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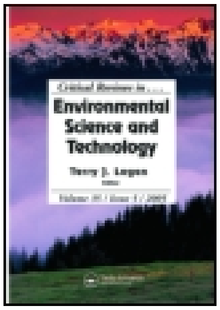

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Acid mine drainage treatment in fluidized-bed bioreactors by sulfate-reducing bacteria: a critical review

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Acid mine drainage treatment in fluidized-bed bioreactors by sulfate-reducing bacteria: a critical review

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ABSTRACT

Acid mine drainage is an important environmental problem related to the release of acidic, sulfate and metal-containing wastewater into the environment. Sulfate-reducing bacteria can be used for treating mining wastewaters and recovering metals in several bioreactor configurations. The fluidized-bed configuration is an efficient way for acid mine drainage remediation due to the high biomass retention, the great resistance to inhibitors and the possibility to use high organic, sulfate and metal loading rates at low hydraulic retention times. Additional research is needed to assess the removal of the organic rest-pollution and the factors that promote a better metal recovery in single-stage processes.

Keywords: Acid mine drainage, wastewater, sulfate-reducing bacteria, fluidized-bed reactors, metal precipitation

1. INTRODUCTION

Wastewaters from mining and mineral processing activities are often characterized by a low pH and high metal and sulfate concentration (Johnson and Hallberg, 2005). Metals such as Cd, Cr, Cu, Pb and Zn are toxic and non-biodegradable pollutants which tend to accumulate in the food chain and are absorbed by living organisms, including the human body causing serious health disorders (Roberts and Johnson, 1978; Zhuang et al., 2009). Besides, they also affect the aesthetic quality of potable water (Gray, 2008). Heavy metal containing wastewaters can be treated using several technologies including adsorption, cementation, coagulation-flocculation, ion exchange, membrane separation or precipitation (Brooks, 1991; Kurniawan et al., 2006; Fu and Wang, 2010). Most industries treat these wastewaters by precipitation with hydroxide or carbonates essentially because of process simplicity and ease of process control (Veeken et al., 2003a). However, this method presents some drawbacks in terms of application and effectiveness as it usually results in the production of unstable metal hydroxides, which are hardly suitable for metal recovery and lead to a greater disposal expense (Tabak and Govind, 2003; Esposito et al., 2006).

Since heavy metals are non-renewable resources, their recovery and reuse is as important as their removal from wastewaters for both economic and environmental reasons (Badmus et al., 2007). Besides a better selective metal recovery, sulfide precipitation has been demonstrated to be superior over hydroxide precipitation as (i) effluent concentrations are orders of magnitude lower (μg/L vs. mg/L), (ii) high reaction rates result in low hydraulic retention times (HRT) and (iii) metal sulfide sludge is more compact and exhibits better settling, thickening and dewatering characteristics than hydroxide sludge (Brooks, 1991; Hammack et al., 1993; Peters et al., 1993; Veeken et al., 2003a).

In recent years, the use of biological processes for treating heavy metal containing wastewaters has gained increasing interest, mainly due to their ability to produce effluents suitable to be discharged into the environment (Janssen et al., 2001). When sulfate is also present in the wastewater stream, biogenic sulfide produced by sulfate-reducing bacteria (SRB) is an important alternative sulfide source for metal precipitation. The removal mechanism is based on the fact that, under anaerobic conditions, SRB can oxidize simple organic compounds using sulfate as terminal electron acceptor which is reduced to sulfide (Lens et al., 2000). Removal of metals is due to the production of highly insoluble precipitates that react with the biogenic H_2S (Lewis and van Hille, 2006; Gallegos-Garcia et al., 2008).

Recently, the development and improvement of several bioreactor configurations lead to effective removal of metals and acidity, either by immobilization of microbes on solid substrate (Cohen, 2006) or keeping microorganisms in suspension. Among the different bioreactor designs, fluidized-bed reactors (FBR) are gaining increasing attention due to their high efficiency in wastewater treatment and other advantages such as a large surface area for microorganism growth and attachment, good mixing and contact between substrate and biomass, high mass transfer and substrate utilization rates, high organic loading rates and greater resistance to inhibitors (Nicolella et al., 1997; Buffiere et al., 2000; Sowmeyan and Swaminathan, 2008b).

This article is an integrated review of current knowledge about the treatment of acid mine drainage in anaerobic FBRs. Preliminary a short overview is given on general aspects concerning *i)* acid mine drainage characteristics and formation, *ii)* typical treatments technologies for AMD wastewaters, *iii)* characteristics of sulfate-reducing activity and i*v)* different bioreactor configurations. The attention is then focused on critical parameters for the start-up, design and long-term operation of this particular type of bioreactor configurations when applied to treat heavy metal- and sulfate-containing wastewaters.

2. AMD AND MINING WASTEWATER: FORMATION AND TREATMENT

2.1 AMD formation

AMD is an outflow of acidic water from coal mines or metal mines. AMD from abandoned mines is a major environmental issue in the United States and other countries wherever mining has been practiced on a large scale (Tabak et al., 2003). This drainage typically contains high concentrations of dissolved metals (Table 1) and more than 3 g/L sulfate (Tabak and Govind, 2003). AMD and mining water characteristics change according to the chemical soil composition the waters pass through. Table 1 gives some examples of the dissolved metal concentrations in Berkeley Pit AMD (USA) (Tabak et al., 2003), in the Gromolo river in Libiola mine area (Italy) (Dinelli et al., 2001; Dinelli and Tateo, 2002), in Kennecott Copper mine AMD (USA) (Buisman et al., 1999) in Queen mine wastewater (USA) (Ashe et al., 2008) and in Leviatham mine wastewater (USA) (Tsukamoto et al., 2004).

The AMD generating processes are currently quite well understood: AMD is generated through a combination of chemical and biological processes by which metal sulfides (such as pyrite) are converted to sulfates and metal hydroxides when exposed to fresh water and oxygen (Tichy et al., 1998; Neculita et al., 2007). Moreover, acid mine drainage formation is further amplified when the reactions are catalyzed by aerobic bacteria such as *Acidithiobacillus ferrooxidans* (Brown et al., 2002). The mechanism for the production of AMD is presented in the following reactions (1-4), which describe the oxidation of pyrite ore as a typical example (Banks et al., 1997; Kaksonen and Puhakka, 2007):

$$2FeS_2 + 7O_2 + 2H_2O \rightarrow 2Fe^{2+} + 4SO_4^{2-} + 4H^+ \qquad (1)$$

The oxidation of ferrous to ferric iron consumes protons:

$$Fe^{2+} + 4H^+ + O_2 \rightarrow Fe^{3+} + 2H_2O \qquad (2)$$

Hydrolysis of ferric iron occurs subsequently which releases protons:

$$Fe^{3+} + 3H_2O \rightarrow Fe(OH)_3 + 3H^+ \qquad (3)$$

The overall sequence of reactions is acid-producing:

$$2FeS_2 + 5H_2O + 8O_2 \rightarrow Fe(OH)_3 + 4SO_4^{2-} + 3H^+ \qquad (4)$$

As shown in the previous reactions, the water pH progressively decreases resulting in mobilization of metals from mine wastes (Neculita et al., 2007). Consequently, disposing AMD without an appropriate treatment leads to environmental contamination (Jong and Parry, 2003). Pollution control of AMD can be achieved by preventing AMD formation and/or collection and treatment of the AMD (Geldenhuis and Bell, 1998; Johnson, 2000).

