Microbiology and Chemistry Interactions in a Biological Sulphate-Reducing Process

Mona Arnold, Jarno Mäkinen, Marja Salo, Malin Bomberg

VTT Technical Research Centre of Finland ltd, P.O.Box 1000 02044 VTT Finland
Mona.Arnold@vtt.fi, Jarno.Makinen@vtt.fi, Marja.Salo@vtt.fi, Malin.Bomberg@vtt.fi

Abstract

In this study, we tested the industrial anaerobic sludge as inoculum for biologic sulphate removal from mine water with high sulphate (app. 10 g/L), but negligible metal concentration originating from a subarctic mine. Because of the remoteness of the mine, ethanol was chosen as substrate due to its easy storage and transportation to remote sites. Experiments were performed in three 0.7L reactors at room temperature. The reactors were operated as upflow anaerobic sludge blanket reactor (Reactor 1), and fluidized bed reactors with different carriers.

Physicochemical parameters (pH, redox, sulphate, sulphide) were measured over six months and the microbiology was analysed by high throughput (HTP) sequencing. The pH in the reactors varied between 5.2 and 7 and the sulphate removal varied from -111% (indicating sulphate production) to 48% removed sulphate. In addition, up to 3.5 g L^{-1} acetate was formed at pH 7.2 – 7.4.

The HTP sequencing revealed that the microbial communities changed markedly from that of the inoculum. SBR communities evolved during the experiments. The most common bacteria in the reactors belonged to sulphide- and thiosulphate- reducing Epsilonproteobacteria, the archaeal population consisted mostly of acetate-utilizing *Methanosaeta* – methanogens. 2 – 15% of the bacterial communities consisted of SRB. In addition, the microbial consortium contained a large variety of organic matter degrading species.

A high input sulphate level and a low pH together with undissociated H_2S may inhibit growth and activity of the SRB. Elevated concentration of acetate may also inhibit the SRB. Analysis of the microbial community gives added insight into the processes. Our study also indicated that parameters normally used for following up an SBR process such as redox and pH, is not necessary sufficient for assuring a functioning process.

Key words: SBR, sulphate removal, chemical indicators, microbiology

Introduction

Biologic sulphate reduction is a widely applied method for sulphate removal from mine waters (Bowell, 2004). The technology relies on sulphate reducing bacteria (SRB), which in anoxic conditions use organic compounds or hydrogen gas as a substrate for reduction of sulphate to hydrogen sulphide (Elliot et al. 1998; Kaksonen et al. 2003; Sahinkaya 2009). The sulphate reduction equations when ethanol is used as a substrate are presented in Eq1 – Eq.3 (Sahinkaya et al. 2011).

$$2CH_3CH_2OH + SO_4^{2-} \rightarrow 2CH_3COO^- + HS^- + H^+ + 2H_2O \quad \text{Eq.1}$$

$$2CH_3COO^- + 2SO_4^{2-} \rightarrow 4HCO_3^- + 2HS^- \quad \text{Eq.2}$$

$$4HCO_3^- + H^+ \rightarrow CO_2 + H_2O \quad \text{Eq.3}$$

Eq.1 and Eq.2 show that ethanol degradation is a two-step process where the sulphate to sulphide conversion is first producing acetate and acidity, followed by acetate degradation and production of bicarbonate and sulphide. If both reactions are proceeding completely, the pH rises due to the bicarbonate conversion to carbon dioxide (Eq.3). Moreover, ideally the sulphate to sulphide conversion takes mainly place in the acetate degradation step (Eq.2).

If sulphate reduction is incomplete, i.e. the reaction described in Eq. 2 is very slow, increasing acetate concentration resulting from Eq. 1 can inhibit the growth of SRB (Kaksonen et al. 2003b, Kaksonen and Puhakka 2007, Sánchez-Andrea et al. 2014). Especially at lower pH values (<4.75) acetate is mostly in the form of highly toxic acetic acid. However, with a diverse microbial community, acetate can be used by other species besides SRB (e.g. methanogens) and the likelihood of inhibition is reduced.

Dissolved metals present in the treated solution reacts with the produced sulphides (Eq 2) and precipitate as insoluble metal sulphides, If metals are not present the produced sulphide should be transformed to a non-toxic form. This can take place by oxidizing the sulphide to elemental sulphur, either via a chemical or biochemical process (Eq.4) (van der Zee et al. 2007).

$$H_2S + 2O_2 \rightarrow S^0 + H_2O \qquad \text{Eq.4}$$

Ethanol has been suggested as an easy substrate for use in biological sulphate removal applications due to its relatively low price, easy availability and transport. E.g. Nagpal et al. (2000) used ethanol as substrate for SRB in a fluidized bed reactor achieving 95 % removal of ~2500 mg/l sulphate with a hydraulic retention time (HRT) of 35 h. Nevertheless, in a similar experiment with a down-flow fluidized bed reactor, Celis et al. (2009) obtained only ca 30 % sulphate reduction. Sulphate loading was 830-1660 $g/m^3 \cdot d$ and HRT was 48 h. In both experiments acetate accumulation was detected in the effluent. Whereas this affected negatively reactor performance in the study by Celis et al. (2009), Nagpal et al.'s (2000) results were not affected by the accumulation of acetate. The difference can be explained by differences in the microbial communities operating in the bioreactors.

