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BIOLOGICAL SULFATE REDUCTION USING – HYDROGEN AND METHANOL AS ENERGY AND CARBON SOURCES FOR TREATING ACID MINE DRAINAGE

[1]Nam, Nguyen Hoang and *[2]Chung, Tran Van

[1]Faculty of Environment Hanoi University of Mining and Geology, Duc Thang, Bac Tu Liem, Hanoi, Vietnam
[2]Department of Analytical chemistry, Institute of Chemistry and materials, Hanoi Vietnam

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ABSTRACT

Acid-mine drainage (AMD) contains high concentrations of heavy metals these contaminants were generally avoided by lime neutralization. However, this method is expensive and generates large amounts of residual sludge. The selective precipitation of metals using H_2S produced biologically by sulfate reducing bacteria has been proposed as an alternative process. in this study, acid mine drainage was treated in a laboratory scale. Bioreactor fed with an H_2 and Methanol was used to treat acid mine drainage. The maximum rate of H_2 transfer suggests that this step should not be a limiting factor. However, increase H_2 flow rate increased the sulfate reduction rate. The replacement of synthetic medium by real effluent had increased sulfate reduction rate about30%. The maximum sulfate reduction rate observed with the real effluent was 0,009 (mmol/L h), corresponding to a residence time of 4.7 day.

INTRODUCTION

Acid mine waste water contains high concentrations of dissolved metals and sulfate, and very low pH of 2.5. Acidic industrial waste water must be treated before discharging into sewage networks. Biological sulfate removal can be used to treat acid mine drainage containing sulfate, metal removal and neutralisation. Sulfate can be removed as elemental sulphur via sulfide as an intermediate product when an energy source is provided. Desalination is achieved by effecting calcium carbonate crystallisation after sulfate removal. Metals are completely removed by precipitation as sulfides. Alkalinity is generated in quantities stoichiometric ally equivalent to the amount of sulfate removed, which allows direct treatment of acid water. The biological sulfate removal process has been developed for over 15 years Maree and Strydom showed that sulfate can be removed in an anaerobic packed-bed reactor using sucrose, pulp mill effluent or molasses as carbon and energy source (Maree, J.P. and Strydom 1985). Metals as iron, nickel, cadmium and lead were completely precipitated as metal sulfides. Maree and Hill showed that a three-stage process can be applied for sulfate removal, using molasses as carbon and energy source in an anaerobic packed-bed reactor (Maree, J.P. and Hill 1989).

Corresponding author: Chung, Tran Van,
Department of Analytical chemistry, Institute of Chemistry and materials, Hanoi Vietnam.

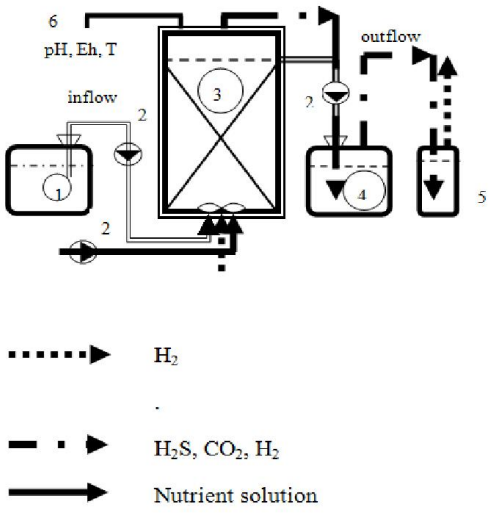
Sulfide can be stripped with a mixture of CO_2/N_2 from the effluent of the anaerobic reactor in a H_2S-stripping stage and residual COD and $CaCO_3$ can be removed in an aerobic final treatment stage. Maree *et al*. showed that when molasses is used as carbon and energy source it can either be utilised in the fermented or unfermented form (Maree, J.P. *et al.* 1991). When molasses is allowed to ferment, acetic acid is the main carbon and energy source for the sulfate reducing bacteria. When molasses is kept sterile in the storage tank, sucrose is the main carbon and energy source with acetic acid as the metabolic end-product. it was concluded that by running two anaerobic sulfate removal reactors in series, sucrose can be fermented to lactate in the first reactor and via acetate to CO_2 in the second reactor. Du Preez *et al*. firstly demonstrated that producer gas (mixture of H_2, CO and CO_2) can be used as carbon and energy source for biological sulfate reduction (du Preez *et al.* 1992). H_2 and CO_2 were utilised as energy and carbon sources, respectively.

