mining. In order to obtain lignite by surface mining, vast amounts of overburden sediments are to be removed, which in 1996, for example, amounted 920 million cubic meters. This overburden is piled up in big dumps or used to fill abandoned workings. During this procedure the overburden is intensively exposed to oxygen, which initiates a bacterially catalyzed oxydation of sulfidic compounds, mostly FeS_2 as pyrite or marcasite (eq. 1).

$$FeS_2 + 3{,}5\ O_2 + H_2O \text{ v } FeSO_4 + H_2SO_4 \qquad \text{Eq. 1}$$

The sulfuric acid formed by this reaction destroys other non-sulfidic minerals present in the overburden sediment, subsequently releasing heavy metals. The seepage waters derived from this process are known as Aacid mine drainage@ (AMD). AMD is characterized by a low pH (as low as 1.5) and high concentrations of sulfate and heavy metals. The contamination of surface waters, ground water reservoirs or soil with AMD is an environmental hazard which must be strongly avoided. Processes have therefore been developed for treating AMD prior to its release into the environment.

Various biological and chemical processes of treating collected AMD have been described previously, for example by Stucki et al. (1993), Maree and du Plessis (1993), Dill et al. (1995). Most of these processes use different types of reactors, stirred tank- as well as fixed bed-reactors.

The biological processes for treatment of AMD make use of the anaerobic bacterial sulfate reduction. In the presence of organic substrates, e. g. alcohols or organic acids, as suitable electron-donors for the bacteria, sulfate is biologically reduced to sulfide (eq. 2).

$$4\,(CH_2O) + FeSO_4 + H_2SO_4 \text{ v } 4\,CO_2 + 4\,H_2O + FeS + H_2S \qquad \text{Eq. 2}$$

Oxidizable gases, such as hydrogen or carbon monoxide, may react in the same way as the organic energy sources mentioned above (eq. 3).

$$4\,H_2 + H_2SO_4 \text{ v } H_2S + 4\,H_2O \qquad \text{Eq. 3}$$

The use of gases has the advantage that no excess organic load appears in the water being treated.

Due to the consumption of protons in these reactions there is an increase in pH and the sulfide formed precipitates dissolved heavy metals as virtually insoluble heavy metal sulfides. The result is an effluent water with reduced concentrations of sulfate and heavy metals and neutral pH.

Because of the high costs of treating collected AMD in reactors by the so-called "pump and treat"-techniques, there was a need to look for cheaper alternatives. We thus conducted pilot scale experiments involving the treatment of AMD in situ at its source inside the overburden dumps. For this purpose we tried to establish zones of active biological sulfate reduction in the sediment by adding organic substrates. In this way, both bacterial catalyzed reactions, sulfide oxidation (equation 1) and sulfate reduction (equation 2) occur in close proximity, in so called "micro-environments". The overall reaction of equation 1 and equation 2 is as follows (eq. 4):

$$FeS_2 + 3{,}5\,O_2 + 4\,(CH_2O) \text{ v } 4\,CO_2 + 3\,H_2O + H_2S + FeS \qquad \text{Eq. 4}$$

This means, that in sediments containing pyrite or other sulfidic minerals the seepage of acid mine water can be avoided by adding a suitable organic substrate to the sediment. We conducted experiments on this new type of in situ-treatment.

The in situ-treatment of overburden sediments leads to changes in the bacterial in situ-community, since composition and diversity of bacterial communities respond to environmental conditions in the habitat and this determines the functional activities in the bacterial community. These changes have to be monitored, not only by legal issues, but also to ascertain that the changes being induced lead to the desired functional activities. These changes can be monitored by classical microbiological methods such as determinations of the "most probable number" (MPN) of different physiological groups.

Considering the great number of not yet cultured - and perhaps non-culturable - bacteria, new approaches are required. For determining the abundance of sequences with phylogenetic significance and for the analysis of bacterial communities, molecular methods are of increasing importance.

We thus attempted to obain an overview of sequence diversity of PCR products of variable sections of 16S rRNA coding genes, and compared these data with the results obtained by classical microbiological methods.

EXPERIMENTAL

We conducted preliminary laboratory experiments with a series of leaching columns, which had been previously described (Becker et al., 1993), and a pilot scale field study to ascertain the effectiveness of this treatment process under outdoor conditions.

As pyrite-containing material we used in both sets of experiments overburden sediment from lignite opencast mining. In the laboratory experiments we used sediment with a maximum sulfur content of 2.0 g/kg and pH values as low as 2.4. Organic substrates used as energy sources were sewage sludge, waste water from a sugar factory, chopped wood and straw.