In this study, ethanol was used as a substrate in biological sulphate removal from sulphate rich water originating from a northern mine. The performance of three bioreactors with different carrier materials was compared by monitoring the physicochemical parameters and characterizing the microbial communities in the reactors with high throughput (HTP) sequencing method.

Methods

Bioreactors. The experimental setup consisted of three anaerobic 0.7 L reactor reactors. Reactor 1 was operated as upflow anaerobic sludge blanket reactor (UASB), while Reactor 2 and Reactor 3 as fluidized bed reactors (FBR). Reactor 2 and Reactor 3 were operated with 10 % fluidization volume and filtralite and activated carbon as carrier materials, respectively. Later, the carrier material for Reactor 2 was changed to 0.5-1.0 particle sized sand, as Filtralite easily blocked the tubes. The sludge blanket and carrier material volume was 0.3 L.

The inoculum for all reactors was anaerobic granular sludge from an industrial waste water treatment plant in Finland. Ethanol was chosen as a substrate, due to proven references (e.g. Nagpal et al (2000) and also because of simple storage and transportation to remote mine site locations. Reactor 1 was inoculated with 300 ml of sludge whereas 60 ml was added to Reactors 2 and 3 together with the carrier materials. The HRT in the experiment was 173 hours, both in the ramp up and actual experimental phase.

The feed solution for the reactors was a mixture of mine water, nutrients and ethanol as substrate, resulting in 8500 – 9000 mg/L SO_4, 56 mg/L KH_2PO_4, 137 mg/L $(NH_4)_2HPO_4$, 11 mg/L ascorbic acid and 11 mg/L yeast extract. The ethanol dosage was calculated based on COD; one gram of sulphate requires 2 grams of COD and the COD of ethanol is 1440 g/L. The ethanol dosage was 15 % of required during the ramp up phase and the first 27 days of the experiment. This was done to prevent any acetate formation in the reactors. On days 28 – 104 the ethanol dosing was increased to 160 % of required to secure enough substrate for efficient sulphate removal. During days 105 – 171 the input sulphate level was decreased to 3000 mg/L by dilution with distilled water. At the same time also the ethanol dosing was decreased to 120 % of required. The reactors were monitored for pH, RedOx, sulphate and sulphide twice per week.

Microbiology. The microbiology of the inoculum and reactors (Day 101 and 140) was determined using DNA based high throughput (HTP) sequencing techniques. Microbial DNA was extracted from

0.5 g inoculum sludge and from 2 mL effluent from the bioreactors using the NucleoSpin Soil DNA extraction kit (Macherey-Nagel) with the SL1 lysis buffer and Enhancer solution. The microbial community composition was determined by amplicon sequencing of the bacterial and archaeal 16S rRNA genes and the fungal ITS region using the Iontorrent platform as described in Rajala et al. (2016). The sequence reads were analysed using the QIIME software version 1.9 (Caporaso et al., 2010) as described in Rajala et al. (2016). Shortly, the sequences were clustered in to Operational Taxonomic Units (OTUs) at 97% sequence similarity and taxonomy was assigned for bacterial and archaeal sequences using the Greengenes database (DeSantis et al., 2006) and for fungi using the UNITE database (Köljag et al., 2013). Principal Coordinates Analysis (PCoA) on the non-normalized OTU data using the Bray-Curtis dissimilarity index was calculated using the PhyloSeq package in R (R Core Team, 2015) and plotted using gplot (Warnes et al., 2016). Abundance profiles of the bacterial, archaeal and fungal communities were visualized using PhyloSeq (McMurdie and Holmes, 2014) in R and plotted with gplot**.**

Results

Performance and chemical aspects. The goal of ramp up phase was to reach negative RedOx-potential values in all reactors. When this criteria was fulfilled, the experiment started (day 0). During the first 20 days, with only 15 % ethanol dosage, the sulphate removal was negative (Figure 1). Then, a drastic drop in the outlet sulphate concentration was observed in all reactors. In reactors 1 and 2 the decrease stopped after a couple of days only and the sulphate removal remained at app. 10 – 20 % and 100 – 250 g/m^{3}d, respectively. The performance of reactor 3 was better; the sulphate removal and reduction rate were app. 45 % and 500 g/m^{3}d. The performance of all reactors improved just before increasing the ethanol dosage to 160 % (day 28). After day 60, in all reactor the outlet sulphate level started to rise strongly, and in reactor 1 the sulphate removal was again negative. Due to technical difficulties with filtratlite in lab scale equipment the carrier material in reactor 2 was changed to sand, and reactor was reinoculated with 200 ml of reactor 3 effluent. Due to the fluctuating sulphate removal rate both sulphate and ethanol concentration in the feed was decreased on day 105. Despite this, the reactors´removal efficiency remained very low.

