Biological sulphate removal from acid mine drainage utilizing producer gas as carbon- and energy source - Process limitations and their resolution -

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ABSTRACT

Many industries especially mining companies are faced with serious problems caused by effluents containing high amounts of sulphate, heavy metals and a low pH. These effluents are known as acid mine drainages (AMD). Due to the fact that AMD is highly corrosive, it results in many economic and environmental problems. These problems can be avoided by using a cheap and effective method of biological effluent treatment. In an anaerobic process AMD is percolated over a packed-bed with an attached biofilm of sulphate reducing bacteria (SRB). The bacteria reduce the sulphate to hydrogen sulphide (H_2S) by using different organic carbon sources. For an optimal performance of the biological process a pH between 6.8 and 7.5 is needed. A treatment of AMD with limestone as a pre-neutralisation step is possible [11], but not necessary in a stable running process [17]. In higher concentrations produced H_2S inhibits the biotransformation [10].

To avoid the problems caused by addition of organic material to the effluent, producer gas as a gaseous carbon- and energy source can be used. With producer gas, the biomass production and the sulphate reduction rate are much lower compared to the addition of organic material such as molasses or sugar [13,17]. This paper shows the resolution of the process limitations using three different reactor types and sizes for the investigations. The process was optimized in a two step scale-up. Four small fed-batch reactors (3 l) were used for different feeding experiments. AMD with sulphate concentrations between 600 - 6000 $mg \cdot l^{-1}$ was recycled in addition with different nutrition elements to CO as main substrate. Results were implemented on a laboratory reactor (15 l) which was running over 150 days and after successful operation, a pilot plant (800 l) was running for 100 days under the same conditions. Investigations with the fed-batch reactors resulted in an additional feeding of nutrients, the process limitations could be resolved and the sulphate reduction rate in the bigger plants was 1.8 - 2.4 $g \cdot l^{-1} \cdot d^{-1}$.

KEYWORDS

Sulphate; producer gas; carbon monoxide; hydrogen; hydrogen sulphide; anaerobic.

INTRODUCTION

The discharge of effluents rich in sulphate is of increasing concern. Discharging industrial effluents containing high sulphate concentrations into surface waters contributes directly to mineralization and corrosion potential of the receiving waters. Sulphate originates from the use of sulphuric acid in manufacturing, chemical and metallurgical processes, or from the oxidation of pyritic material in either sulphidic ores or coal ore bodies under aerobic conditions, like in overburden dumps e.g. Unacceptable high concentrations of sulphate may occur in cooling water due to evaporation.

Generally, sulphate levels less than 200 to 500 $mg \cdot l^{-1}$ are acceptable for discharge into public streams. The Municipality of Johannesburg and water authorities in the Transvaal area allows the discharge of water with high sulphate concentrations (e.g. 2000 mg/l^{-1}) into sewer systems or rivers. The main reason for the acceptance is the fact that the ratio of sulphate-rich water produced by industrial activities (e.g. mining, chemical, and power stations) to surface water, is high in that region. It is expected that as soon as proven technologies are available for the removal of sulphate at an acceptable cost, legislation would be enforced to prevent the discharge of waters with high sulphate concentrations into the receiving waters bodies.

Sulphate can be removed from water by desalination processes such as reverse osmosis and ion exchange, but these are costly. The biological sulphate removal from AMD with sulphate reducing bacteria is, in comparison with the costs of chemical purification, far more economical. Sulphate reducing bacteria (SRB) oxidize organic compounds like lactate or acetate, with sulphate as electron acceptor being reduced to sulphide:

$$8\ CH_3COO^- + 8\ SO_4^{2-} + 16\ H^+ \longrightarrow 16\ CO_2 + 16\ H_2O + 8\ HS^-$$

This reaction consumes protons leading to an increase in pH of the treated water up to a final pH of 7.0 - 7.5. Heavy metals are precipitated by produced H_2S as virtually insoluble heavy metal sulphides. Hence, increasing attention has been given to the process of biological sulphate removal by Maree et al. and Naeveke et al. Sulphate can be removed biologically provided that a suitable energy source is available, e.g. lactic acid (Middleton and Lawrence [19]), wood dust and sewage sludge (Butlin et al. [7], [8]; Conradie and Grütz [9]; Knivett [14]; Sadana and Morey [21]; Tuttle et al. [22]). Good sulphate removal was obtained for all carbon sources, but long retention times of 5-10 days were required.