Visser investigated the competition between sulfate reducing bacteria (SRB) and methanogenic bacteria (MB) for acetate as energy and carbon source in an upflow Anaerobic Sludge Blanket (UASB) reactor (Visser 1995). He found that at pH values less than 7.5, SRB and MB are equally affected by the presence of H_2S, while at higher pH values SRB out-compete MB. Van Houten showed that sulfate can be reduced to H_2S at a rate of 30 g SO_4/(l.d) when H_2/CO_2 is used as carbon and

energy source and employing pumice or basalt particles to support bacterial growth in a fluidised-bed reactor (Van Houten, *et al.* 1996). He found that the pH should be between 6.5-8.0; the temperature should be between 20 - 35ºC; the H_2S concentration should be less than 450 mg/l; the system should be completely anaerobic; the biomass should be immobilized and the retention of the active biomass should be high; the gas should be in the ratio: H_2/CO_2, 80%:20%; the hydrogen mass transfer should be maximized; there should be a high gas hold-up (through the system recycle) and there should be a low bubble diameter (small gas bubbles). Geldenhys *et al.* demonstrated that hydrogen can be generated on-site to provide it cost-effectively (Geldenhuys *et al.* 2003). Eloff at al. showed that a venture device can be used to introduce hydrogen gas into the system as the energy source, while geotextile (a coarse, fibrous material, used in road construction). can be used as a support material for SRB growth (Eloff *et al.* 2003).

MATERIALS AND METHODS

Experimental set up



(1 - feeding tank; 2 - Pump; 3 - anaerobic reactor; 4 – outflow tank; 5 - Gaswashingbottle; 6 – meter?)

Fig. 1. Scheme of the laboratory reactor used in this study

The experimental set-up is shown in Figure 1. The laboratory bioreactor (working volume of 2.0 L) was made of glass. The temperature of reactor maintained at 30°C by a water batch.
A synthetic AMD was prepare according to (Bissinger *et al.* 2000). the of 560 mg L^{-1} sulfate, 116 mg L^{-1} iron, 3,6 mg L^{-1} zinc, 10,3 mmol L^{-1} acidity as NaOH eq. 11 mmol L^{-1} and a pH of 2.7. The inflowwas continuously supplied into the reactor by a pump. H_2 gas flow into the reactor system via the system control valve and flow is determined by a gas flow measuring device H_2. Produced gas is output directed into water tank and continued absorption by liquid $Zn(CH_3COO)_2$ in NaOH. The pH-electrode, redox-electrode, temperatur-electrode were inserted into the bioreactor and connected to a meter online.

Biomass

200 g of centrifuged sludge was put into 1 litter-glass bottle containing 800 ml of AMD. 5 ml of MeOH 100% was added into the bottle, and H_2 was injected into the solution during 30 minutes then the bottle was tied closely. It is put into the shaking machine which is placed in the thermoregulation room at 20^0 C during 2 weeks. After that, the diluted AMD (1:5) was put into reactor and 1L/h H_2 was injected during 1 week. The aim of this investigation was to demonstrate the performance of the integrated process consisting of a heating unit, anaerobic reactor for sulfate reduction, H_2S-stripping and stabilization stages.

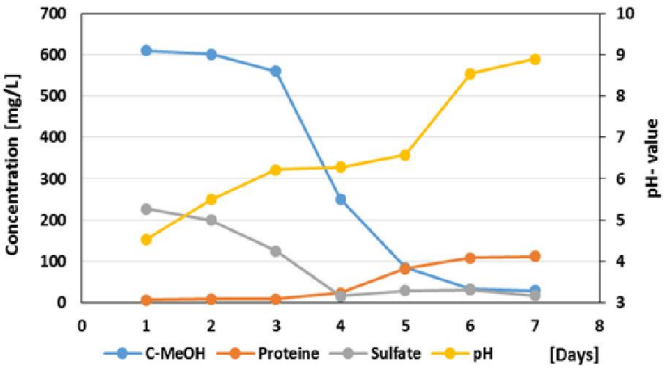


Fig. 2. Growth (increase in protein concentration) and reduction of methanol and sulfate concentration in the enrichment culture

Medium

The growth medium contained show in the table 1 and 2, acidic trace elements, 1 mL/L; and alkaline trace elements, 1 mL/L. All chemicals used were of extra pure quality and supplied by Merck (Darmstadt, Germany)