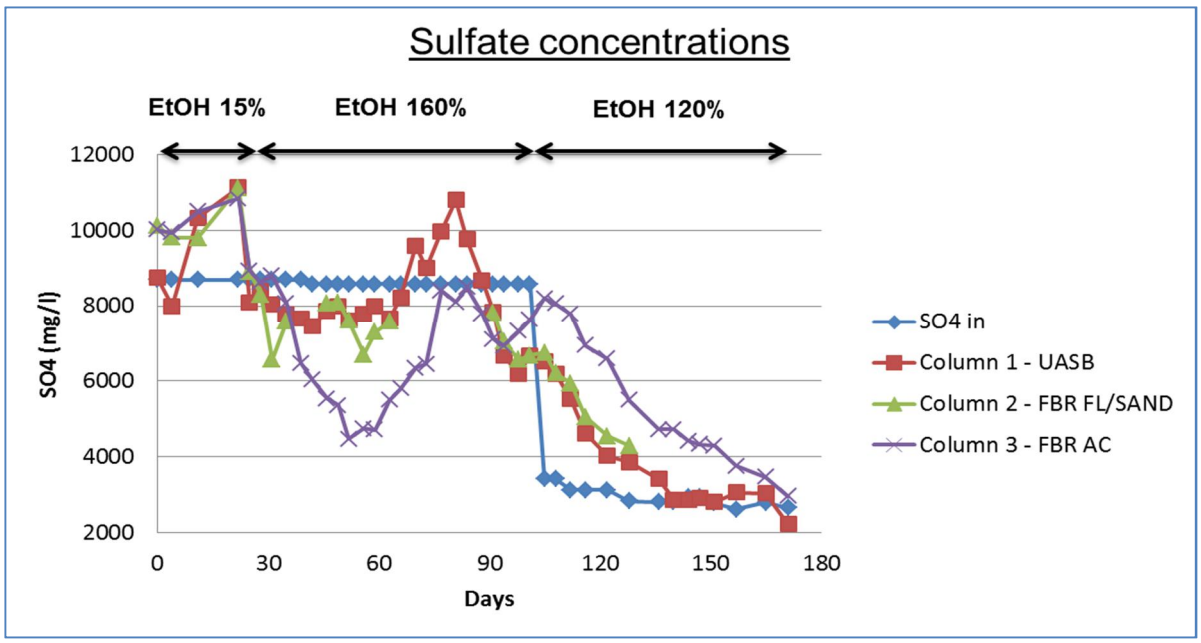

Figure 1 *Sulphate concentrations in influent and effluents during the experiment. Ethanol dosage rate compared to required theoretical need for complete reduction on sulphate according to COD.*

When ethanol dosage was elevated to 160% (day 28), the pH of reactors started to decrease (Figure 2). In reactor 1 the decrease was remarkable, from pH 7.1 to 6.2 between days 28-39. On day 39, the

acetate concentration in reactor 1 was 6.8 g/L, indicating that acetate was accumulating to the system and leading to pH decrease (Eq1, Eq2). The pH continued to decrease and therefore $NaHCO_3$ was added to reactor 1 between days 81-88 (total of 3 g) to raise the pH. Simultaneously, the acetate level was 2.2-3.4 g/L. The pH decrease in reactor 3 was less dramatic and increased without $NaHCO_3$ addition to pH >7 on day 84 when the acetate concentration was 100 mg/L.

