

4. Articles

*Sovint, repetiria allò d'aquell personatge de novel·la:
«Proveu que dic la veritat!».
Però no com un repte, sinó com una súplica*

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Article I: Treatment of Acid Mine Drainage by Sulphate-Reducing Bacteria Using Permeable Reactive Barriers: A Review from Laboratory to Full-Scale Experiments

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Review

Treatment of acid mine drainage by sulphate-reducing bacteria using permeable reactive barriers: A review from laboratory to full-scale experiments

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Abstract

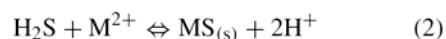
Acid mine drainage *in-situ* bioremediation has in the last decades drawn the attention in the field of environmental biotechnology. The most recent treatment technique are the permeable reactive barriers using sulphate-reducing bacteria. This view describes the basis of many of the current approaches to use sulphate-reducing bacteria in acid mine drainage treatment, from laboratory to full-scale realisations, and the limitations encountered when applied to full scale applications.

1. Introduction

Since Tuttle and co-workers (Tuttle et al. 1969) suggested the use of sulphate reducing bacteria (SRB) for the treatment of acid mine drainage (AMD), which is characterised by high acidity and high levels of metals and sulphates, the number of studies demonstrating the effectiveness of biogenic sulphide generation as an alternative to chemical sulphide has grown considerably till the present time. SRBs are a group of anaerobic bacteria (e.g., *Desulfovibrio*, *Desulfotomaculum*) that can reduce sulphate to form sulphide without significant assimilation of the sulphur into cell biomass (Postgate 1979). The overall sulphate reduction process can be represented by the equation:



where CH₂O represents an organic compound. The sulphide can precipitate with many of the metals present in an AMD, such as Fe, Zn or Cu:



where M includes metals such as Fe, Zn, Ni and Pb. Since the mass involved in reaction (1) is usually higher than the mass of metals involved in reaction (2), the overall process leads to an increase in alkalinity and the pH value of water. The activity of SRBs can, therefore, be very effective for the treatment of AMD.

The SRBs have specific environmental requirements which must be met to enable sulphate-reducing activity, such as an anaerobic environment (redox potential around –200 mV are generally needed), pH values greater than 5, the presence of an organic substrate or H₂ to be oxidised as the energy source (*electron donor*) and the presence of an appropriate sulphur species (as sulphate) to be reduced (*electron acceptor*), and a physical support on which the SRBs can be immobilised. Until recently, the use of SRBs was limited to *ex-situ* treatment in bioreactors, but latterly attention has focused on the application of SRBs in *in-situ* passive systems, such as artificial wetlands and, more recently, permeable reactive barriers (PRB). A permeable reactive barrier consists of the installation into the aquifer of an appropriate

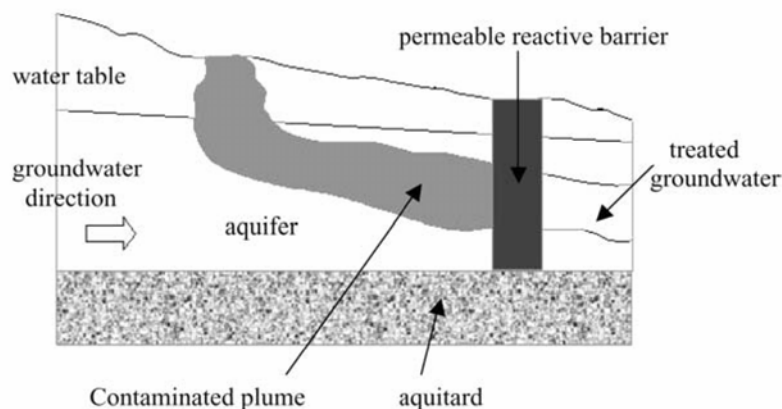


Figure 1. Schematic representation of a permeable reactive barrier.

reactive material able to induce physico-chemical and biological processes that remediate contaminated groundwater that flows through it. Figure 1 represents a scheme of a permeable reactive barrier. Once established, PRBs can theoretically be left to themselves without maintenance.