A possibility to avoid the release of AMD in the environment is an in-situ treatment of the water. A long term study over a 18 months period, where different organic materials were mixed with dump material and flooded with AMD, showed, that an initiation of sulphate reduction in the oxygen free layers of the soil was obtained. By adding waste water of a sugar beet plant as additional carbon- and energy source the sulphate reduction carried on over the complete experimental period. (Becker et al. [3], [4]).

Experiments with stirred tank reactors showed low sulphate reduction rates compared to packed bed reactor results (Dill [10]). Using stirred tank reactors for sulphate reduction, a major part of the SRB could be found grown up as a biofilm on the reactor surfaces. For studies over a period longer than 30 days with nitrogen- and phosphorus free AMD an addition of ammonia and phosphor is necessary to avoid a limitation of the sulphate reduction process (Marschall [18]). For higher sulphate reduction rates, reactors with a big surface as support media for the biofilm, like packed-bed-, membrane- or fluidized-bed reactors should be used. Figure 1 shows a scheme of the formation of AMD in overburden dumps and the possible on-site treatment by SRB in reactors.

Upflow packed bed reactors were used by Maree and Strydom [15] to establish well-developed microbial biofilms for sulphate removal from mine water with either sugar, pulp mill effluent or sewage as energy sources. They concluded that 1.6 mg sugar, 16.7 ml of spent sulphite liquor or 172 ml raw sewage sludge were necessary to remove 1 800 $mg \cdot l^{-1}$ sulphate.

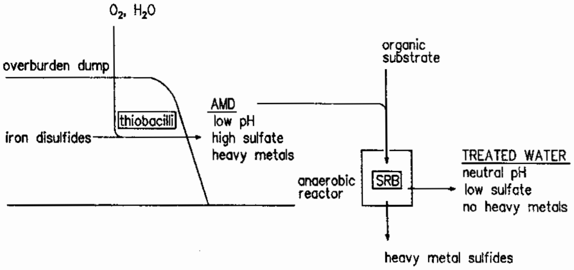


Figure 1: formation of AMD by bacterial sulphide oxidation in overburden dumps and proposed treatment of AMD by SRB in reactors

Maree et al. [16] showed that a three stage process (anaerobic - aerobic - anaerobic) employing upflow packed bed reactors (or sludge blanket reactors) for anaerobic treatment, and an activated sludge system for aerobic treatment, could be used for producing reusable water from mining effluents. Sulphate was reduced form 2.5 g/l to less than 0.5 g/l with concomitant removal of H_2S, carbonates, complexed cyanides, phenol and heavy metals. Molasses was used as energy source. Olthof et al. [20] described the "Biosulfix" process in which H_2S gas produced during sulphate reduction is absorbed in sodium hydroxide and converted to sodium bisulphide. The later product can be reused in pulp manufacturing. Buisman [5] showed that H_2S produced during biological sulphate reduction can be oxidised to elemental sulphur only (and not oxidized totally to sulphate) provided that the oxygen level in the process is kept low.

A major disadvantage of biological sulphate removal from effluents by addition of organic carbon sources as substrate, is the high residual organic carbon content or the release of coloured water [17], which requires downstream treatment after the anaerobic reactor. This disadvantage was overcome by replacing organic carbon sources with producer gas (a mixture of CO_2, CO, H_2 and N_2) (Du Preez et al. [12], [13]). Producer gas is a cheap carbon- and energy source, available from different gasification processes in sufficient quantities for the treatment of large flows. If producer gas is used as carbon- and energy source instead of organic material, the VSS concentration on the packed bed is low and the sulphate reduction rate is limited [13,17].

The purpose of this investigation was to demonstrate the technical feasibility of the biological sulphate reduction process, after removal of the process limiting factors.

Specific aims of the study were to determine:

- The different rates of sulphate reduction on pilot scale using producer gas or carbon monoxide as main energy source.
- The effect of H_2S removal from the recycled gas, through absorption, on the sulphate reduction rate.
- The effect on the sulphate reduction rate by adding different feeding solutions like yeast extract, trace elements and small amounts of lactate to the anaerobic reactor.

MATERIALS AND METHODS

Four small fed-batch reactors (volume 3 l) and a laboratory reactor (volume 15 l) were used. The added feedstock was artificial AMD, containing 3000 $mg \cdot l^{-1}$ sulphate, 500 $mg \cdot l^{-1}$ iron, ammonia and phosphate (chemical composition: see below).