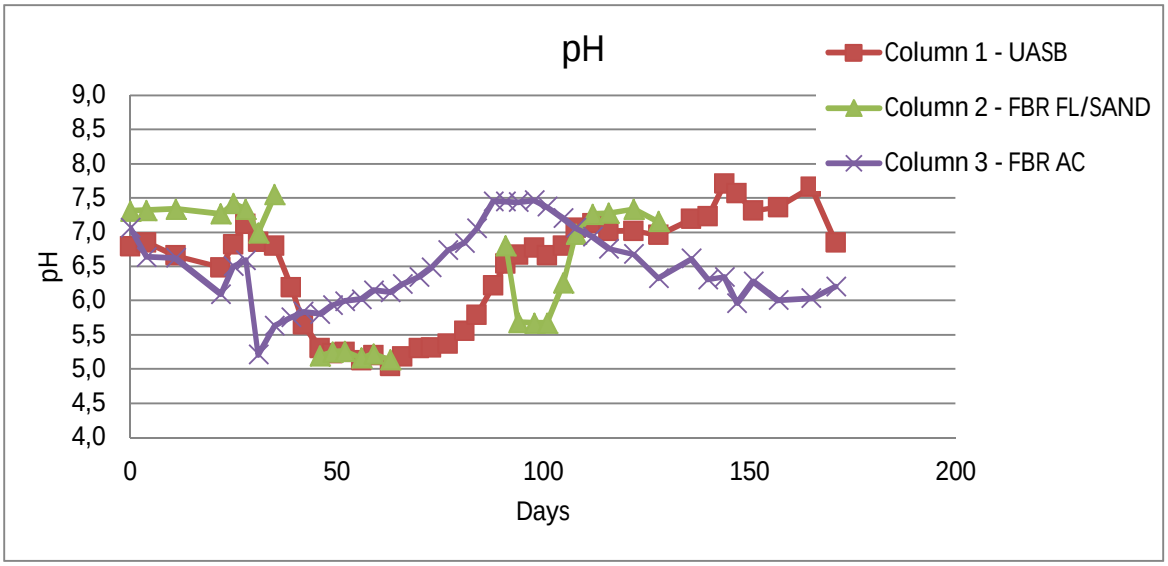

Figure 2 *pH in effluents during the experiment.*

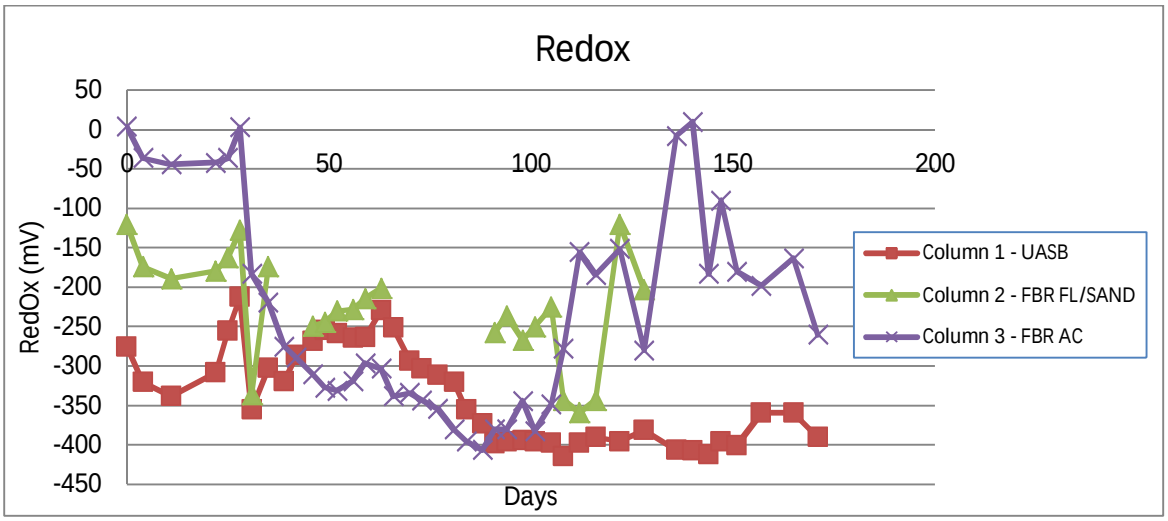

Figure 3 *Redox effluents during the experiment.*

Sulphide formation started practically on day 30 alongside with improved sulphate removal (Fig 4). After day 70, the sulphide level in reactor 1 rapidly increased and fluctuated between 100-300 mg/L; whereas the sulphide level in reactor 3 remained stable at <100 mg/L. Sulphide production did not seemingly correlate with the effluent sulphate level (Figure 1). One possible reason for the lower sulphide concentration in reactor 3 is adsorption to the carrier material activated carbon, but this does not fully explain why sulphide levels decreased over time.