2. SRB experience under batch systems

An understanding of the geo-biological processes may improve the design of PRBs incorporating bacterial sulphate-reduction for the treatment of AMD.

A significant body of knowledge on the sulphate reducing process has been developed on the basis of its application in continuous systems (e.g., industrial reactors). Typically, this knowledge has been achieved in a first step by using batch experiments (Christensen et al. 1996; Dvorak et al. 1992; Hammack et al. 1992, 1994; Glombitza 2001; Harris et al. 2001; Kolmert et al. 2001; Waybrant et al. 1998; Webb et al. 1998; White et al. 1996a).

2.1. Inoculum source

In these experiments, the SRB inoculum source is an environmental organic sample (e.g., creek sediment, sewage sludge or animal manure) eventually accompanied by additional organic matter (compost, vegetal wastes, wood, whey ...) as carbon and nutrients sources to stimulate bacterial activity. Using natural sources or mixtures of them has several advantages over pure bacterial cultures: they are less liable to be contaminated from other micro-organisms, they can oxidise more completely carbon sources than pure

cultures, they contain a consortium of bacteria that can facilitate the development of reducing conditions, and they are more easily available (Waybrant et al. 1998; White et al. 1996a). The microbial community can be pre-grown, cultivating it in a rich-nutrient medium, with incubating times of up to several even weeks (Harris et al. 2001, Webb et al. 1998). Once the community is enriched and maintained, it is inoculated into the treatment system to start the experiment. Inoculation of organic matter with this incubated enrichment does not seem necessary to initiate the sulphate-reduction process, but it can be convenient to minimise the initial acclimation period (Harris et al. 2001).

2.2. Electron donor

A variety of natural organic substrates have been tested as carbon and nutrient source, including sewage sludge, leaf mulch, wood chips, animal manure, vegetal compost, sawdust (Waybrant et al. 1998), mushroom compost (Dvorak et al. 1992; Hammack et al. 1992, 1994), whey (Christensen et al. 1996), ryegrass (Harris et al. 2001) and other agricultural and food processing wastes. Synthetic organic compounds have also been evaluated, essentially small molecular weight compounds, like acetate, lactate, propionate, oxalate, methanol, ethanol, glycerol, glucose (Glombitza 2001; Kolmert et al. 2001; Webb et al. 1998; White et al. 1996a). Among these ethanol seems to be the most cost-effective carbon source (Kolmert et al. 2001; White et al. 1996a). H_2 can also serve as electron donor for many SRB (Foucher et al. 2001), although its application is not a general practice.

Table 1. Summary of laboratory and full-scale experiments on PRB

Source of organic matter	System	Sulphate conc (ppm)	t _R (days)	Additional carbon source	Sulphate removal rate (mg/L·d) ¹	Sulphide production rate (mg/(L·d)) ¹	Reference
SRB culture, cow manure, whey	batch	1000	—	none	3–4.4	1–1.5 (total produced) 0–0.63 (effl. content)	Christensen et al. (1996)
Creek sediment, sewage sludge, leaf mulch, wood chips, sheep manure, sawdust, cellulose, sand, limestone	Batch	1200–4600	—	none	4,6–109	n.a.	Waybrant et al. (1998)
SRB culture	batch	n.a.	—	—/glycerol/acetate/ lactate/propionate	n.a.	200–550 (with glycerol)	Webb et al. (1998)
Mushroom compost (composted straw, hay, horse and poultry manure ground corncobs, gypsum, limestone)	column	2000	0.5	no lactate lactate period (3500 mg/L)	0 30–100	n.a. n.a.	Hammack et al. (1992)
SRB culture	column	1920	2.1	glycerol/lactate/ethanol	250–300	250	Kolmert et al. (2001)
Creek sediment, vegetal compost, limestone	column	100/500	0.6	no C source acetate (1g/L) oxalate (1g/L)	0 27	n.a. 6	not published
Mushroom compost	continuous bioreactor sulphide recirculation	n.a.	4.1	lactate	n.a.	413	Hammack et al. (1994)
Mushroom compost	continuous bioreactor	1000	5–17	None	21–32	7.5	Dvorak et al. (1992)
Municipal compost, leaf compost, wood chips, limestone, silica sand	full-scale barrier	2000–4000	90	none	15 (1st year) 10 (3rd year)	n.a.	Benner et al. (2002)
Leaf compost, pea gravel, limestone	full-scale barrier	n.a.	6	none	n.a.	3.7	Ludwig et al. (2002)

n.a.: not available.