Analytical methods

MeOH was determined with a gas chromatograph (Haedspace-Gaschromatographie) using a method adapted from (Uematsu *et al.* 2002). Anions (Chlorid, Nitrit, Nitrat, Sulfat) were determined with an IC equipped with a AG4A-SC/AS4A-SC (DIONEX 100). Sulfide was determined photometrically using a method adapted from Siegel (1965). Gas phase composition was determined with a gas chromatograph (Fisons Instruments GC 8000) equipped with two columns: 1.5 m X *Y4'* Teflon packed with Chromosorb 108 (60 to 80 mesh), and 1.2 m X *Yirr* stainless-steel packed with mol. sieve 5A (60 to 80 mesh). TOC, TIC were determined Infrarot-Spektrometrie equipped with detector UV using a method adapted from DIN EN 1484: 1997-08. Hydrogen, methan, nitrogen, oxygen were determined with a gas chromatograph equipped with detector (WLD), using a method adapted from Köhler 2000. Protein was determined with photometrially using a method adapter from Bradford 1976. Heavy metals were determined with photometrially and AAS.

RESULTS

Variation of sulfate reduction during cultivation period

The results of the first experiment are shown in Figure 2. Fig. 3. growth (increase in protein concentration) and reduction of methanol and sulfate concentration in the enrichment culture In this experiment the inoculation in sulfate reducing medium

(SRM) were observed in the culture SRBMM 111-2 (with methanol as carbon source, a gas as electron donator source feed of 1 Ln/h was supplied) a decrease in methanol and sulfate concentration and an increase in protein concentration and the pH was observed. During the inoculation in the SRM, a black precipitate was formed (iron sulfide).

The studies with the enrichment culture SRBMM 111-2 were carried out with 1 Ln/h hydrogen-gassing rate, but different concentrations of methanol and at different hydraulic retention times (HRT) (flow rates) of the model waste water (artificial AMD), namely:

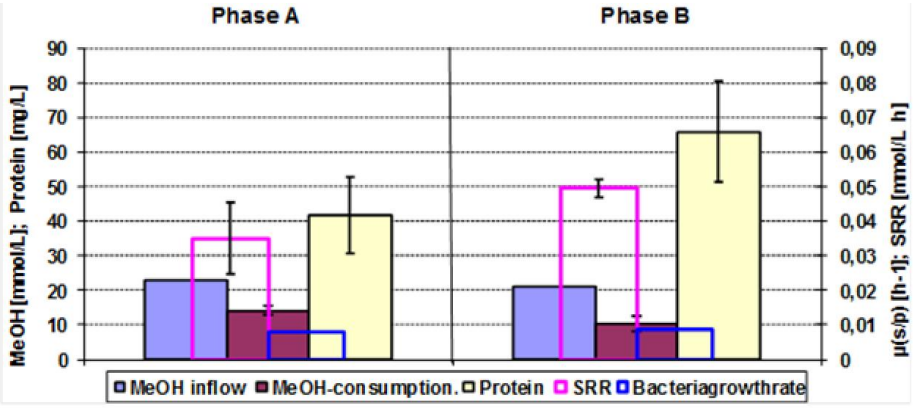


Fig. 4. Relations between methanol and methanol concentration in feed consumption, protein concentration, sulfate reduction rate (SRR) and Bacterial growth rate (µ(s/p)) in phase A and phase B), (hydrogen flow

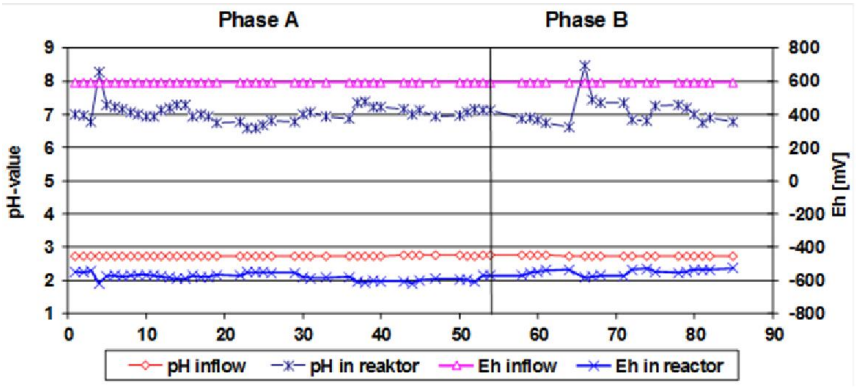


Fig. 5. relationships between redox potential and pH in the reactor with methanol as a carbon source during phase A and phase B