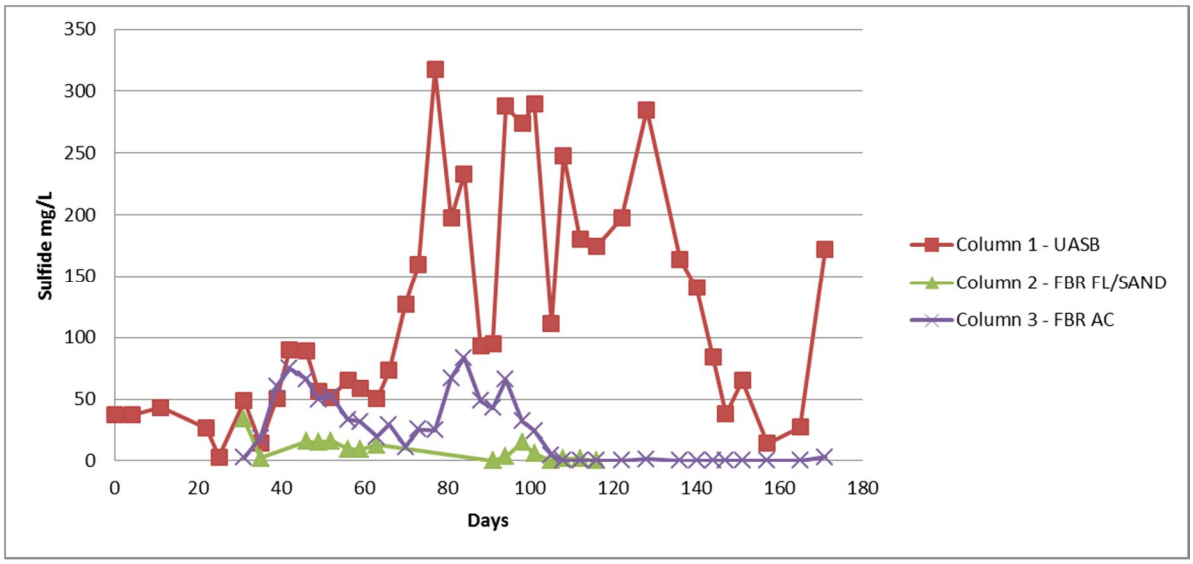

Figure 4 *Sulphide concentration in effluents*

Microbiology. The bacterial, archaeal and fungal communities of the inoculum and of the bioreactors after 101 days from all reactors and after 140 days of operation from reactors 1 and 3 were examined using high throughput amplicon sequencing. In general, the microbial communities changed quite markedly from that of the inoculum over time (Figure 5).

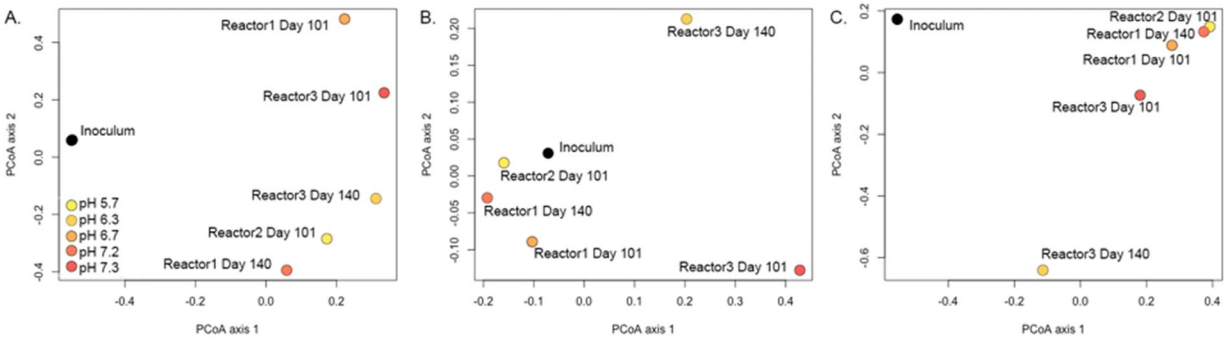

Figure 5 *PCoA plots of the A) bacterial, B) archaeal, and C) fungal communities identified by HTP sequencing from the inoculum and after 101 and 140 days of reactor operation. The reactor samples are coloured according to pH as indicated in the legend in A.*

The most abundant bacteria in the original inoculum were organic matter degrading Aminicenantes (OP8, 49%) and thiosulphate-reducing Caldiserica. In the reactors the bacterial communities changed to contain mostly proteobacterial groups (56 – 80%) and Bacteroidetes (4 – 32%). In Reactors 1 and 3 the proteobacteia belonged to sulfur-oxidizing *Sulfuricurvum*, *Sulfurospirillum*, *Thiobacillus*, *Thiofaba*, and *Thiomonas*, 24 and 80% of the bacterial community (Fig. 6). In Reactor 2 (day 101) the most abundant bacteria belonged to undetermined gammaproteobacterial groups (25%) and the alphaproteobacterial *Ochrobactrum* (20%), which is also known to oxidize reduced sulphur species. The SRB detected in the reactors belonged to the deltaproteobacterial groups *Desulfobulbus*, *Desulfomicrobium*, *Desulfovibrio* and *Desulfuromonas* and contributed between 2 and 15% of the bacterial community. Small amounts of Firmicutes SRB belonging to the *Desulfotomaculum* genus were detected in Reactor 2 after 101 (0.2%) and in Reactor 3 after 140 days of operation (0.5%). The archaeal communities in the inoculum and in all reactors consisted mostly of methanogenic *Methanosaeta*, which uses acetate for its methane production. Reactor 3 at 101 days of operation, however, sported a dominance of CO_2 and H_2 utilizing methanogens belonging to the *Methanobacterium*, which otherwise was present at only low abundance in all samples. Most of the

fungi detected in the inoculum and reactors belonged to different groups of Ascomycota (51– 97%). However, Reactor 1 and 2 also contained a considerable amount of Basidiomycota (26 – 47%).