2.2 AMD treatment

Traditional AMD treatment systems use alkali to increase the water pH and to precipitate metals as hydroxides and carbonates (Cravotta and Trahan, 1999; Santos et al., 2004). A method to add alkalinity to acidic water is through its direct contact with limestone according to the following reactions (Nairn et al., 1992; Gazea et al., 1996):

$CaCO_3 + 2H^+ \rightarrow Ca^{2+} + H_2CO_3$ (5)

$CaCO_3 + H_2CO_3 \rightarrow Ca^{2+} + 2HCO_3^-$ (6)

This treatment is hardly effective when the influent contains appreciable amounts of ferrous iron and it is in contact with limestone in an oxidizing environment, as limestone is quickly coated with iron precipitates slowing down its dissolution and alkalinity production (Gazea et al., 1996). Other drawbacks are: *i)* high costs for the large amount of chemicals used, *ii)* the inefficient removal of sulfate and *iii)* the production of high bulky sludge volumes that require further treatment and high disposal costs (Kurniawan et al., 2006; Aziz et al., 2008).

Other technologies such as ion exchange, reverse osmosis and electro-dialysis are available to treat AMD waters but they are expensive and not commonly used (Prasad et al., 1999). Kurniawan et al. (2006) overview all possible physico-chemical treatments (including coagulation-flocculation, air flotation, ion exchange, membrane filtration and adsorption) for heavy metal-containing wastewaters evaluating metal removal efficiencies and list the advantages and drawbacks of each technology. However, all these methods require high capital investments and have generated sludge disposal problems due to the need of drying facilities and further treatment (Cohen, 2006; Aziz et al., 2008).

Therefore, the stabilization of metals is preferred in the form of sulfide precipitates because the solubility products of most metal sulfides are lower than those of metal hydroxides (Luther et al., 1996) and sulfide can be naturally produced in biosulfidogenic reactors (Hammack et al., 1993; Bhagat et al., 2004; Esposito et al., 2006).

2.3 Sulfate reduction biotechnology

2.3.1 Basic principles

Bacterial sulfate reduction is considered as an important bio-process for removing metals from metal-mine drainage (Tuttle et al., 1969; Wakao et al., 1979; Herlihy and Mills, 1985; Hedin et al., 1989). Sulfate-reducing bacteria (SRB) are a unique group of prokaryotes that, under anaerobic conditions, oxidize organic compounds using sulfate as electron acceptor and thereby generate hydrogen sulfide and bicarbonate ions (Postgate, 1984; Dvorak et al., 1992):

$$3SO_4^{2-} + 2CH_3CHOHCOOH \rightarrow 3H_2S + 6HCO_3^- \qquad (7)$$

The biogenic hydrogen sulfide reacts with contaminant metals to remove them from solution as insoluble metal sulfides:

$$H_2S + M^{2+} \rightarrow MS(s) + 2H^+ \qquad (8)$$

where M stands for the general metal. Bicarbonate ions react with protons to form CO_2 and water and remove acidity from solution as CO_2 gas:

$$HCO_3^- + H^+ \rightarrow CO_2\,(g) + H_2O \qquad (9)$$

In case of autotrophic SRB, carbon dioxide is used as carbon source and molecular hydrogen is used as energy source (Widdel, 1988; van Houten et al., 1994; Esposito et al., 2003):

$$4H_2 + SO_4^{2-} + 2H^+ \rightarrow H_2S + 4H_2O \qquad (10)$$

2.3.2 Organic electron donors for heterotrophic SRB

Generally acid mine drainage contains very low concentrations of dissolved organic carbon (Kolmert and Johnson, 2001). For this reason, the additional organic carbon source to be added as electron donor determines the overall costs of the sulfate reduction bio-process (Gilbert et al., 2004; Zagury et al., 2006). The choice of the substrate is based on different criteria: *i)* the ability of SRB to utilize the organic substrate, *ii)* the sulfate load to be reduced and the cost of the substrate per unit of H_2S produced, *iii)* the availability in sufficient quantities and *iv)* the remaining pollution load from the incompletely degraded substrate (van Houten et al., 1994; Dries et al., 1998; Dijkman and Buisman, 1999). In Table 2 the main organic electron donors used for sulfate reduction are listed with their respective advantages and shortcomings (for more complete reviews, see Liamleam and Annachhatre, 2007; Kaksonen and Puhakka, 2007).

2.3.3 Toxic effects

Several studies have been conducted to assess the inhibitory effects of particular factors such as toxic metals, dissolved H_2S, organic matter and low pH to biological sulfate reduction. Heavy metals can affect the SRB metabolism by deactivating the enzymes and denaturing the proteins (Cabrera et al., 2006). Depending on the metal concentration, the effect can be different such as *i)* inhibition of the bacterial growth (Table 3), *ii)* extension of the lag phase in sulfide production, *iii)* decrease in sulfate-reducing activity and *iv)* decay of bacteria (Sani et al., 2001; 2003; Cabrera et al., 2006). The inhibitory effect of H_2S and organic acids is caused when these substances are present as unionized compounds because only neutral molecules permeate the cell membrane (Oleszkiewicz et al., 1989; Okabe et al., 1992 Reis et al., 1992; Kolmert et al., 1997; Hulshoff Pol et al., 2001; Kimura et al., 2006). Undissociated acids enter the bacterial cell, acidify the cytoplasm and lead to bacterial death at high concentrations (Kimura et al., 2006). Low pH values increase toxicity as they promote the formation of undissociated sulfide (Hulshoff Pol et al., 2001; Willow and Cohen, 2003) and non-ionized organic acids (Kimura et al., 2006).

2.4 Bioreactor configurations

As a consequence of the increasing interest in applying biological processes for AMD and mine wastewater treatment, many different bioreactor designs have been developed (for a more detailed analysis, see Speece, 1983; Hulshoff Pol et al., 2001; Lens et al., 2002; Kaksonen and Puhakka, 2007), including *i)* continuously stirred tank (CSTR) reactors (Barnes et al, 1991; White and Gadd, 1996a), *ii)* packed-bed (PBR) reactors (Maree and Strydom, 1985, 1987; Elliott et al., 1998; El Bayoumy et al., 1999; Kolmert and Johnson, 2001; Jong and Parry, 2003), *iii)* gas-lift (GLR) reactors (van Houten et al., 1994; Weijma et al., 2002; Esposito et al., 2003; Bijmans et al., 2009; Esposito et al., 2009), *iv)* up-flow anaerobic sludge blanket (UASB) reactors (Lettinga et al., 1980; de Lima et al., 1996; Omil et al., 1996; Kalyuzhnyi et al., 1997; Vallero et al., 2003; 2004; Jhung and Choi 1995), *v)* membrane biological (MBR) reactors (Chuichulcherm et al., 2001; Mack et al., 2004; Vallero et al., 2005) and *vi)* fluidized-bed (FBR) reactors (as described more in detail below). Figure 1 gives a schematic representation of the different reactor configurations (from *i* to *v*), whereas Table 4 overviews benefits and drawbacks.