Based on data obtained in the preliminary experiments, the pilot scale study was performed under outdoor conditions in test dumps: wooden containers, 3.4 m^3 in size, filled with overburden material with a sulfur content of 6 g/kg and a degree of sulfur pre-oxidation of about 30 % (pH 2.6). In the treated dumps, the overburden was mixed with 10 % (v/v) of organic substrates, different kinds of sewage sludge and compost material of varying age. In one of the test dumps the substrate (compost) was added in layers. One untreated dump was used as a control,while another dump was treated only with waste calcium carbonate.

The dumps were watered by an automatic sprinkler system adjusted to supply 1250 L/m^2 per year, double the amount of natural rain fall in Northern Germany. The test dumps were run for a total period of 18 months, for 5 months of which they were completely frozen.

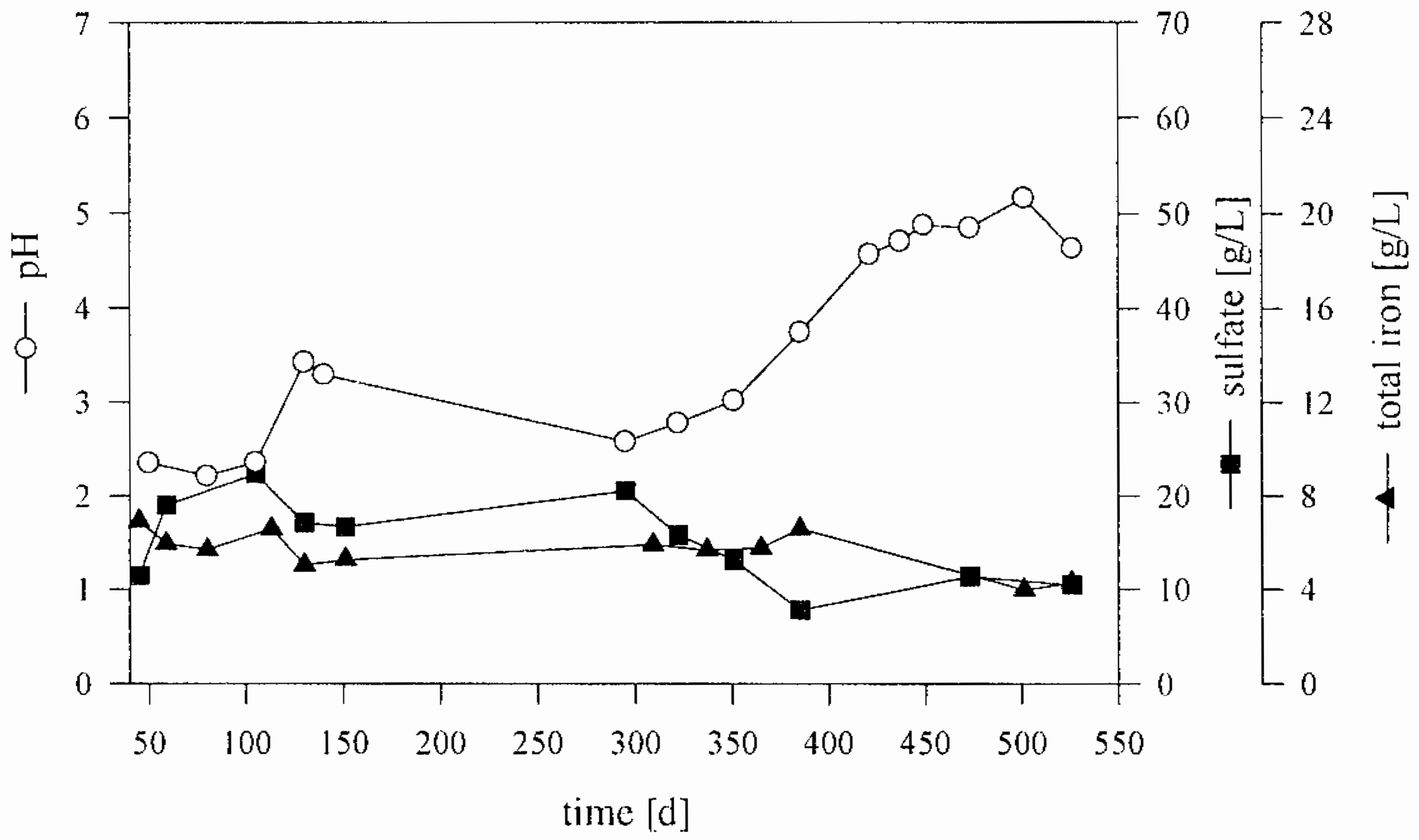
Effluent, seepage water from the test dumps, was analyzed for pH value and redox potential (Eh), as well as for the concentrations of sulfate, total iron and dissolved organic compounds (DOC). Shifts in the bacterial community were monitored both by determination of the MPN of different physiological groups of bacteria (S-oxidizers, Fe-oxidizers, sulfate reducing bacteria) and by characterization of the 16S rDNA pattern obtained in Temperature Gradient Gel Electrophoresis (TGGE) following cell- and DNA isolation and Polymerase Chain reaction (PCR). The variation of patterns generated by sequence specific separation of 16S rDNA fragments using TGGE were compared with the results obtained from traditional MPN determinations.