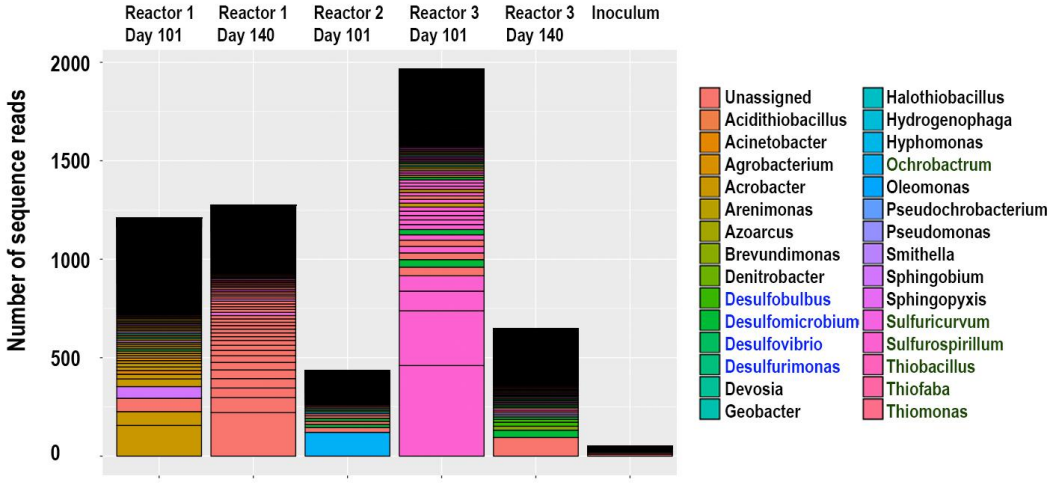

Figure 6 *The abundances of sequence reads belonging to Proteobacterial genera detected by the HTP sequencing. Names of sulphate reducing genera are indicated in blue and sulphur oxidizing genera in green text. The Y-axis show the absolute number of proteobacterial sequence reads obtained from the samples and the OTUs are ordered according to abundance, with the biggest OTUs at the bottom of each reactor. The taxonomic groups are shown by the colour charts to the right in the figure.*

Discussion

In order for SRB to efficiently reduce sulphate, specific environmental conditions need to met. These are low redox potential and availability of suitable electron donors. One such electron donor is ethanol, which has been used with variable results in previous studies (e.g. Napgal et al., 2000; Celis et al., 2009). The down side with using ethanol is the first step of ethanol oxidation (Eq1), where acetate is produced. Napgal et al. reported accumulation of acetate as high as 1.47 – 2.655 g/L without detecting any inhibition in the sulphate removal rate. However, as in our study, Celis et al. (2009) found that the sulphate reduction process was hampered as acetate accumulated.

The initial sulphate concentration affects biologic sulphate reduction. Optimal SRB growth has been observed in approximately 2500 mg/L sulphate concentrations (Al-Zuhair et al. 2008; Sahinkaya et al. 2011). When the sulphate concentration is higher, the reduction rate decreases and acetate accumulation is possible, leading to a risk of increased inhibition and process failure (Sahinkaya, 2009). In our case the sulphate concentration of the mine water was 8500 – 9000 mg/L, which is almost 4 times higher than reported for optimal growth of SRB. Thus, a combination of a high concentration of sulphate and ethanol as electron donor did probably result in the accumulation of acetate and malfunction or the biological process.

SRB need anoxic conditions with negative redox potential of at least -150 to -200 mV for their metabolism to function properly (Postgate, 1979; Gibert et al. 2002). If the redox potential is higher, for example in the presence of oxygen, sulphate remains unaffected and no sulphide is produced (Delaune and Reddy 2005). In our experiments the redox stayed between -400 and -100 mV. The highest value occurred in reactor 3, but there was no obvious correlation between the redox and the reactor performance. In addition sulphate reduction usually works best at pH 7 – 8 (Kaksonen 2004; Moosa and Harrison 2006), although there have been experiences with comparable sulphate reduction even in very acidic (pH 4) environments (Elliot et al. 1998). In our experiments the pH varied between 5.5 and 7.5 which are in acceptable limits. Anyway in combination with elevated acetate levels the lower values seemed to be detrimental to our processes.