¹Rates values estimated from raw data.

All studies have concurred that the nature of the organic matter is a determinant factor on the biotreatment efficiency. Therefore, one of the objectives to the design of permeable reactive barrier is the selection of one or some available organic substrates to determine which are the most adequate to stimulate SRB activity. There are, at present, only few detailed studies that have compared different organic sources (Table 1). Waybrant et al. (1998) found that mixtures containing multiples organic matter sources show higher sulphate-reducing rates than those corresponding to single sources. Thus, for a water with a high SO_4^{2-} content (3300 mg/L), a mixture containing up to five different kind of organic matter (sewage sludge, leaf mulch, wood chips, sheep manure and sawdust), attained a sulphate removing level of

almost 100% in 20 days. Christensen et al. (1996) obtained similar results with mixtures containing vegetal compost and cow manure. Sulphate reduction rates varied from 5 to 300 mg/L·d (Table 1).

2.3. pH neutralisation and metal and sulphate removal

Initial pH ranged between 3 and 6, and in all cases the oxidation of the organic matter corrected it to near neutral values.

Metals evaluated in these studies include Fe, Ni, Zn, Al, Mn, Cu, U, Se, As, V (Christensen et al. 1996; Dvorak et al. 1992; Gu et al. 1998; Hammack et al. 1992, 1994; Waybrant et al. 1998). Initial concentrations ranged from 0.5 to 6 mg/L for U to hundreds of

mg/L for Fe or Ni. In most cases metal removing levels are greater than 95%.

Metal removal studies have shown that this step is not a simple process and that formation of different precipitates (sulphides, hydroxides, carbonates) as well as adsorption processes on mineral surfaces are taking place. Processes related to the generation and precipitation of metallic sulphides are responsible for the removal of divalent metals like Fe, Cu, Cd, Ni, Zn (Christensen et al. 1996; Dvorak et al. 1992; Gu et al. 1998; Hammack et al. 1992, 1994; Waybrant et al. 1998) whereas it seems that removal of trivalent metals (Al, Fe³⁺) results from hydroxides and (oxy)hydroxides precipitation (Christensen et al. 1996; Dvorak et al. 1992). In more alkaline environments carbonates may also become significant phases. Manganese is not removed by biogenic sulphide treatment, but as manganese carbonate (MnCO₃) (Waybrant et al. 1998). In relation to the mineralogy of iron sulphides, SEM and XRD analysis confirm the presence of amorphous FeS precipitates, which may eventually recrystallise to mackinawite (Fe_{1+x}S), a precursor of pyrite (FeS₂) (Herbert et al. 1998). Webb et al. (1998) concluded that Fe sorption on organic matter may also be an important process in the elimination of Fe.

The mechanisms of metal removal and more interestingly, how these might differ from one species to another, remains to be determined for each particular AMD to be treated.

Sulphates have been studied in concentrations from 1000 to 3600 mg/L and, in batch systems, almost 100% removal has been reported.