RESULTS

Our laboratory experiments with the leaching columns showed bacterial sulfate reduction to occur in "fresh", non-oxidized sediment with high sulfur content (2.0 g/kg, pH 5.1), as well as in oxidized sediment with low sulfur content (0.01 g/kg, pH 6.0), that means in sediments with moderate pH-values and relatively low initial sulfate concentrations.

In oxidized sediments with high sulfur content, i.e. with a low pH of 2.4 and in which all sulfur (2.0 g/kg) had been oxidized to sulfate, sulfate reduction started after the pH of the the column had been increased with limestone or calcium hydroxide. The pH of column effluents was about 8.0, while sulfate concentration decreased from 2.5 to 0.56 g/L. Heavy metals were completely absent, having been removed from the effluent through precipitation as sulfides.

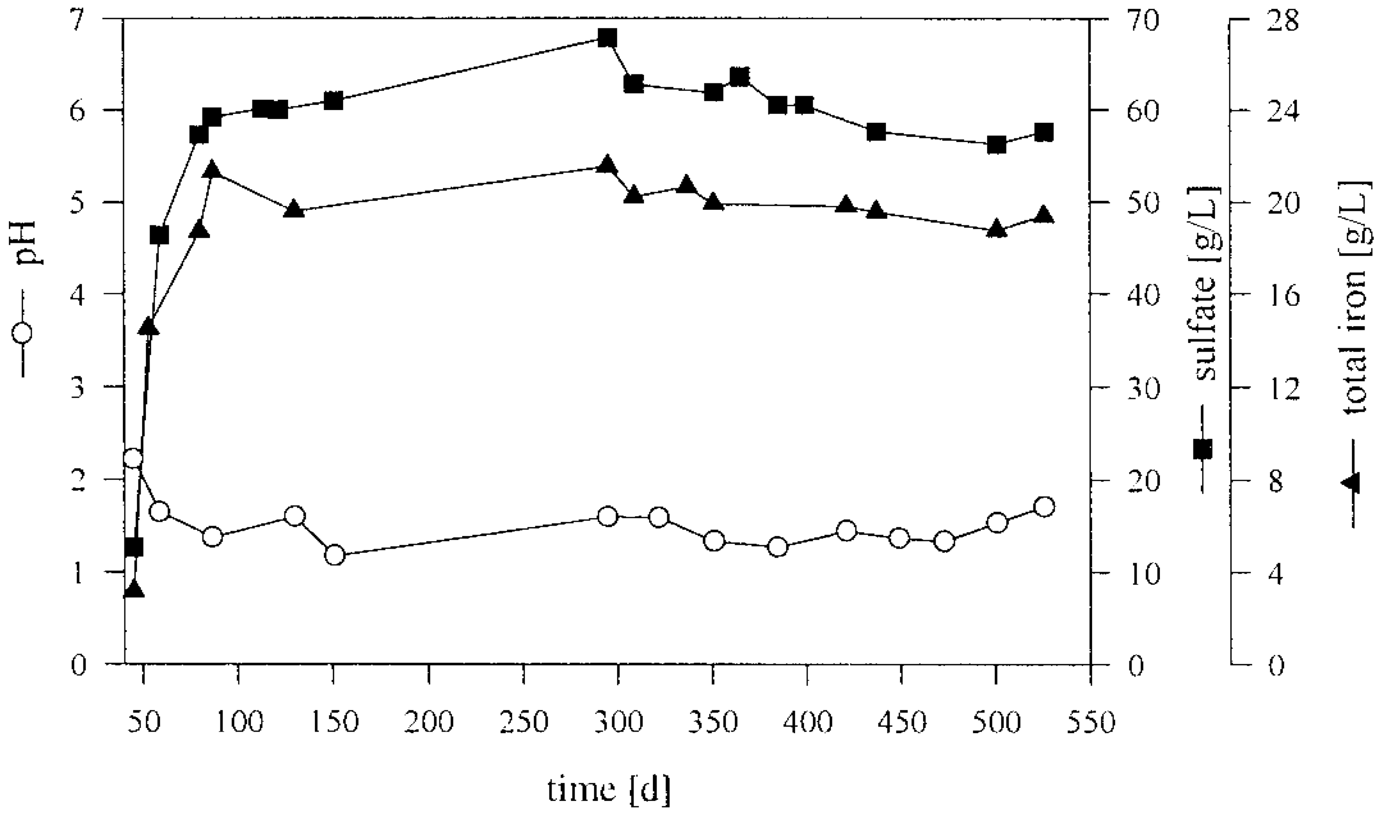
According to the results of this preliminary laboratory study, bacterial sulfate reduction occurred in test dumps of overburden sediment under outdoor conditions inside the sediment (sulfur content 6 g/kg) after a period of 12 months (with an active period of 7 month), but only in the dump containing dewatered and lime-treated sewage sludge. In the zone of sulfate reduction (the water saturated zone at a depth of 0,65 - 0.95 m), pH increased from 2.4 to 7.0 and the redox potential Eh decreased from + 630 mV to - 190 mV. When the study was terminated, the concentrations of sulfate and total iron in treated dump effluent were 7 g/L and 4 g/L, respectively (fig. 1), while in the untreated control dump the concentrations of sulfate and total iron were about 60 g/L and 20 g/L, respectively (fig. 2). Here, the seepage water remained nearly unchanged during the whole course of the study, with constantly high concentrations of sulfate and iron, and pH values between 1.5 and 2.0. The sediment showed a pH of about 2.4 and a redox potential Eh of + 610 mV. The dump treated with waste calcium carbonate (fig. 3) showed decreases in the concentration of both sulfate and iron, while pH of the seepage water was about 2.5. Inside the sediment, pH was 5.5 and redox potential Eh + 340 mV.