High sulphate concentration may result in elevated redox potential and lowered pH and thus diminish the sulphate reduction activity (White and Gadd 1996). These environmental changes may also favour other microbes besides SRB to accumulate in the reactor (Oyekola et al. 2010).This is in agreement

with our results, as 2-15% of the identified bacterial sequences belonged to SRB. However, as the current literature on full analysis of microbial communities in SBR is very scarce, it is very difficult to evaluate in detail the process performance vs microbial abundancy. The presence of high amounts of acetate-utilizing methanogens and the relatively low abundance of SRB is consistent with the low sulphate reduction activity observed in the reactors. The growth of SRB that are not able to oxidize acetate is generally inhibited by increasing acetate accumulation. In addition, the high abundance of sulphur oxidizing Epsilonproteobacteria, such as *Sulfurispirillum, Sulfuricurvum* and *Sulfurivorum*, may also have affected the processes in the reactor. These bacteria oxidize reduced sulphur species to sulphate or sulfuric acid.

Oyekola et al. (2010) pointed out that if the sulphide levels are moderately high, the redox potential is likely to be more negative, and thus counteracting the possible inhibiting effect of high sulphate. However, sulphide can also cause inhibition in higher concentration. Depending on the pH, sulphide occurs in as different species with different inhibitory effect. Generally, SRB are less sensitive to total sulphide when the pH is increased from 6.8 to 8.0 and more sensitive to the undissociated sulphide (H_2S) concentration (pH < 6).

Mine waters commonly contain only small amounts of organic matter and an external carbon source and electron donor needs to be provided for biological sulphate reduction. Numerous options for substrates are available, and ethanol is one of the commonly used ones, mainly because of relatively low cost and good suitability for a wide range of SRB. However, as in our case the possibility of incomplete oxidation and the production of inhibitory acetate is a risk.

Conclusions

A high input sulphate level and a low pH together with undissociated H_2S may inhibit growth and activity of the SRB. Elevated concentration of acetate may also inhibit the SRB. Analysis of the microbial community gives added insight into the processes. Our study also indicated that parameters normally used for following up an SBR process such as redox and pH, is not necessary sufficient for assuring a functioning process.

Despite the easy accessibility of ethanol as electron donor for biological sulphate removal processes, the ethanol in our case was incompletely oxidized, which resulted in acetate accumulation in the reactors. In future experiments a different source of electron donor may be needed in order to promote growth and activity of SRB.

References

Al-Zuhair, S., El-Naas, M.H., Al-Hassani, H. 2008. Sulphate inhibition effect on sulphate reducing bacteria. Journal of Biochemical Technology 1, 2, 39-44.

Bowell, R. J. (2004): A review of sulphate removal options for mine waters. – In: Jarvis, A. P., Dudgeon, B. A. & Younger, P. L.: mine water 2004 – Proceedings International Mine Water Association Symposium 2. – p. 75-91, 6 Fig., 7 Tab.; Newcastle upon Tyne (University of Newcastle).

Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., Fierer, N., Gonzalez Pena, A., Goodrich, J. K., Gordon, J. I., Huttley, G. A., Kelley, S. T., Knights, D., Koenig, J. E., Ley, R. E., Lozupone, C. A., McDonald, D., Muegge, B. D., Pirrung, M., Reeder, J., Sevinsky, J. R., Turnbaugh, P. J., Walters, W. A., Widmann, J., Yatsunenko, T., Zaneveld, J. and Knight, R. (2010). QIIME allows analysis of high-throughput community sequencing data. Nature Methods. 7, 335-336.

Celis, L. B., Villa-Gomez, D., Alpuche-Solis, A. G., Ortega-Morales, B. O., Razo-Flores, E. (2009). Characterization of sulphate-reducing bacteria dominated surface communities during start-up of a down-flow fluidized bed reactor. The Journal of Industrial Microbiology and Biotechnology 36, 111-121.

Delaune, R. D., Reddy, K. R. (2005). Redox Potential. Encyclopedia of Soils in the Environment. D. Hillel (ed). Academic Press. 366-371.

DeSantis, TZ.; Hugenholtz, P.; Larsen, N.; Rojas, M, Brodie.; EL, Keller, K.; Huber, T.; Dalevi, D.; Hu, P.; Andersen, GL. (2006) Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. Appl. Environ. Microbiol., Vol 72, 5069-5072

Elliot, P., Ragusa, S., Catcheside, D. (1998). Growth of sulphate-reducing bacteria under acidic conditions in an upflow anaerobic bioreactor as a treatment system for acid mine drainage. Water Research 32, 3724-3730

Gibert, O., de Pablo, J., Cortina, J.L., Ayora, C. (2002) Treatment of acid mine drainage by sulphate-reducing bacteria using permeable reactive barriers: A review from laboratory to full-scale experiments. Reviews in Environmental Science and Biotechnology 1, 4, 327-333.