Fed-batch reactors

For the investigations with additional nutrients four fed-batch reactors were used. The reactors had a height of 0.8 m and a diameter of 0.09 m. They were filled with 3 different kinds of packed bed material and inoculated with sewage sludge of different ages (see table 1). The volume of the packed bed was 3 l, covered by 0.75 l reactor liquid, which was in total also 3 l.

The reactor liquid was recycled by a pump with hydraulic retention times of 4.0 to 0.7 h. AMD and the tested additional nutrients were added by releasing the liquid reactor content out of the reactor and exchange 0.75 l of reactor liquid against AMD. The new solution was mixed well and returned into the reactor. Carbon monoxide or producer gas was bubbled through the reactor from the bottom hourly. If the experiments were made with organic compounds like lactic acid or molasses as carbon- and energy source, nitrogen gas as protection gas was used.

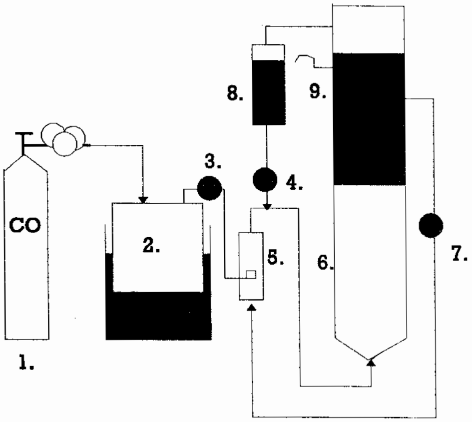
Experiments were made with artificial AMD, the sulphate concentrations were between 600-6000 $mg \cdot l^{-1}$ in the reactor liquid. pH, sulphide and alkalinity were measured in the beginning and at the end of an experiment, sulphate was measured every 2-4 hours over 24 hours. The removal of sulphate was expressed as kinetic of sulphate removal (removal of sulphate [$mg \cdot h^{-1}$]).

Laboratory reactor

A laboratory plant was running over a period of 150 days with carbon monoxide as main energy source. The laboratory reactor was used as a first scale-up step, where the results from the fed-batch reactors were implemented in, by testing the effect of different additional nutrient solutions on the sulphate reduction in the 15 l scale.

Samples were taken daily and analyzed for sulphate and pH, weekly alkalinity, sulphide, calcium, ammonia, phosphate and iron were analyzed. Complete analyses of VSS and acetate were carried out every fortnight.

Figure 2 shows a schematic diagram of the laboratory reactor. The reactor had a diameter of 150 mm, a height of 1 500 mm and a volume of 15 l. It was filled with clay rings (see table 1) as support media for bacterial growth. The biologically active film was established on the packed bed by inoculating the reactor with fresh sludge from an anaerobic digester 24 months previously. The AMD was fed from the bottom with a feed rate of 1.5-15 $l \cdot d^{-1}$. Carbon monoxide was also fed with a feed rate of 20-80 $ml \cdot min^{-1}$ from the reactor bottom and recycled in a side stream, where H_2S was removed from the excess gas, leaving the reactor, by bubbling it through 43% ferric solution.



1.	Carbon monoxide cylinder	2.	Gas reservoir
3.,4.	Gas pumps	5.	Diffuser
6.	Packed bed	7.	Water recirculation pump
8.	Ferric solution for H_2S removal	9.	Overflow

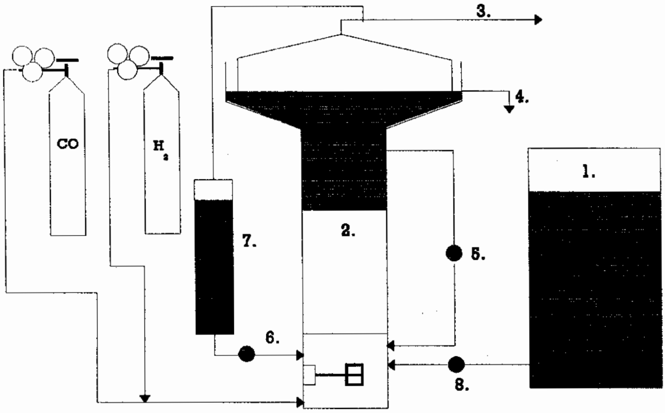
Fig. 2: Schematic diagram of the laboratory reactor for biological sulphate removal used as intermediate step for the scale-up on the pilot plant

Pilot plant

Together with the laboratory plant a pilot plant was also operated in a similar way for a total period of 100 days. During the first 20 days the reactor was stabilised by feeding fermented molasses as main energy- and carbon source. Then the reactor was running over 60 days with carbon monoxide (CO) by adding small amounts of fermented molasses, yeast extract and trace minerals. Over the remaining 20 days the feed gas was a mixture of hydrogen and carbon monoxide (H_2/CO) in a ratio of 50:50.