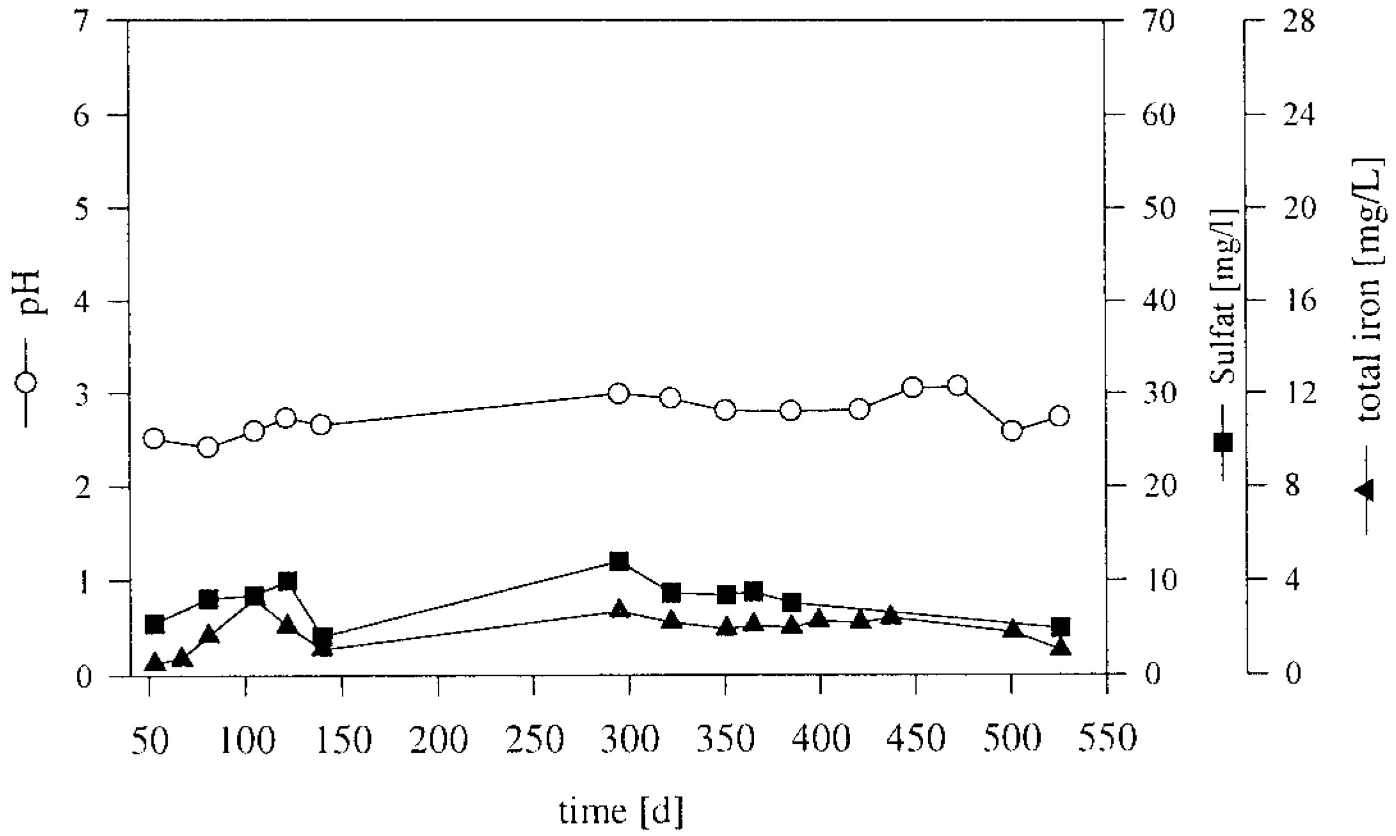
Although bacterial sulfate reduction did occur, the concentration of sulfate reducing bacteria did not significantly increase in the treated dump. The concentrations of SRB were about 10^6 g^{-1} during the whole experiment. In the untreated control, a SRB concentration of about 10^2 g^{-1} was found, while in the dump treated with calcium carbonate the SRB concentration was 10^1 g^{-1} (fig. 4)