3. FLUIDIZED-BED REACTORS

Fluidized-bed reactors (FBR) are an important alternative biofilm process for biological wastewater treatment (Nikolov and Karamanev, 1991). In FBRs, a recirculation flow fluidizes small carrier particles and induces extensive cell immobilization, thereby achieving a high reactor biomass hold-up and a long cell residence time (Shieh and Hsu, 1996). Literature is rich in scientific work on this technology since, besides sulfate reduction and metal removal, FBRs have been applied abundantly for denitrification (Green et al., 1994), nitrite removal (Boehler and Haldenwag, 1991), anaerobic digestion (Heijnen et al., 1989; Anderson et al., 1990; Garcia-Calderon et al., 1998a; Buffiere et al., 2000; Arnaiz et al., 2003; Sowmeyan and Swaminathan, 2008a; 2008b) and the removal of chlorinated phenols (Puhakka and Järvinen, 1992; Wilson et al.,1997), chlorinated aliphatic compounds (Niedzielski et al., 1989) and aromatic hydrocarbons (Shimodaira and Yushina, 1983; Voice et al., 1992).

3.1 Fluidization typologies

3.1.1 Classical fluidization

In the traditional up-flow fluidization, the solid particles have a higher density than the liquid and are fluidized by the liquid stream, pumped at the bottom of the reactor and flowing in the opposite direction of gravity.

The fluidized-bed bioreactor design has a series of advantages compared to other anaerobic reactor concepts (Iza, 1991):

- an efficient biomass retention that allows high mass transfer and reaction rates (Speece, 1983; Shieh and Keenan, 1986; Marin et al., 1999);
- higher organic loading rates (OLRs) and lower hydraulic reaction times (HRTs) permit lower reactor sizes (Garcia-Calderon et al., 1998a);
- large surface area for biofilm formation due to the fluidization of the bed (Anderson et al., 1990; Diez-Blanco et al., 1995);
- good mixing and contact between substrate and biomass (Nicolella et al., 1997);
- great resistance to inhibitors due to the recycle flow that dilutes high influent concentrations (Marin et al., 1999; Kaksonen et al., 2003a; 2003b; 2004b; Sahinkaya et al., 2007a, 2007b);
- high efficiencies in terms of sulfate reduction (Celis-Garcia et al., 2007) and metal precipitation (Kaksonen et al., 2003a; Sahinkaya 2007b).

3.1.2 Inverse fluidization

The inverse (or down-flow) fluidized-bed reactor (IFBR or DFFBR) is a particular fluidized-bed reactor configuration. Figure 2 shows a schematic representation of FBR and IFBR reactor design. The IFBR configuration utilizes small particles with a lower specific density than the specific density of the water, thus particles float and are expanded downward by the liquid flow (Sowmeyan and Swaminathan, 2008b). In the case of gas production, it also contributes to bed expansion and this phenomenon is called pseudo-fluidization (Arnaiz et al., 2003). However, other authors have found that gas formation has little effect on the hydrodynamic behavior, and therefore it is possible to describe the bed in an IFBR like a two-phase solid-liquid system (Diez-

Blanco et al., 1995). Down-flow fluidization has further advantages compared to classical fluidization such as:

- it allows the recovery of solid products, such as metal sulfides, at the bottom of the reactor. In this way, the biofilm developing on the top of the reactor remains separated from the metal precipitates (Celis-Garcia et al., 2007; Kaksonen and Puhakka, 2007; Villa-Gomez et al., 2011);
- it is not prone to clogging (Garcia-Calderon et al., 1998b);
- it has a lower energy requirement (Castilla et al., 2000).

3.2 Carrier material

Table 5 shows the characteristics and the fluidization velocities of different materials used as support for the growth of the microbial species in several fluidized-bed applications. Many materials, with density higher than water, such as porous glass beads, granular activated carbon (GAC), silicate mineral sand, celite particles and polymeric granules covered with iron dust have been used and tested as packing materials in classical FBRs, showing all good biomass attachment capabilities with different reactor operating conditions.

Nagpal et al. (2000a) used spherical glass beads as biomass carrier particles. They were shown to be very suitable due to their roughness and porosity characteristics since the surface of the clean particles had numerous craters (10-30 μm wide and 5 μm deep). The effect of liquid velocity on bed fluidization was studied both with clean beads and with biomass-loaded beads and no significant change in bed expansion were observed (v=145.8m/h and v=151.2m/h with clean and biomass-loaded beads, respectively).

Voice et al. (1992) used scanning electron microscopy to study the microbial attachment to the external surface of granular activated carbon. A uniform biofilm over the entire surface area was formed and not only in the biggest crevices of the material. This resulted from the ability of the activated carbon to concentrate nutrients necessary for microbial growth and to provide a well-protected environment from fluid shear forces.

Sand particles have been shown to guarantee a very good biomass attachment as well. Kaksonen et al. (2003a) could test their sulfate-reducing and metal-precipitating FBRs by increasing the recirculation flow up to 30% using a silicate filtralite support. In a denitrifying fluidized bed treatment, Green et al. (1994) observed an unexpected biofilm thickness growth (up to 200 µm). In order to lose part of the biomass, the authors were obliged to decrease the HRT in their reactor twice a day by increasing the inlet flow and thus also the fluid shear forces. The excessive growth of microorganisms increases the biofilm thickness which limits diffusion of the substrate to the deeper biofilm layers. Starvation of microorganisms at the base of the biofilm causes the detachment of pieces of biofilm and leads to ineffective bioreactor operation. In anaerobic conditions, the maximum biofilm thickness at which no diffusional limitation occurs has been observed to be about 100 µm in a liquid-solid IFBR (Karamanev and Nikolov, 1996).

Using a support of polymeric granules covered with iron dust, the performance of a FBR was higher than a PBR in terms of sulfate reduction efficiency (Somlev and Tishkov, 1992). The higher friction among the particles in the fluidized-bed treatment did not affect the biomass growth on the carrier material. Puhakka and Järvinen (1992) observed that the microbial cells entrapped within the pores of a celite carrier were well protected from the shear forces and carrier collisions. Furthermore, the celite carrier also showed to have a high persistence to the mechanical friction caused by the up-flow velocity.

In down-flow fluidization, floatable materials such as polyethylene, polypropylene, polystyrene, perlite and cork are used (Garcia-Calderon et al., 1998b; Castilla et al., 2000). In IFBRs biomass accumulation makes particles heavier, increasing particle density and bed expansion. If there is an excess of biomass accumulation, the density of the particles can attain 1000 kg/m^3 and particles may be washed out from the reactor (Buffiere et al., 2000). Bacteria which carry out the anaerobic digestion of wastewater, stick to the fluidized particles, modifying their density, size, shape and therefore the hydrodynamic behavior. Thus, it is important to fit the recirculation ratio to the amount of attached microorganisms in order to maintain a fixed expansion of the bed (Garcia-Calderon et al., 1998a). Celis et al. (2008) showed that at lower superficial velocities (10 m/h), a high amount of biomass (1.29 gVSS/l) attached to the upper part of the plastic support where the fluidization was lower, while no biofilm grew in the lower part of the bed where the fluidization was higher. Increasing the superficial liquid velocity up to 15.2 m/h, a uniform expansion of the carrier material was achieved but leading to detachment of part of the biomass (0.80 gVSS/l).