Samples were taken daily and analyzed for sulphate and pH, weekly alkalinity, sulphide, calcium, ammonia, phosphate and iron were analyzed. Complete analyses of VSS and acetate were carried out every fortnight.

Figure 3 shows a schematic diagram of the pilot plant. The anaerobic reactor had a diameter of 500 mm and a height of 4 200 mm. It was filled with 200 l of pelletized ash (see table 1) as support medium for bacterial growth. A biologically active film was established on the pebble medium by inoculating the reactor with anaerobic sludge from a sulphate reducing laboratory plant 20 months previously. The temperature was kept constant at 35°C by means of a thermostat. Carbon monoxide\Hydrogen gas was introduced into the anaerobic reactor from the reactor bottom and was recirculated in a side stream downwards, through 43% ferric solution for H_2S removal from the gas stream. The amount of gas fed was about 300 - 600 ml·min^{-1}. AMD was pumped from a 1 500 l feedstock tank to the anaerobic reactor at a rate between 40 and 400 l·d^{-1}. The flowrate of the water recycled was 1.0 l·min^{-1}.



1.	Feed reservoir	2.	Anaerobic packed-bed reactor
3.	Gas outlet	4.	Outflow
5.	Water recirculation pump	6.	Gas recirculation pump
7.	Ferric solution	8.	Water feed pump

Fig. 3: Schematic diagram of the pilot plant used for biological sulphate removal

The chemical composition of the feedstock was as follows:

514 mg·l^{-1} H_2SO_4 (97%), 2490 mg·l^{-1} $FeSO_4 \cdot 7\ H_2O$,
640 mg·l^{-1} $MgSO_4 \cdot 7\ H_2O$, 0.02 ml H_3PO_4, 190 mg·l^{-1} NH_4Cl.

For the fed-batch experiments with AMD containing 6000 mg·l^{-1} SO_4 the amount of all chemicals were doubled. As a modified water AMD with 2000 mg·l^{-1} $CaSO_4$ instead of $FeSO_4$ was used over 60 days period in the pilot plant.

Table 1 shows a comparison of the reactors used for the investigations.

Reactor Type	Material of packed bed	Inoculation material	Dimensions (ϕ; height)	Volume	Pore volume of packed bed
Fed-batch reactor I	Clay rings	Material from the laboratory reactor	0.09 m; 0.80 m	3 l	65 %
Fed-batch reactor II	Ceramic rings	Fresh Sewage sludge	0.09 m; 0.80 m	3 l	75 %
Fed-batch reactor III	Ceramic rings	Enrichment culture from fresh Sewage sludge	0.09 m; 0.80 m	3 l	75 %
Fed-batch reactor IV	Pelletized ash	Material from the pilot plant	0.09 m; 0.80 m	3 l	50 %
Laboratory reactor	Clay rings	Sewage sludge*)	0.15 m; 1.50 m	15 l	65 %
Pilot plant	Pelletized ash	Sewage sludge**)	0.50 m; 4.20 m	800 l	50 %

*) inoculated 24 months ago; worked over a period of 20 month
**) inoculated 20 months ago; worked over a period of 12 month

Table 1: Comparison of the different reactors used for the investigations

Results of the fed-batch reactor investigations were implemented on the laboratory reactor which was run in a continuous-flow mode. After successful studies over a two week period with the laboratory reactor, the results were also implemented on the continuously running pilot plant.

Analytical

Manual determinations of sulphate, sulphide, alkalinity, calcium and pH were carried out according to analytical procedures as described in Standard Methods (APHA, 1985) [1]. Ammonia and phosphate were determined by colorimetric methods. With the exception of sulphide, all the analyses were carried out on filtered samples.