The community patterns determined during the course of the study by TGGE analysis are shown in figure 5. Using a set of universal primers (fig. 5 A), complex and reproducible band patterns of 16S rDNA fragments from directly extracted microbial communities (lane A 4-11) were obtained. Some of the bands (A 8, A 22/23, A29/30) show an intensity shift in time, indicating a succession in the community. The patterns resulting from MPN cultures of SRB (lane A 1-3) are less complex and less unique, and show bands (A 3, A 5-11) which are not found using the non-cultivated approach.

The use of a primer set "specific" for SRB led to different patterns of reduced complexity (fig. 5 B). In contrast to the patterns described above, two bands (B 2, B 20) obtained from the SRB cultures correspond to bands of the directly extracted community. Due to the selectivity of the SRB medium, bands B 8-19 do not appear in lane B 1-3. The shift in the microbial community can also be observed in these patterns and corresponds with the increase in bacterial sulfate reduction.



pH
sulfate [g/L]
total iron [g/L]
time [d]
0
1
2
3
4
5
6
7
0
10
20
30
40
50
60
70
0
4
8
12
16
20
24
28
50
100
150
200
250
300
350
400
450
500
550



pH
Sulfat [mg/l]
total iron [mg/L]
time [d]
0
1
2
3
4
5
6
7
0
10
20
30
40
50
60
70
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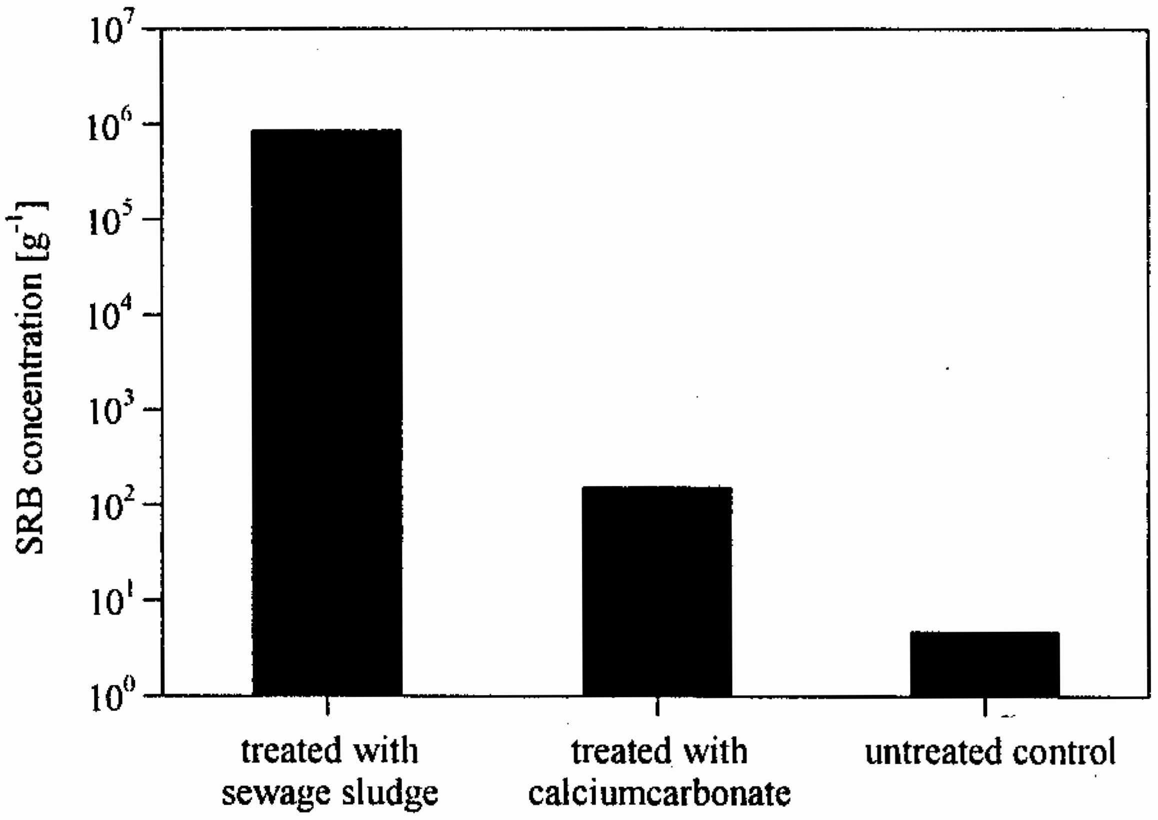

DISCUSSION

The bacteria responsible for the oxidation of sulfidic compounds according to equation 1, and thus for the production of AMD from sulfidic materials, are acidophilic bacteria. They are well adapted to acidic conditions and can withstand as low as about pH 1. The bacteria able to carry out the reverse reaction, biological reduction of sulfate, are neutrophilic bacteria which are inhibited at pH values lower than about 5. It therefore seemed questionable, as to whether biological sulfate reduction could be initiated in overburden material from lignite mining or other materials leading to the seepage of AMD.

On the other hand, it is known that in many habitats, acidophilic sulfur oxidizing bacteria and neutrophilic sulfate reducing bacteria (SRB) are active in close proximity to one another (Graff and Näveke, 1985, Binder and Graff, 1995). In accordance with these earlier findings, our current investigations have shown that even in quite acidic sediments, viable SRB were present and detectable both by cultural and molecular-biological methods.