Biomass accumulation is not the only parameter affecting bed expansion because other substances can precipitate onto the surface of the carrier material. Villa-Gomez et al. (2011) observed that metal precipitates can be located in the biofilm especially when the sulfide concentration is low in the reactor mixed liquor. In these conditions, the supersaturation and the precipitation of the metal sulfide fines occur in the biofilm because sulfide is present in higher concentrations around its surface.

Garcia-Calderon et al. (1998b) tested different carrier materials and calculated the minimum fluidization velocities: perlite particles (Φ=0.968mm, v=2.3m/h), polyethylene spheres (Φ=3.6mm, v=13.2m/h), polypropylene spheres (Φ=3.6mm, v=8.6m/h) and ground cork particles (Φ=0.92mm, v=6.24m/h). Perlite was chosen among the four materials due to the lowest minimum fluidization velocity and due to the presence of sharp angles and crevices on its surface where microorganisms tend to grow up better (Garcia-Calderon, 1998a).

Celis-Garcia et al. (2007) used low density polyethylene pellets with 0.4mm mean diameter as biomass carrier material for an IFBR, without any kind of treatment of the polyethylene surface. Choi and Shin (1999) mentioned that polyethylene has hydrophobic surface properties, which are not good for the immobilization of microbes. Therefore, they modified the surface properties of the polymer substrate from hydrophobic to hydrophilic by treating the polyethylene surface with chlorosulfonic acid. To increase the polyethylene surface area, Villa-Gomez et al. (2011) crushed 3 mm diameter-beads with sand in a blender promoting a better biomass attachment.

3.3 SRB community composition

In many studies the composition of the microbial community was shown to influence the performance of anaerobic reactors (Briones et al., 2007). The interactions among the different microbial communities present in a biofilm are strongly related to the ability of microorganisms to attach to the support at the operational conditions applied to the reactor (Celis et al., 2008).

Microbial diversity improves the robustness and the flexibility of the bioreactor, increasing its performance under sudden changes of the environmental conditions, e.g. organic matter, metal or acidity shocks (von Canstein et al., 2002).

Sulfate-reducing FBR communities enriched and maintained with single electron donors (ethanol or lactate) consist of different species of bacteria. Clone libraries and denaturing gradient gel electrophoresis (DGGE) analysis showed significant differences between bacterial communities in reactors fed with lactate and ethanol, respectively (Kaksonen et al., 2004a). A large number of *Proteobacterium* sequences was found in the ethanol-fed community, whereas in the lactate-fed reactor sequences clustering with the *Nitrospira* phylum were the most abundant. Moreover, from each reactor, sequences related to sulfate reducers such as *Desulfobacca acetoxidans* and *Desulforhabdus amnigenus* were found explaining why acetate accumulation did not affect the FBR performance in terms of COD and sulfate removal (Kaksonen et al., 2003a; 2003b).

Sahinkaya et al. (2007b) analyzed the microbial communities present in their applications at psychrophilic and thermophilic conditions. The arctic mine enrichment culture used for augmenting the reactor operated at 8°C was dominated by the species *Desulfomicrobium apsheronum* able to incompletely oxidize organic compounds to acetate. Long-term operation in the presence of acetate did not enrich acetate-oxidizing bacteria. At 65°C, the community changed because the frequent failures and recoveries of the process. Under these temperature conditions, sequence analysis of DGGE fragments revealed the presence of different species and many of them were not affiliated to SRB (Sahinkaya et al., 2007b).

3.4 Effect of operational conditions on FBR performance treating AMD and mine wastewaters

3.4.1 Fluidization degree

Table 6 shows the operational conditions of the AMD-treating FBR studies reviewed in this article. The fluidization degree of the carrier affects the start-up and maintenance of the FBR-process in a different way. In terms of sulfate reduction, sulfide production and effluent alkalinity, the start-up phase of a FBR, using silicate mineral sand as carrier material, with a 10% fluidization degree is superior to FBRs operated at 20% and 30% fluidization degrees (Kaksonen et al., 2003a). At low recycling ratios, smaller attrition supports faster biofilm growth on the carrier material promoting a faster increment in reactor performance. On the contrary, the FBRs with 20-30% fluidization degrees performed better at the highest loading rates (sulfate loading rate = 3400 ± 200 mg/L · day, organic loading rate = 880 ± 20 mg/L · day) reaching about 80% of sulfate reduction efficiency. High recycling ratios allow the treatment of low pH (2.5 ± 0.1) wastewaters contaminated with high Zn concentrations (up to 230 mg/L), since the dilution of metal concentrations and acidity is efficient and mass transfer between the liquid phase and biofilm is improved (Kaksonen et al., 2003a).

3.4.2 Environmental conditions

3.4.2.1 pH

Strong acidic wastewaters (pH=2.5-3) like AMD can be completely neutralized to 7.5-8.5 by the alkalinity produced by SRB during lactate or ethanol oxidation in a FBR (Kaksonen et al., 2003a). This is possible due to the alkalinity produced by the SRB and the recycle flow that dilutes influent concentrations and acidity so that SRB are active in a pH range close to that of the treated effluent. The pH increases although metal precipitation also produces acidity according to the following reaction (Kaksonen et al., 2003b):

$$H_2S + M^{2+} \rightarrow MS(s) + 2H^+ \quad (11)$$

The pH increases up to values around pH 8 due to the production of HCO_3^- by the microbial metabolism. This has also the advantage to reduce the amount of non-ionized sulfide, which is the most toxic form of sulfide (Hulshoff Pol et al., 2001).

3.4.2.2 Temperature

Sulfate-reducing activity is strongly limited by low temperature (Tsukamoto et al., 2004). Biogenic alkalinity is hardly produced at psychrophilic conditions (T=8°C) because of the incomplete oxidation of the electron donor (e.g. lactate or ethanol) to acetate, thus resulting in the need for an external $NaHCO_3$ addition to the feed solution in order to guarantee pH conditions favorable for the bacteria (Sahinkaya et al., 2007a). The performance of the biological process does not reach high COD removal and sulfate reduction efficiencies (Sahinkaya et al., 2007a), despite that the inoculation of several enriched cultures of SRB originate from cold temperature mining areas were enriched on a mix of ethanol and lactate at 7°C (Karnachuk et al., 2005).

At thermophilic conditions (65°C), sulfate-reducing activity is shown to be faster (Sahinkaya et al., 2007a). They observed a 99.9, 46 and 29% ethanol, sulfate and acetate removal efficiency, respectively, just after 6 days of reactor operation. The average sulfate reduction and acetate oxidation rates were three and four times higher, respectively, at 65 than at 8°C. The biological process was carried out by particular species of thermophilic SRB (Kaksonen et al., 2006) that showed higher degradation yields in long-term operation but, however, did not respond immediately to sudden changes in influent pH and loading rates, resulting in a temporary decrement of electron donor consumption and sulfate reduction efficiencies (Sahinkaya et al., 2007b).

Both at psychrophilic and thermophilic conditions, enough biogenic sulfide can be produced in FBRs to achieve a complete metal precipitation. In the study of Sahinkaya et al. (2007a), iron was completely removed as iron sulfide at loading rates of 90 and 60 mg/L·day at 65°C and at 8°C, respectively.