RESULTS AND DISCUSSION

Feeding experiments with fed-batch reactors. Start concentrations of sulphate used in the fed-batch reactors were between 600-6000 $mg \cdot l^{-1}$. The sulphate concentrations in treated waters decreased from 100 % down to less than 10% during 24 hours, using sulphate concentrations of 600-2500 $mg \cdot l^{-1}$ while the average VSS was 4.0 $g \cdot l^{-1}$. Using AMD with sulphate concentrations higher than 2500 $mg \cdot l^{-1}$ the retention time for 90% of sulphate removal was between 30-45 hours.

The VSS concentration increased during the experiments from 2.5 up to 4.7 $g \cdot l^{-1}$ with the feeding of yeast extract. The increase of biomass in the reactors with material from the bigger plants (FB I, FB IV) was lower (from 3.6 to 4.5 g·l^{-1}) than in the other reactors inoculated with fresh material (from 1.0 to 4.7 g·l^{-1}).

Figure 4 shows a kinetic of sulphate removal with an additional feeding of yeast extract and lactate with CO as main carbon source.

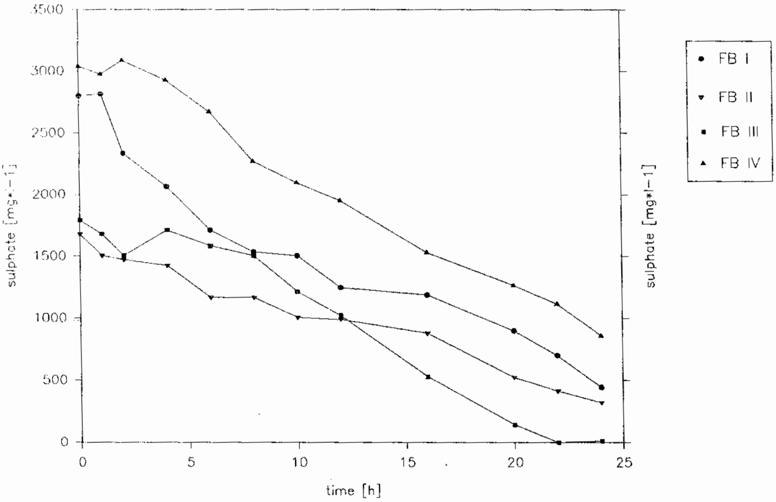


Fig. 4: Removal of sulphate in the fed-batch reactors using an additional feeding while CO is the main energy source; yeast extract: 1.5 g·l^{-1}, lactate: 0.5 g·l^{-1}

Sulphate removal in the laboratory reactor. Sulphate in the treated water decreased gradually from 3000 to 250 mg/l (as SO_4). The volumetric sulphate reduction rate was 1.8 - 2.4 g·l^{-1}·d^{-1} SO_4. As the volatile suspended solids (VSS) concentration was 5.0 g·l^{-1}, the average specific sulphate reduction rate was 0.44 g SO_4·g^{-1} VSS·d^{-1}.

If the reactor was working unstable, shown by an alkalinity less than 500 mg·l^{-1} $CaCO_3$ and sulphate concentrations over 250 mg·l^{-1}, the system could be stabilised by adding 60 mmol·l^{-1} lactic acid to the reactor liquid. During 24 hours the alkalinity increased up to 3500 mg·l^{-1} $CaCO_3$, the sulphate concentration in the effluent decreased below 50 mg·l^{-1}.

Figure 5 shows the sulphate reduction in the laboratory reactor in the set up during the days 20-50, showing the concentrations of sulphate, sulphide, alkalinity and the pH. The results for the pilot plant were quite similar.

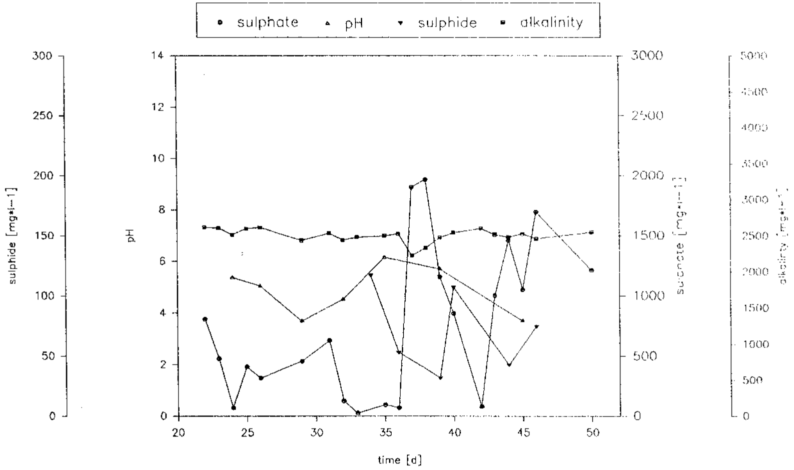


Fig. 5: Removal of sulphate in the laboratory reactor during the set up (day 20-50). Until day 42 the reactor was feed with lactate as main energy source. At day 36 the daily flow rate was increased from 5 to 13 $l{\cdot}d^{-1}$. Since day 43 CO was used as the main energy source.