3. SRB performance under continuous flow systems

The study of the sulphate-reduction in continuous flow systems is more complex. Several attempts have showed the need of considering key factors, not considered in batch experiments. The influence of other parameters such as flow rate or residence time may be noticeable on the biotreatment efficiency. Residence times that are too short do not allow bacteria to generate enough sulphide to precipitate metals and enough alkalinity to neutralise the acidity. Pilot experiments using bioreactors with mushroom compost (mixture of manure, hay, straw, corn cobs, wood chips, gypsum and limestone) showed a 99% decrease in sulphide concentration when the flow rate

was doubled (residence times from 17 to 7 days; Dvorak et al. 1992). Thus, it is necessary to apply appropriate conditions to allow maximal bacterial activity. This can be reached by increasing residence times (which implies a flow rate decrease or an increase of the thickness of the supporting material to be crossed) or by stimulating bacterial activity. Considering the PRB application, the water flow value is fixed by the aquifer, and it is thus not a variable that can be optimised. Dimensions of PRB can require large amounts of material, which may entail prohibitive costs. Thus, increasing the barrier thickness is not presented as a good option. An alternative can be the stimulation and acceleration of bacterial activity (Hammack et al. 1992, 1994; White et al. 1996a,b).

Hammack et al. (1992) studied the effect of the continuous addition of lactate in the treatment of a water rich in Ni (50–1000 mg/L) and sulphate (2000 mg/L) in column experiments with an organic matter based on mushroom compost. Based on a 12-h residence time, only a slight decrease of nickel and sulphate was observed, but a lactate supplementation in the influent (3500 mg/L) lowered Ni and sulphates concentrations to <50 mg/L and <600 mg/L, respectively. When lactate supplementation was suspended, effluent concentrations increased to their initial levels. The positive response of the system to lactate supplementation indicates that sulphate reduction was carbon limited. Similar results were obtained when studying the SRB efficiency on groundwater treatment scenarios with high groundwater velocity, i.e., >1 m/d (data not published). Experiments in columns were carried out to evaluate an organic mixture (sediment creek, vegetal compost, limestone) for the treatment of an acidic water containing sulphate (500 mg/L) and Fe (10 mg/L), at a residence time of 12 hours. Initially, no evidence of bacterial activity was observed. When acetate and oxalate were added to the inflow (1000 mg/L), dramatic changes occurred: redox potentials dropped to –200 mV, sulphate concentrations decreased by 50%, effluent acquired a strong hydrogen sulphide odour (confirmed by an increase of the sulphide concentration), indicating that bacterial sulphate reduction was taking place. The maximum sulphide generation rate was 6 mg/L·d, as shown in Table 1.

The SRB activity can be optimised by using processes that slowly release organic substrates. Alternatives, such as encapsulation or pelletisation of those organic substrates, are current under development at laboratory scale (Rust et al. 2002; Stoessell et al.

2001). The application of these materials on PRB may provide an alternative solution to increasing the thickness of the barrier if a higher residence time in the PRB is required.

With respect to the sulphate, an initial decrease of its concentration can be observed. This removal is not due to the microbial activity but to other factors like adsorption or co-precipitation of sulphates on aluminium and ferric(oxy)hydroxide precipitates. This process is important as long as reducing conditions are not reached. (Christensen et al. 1996). Amorphous Al-Fe-(oxy)hydroxides containing metals and sulphates can be observed ubiquitously under scanning electron microscopy (Gibert et al. 2001). When the redox potential drops and reducing conditions evolve, a sulphate re-dissolution takes place and even an increase of its effluent concentration can be observed (Christensen et al. 1996).

4. SRB experience at field scale on permeable reactive barriers

The use of the SRB process as treatment strategies on PRB for acid mine drainage abatement at field scale was first proposed by Blowes et al. (1995). A full-scale reactive barrier based on biogenic sulphide generation to remediate AMD was installed in 1995 at the Nickel Rim mine site near Sudbury, Ontario (Canada). Nickel Rim AMD contains 2000–4000 mg/L sulphate, 200–1200 mg/L Fe and a pH value of 6. The reactive material used in the barrier contained a mixture of pea gravel (50%) and a reactive mixture (50%) containing municipal compost (40%), leaf compost (40%), wood chips (18%) and limestone (2%). The barrier dimensions were 3.6 m depth, 15 m length and 4 m thick, with an estimated average residence time of 90 days (Benner et al. 2002). The PRB installation was combined with an extensive monitoring system at the location. This provided analytical data on both physical parameters (temperature, groundwater velocity, etc.) and chemical parameters (pH, alkalinity, sulphates, sulphides, metals, etc.). Up till now, both sulphate and iron removal have occurred, and the basic mechanisms involved were Fe sulphide formation under Fe mackinawite was observed (Herbert et al. 2000).