3.4.3 HRT

Several studies assessed the effect of the HRT on a FBR treating a heavy metal and sulfate containing wastewater. In almost all the studies, a higher HRT is applied at the beginning of the experiments to enhance the contact time between the microorganisms and the support (Celis et al., 2008). This results in a significant biomass immobilization on the carrier material. Then, in order to test the robustness of the reactors, the HRT is quickly or gradually decreased. If a significant biofilm development is attained on the carrier support, pseudo-steady states are reached in a short period of time after operational HRT changes (Celis-Garcia et al., 2007).

Kaksonen et al. (2004b) demonstrated that an acidic fed FBR can be successfully implemented at low HRTs (6.5 h) if the HRT is gradually decreased. Effluent dissolved zinc and iron concentrations were below 0.1 mg/L during stable performance of the reactor at HRT=6.5 h

(Kaksonen et al., 2004b). However, a sudden decrease of the HRT from 9.7 h to 7.3 h resulted in a decrease of the reactor performance since higher metal concentrations were detected in the effluent solution. Effluent pH and sulfide concentration decreased from 8 to 5 and from 200 to 50 mg/L, respectively, whereas effluent iron concentration increased up to 10 mg/L. In contrast, Villa-Gomez et al. (2011) did not observe a relevant variation in metal precipitation with a sudden change of HRT from 24 to 9 hours in two IFBR treating a wastewater containing Zn, Cu, Pb and Cd operated at different sulfide concentrations. This was most likely due to the neutral feed pH that Villa-Gomez et al. (2011) used in their applications that always guaranteed enough sulfide to achieve an almost complete metal precipitation.

In terms of sulfate reduction performance, Nagpal et al. (2000a) observed that a gradual decrease of the HRT from 35 h to 5 h led to a decrease of the sulfate reduction efficiency (from 90% to 65%) in the reactor at the highest influent sulfate concentration of 2.5 g/L. The maximum sulfate reduction rate was reached (6.33 g/L · day) at an HRT of 5 h, showing that the liquid–solid fluidized-bed process responded well to the gradual HRT decrease.

At low HRTs, incomplete ethanol oxidation resulted in an increase of the acetate effluent concentration, especially when the HRT was decreased below 12 h (Kaksonen et al., 2004b). Acetate is produced as an intermediate product in ethanol oxidation according to reaction (11) (Dries et al., 1998, Nagpal et al., 2000b; Kaksonen et al., 2003b):

$$2CH_3CH_2OH + SO_4^{2-} \rightarrow 2CH_3COO^- + H_2S + 2H_2O \qquad (12)$$

Acetate oxidation is important in AMD treatment because it produces more alkalinity (reaction 12) and because acetate represents the most common organic rest pollution of the supplied electron donor (Kaksonen et al., 2004b):

$$CH_3COOH + SO_4^{2-} \rightarrow 2HCO_3^{-} + H_2S \qquad (13)$$

Nevertheless, acetate oxidation is the rate limiting step in biological AMD wastewater treatment (Kaksonen et al., 2003b). Acetate tends to accumulate in the FBRs, decreasing the COD removal efficiency resulting in a high organic rest pollution. As described in the sections below, efforts have been done to increase acetate removal efficiencies without satisfactory results (Sahinkaya et al., 2007b).

Sahinkaya et al. (2007a) showed that a higher HRT (1 day) was necessary to carry out their experiments at 65°C. Furthermore, after an intentional sudden decrease of the feed pH from 4.3 to 3.7, the authors needed to increase the HRT to 1.5 days to avoid the failure of the fluidized-bed process. Hence, increasing the HRT is a good solution to recover a process that is failing.

3.4.4 Type of electron donor used for sulfate reduction

Lactate has been shown to give higher biomass yields compared to ethanol and to enhance the rapid colonization of a fresh carrier material. For this reason, in several studies lactate is used during the start-up of the reactors to promote the sulfate-reducing biofilm development before to be replaced by ethanol (Nagpal et al. 2000a; Gallegos-Garcia et al., 2008; Celis et al., 2008). However, in large-scale operations, the use of lactate would result in high operational costs (Nagpal et al., 2000b).

Villa-Gomez et al. (2011) reported a maximum lactate removal rate of 1.4 g/L · day (53% COD consumption percentage) during the IFBR operation with a COD/SO_4^{2-} ratio of 1, at a HRT of 9h and with metals in the feed solution. Under these conditions, sulfate reduction rates were not the highest (1.0 g/L · day) but metal precipitation was almost complete (27 mg/L · day) for Zn, Cu, Pb and Cd. The highest achieved sulfate reduction rates were over 2.0 g/L · day in Kaksonen et al.'s study (2003a) using lactate as electron donor. The metal precipitation rates obtained were 250-350 mg/L · day for Zn and 80 mg/L · day for Fe at a HRT of 16h. Residual lactate concentrations were around 25 mg/L as DOC (over 90% removal).

Kaksonen et al. (2003b) observed that the efficiency of an ethanol fed-FBR to precipitate metals and to increase the pH to neutrality was similar to that reported with a lactate-fed FBR (Kaksonen et al., 2003a), but a greater percentage of ethanol was used for sulfate reduction (77-95%) compared to lactate in the lactate-fed bioreactor (60-75%). In contrast, Gallegos-Garcia et al. (2008) obtained lower COD and sulfate removal efficiencies (50% and 35%, respectively) with ethanol as carbon source. The highest sulfate reduction rate was found to be around 1.0 g/L · day and the residual electron concentration was 1.25 g/L as COD. Acetate accumulated in solution up to 1.4 gCOD/L resulting in a high organic rest pollution, but enough sulfide was produced to precipitate all the metals.

Acetate has also been studied as an electron donor for sulfate reduction in FBRs, both as sole carbon source (Sahinkaya et al. 2007b) or with a mixture of propionate and butyrate (Celis-Garcia et al., 2007). Sahinkaya et al. (2007b) obtained different results in terms of reactor performance for the 8°C and the 65°C FBR. At psychrophilic conditions, no acetate oxidation and sulfate reduction occurred whereas, at thermophilic conditions, the acetate removal efficiency even reached 67% most probably due to thermophilic SRB enriched on acetate, lactate and ethanol (Kaksonen et al., 2006). At 65°C, the lowest acetate concentration detected in the effluent was 231 mg/L and the sulfate reduction rate reached 573 mg/L · day.

Celis-Garcia et al. (2007) observed that, using a mixture of acetate, propionate and butyrate as electron donor, a high COD removal percentage (93%) could be achieved but the COD removed by SRB was three times lower than the COD removed by methanogens. In order to increase the SRB activity, acetate was replaced by lactate in the feed and the lactate concentration was gradually increased during time. At the end of operation, the residual COD concentration averaged 350 mg/L, the sulfate reduction efficiency was 75% and the highest removal rates were around 5.5 g/L · day. SRB consumed 80% of the removed COD showing that methanogens were still present in the biofilm and are difficult to wash out, especially when a heterogeneous anaerobic sludge is used as biomass source.