Kaksonen, A. (2004). The performance, kinetics and microbiology of sulfidogenic fluidized-bed reactors treating acidic metal- and sulphate-containing wastewater (Doctoral dissertation). Publication 489, Tampere University of Technology.

Kaksonen, A. H. and Puhakka, J. A. (2007). Sulphate Reduction Based Bioprocesses for the Treatment of Acid Mine Drainage and the Recovery of Metals. Engineering in Life Sciences 7, 6, 541–564.

Kaksonen, A. H., Franzmann, P. D., Puhakka, J. A. (2003b). Performance and ethanol oxidation kinetics of a sulphate-reducing fluidized-bed reactor treating acidic metal-containing wastewater. Biodegradation 14, 207–217.

Kaksonen, A.H., Riekkola-Vanhanen, M.-L., Puhakka, J.A. (2003) Optimization of metal sulphide precipitation in fluidized-bed treatment of acidic wastewater. Water Research 37, 255-266.

Kõljalg U, Nilsson, RH, Abarenkov, K, Tedersoo L, Taylor, AFS, Bahram, M, Bates ST, Bruns TD, Bengtsson-Palme, J, Callaghan, TM, Douglas B, Drenkhan, T, Eberhardt U, Dueñas, M, Grebenc T, Griffith GW, Hartmann M, Kirk PM, Kohout, P, Larsson E, Lindahl, BD, Lücking, R, Martín MP, Matheny PB, Nguyen, NH, Niskanen, T, Oja, J, Peay KG, Peintner, U, Peterson M, Põldmaa K, Saag L, Saar, I, Schüßler, A, Scott, JA, Senés, C, Smith, ME, Suija A, Taylor DL, Telleria MT, Weiß M, Larsson, K-H. (2013) Towards a unified paradigm for sequence-based identification of Fungi. Molecular Ecology Vol 22, 5271-5277.

McMurdie and Holmes (2014) Shiny-phyloseq: Web Application for Interactive Microbiome Analysis with Provenance Tracking. Bioinformatics (Oxford, England) 31(2), 282–283.

Moosa, S. and Harrison, S. T. L. (2006). Product inhibition by sulphide species on biological sulphate reduction for the treatment of acid mine drainage. Hydrometallurgy 83, 14, 214-222.

Nagpal, S., Chuichulcherm, S., Peeva, L., Livingston, A. (2000). Microbial Sulphate Reduction in a Liquid–Solid Fluidized Bed Reactor. Biotechnology and Bioengineering 70, 4, 370-380.

Oyekola, O.O., van Hille, R.P., Harrison, S.T.L. (2010) Kinetic analysis of biological sulphate reduction using lactate as carbon source and electron donor: Effect of sulphate concentration. Chemical Engineering Science 65, 16, 4771-4781.

Postgate, J.R. (1979). The Sulphate Reducing Bacteria. Cambridge University Press, Cambridge.

R Core Team (2015). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Rajala P, Bomberg M, Huttunen-Saarivirta E, Priha O, Carpén L. (2016) Influence of chlorination and choice of materials on fouling in cooling water system under brackish seawater conditions. Materials 9, in press

Sahinkaya, E. (2009) Biotreatment of zinc-containing wastewater in a sulfidogenic CSTR: Performance and artificial neutral network (ANN) modelling studies. Journal of Hazardous Materials 164, 105-113.

Sahinkaya, E., Gunes, F.M., Ucar, D., Kaksonen, A.H. (2011) Sulfidogenic fluidized bed treatment of real acid mine drainage water. Bioresource Technology 102, 583-689.

Sánchez-Andrea, I., Sanz, J.L., Bijmans, M.F.M., Stams, A.J.M. (2014) Sulphate reduction at low pH to remediate acid mine drainage. Journal of Hazardous Materials 269, 98-109.

van der Zee, F.P., Villaverde, S., García, P.A. & Fdz.-Polanco, F. (2007). Sulfide removal by moderate oxygenation of anaerobic sludge environments. Bioresource Technology 98, 3, 518-524.

Warnes GR, Bolker B, Lumley T. (2016) gplots: Various R programming tools for plotting data. R package version 2.6.0.

White, C., Gadd, G.M. (1996) Mixed sulphate-reducing bacterial cultures for bioprecipitation of toxic metals, factorial and response-surface analysis of the effects of dilution rate, sulphate and substrate concentration. Microbiology, 142, 2197–2205