Recently, Benner et al. (2002) critically summarised the critical performance data on the changes of the sulphate and iron reduction rates. Factors as temperature, organic matter ageing as well as the

hydrodynamic aspects of creation of slow and fast flow lines were critically discussed. After 3 years of installation, the overall rate of sulphate reduction within the barrier is 40 mmol/L-a, 30% of the initial value, removing <1000 mg/L sulphate (Benner et al. 1997, 1999, 2002). However, due to the high levels of sulphates and iron in the groundwater to be treated PRB could provide long-term treatment efficiency and ultimately remediate the aquifer. The potential importance of variations in hydraulic conductivity and temperature fluctuations has shown as key parameters for the design of the PRB.

A second full-scale SRB application on PRB to treat AMD was started in Aznalcóllar (Spain) in November 2000 (Carrera et al. 2001). Groundwater to be treated contained 1000 mg/L sulphate, 30 mg/L zinc, 30 mg/L Cu and pH values between 3 and 4. The barrier, 4.5 m depth, 110 m length and 1.4 m thickness, was divided into three modules, each 30 m long, containing different proportions of limestone, organic compost and zero-valent iron (Fe⁰). The inclusion of iron aggregates on the reactive mixtures was postulated as a mechanism to promote bacterial activity via hydrogen production from water hydrolysis (Gu et al. 1999). Module 1 contained 50% limestone, 30% of municipal compost and 20% sewage sludge, module 2 contained 50% limestone, 50% compost and a small amount of zero-valent iron cast, and module 3, 66% limestone and 33% compost. Till the present time, module 1 has revealed to be not permeable enough to groundwater flow. In module 2 and 3 acidity was corrected to near neutral values and metal reduction is higher than 90%, whereas sulphate reduction is not significant. Metal reduction is attributed to co-precipitation in Fe-Al (oxy)hydroxides phase, caused by the pH increase due to calcite dissolution (Carrera et al. 2001).

Ludwig et al. (2002) reported the installation of a third compost-based PRB to treat a heavy-metal contaminated plume in Vancouver, British Columbia (Canada). Groundwater entering the barrier had an average pH of 6.39 and metal concentrations ranged between 3.63 mg/L for Cu and 0.005 mg/L for Co. Data on sulphate concentrations were not reported. The barrier was 6.5 m depth, 2.5 m width and 10 m length. The reactive mixture used in the barrier consisted of 15% leaf compost, 84% pea gravel and 1% limestone. After 21 months of the barrier installation, the pH of treated water had an average value of 6.86 and the metal removal levels were greater than 80%. Biogenic sulphide generation and thereby metal

precipitation is regarded as the main metal removing mechanism in the barrier, although positive Eh values are measured both in the up-gradient and down-gradient side of the barrier. These considerations appear to be in contradiction with studies reporting that "SRB conduct their major metabolic oxidations within a redox span whose positive end is around Eh -150 to -200 mV" (Postgate 1979). These environmental requirements concerning the need of negative Eh values for SRB activity are also reported by Lyew et al. (1997) and White et al. (1996b).

A recent published paper reports the design of a fourth organic-based PRB to treat colliery spoil leachates in Shilbottle Colliery, near Newcastle upon Tyne (UK) (Amos et al. 2003). Different media mixtures containing varying proportions of cattle slurry screenings, green waste compost, calcite limestone and pea gravel have been tested in laboratory-scale batch experiments in order to determine appropriate mixture proportions for use in PRB. A mixture containing 50% limestone chips, 25% slurry screenings and 25% compost was provisionally assumed as the most optimal in terms of reactivity and permeability.