Their metabolic activity could be increased by adding suitable organic substrates. Starting from "micro-environments" with appropriate physico-chemical conditions, zones of active biological sulfate reduction extended to areas where conditions were previously not suitable. Sulfate formed by bacterial oxidation, for example,. at the top of the dumps due to oxygen bearing rain water, is rapidly reduced within the dumps in anaerobic zones of bacterial sulfate reduction. Because of the limited oxygen supply deeper in the sediment, oxidation processes come to an end.

In our outdoor experiments, sulfate concentration in effluent from the treated dumps was decreased by about 90 %, while iron concentration was reduced by about 80 %. Despite a 5-months period during which the dumps were completely frozen, the relatively short "vegetation period" of 7 months was sufficient for the initiation of a viable sulfate reduction inside the fairly acidic dump material. The properties of effluent from the treated dump shifted from being those of real AMD to those of slightly acidic water, while physicochemical conditions inside the dump changed from acidic and highly oxidizing conditions favourable for acidophilic bacteria to neutral and more-or-less reducing conditions.

In figure 6, an Eh-pH-diagramm shows the areas of stability for different iron compounds and ions in the system Fe - H_2S - CO_2 - H_2O (Krauskopf, 1979). To this diagramm the areas of activity for the sulfide oxidizing thiobacilli and for the sulfate reducing bacteria are added, according to Baas Becking and Wood (1955).

The arrows indicate shifts of pH and Eh measured in the sediment during the study, the upper short arrow for the dump treated with calcium carbonate, the lower long arrow for the dump treated with sewage sludge. It can be easily seen that coincidental with the change in the areas of bacterial activity, there is also a change in the stability fields of iron-compounds. pH-Eh-conditions in the dump containing sewage sludge changed from the activity area of the sulfide oxidizing bacteria to that of the sulfate reducers and concomitantly from soluble iron ions to the virtually insoluble compounds FeS and $FeCO_3$. The shift observed in the dump treated with carbonate did not lead to a change in the area of bacterial activity, nor to a change in the stability field of the iron species.