3.4.5 Organic, sulfate and metal conversion rates and influent COD/SO_4^{2-} ratios

Increasing the organic and sulfate loading rates are two ways to test the robustness of FBRs. Generally, under mesophilic conditions, sulfate-reducing FBRs operate well under gradual intentional increase in sulfate and DOC loading rates up to 3500 and 900 mg/L · day, respectively, since increments of sulfate reduction and electron donor removal percentages have been observed (Kaksonen et al., 2003a). Furthermore, with increasing Fe, Zn and Cd loading rates of 320, 220 and 20 mg/L · day, respectively, metal precipitation has been shown to be almost complete (Gallegos-Garcia et al., 2008).

At thermophilic conditions, after an increase of sulfate and ethanol loading rates from 1000 to 1500 mg/L · day and from 320 to 485 mg/L · day, respectively, the FBR did not respond immediately (Sahinkaya et al., 2007a). The authors observed that the performance of the reactor initially decreased both in terms of sulfate reduction (down to 25%) and ethanol oxidation (down to 40%), but after just 10 days, the process recovered and a complete oxidation of ethanol and a 60% reduction of sulfate were observed. The sulfide concentration was enough to guarantee a complete precipitation of iron at an iron loading rate of 90 mg/L · day.

The three most important organic substrates studied for AMD treatment in fluidized-bed reactors are lactate, ethanol and acetate. According to the respective sulfate reduction reactions, the theoretical COD/SO_4^{2-} ratio (gram/gram) needs to be 0.67 for all the three substances to achieve complete sulfate reduction (Nagpal et al., 2000b; Kaksonen et al., 2003a; Velasco et al., 2008). Several authors investigated the performance of fluidized-bed treatment at different COD/SO_4^{2-} ratios.

Under conditions of sulfate in excess (COD/SO_4^{2-} ratio of 0.11-0.20), using lactate and ethanol as carbon sources, Kaksonen et al. (2003a; 2003b) obtained an average sulfate reduction efficiency of 70% and the biogenic sulfide produced was enough to precipitate almost all the metals. The dissolved metal concentration in the reactor was detected below 0.1 mg/L.

According to Celis-Garcia et al. (2007), the optimal COD/SO_4^{2-} ratio for sulfate reduction with lactate as electron donor was exactly the stoichiometric one. Increasing the sulfate loading rate from 1.5 up to 7.3 gSO_4^{2-}/L · day keeping the organic loading rate stable, sulfate reduction became the main biological process occurring in the reactor with a strong enrichment of SRB in the biofilm.

In a different way, Celis et al. (2008) increased the organic loading rate, keeping the influent sulfate concentration constant. That resulted in higher acetate concentrations and, thus, in a worse organic matter removal performance. Indeed, Villa-Gomez et al. (2011) noticed that, in a IFBR fed with a COD/SO_4^{2} ratio of 5 (g/g), the COD removal efficiency was just around 30% due to the formation of acetate. Nevertheless, sulfate-reducing activity occurred at the highest conversion rates (2000 mg/L · day at a HRT of 9h), the sulfide production reached a value of 648 mg/L and 87% of the COD was consumed by the SRB.

Low electron donor concentrations are thus sufficient to obtain an almost complete metal precipitation as sulfide is produced in enough quantities and metal sulfide solubility products are very low. This results in a cheaper process since organic matter has always to be added as an external source in AMD treatment. On the contrary, if the aim is to optimize sulfate reduction, the COD/SO_4^{2} ratio has to be increased at least to the stoichiometric value, even if it is recommendable to operate the reactors at an excess of electron donor. In this way, even if other fermentative reactions occur or acetate accumulates, enough electron donor is available for the sulfate-reducing activity.

3.4.6 Metal recovery

Biological sulfate reduction and metal precipitation using biogenic H_2S can be applied in single-stage or separated unit processes (for a review see Hao, 2000). FBRs and IFBRs are a single-stage process where sulfate-reducing activity and metal precipitation occur at the same time in the same reactor (Celis-Garcia et al., 2007). As described in the different sections above, in all the studies mentioned metal precipitated as sulfides almost totally and they can be found onto the surface of the biofilm, as fines in solution (Kaksonen et al., 2003a) or settled to the bottom of the reactor (Villa-Gomez et al., 2011). Furthermore, as non-renewable resources, metal recovery is as important as their removal from wastewaters (Badmus et al., 2007) and especially selective metal recovery is an appealing challenge for future research.

Villa-Gomez et al. (2011) quantified the metals accumulated at the bottom of the reactors in their IFBR applications. The highest metal recovery percentages were 49.4%, 44.2%, 60.3% and 47.4% for Zn, Cu, Pb and Cd, respectively, but they could not recover the metals in a selective way. In fact, although IFBR characteristics allow metal precipitates to settle down, it is impossible to control the sulfide concentration. Selective metal recovery can be regulated by using different sulfide concentrations (Veeken et al., 2003b; Sampaio et al., 2009) and, at the current state of research, this is only possible in a separated process unit also to avoid metal toxicity in the biological system (Utgikar et al., 2002; Kaksonen et al., 2004b).

3.4.7 Sulfide inhibition

Kaksonen et al. (2004b) carried out batch kinetic experiments by stopping the continuous flow in the FBR to study the effect of the dissolved sulfide on ethanol and acetate oxidation in FBRs. For each batch experiment, sulfate was added in excess (3 times as much as stoichiometrically required). Starting from a noncompetitive inhibition model (eq. 14), the inhibition constants (k_I) for acetate and ethanol, added as electron donors, were obtained:

$$v = \frac{v_{\max} \cdot S}{(k_m + S) \cdot (1 + \frac{I}{k_I})} \quad (14)$$

(where v = oxidation velocity, v_{max} = maximum oxidation velocity, S = initial substrate concentration, k_m = Michaelis-Menten constant, I = inhibitor concentration and k_I = inhibition constant). H_2S inhibition constants for ethanol and acetate oxidation were 84 mgS-H_2S/l and 124 mgS-H_2S/l respectively, showing that ethanol oxidation is more affected by sulfide than acetate oxidation.

In contrast, Celis-Garcia et al. (2007) showed that, although the sulfide concentration reached a value of 1215 mg/L, both lactate consumption and sulfate reduction were not affected by the high sulfide production and kept stable around 90% and 75%, respectively. The high recirculation rate may have contributed to the formation of a biofilm able to tolerate high total sulfide concentrations without any apparent toxic effect.

3.4.8 Inhibition by heavy metals

High recycle ratios of FBRs allow treatment of wastewater with high concentrations of zinc (240 mg/L) and iron (58 mg/L) without inhibitory effects because of its dilution effect. This is due to the great capacity of the FBR to dilute high influent concentrations and to let bacteria be exposed to concentrations much lower than the influent concentrations (Kaksonen et al., 2003a; 2003b). Furthermore, the biogenic sulfide produced by the SRB quickly reacts with metals leading to the formation of metal sulfide particles less toxic and bioavailable for the bacteria (Isa et al., 1986a; 1986b).

The injection of zinc, iron and cadmium, added at maximum loading rates of 220, 320 and 20 mg/L·day, respectively, does not affect COD removal and sulfate reduction efficiencies in a sulfate-reducing IFBR (Gallegos-Garcia et al., 2008). Similarly, zinc, copper, lead and cadmium added each in a concentration of 10 mg/L, do not influence the sulfate-reducing activity of biomass grown in IFBRs of Villa-Gomez et al. (2011).