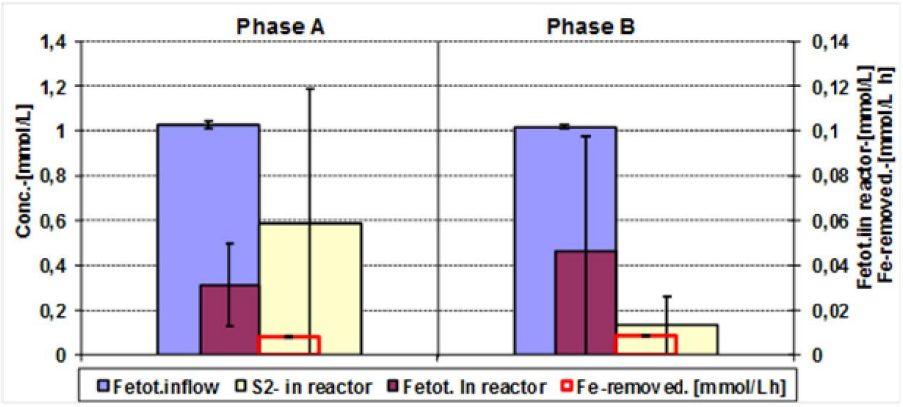


Fig. 6. Relations between iron and iron sulfide concentration and removal rate in the reactor with methanol as a carbon substrate for phase A (HRT of 5.2 days) and phase B (HRT of 4.7 days)

Bacterial growth and sulfate reduction rate

It was the characterization of bacterial growth of the enrichment cultures under continuous fermentation conditions at different hydraulic retention time (HRT) (phase).

- Phase A: HRT of 5.2 days and MeOH concentration in the inflow of 23.16 mmol/L.
- Phase B: HRT of 4.7 days and MeOH concentration in the inflow of 21.13 mmol/L.

The results (Figure 3) show an average methanol consumption of 14.05 ± 1.3 mmol/L in phase A and only of 10.27 ± 2.1 mmol/L in phase B. This corresponds to a share of 61% (phase A) and 49% (phase B) of the methanol fed. The average protein concentrations in phase A and B were 41.56 ± 11.6 mg/L and 65.79 ± 14.48 mg/L respectively. The bacterial growth rates were very similar with 0.008 h^{-1} (phase A) and 0.009 h^{-1} (phase B). The average SRR with methanol as the substrate were amounted to 0.035 for phase A and 0.05 mmol/L.h for phase B. The SRR were in the experimental phase A in the range of 0.02 to 0.037 mmol/L.h and in the experimental phase B from 0.028 to 0.07 mmol/L.h

Redox potential and pH values

Kümmen and Papp (1990), Köhler and Völsgen (1998) show evident prior to sulfate reduction processes favorable conditions in the reactors, so it must be presented from predominantly a negative redox potential below -217 mV (Köhler and Völsgen 1998; Kümmel and Papp 1990). The investigations in the enrichment culture SRBMM 111-2 (with methanol as a carbon substrate) show results also changes the pH and redox potential of the microbial sulfate reduction processes (see Figure 4). In phase A, the redox potential was relatively stable with increased -581 ± 20 mV and the pH from 2.7 to 7.04 ± 0.28. In Phase B, the redox potential was comparable (-552 ± 18 mV) and the pH changed only slightly to 7.08 ± 0.42. The highest pH value during the period was 8.47 and the lowest 6.59. Through the resulting sulfides or by the carbonate, the pH is increased and heavy metal ions are removed by the sulfide precipitates

Heavy metal removal rate in the reactor using the example of iron

Due to the low solubility of heavy metal sulfites of heavy metal removal was carried out from the solution by the resulting in microbial sulfate reduction sulfides. Figure 5 shown the average iron and sulfide concentrations in the inlet or in the reactors. The obtained experimental results (see Figure 5) showed a similar strong iron removal rates in both phases. The iron removal rates were 0.008 mmol/L.h (0.44 mg/L.h) and 0.009 mmol/L.h (0.48 mg/L.h) in phase A and B, whereas the iron concentration in the fermentation solutions were very low at 1,76 ± 1.03 mg/L (0.03 mmol/L) in phase A and 2.59 ± 2.85 mg/L (0.046 mmol/L) in phase B. Nevertheless, the concentration of dissolved total sulfide in phase A was 0.59 ± 0.6 mmol/L and in phase B only 0.13 ± 0.12 mmol/L.