Nevertheless, treatment with sewage sludge did not restore the initial conditions found in the overburden sediment prior to its movement: The sulfidic compound present in the overburden is FeS_2, originally formed by early diagenetic processes inside the sediment. The products released by bacterial sulfate reduction are FeS and H_2S, according to equation 2. Currently we have no data on how fast the reaction of these two compounds to FeS_2 will take place in overburden dumps. This remains to be determined. So, the net chemical change is the conversion of FeS_2 to FeS and H_2S, at the expense of organic substrates, which are oxidized to CO_2.

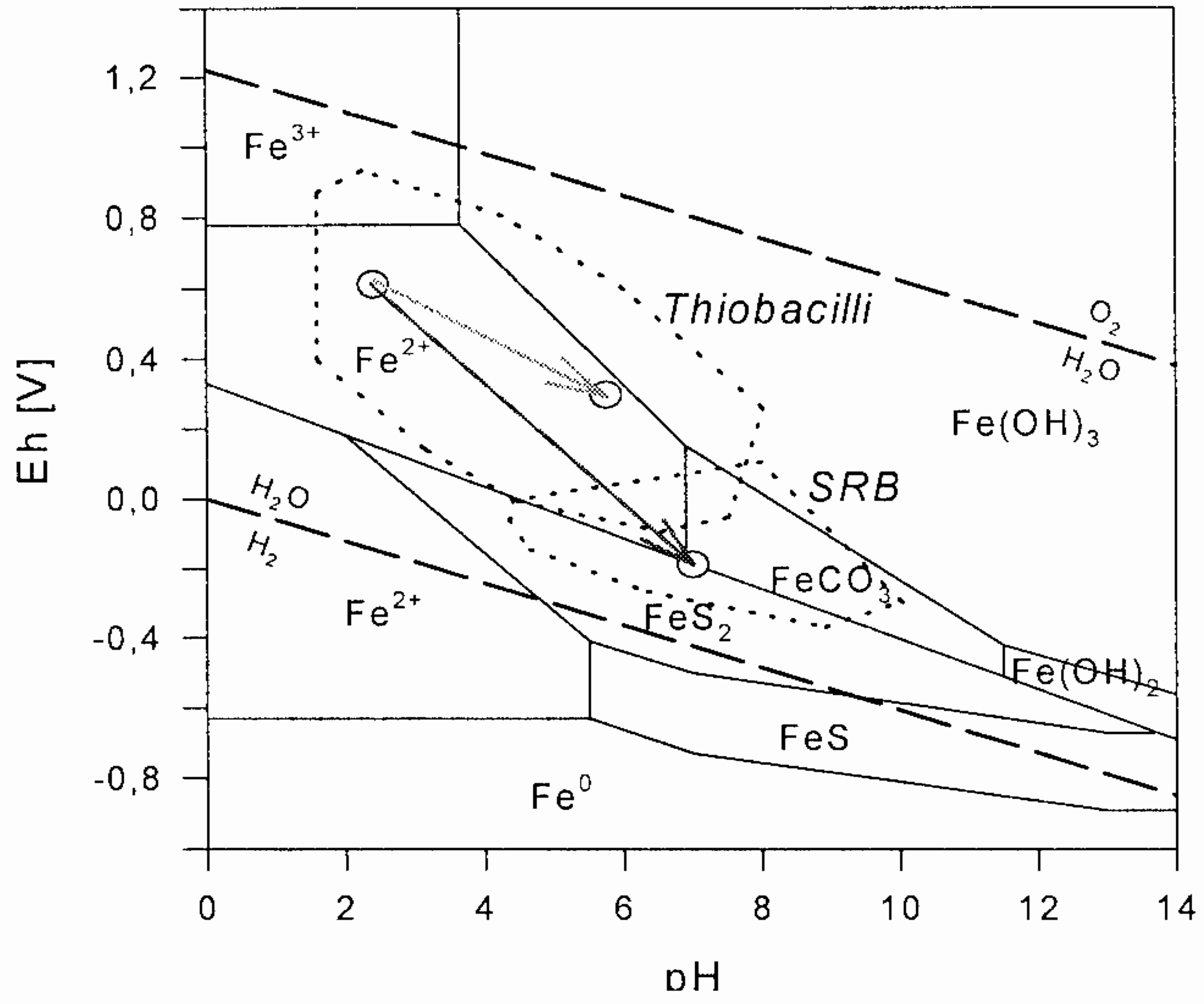

Fig. 6: Eh-pH-diagramm for the system Fe - H_2S - CO_2 - H_2O and areas of activity of related bacteria Arrows indicate the shifts having occured in the treated dumps.
upper short arrow: dump with calcium carbonate, lower long arrow: dump with sewage sludge