On the contrary, Sahinkaya et al. (2007b) observed that metals can be toxic for SRB at 65°C even at very low concentrations. During the thermophilic FBR operation, the authors supplemented a trace element solution to the feed to overcome possible limitations in reactor performance. Unfortunately, this addition caused a quick decay in FBR efficiency since effluent acetate concentrations increased while sulfate reduction, dissolved sulfide concentration and effluent alkalinity decreased till the trace elements were excluded from the feed solution and the process slowly recovered.

4. CONCLUSIONS AND SUGGESTIONS FOR FUTURE RESEARCH

1. SRB bioprocesses for the treatment of AMD contaminated waters have been developed because of their advantages over traditional remediation techniques. Several bench-scale bioreactor types have been studied and they offer stable performance and control of the process. FBRs and IFBRs are flexible reactor configurations since they can be operated for several contaminated wastewater types, under different fluidization conditions and with many possible carrier materials.
2. Several materials have been tested as carrier in fluidized-bed applications and most of them are shown to be suitable for growing microbial communities. The choice of the material is very important especially depending on the fluidization conditions that have been decided to use. Nowadays, the evolution of the research is towards the development of the IFBRs and so towards the use of floatable materials as carrier. Further efforts have

to be done in this direction to find a material with better surface properties for biomass immobilization and to avoid the settling of the material when the biofilm is developing. Polymeric support pellets do not give complete guarantees at the current state of the art.

3. FBR characteristics permit a faster biofilm formation at lower recycle rates. At higher recycle rates, removal of metals, sulfate and acidity is very efficient because mass transfer between the liquid phase and biofilm is improved. Gradual decreases of the HRT are advisable because they do not affect metal precipitation and the reactor performance in terms of removal rates. In contrast, the removal efficiencies decrease with decreasing HRT because of the incomplete extent of the electron donor oxidation reactions.
4. Psychrophilic and thermophilic conditions strongly affect the biological process and FBR performances are appreciably lower than under mesophilic conditions. The response to intentional changes in the influent solution is not immediate, acetate accumulates in solution and its oxidation is difficult to obtain. Furthermore, thermophilic bacteria appear to be more susceptible to metal toxicity even at low metal concentrations. Additional research is recommended in this direction.
5. Acetate oxidation is found to be the rate limiting step in sulfate reduction for AMD wastewater treatment. Acetate tends to accumulate in the FBRs decreasing the COD removal efficiency and the biogenic alkalinity production. At the current state of the research, acetate seems to be quite impossible to be removed with high performances in sulfidogenic applications. The suggestion for not having acetate in solution is to use low COD/SO_4^2 ratios and enriched SRB cultures rather than heterogeneous sludge as inoculum. Under these conditions, enough biogenic sulfide is produced to precipitate

metals and almost all the electron donor is consumed by complete oxidation. At higher COD/SO_4^{2} ratios, the process shows higher sulfate removal percentages, but it results in a higher organic rest pollution.

6. As organic carbon source, lactate is recommended to get higher biomass yields and to neutralize better the system since alkalinity is produced, although lactate is partially oxidized to acetate. However, ethanol is cheaper than lactate and it guarantees better performance than lactate especially in terms of sulfate reduction efficiency.

7. Metal precipitation is almost complete in all the studies mentioned, but it is not possible to obtain a selective metal recovery inside a fluidized-bed reactor. Multi-stage process configurations have to be used to guarantee the best conditions for sequential separation and metal recovery.

8. High sulfide concentrations and metal loads are shown to not affect the biological sulfate-reducing activity and metal removal efficiencies thanks to the FBR hydrodynamic characteristics. However, literature lacks metal and sulfide toxicity studies in sulfate-reducing fluidized-bed reactors.

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Legends to tables

Table 1 – Typical AMD and mining wastewater composition (the unit is mg/L)

Table 2 – Carbon sources used for biological sulfate reduction

Table 3 – Toxic metal concentrations completely inhibiting sulfate-reducing activity

Table 4 – Benefits and drawbacks of different bioreactor configuration operated for biological sulfate reduction

Table 5 - Fluidization velocities of different carriers used in up-flow and down-flow fluidized-bed reactors treating different types of wastewater (divided by fluidization typology)

Table 6 – Operational conditions in AMD wastewater-treating FBRs and IFBRs

Table 1

Compound	Berkeley Pit AMD (Tabak et al., 2003a)	Gromolo river (Dinelli et al., 2001)	Kennecott Copper AMD (Buisman et al., 1999)	Queen mine wastewater (Ashe et al., 2008)	Leviatham mine water (Tsukamoto et al., 2004)
Al^{3+}	293	230	2412	3950	-
Cu^{2+}	223	175	44	-	0.7
Mn^{2+}	223	9.15	200	1620	-
Fe^{2+}	514	775	512	2500	220
Zn^{2+}	630	35	82	930	0.9
Cd^{2+}	1.38	-	-	-	-
Ni^{2+}	2.14	8.5	-	-	0.5
As^{3+}	0.512	-	-	-	30
Co^{2+}	1.51	-	-	-	-
Cr^{3+}	-	1.23	-	-	-
Na^{+}	213	442	-	-	-
Mg^{2+}	-	1080	2640	2890	-
Ca^{2+}	-	333	-	-	-

Table 2

Carbon source	Advantages (+) / Shortcomings (-)	References
Formate	(+) Most of SRB that use hydrogen are able to oxidize formate as sole source of carbon	Widdel (1988)
	(-) Methanogens can predominate on SRB at thermophilic conditions	Vallero et al. (2004)
Methanol	(+) Cost effective	Glombitza (2001)
	(+) Predominance of SRB at high temperatures	Vallero et al. (2003)
	(-) Very few SRB species oxidize methanol	Widdel (1988)
	(-) Slow SRB growth at mesophilic conditions	Weijma and Stams (2001)
Ethanol	(+) High sulfate conversion efficiencies	Kaksonen et al. (2003b)
	(-) Accumulation of acetate as intermediate	Kaksonen et al. (2004b); Gallegos-Garcia et al. (2008)
Lactate	(+) High alkalinity production and biomass growth yields	Kaksonen et al. (2004a)

	(-) High cost	Nagpal et al. (2000b)
	(-) Accumulation of acetate as intermediate	Oyekola et al. (2009)
Acetate	(-) Methanogens generally outcompete SRB	Yoda et al. (1987)
	(-) Difficult to oxidize even with acetate-enriched cultures	Sahinkaya et al. (2007b)
	(-) Main responsible of organic rest pollution	Lens et al. (1998)
Fatty acids mixture	(+) Propionate is mainly oxidized by SRB at high sulfate concentrations	Visser et al. (1993)
	(-) Competition between methanogens and SRB even at high sulfate concentrations	Visser et al. (1993); Celis-Garcia et al. (2007)
	(-) Production of acetate as intermediate	Celis-Garcia et al. (2007)
Glucose and fructose	(+) Easily degraded and production of hydrogen as interspecies	(Klemps, 1985)
	(-) Production of volatile fatty acids as intermediates: competition between SRB and methanogens and decrease of pH	White and Gadd (1996b)
Aromatic	(+) Degradation of dangerous	Harms et al. (1999);

hydrocarbons	substances such as phenols and benzene compounds	Lin and Lee (2001)
	(-) Low free energy change	Widdel (1988)
Molasses	(+) Ready availability and low costs	Maree and Strydom (1987)
	(-) Presence of non-biodegradable content	Annachhatre and Suktrakoolvait (2001)
Organic waste reactive mixtures	(+) High sulfate reduction rate because of high carbon content	Waybrant et al. (1998; 2002); Cocos et al. (2002)
	(+) Cost effective	Prasad et al. (1999)
	(-) High organic rest pollution	Glombitza (2001)
	(-) Synergism with other microbial groups	Kuyucak and St-Germain (1994)