5. Conclusions

It can be concluded that:

- The use of SRBs might be feasible for treatment of acid mine drainage. The choice of an efficient, widely available organic matter is fundamental to reduce the price of the process and improve the treatment efficiency. The laboratory results show that mixtures containing multiple sources of organic matter appear to be the best candidates. It remains to highlight the need to supplement a simple carbon substrate to satisfy nutrient requirements in order to maintain bacterial activity.
- Bacterial sulphate reduction seems to be reaction rate limited. It is thus necessary to give a sufficient residence time. Therefore results from batch experiments can not be easily extrapolated to continuous flow systems as permeable reactive barriers.
- Bacterial sulphate reduction and consequent metal precipitation is not the only source of metal removal. Other processes more related to the pH increase than the redox potential decrease can produce significant metal precipitation as hydroxides and carbonates.

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References

- Amos PW & Younger PL (2003) Substrate characterisation for a subsurface reactive barrier to treat colliery spoil leachate. *Water Res.* 37: 108–120
- Benner SG, Blowes DW & Ptacek CJ (1997) A full-scale porous reactive wall for prevention of acid mine drainage. *Ground Water Monit. R.* 17: 99–107
- Benner SG, Blowes DW, Gould WD, Herbert RB Jr & Ptacek CJ (1999) Geochemistry of a permeable reactive barrier for metals and acid mine drainage. *Environ. Sci. Technol.* 33: 2793–2799
- Benner SG, Blowes DW, Ptacek CJ & Mayer KU (2002) Rates of sulfate reduction and metal sulfide precipitation in a permeable reactive barrier. *Appl. Geochem.* 17: 301–320
- Blowes DW & Ptacek CJ (1995) System for treating contaminated groundwater. United States patent number 5,362,394. US patent office
- Blowes DW, Ptacek CJ, Benner SG, McRae CWT, Bennett TA & Puls RW (2000) Treatment of inorganic contaminants using permeable reactive barriers. *J. Contam. Hydrology* 45: 123–137
- Carrera J, Alcolea A, Bolzicco J, Knudby C, Ayora C et al. (2001) An experimental geochemical barrier at Aznalcóllar. In: Thornton S & Oswald S (Eds) *Proceedings of the 3rd International Conference on Groundwater Quality*, 18–21 June 2001 (pp 407–409). Sheffield, UK
- Chen C, Mueller RF & Griebel T (1994) Kinetic analysis of microbial sulfate reduction by *Desulfovibrio desulfuricans* in an anaerobic upflow porous media biofilm reactor. *Biotech. Bioeng.* 43: 267–274
- Christensen B, Laake M & Lient T (1996) Treatment of acid mine water by sulfate-reducing bacteria: results from a bench scale experiment. *Water Res.* 30(7): 1617–1624
- Dvorak DH, Hedin RS, Edenborn HM & McIntire PE (1992) Treatment of metal contaminated water using bacterial sulfate reduction: results from pilot-scale reactors. *Biotech. Bioeng.* 40: 609–616
- Foucher S, Battaglia-Brunet F, Ignatiadis I & Morin D (2001) Treatment by sulfate-reducing bacteria of Chessy acid-mine drainage and metals recovery. *Chem. Eng. Sci.* 56: 1639–1645
- Gibert O, de Pablo J, Cortina JL & Ayora C (2001) Evaluación de materia orgánica como material para su utilización en barreras permeables reactivas. In: Medina A, Carrera J & Vives L (Eds) *Proceedings of the Conference "Las caras del agua subterránea"*, September 2001 (pp 43–48). Barcelona, Spain
- Glombitza F (2001) Treatment of acid lignite mine flooding water by means of microbial sulfate reduction. *Waste Manage.* 21: 197–203
- Gu B, Liang L, Dickey MJ, Yin X & Dai S (1998) Reductive precipitation of uranium(VI) by zero-valent iron. *Environ. Sci. Technol.* 32: 3366–3373
- Gu B, Phelps TJ, Liang L, Dickey MJ et al. (1999) Biogeochemical dynamics in zero-valent iron columns: Implications for