Surprisingly, the sulfate and iron concentrations in the dump treated with calcium carbonate also decreased markedly. But here, pH and redox conditions did not change, so that the effluent remained very acidic. Probably, the decrease in sulfate and iron concentrations is due to abiotic chemical precipitation reactions. It seems likely that the concentrations will rise again after depletion of the carbonate stock. Sulfate reducing bacteria are present in the sediment but are inactive due to the lack of oxidizable substrate. In our preliminary laboratory experiments, we found that in pre-oxidized, i.e. fairly acidic sediment, sulfate reduction starts only after a slight increase of the pH. This can easily be done by adding dewatered and lime treated sewage sludge. The success of the process depends mainly on the degree of pre-oxidation of the overburden being treated and on the organic substrates being added. Suitable substrates are those which provide alkalinity together with good degradability.

Concerning monitoring of the changes in the microbial in situ-community, we found TGGE to be a useful tool in the analysis of complex habitats, avoiding the drawbacks of having to cultivate the bacteria under investigation. Comparing the traditional cultivating method of MPN determination and TGGE, the molecular biological technique provides a more detailed community analysis. In addition, the beginning of bacterial sulfate reduction, as indicated by chemical analysis of the effluent, was not detectable as a community shift in the MPN determinations. Using the molecular biological approach, the shift in the microbial community could be observed in the obtained patterns as corresponding with the increase in bacterial sulfate reduction. Furthermore, molecular biological investigations provide a more detailed insight into the microbial community than the classical MPN determinations. They provide information on distinct species or strains, whereas MPN determinations give information on physiological groups of bacteria.

We are convinced that many problems and costs resulting from the seepage of acid mine water could be avoided by treating the overburden in situ instead of collecting and treating the AMD after its release from the dumps. We plan to carry out a large scale treatment experiment using the technique described in this paper in the near future.

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LITERATURE

1. Baas Becking, L.M.G., Wood, E.J.F. (1955) Biological processes in the estuarine environment Kon. Ned. Akad. Weten. Proc. B. 58, 160-181
2. Becker, B., Graff, M., Näveke, R. (1993) Initiation of bacterial sulfate reduction in acidic overburden dumps from lignite surface mining 3. Biennial Conference of the Water Institute of Southern Africa, Durban, South Africa, 1993
3. Becker, B., Engelen, B., Lüdemann, H., Graff, M., Näveke, R., Backhaus, H. (1997) Characterizing the treatment of acid mine drainage with sulfate reducing bacteria by fingerprinting the associated microbial communities in TGGE VAAM -Jahrestagung, Hamburg, 1997
4. Binder, G., Graff, M. (1995) Microbial induced corrosion at hydraulic steel structures Materials and Corrosion **46**, 639-648
5. Dill, S., Du Preez, L., Graff, M., Maree, J. (1995) Biological sulphate removal from acid mine drainage utilising producer gas as carbon- and energy source Water Sewage and Effluent **15**, 43-44
6. Graff, M., Näveke, R. (1985) Microbial Metabolism in the Lake Emma near Helmstedt (Lower Saxony) Braunschw. Naturk. Schr. **2**, 355-372
7. Graff, M. (1997) Biotechnische Verfahren zur Behandlung und Vermeidung schwefelsaurer Grund- und Bergbauwässer Umwelttechnik Forum **12**, 2, 17-18
8. Krauskopf, K.B. (1979) Introduction to Geochemistry McGraw-Hill Int. Series, 2. Edition, New York, 1979
9. Maree, J.P., du Plessis, P. (1993) Neutralisation of acidic effluents with limestone WRC Report No. 355/1/94
10. Muyzer, G., De Waal, E.C., Uitterlinden, A.G. (1993) Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S Rrna Appl. Environ. Microbiol. **59**, 695-700
11. Stucki, G., Hanselmann, K.W., Hürzeler, R.A. (1993) Biological sulfuric acid transformation: Reactor design and process optimization Biotechnology and Bioengineering **41**, 303-315