Sulphate removal in the pilot plant. Sulphate in the treated water decreased gradually from 3000 to 200 $mg{\cdot}l^{-1}$, except on one occasion. A decrease in sulphate removal on day 45 was due to a leak in the gas feed line.

The volumetric sulphate reduction rate was 1.7 - 2.2 $g{\cdot}l^{-1}{\cdot}d^{-1}$ SO_4. As the volatile suspended solids (VSS) concentration was 4.5 $g{\cdot}l^{-1}$, the average specific sulphate reduction rate was 0.43 g $SO_4{\cdot}g^{-1}$ $VSS{\cdot}d^{-1}$. The volumetric sulphate reduction rate compares favourable with that of other systems [6],[12],[17],[19],[21]. The maximum volumetric rate of sulphate reduction achieved was 2.4 g $SO_4{\cdot}g^{-1}$ $VSS{\cdot}d^{-1}$. The process was running stable over a 70 day period where heavy metals were removed totally.

The reactions responsible for sulphate reduction if H_2 and CO are used as energy sources are the following:

$$4H_2 + SO_4^{2-} \rightarrow S^{2-} + 4H_2O \qquad (1)$$

$$4CO + SO_4^{2-} \rightarrow S^{2-} + 4CO_2 \qquad (2)$$

Organisms responsible for reactions (1) and (2) could be Desulfovibrio vulgaris (Badziong and Thauer [2]) and Desulfovibrio desulfuricans (Yagi and Tamiya [23]) respectively.

From equations (1) and (2), it is calculated that stoichiometrically 0.93 l H_2 or CO (equivalent to 0.083 g H_2 and 1.17 g CO) for the reduction of 1 g SO_4 to sulphide (H_2S) is required. The utilisation efficiencies of CO and a mixture of H_2/CO were determined experimentally to be 1.27 l·g^{-1} SO_4 for CO and 1.45 l·g^{-1} SO_4 for CO + H_2. For sulphate reduction CO gas was better utilised than the mixture of CO and H_2, possibly due to the fact that CO has got a higher solubility compared to H_2 (23.2 ml·l^{-1} CO in water versus 16.7 ml·l^{-1} H_2 in water). Second reason could be greater leakages of H_2 through the pipe lines than of CO, due to the smaller molecular size of H_2.

CONCLUSIONS

Former studies of Maree et al. [13] have shown that producer gas is a viable energy source for the biological reduction of sulphate from industrial effluents. Fed-batch experiments showed that the additional feeding of yeast extract increased the biomass production of the SRB about 20% and the VSS in the reactors increased from 3.7 g·l^{-1} to 4.5 g·l^{-1}. The sulphate reduction rate could be increased about 50% by adding a mixture of yeast extract, lactate and trace minerals, increasing from 1.2 g l^{-1}·d^{-1} SO_4 to 1.8 g l^{-1}·d^{-1} SO_4 in a stable working system, reducing the fed amount of 3000 mg·l^{-1} sulphate to 250 mg·l^{-1} in 30 hours.

Following these results, a two step scale-up on the 800 l pilot plant was made by using the 15 l laboratory reactor as an intermediate step. Both systems were run over a 70 day period with a sulphate reduction rate of 1.7 to 2.2 g·l^{-1}·d^{-1}, releasing the treated AMD with concentrations less than 250 mg·l^{-1} sulphate. Heavy metals were removed totally in this process.