DISCUSSION

Using of hydrogen as an electron donor for the dissimilatory sulfate reduction

Decisive for the selection of hydrogen as an electron donor in this study were criteria such as the relatively low price and low potential for secondary contamination of treated acid mine water. In general, neutralization and heavy metal removal processes in AMD can be realized through a number of different processes. the different organic electron donator source for dissimilatory sulfate reduction were examined, such as methanol (Tsukamoto and Miller 1999; Walther 2001), ethanol (Muyzer and Stams 2008; Stucki *et al.* 1993; Widdel *et al.* 2007), lactic acid, glycerol (Kolmert and Johnson 2001), acetic acid, sucrose + peptone (Yamaguchi *et al.* 1999) and sugar (Maree, J.P. and Strydom 1985). The application of these organic compounds has the disadvantage that often remain residual concentrations in the effluent water and they are relatively expensive. Therefore, using organic waste products could reduce the treatment cost. Hydrogen, with its relatively low price, the advantage of achieving high rates of sulfate reduction (Fongsatitkul *et al.* 2009; van Houten, B.H.G.W. *et al.* 2006; Van Houten, R. T. *et al.* 1994; Van Houten, R. T. *et al.* 1996). A comparison of the environmental and economic aspects of various methods of H_2 production shows that a decentralized production of H_2 can be quite economical and environmentally friendly (Kothari *et al.* 2008).

The method is based on the process of autotrophic dissimilatory sulfate reduction using the following equation.

$$4H_2 + SO_4^{2-} + H^+ \rightarrow HS^- + 4H_2O$$

The calculations show that the cost of "EURO (H_2)/kg SO_4" are the sulfate elimination much less than 1 €. According to a report by Herrera *et al*. (1997) could be saved U.S. $ 132 million each year through the use of H_2 as an electron donor for the treatment of AMD from the copper mines in Chile, when would replace lactate by H_2 (Herrera *et al.* 1997). In the literature on treatment rich sulfate waste water and AMD by the autotrophic microbial sulfate reduction using a mixed gas of hydrogen as an electron donator and CO_2 as a carbon source for bacterial growth have been described different methods and types of reactors, such as the fluidized bed reactor (Bilek *et al.* 2007), gas-lift reactor (van Houten, B.H.G.W. *et al.* 2006; Van Houten, R. T. *et al.* 1994; Van Houten, R. T. *et al.* 1996) and batch reactor (Herrera *et al.* 1997). An evaluation of different reactor types and methods for their ability to sulfate reduction, the hydrogen consumption, hydrogen flow rate, flow control, the ratio of H_2/CO_2 in the supply of water and the HRT could not be done practical for most.

pH-value

The scope of this work in the investigated systems also showed a strong influence on the pH value in the treatment of used water model. In reactor, the average pH values increased from 2.7 to 7.04 ± 0.28 at a residence time of 5.2 days (trial phase A) and 7.08 ± 0.42 at a residence time of 4.7 days (trial phase B). This pH environment is suitable for growing up of bacterian which providing good condition for process of dissimilatory sulfate reduction (Bilek *et al.* 2007). There was a significant influence of carbon source on the raising of the pH. Generally it can be concluded that the treatment of AMD respectively rich sulfate wastewaters was in the autotrophic dissimilatory sulfate reduction processes using H_2 as the electron donor a significant influence on the pH in the bioreactor systems. In all reactor systems were obtained, as expected, a very sharp rise in pH. A general problem of mine water with very low pH is the low carbon content, which are required for microbial growth. The solubility of CO_2 is very limited. Thus, at a pH of 3.0, the solubility at 20°C for up to only about 40 mg/L. Moreover, in the most acidic mine

drainage, and the concentrations of organic carbon compounds are extremely low. Consequently, to be added with the use of H_2 for the autotrophic dissimilatory sulfate reduction and CO_2 or an organic carbon source. In the case of the use of an organic carbon source by heterotrophic bacteria is formed CO_2, which then also stands for the autotrophic sulfate reducing bacteria are available.