- permeable reactive barriers. *Environ. Sci. Technol.* 33: 2170–2177
- Hammack RW & Edenborn HM (1992) The removal of nickel from mine waters using bacterial sulfate reduction. *Appl. Microbiol. Biot.* 37: 674–678
- Hammack RW, Edenborn HM & Dvorak DH (1994) Treatment of water from an open-pit copper mine using biogenic sulfide and limestone: A feasibility study. *Water Res.* 28(11): 2321–2329
- Harris MA & Ragusa S (2001) Bioremediation of acid mine drainage using decomposable plant material in a constant flow reactor. *Environ. Geol.* 40: 1192–1204
- Herbert RB Jr, Benner SG, Pratt AR & Blowes DW (1998) Surface chemistry and morphology of poorly crystalline iron sulfides precipitated in media containing sulfate-reducing bacteria. *Chem. Geol.* 144: 87–97
- Herbert RB Jr, Benner SG & Blowes DW (2000) Solid phase iron-sulfur geochemistry of a reactive barrier for treatment of mine drainage. *Appl. Geochem.* 15: 1331–1343
- Kolmert Å & Johnson DB (2001) Remediation of acidic waste waters using immobilised, acidophilic sulfate-reducing bacteria. *J. Chem. Technol. Biotechnol.* 76: 836–843
- Ludwig RD, McGregor RG, Blowes DW, Benner SG & Mountjoy K (2002) A permeable reactive barrier for treatment of heavy metals. *Groundwater* 40(1): 59–66
- Lyew D & Sheppard JD (1997) Effects of physical parameters of a gravel bed on the activity of sulphate-reducing bacteria in the presence of acid mine drainage. *J. Chem. Technol. Biotechnol.* 70: 223–230
- Montgomery AD, McInerney MJ & Sublette KL (1990) Microbial control of the production of hydrogen sulfide by sulfate-reducing bacteria. *Biotech. Bioeng.* 35: 533–539
- Morrison SJ, Metzler DR & Carpenter CE (2001) Uranium precipitation in a permeable reactive barrier by progressive irreversible dissolution of zerovalent iron. *Environ. Sci. Technol.* 35: 385–390
- Okabe S, Nielsen PH & Characklis WG (1992) Factors affecting microbial sulfate reduction by *Desulfovibrio desulfuricans* in continuous culture: Limiting nutrients and sulfide concentration. *Biotech. Bioeng.* 40: 725–734
- Postgate (1979) *The Sulphate Reducing Bacteria*. Cambridge University Press, Cambridge
- Rose PD, Boshoff GA, van Hille RP, Wallace LCM, Dunn KM & Duncan JR (1998) An integrated algal sulphate reducing high rate ponding process for the treatment of acid mine drainage wastewaters. *Biodegradation* 9: 247–257
- Rust CM, Aelion CM & Flora JRV (2002) Laboratory sand column study of encapsulated buffer release for potential in situ pH control. *J. Contam. Hydrology* 54: 81–98
- Stoessel RK, Easley DH & Yamazaki GP (2001) Denitrification and phosphate removal in experiments using Al stearate. *Ground Water Monit. R. Spring*: 89–96
- Tuttle LH, Dugan PR & Randles CI (1969) Microbial sulfate reduction and its potential utility as an acid mine water pollution abatement procedure. *Appl. Microbiol.* 17(2): 297–302
- Warren LA & Ferris FG (1998) Continuum between sorption and precipitation of Fe(III) on microbial surfaces. *Environ. Sci. Technol.* 32(15): 2331–2337
- Waybrant KR, Blowes DW & Ptacek CJ (1998) Selection of reactive mixtures for use in permeable reactive walls for treatment of mine drainage. *Environ. Sci. Technol.* 32(13): 1972–1979
- Webb JS, McGuinness S & Lappin-Scott HM (1998) Metal removal by sulphate-reducing bacteria from natural and constructed wetlands. *J. Appl. Microbiol.* 84: 240–248
- White C & Gadd GM (1996a) A comparison of carbon/energy and complex nitrogen sources for bacterial sulphate-reduction: Potential applications to bioprecipitation of toxic metals as sulphides. *J. Industrial Microbiol.* 17: 116–123
- White C & Gadd GM (1996b) 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