REFERENCES

1. APHA: Standard methods for the examination of Water and Waste water treatment, 12th Ed., American Public Health Association, New York (1985)

2. Badziong W., Thauer R.K.: Growth yields and growth rates of Desulfovibrio vulgaris (Marburg) growing on hydrogen plus sulfate and hydrogen plus thiosulfate as the sole energy sources. Arch Microbiol., Vol.117, pp.209-214 (1978)

3. Becker B.: Untersuchungen zur Initiation bakterieller Sulfatreduktion in Deckgebirgsmaterialien von Braunkohletagebauen, Diploma Thesis, Technische Universitaet Braunschweig (1991)

4. Becker B., Graff M., Naeveke R.: Initiation of bacterial sulfate reduction in acid overburden dumps from lignite surface mining, 3. Biennial Conference of the Water Institute of Southern Africa, Durban, RSA, Vol.1, pp.78-79 (1993)

5. Buisman C.J.N.: Biotechnological sulphide removal with oxygen, PhD Thesis, Agricultural University of Waginingen (1989)

6. Byrgess S.C., Wood L.B.: Pilot plant studies in the production of sulphur from sulphate enriched sewage sludge, J. Sci. Food Agric., Vol.12, pp.326-341 (1961)

7. Butlin K.K., Adams M.E., Thomas M.: The isolation and cultivation of sulphate reducing bacteria, J. gen.Microbiol., Vol.27, p.158 (1949)

8. Butlin K.K., Selwyn S.C., Wackerby D.S.: Microbial sulphide production from sulphate enriched sewage sludge, J. appl. Bact., Vol.23, p.158 (1960)

9. Conradie P.J.A., Grütz P.W.E.: The treatment of acid mine waste in admixture with raw sewage sludge in an anaerobic digester, Report to the Chamber of Mines (File No. W6/534/3), National Institute for Water Research, Pretoria (Restricted) (1973)

10. Dill S.: Untersuchungen zur Behandlung schwefelsaurer schwermetallhaltiger Grubenwaesser mit sulfatreduzierenden Bakterien in kompakten Anlagen, Diploma Thesis, Technische Universitaet Braunschweig (1992)

11. Dill S., Graff M., Naeveke R.: Microbial remediation of metalliferous acid dump seepage - Laboratory experiments with packed bed and stirred tank reactors -, International Symposium on Subsurface Microbiology, Bath, Great Britain, **H-25** (1993)

12. Du Preez L.A., Maree J.P., Jackson-Moss, C.A.: Biological sulphate removal from Mining effluents by utilising producer gas as energy source, Proc. of the Int. Mine Water Conf., Ljubliana, Yugoslavia (1991)

13. Du Preez L.A., Odendaal J.P., Maree J.P., Ponsonby M.: Biological removal of sulphate from industrial effluents using producer gas as energy source, Environm. Tech., Vol.13, pp.875-882 (1992)

14. Knivett V.A.: The microbial production of Vitamin B_{12} and sulphate from sewage, Prog. Indust. Microbiol., Vol.2, p.20 (1960)

15. Maree J.P., Strydom W.F.: Biological sulphate removal from a packed bed reactor, Water Research, Vol.19(9), pp.1101-1106 (1985)

16. Maree J.P., Gerber A., Strydom W.F.: A biological process for sulphate removal from industrial effluent, Water SA, Vol.12(3), pp.139-144 (1986)

17. Maree J.P., Hulse G., Dods D., Schutte C.E.: Pilot plant studies on biological sulphate removal from industrial effluent, Wat. Sci. Tech., Vol.23, pp.1293-1300 (1991)

18. Marschall, A.: Untersuchungen zur Behandlung schwefelsaurer metallionenhaltiger Sickerwaesser sulfidischer Genese mit bakterieller Desulfurikation in einem homogen durchmischten Ruehrreaktor, Diploma Thesis, Technische Universitaet Braunschweig (1993)

19. Middleton A.C., Lawrence A.W.: Kinetics of microbial sulphate reduction, J. Wat. Pollut. Control Fed., pp.1659-1670 (1977)

20. Olthof M., Kelly W.R., Oleszkiewicz J., Weinreb H.: Development of anaerobic treatment process for waste waters containing high sulphates, Purdue Industrial Waste Conference, p.16 (1985)

21. Sadana J.C., Morey A.V.: Microbial production of sulphide from gypsum, J. Scient. Indust. Res., Vol.21C, p.16 (1962)

22. Tuttle J.H., Dugan P.R., Ranles C.I.: Microbial sulphate reduction and its potential utility as an acid mine water pollution abatement procedure, Appl. Microbiol., Vol.17, pp.297-302 (1969)

23. Yagi T., Tamiya N., Biochem. Biophys. Acta, Vol.65, pp.508-509 (1962)