Specific sulfate reduction rate

As a key criterion for evaluating the effectiveness of the treatment of AMD and sulfate-rich wastewater is considered the sulfate reduction rate (SRR). The obtained results in different studies increased: SRR is depending on both source of the carbon and electron donator source and function of the operating conditions. The obtained results in this work shows in a function of the operating conditions of different sulfate reduction rates in the treatment of an artificial AMD. Depending on the carbon source was evident a clear influence in the SRR. It was found that despite the same gassing with hydrogen (1 L/h) have different SRR have been achieved. The values in the reactor (with methanol as carbon source) amounted to only about 0.035 mmol/L.h (3.36 mg/L.h) (phase A) and 0.05 mmol/L.h (4.8 mg/L.h) (phase B). They were both experimental phase (A and B) from about 45 mg/L (see fig.4). However, the carbon consumption was in the reactor (methanol carbon 14.05 ± 1.3 mmol/L in phase A and 10.27 ± 2.1 mmol/L in phase B).

Is due to the difference between the various investigations into the sulfate reduction rates suggests had emerged that the examiner is another bacteria coenosis. This showed a lower conversion rate. In the reactor probably a fraction of hydrogen or methanol could be used for methane formation (Preuß 2004). The results show that the SRR dependent strongly on the reactor type, on the H_2 flow amount etc. on the bacterial community. Literature data show that the SRR in the gas-lift reactor (625-1,250 mg/L.h) (van Houten, B.H.G.W. *et al.* 2006; Van Houten, R. T. *et al.* 1994; Van Houten, R. T. *et al.* 1996) and in a fixed bed reactor (from 120 to 200 mg / L h) (Foucher *et al.* 2001) is much higher than in a batch reactor (from 32 to 83 mg/L.h) (Herrera *et al.* 1997). Generally shows that for an evaluation of the performance of autotrophic microbial sulfate reducing reactor systems is the reaction volume specific SRR of crucial significance. This value reflects the kinetics of sulfate reduction processes in the bioreactor. This parameter provides initial estimates for the scale-up from laboratory to be made large scale.

Heavy metal concentration

By sulfate reduction was formed sulfide in the solution. This work reached an average concentration of total sulfide from 20 to 65 mg/L. So that the dissolved heavy metal ions from the water phase were removed substantially in the form of heavy metal sulfides. The residual concentration of dissolved heavy metals, such as iron was in the processes in experimental phases (A and B) is very low (See Fig. 5). Generally it can be concluded that the strong reduction activity of the hydrogen as electron donor for the microbial sulfate reduction can play crucial role in the application for the treatment of AMD. On the one hand was expected the increase in pH by the sulfide formation during the autotrophic microbial sulfate reduction processes, on the other hand showed that from hydrogen and CO_2, an organic carbon source such as Acetate was formed. This process must be in the technical procedures to minimize. The lower cost of H_2, the high SRR or the H_2-consumption high yield compared to other substrates are characterized H_2 as a substrate with a large technical potential for the development process and with lower operating costs for the treatment of AMD and sulfate-containing industrial effluents.

Conclusions

The use of hydrogen as an electron source for microbial dissimilatory sulfate reduction is increasing interest in recent years. The distinct advantage of such purification process is usually in the low effort in the implementation and operation, in particular, hydrogen would cost relatively little compared to organic carbon compounds and achieved high rates of sulfate reduction.

- Through the use of hydrogen, acid mine drainage and heavy metal contaminated, sulfate containing wastewater is treated. The basic principle is based on a coupling of microbial sulfate reduction, precipitation processes, which are electron donors for sulfate reduction comes from the release of organic compounds by hydrogen dosing.
- The proton removal takes place by protonation of sulfide to HS^- and H_2S, which in turn removes heavy metals by sulfide precipitation. These processes have been realized under certain conditions and by different systems on a laboratory scale with the microbial dissimilatory sulfate reduction.
- The efficiency of the use of hydrogen as electron donator source for the microbial dissimilatory sulfate reduction in combination with organic compounds such as Methanol as carbon substrates for bacterial growth and HRT in bioreactors for the treatment of the inserted artificial AMD was very efficient. By the sulfide formation, protons and metal ions were removed from the solution. The average pH values increased in the bioreactor of 2.7 in the inflow to 7-8 at the end. In the presence of relatively high residual concentrations of sulfide, the residual concentration of heavy metals (e.g. iron) in the effluent was negligible, so iron was removed under these conditions, almost completely.
- In the continuous cultivation in the reactor reached the bacterial growth rate values of only 0.008 (phase A) and 0.009 h^{-1} (phase B) and the average sulfate reduction rate was 0.035 (phase A) and 0.05 mmol/L h (phase B).

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