Table 3

Heavy metal	**Conc. (mg/L)**	**SRB species**	**References**
Cd	6	SRB in Los Angeles County sewerage	Morton et al. (1991)

	> 22.4	SRB mixed and pure cultures	White and Gadd (1998)
	112	SRB mixture in manure sludge	Ueki et al. (1991)
Cr (III)	15	*Desulfovibrio vulgaris*	Cabrera et al. (2006)
Cu	1.9	*Desulfovibrio desulfuricans G20*	Sani et al. (2001)
	12	Mixed culture acetate-utilizing SRB	Utgikar et al. (2001)
	> 22.4	SRB mixed and pure cultures	White and Gadd (2000)
Ni	10	*Desulfovibrio desulfuricans*	Poulson et al. (1997)
	59	SRB mixture in manure sludge	Ueki et al. (1991)
Pb	2.1	*Desulfovibrio desulfuricans G20*	Sani et al. (2001)
	25	SRB in Los Angeles County sewerage	Morton et al. (1991)
Zn	13	*Desulfovibrio desulfuricans*	Poulson et al. (1997)
	20	Mixed culture acetate-utilizing SRB	Utgikar et al. (2001)
	20	*Desulfovibrio vulgaris* and *Desulfovibrio sp.*	Cabrera et al. (2006)
	65	SRB mixture in manure sludge	Ueki et al. (1991)

Table 4

Bioreactor type	Benefits (+) / Drawbacks (-)	References
CSTR	(+) Consistency and reliability	Barnes et al. (1991)
	(-) High SRT result in high reactor volumes	Barnes et al. (1991)
	(-) Frequent active biomass washout	Lens et al. (2003)
PBR	(+) High SRT result in mower reactor volumes than CSTRs	Barnes et al. (1991)
	(+) Possibility to be operated both in up-flow and down-flow modalities	Jong and Parry (2003); Zaluski (2003)
	(-) Frequent clogging	Anderson et al. (1990)
	(-) High pressure for pumping the flow	Anderson et al. (1990)
GLR	(+) High mass transfer of the substrates into the bacterial agglomerates	Dijkman and Buisman (1999)
	(+) Very good mixing	Dijkman and Buisman (1999)
	(+) High rate biological kinetics if H_2 is used as electron donor	Van Houten et al. (1994; 1997)
	(-) High pressure needed for pumping the gaseous substrates inside the	Lens et al. (2002)

	reactor	
UASB	(+) Biomass good settling capability	Lettinga et al., (1980)
	(+) No clogging	Omil et al. (1996)
	(+) No carrier material if compared to PBR	Speece (1983)
	(-) Possibility of biomass washout	Vallero et al. (2003)
	(-) High susceptibility to the influent characteristics	Jhung and Choi, 1995
MBR	(+) No need for sedimentation basin	Mack et al. (2004)
	(+) High biomass retention result in high substrate degradation rates	Mack et al. (2004)
	(+) Possibility to prevent direct contact between metals and SRB in a single basin	Chuichulcherm et al. (2001); Manconi and Lens (2009)
	(-) High cost to overcome the trans-membrane pressure	Fedorovich (2000)
	(-) Periodic backwash because of the deposition of aggregates on the membrane surface	Tabak and Govind (2003); Vallero et al. (2005)

Table 5

Carrier	**v (m/h)**	**Application**	**Author**
Up-flow FBR			
Mineral Manville Celite R-633 particles	9.2-12.8	Aerobic treatment of polychlorinated phenols	Puhakka and Järvinen (1992)
0.75 mm granular activated and non-activated carbon	17.4-36.6	Treatment of volatile aromatic hydrocarbons	Voice et al. (1992)
0.86 mm sand particles	16.8-56	Denitrification	Green et al. (1994)
Polymeric granules cover with iron dust	186	Sulfate reduction	Somlev and Tishkov (1992)
0.1-0.2 cm porous glass beads	145.8-151.2	Sulfate reduction	Nagpal et al. (2000a)
0.5-1 mm silicate mineral Filtralite sand	29	AMD treatment	Kaksonen et al. (2004b)
Down-flow FBR			
4.3 mm polyethylene cylinders	18-28	Hydrodynamic study	Hihn (1992)

cylinders			
3.2 mm synthetic foam cylinders	20-30	Hydrodynamic study	Hihn (1992)
3.6 mm polyethylene spheres	8.6-13.2	Hydrodynamic study	Garcia-Calderon et al. (1998b)
0.92 mm cork particles	6.2	Hydrodynamic study	Garcia-Calderon et al. (1998b)
3.6 mm foamed polypropylene spheres	39	Aerobic treatment of oil refinery wastewater	Shimodaira and Yushina (1983)
2-6 cm foamed polystyrene particles	45-60	Nitrite removal	Boehler and Haldenwag (1991)
0.968 mm perlite particles	2.3	Anaerobic digestion of wine distillery wastewater	Garcia-Calderon et al. (1998a; 1998b)
0,175 mm spherical granular silica particles	5.4	Anaerobic digestion of dairy wastewater	Arnaiz et al. (2003)
0.4 mm low density polyethylene pellets	10.9	AMD treatment	Celis-Garcia et al. (2007)

polyethylene pellets			(2007)
0.5 mm low density polyethylene fine particles	18.6	AMD treatment	Celis et al. (2008); Gallegos-Garcia et al. (2008)

Table 6

Fluidization degree (%)	**Temperature (°C)**	**HRT (h)**	**Organic carbon source**	**Organic loading rate (g/L · day)**	**Sulfate loading rate (g/L · day)**	**COD/SO_4^{2-} (g/g)**	**Metal loading rate (mg/L · day)**	**Reference**
-	-	5-55	Lactate/ethanol	1.7-9.6	0.87-12	0.8-1	-	Nagpal et al (2000a)
10-20-30	35 controlled	16	Lactate (DOC)	0.65-0.88	2.45-3.4	0.2	350 Zn 86 Fe	Kaksonen et al. (2003a)
20	35 controlled	16	Lactate (DOC) Ethanol (DOC)	0-0.25 0-0.38	1.6-3.7	0.11 0.11	500 Zn 85 Fe	Kaksonen et al. (2003b)
20	35 controlled	6.1-20.7	Ethanol (DOC)	0.8-2.7	2.32-7.87	0.3	200-650 Zn 115-400 Fe	Kaksonen et al. (2004b)
20	8 and 65 controlled	24	Ethanol (DOC) Acetate	0.32-0.48 0.5-0.7	1-1.5	0.32-0.38	45-100 Fe	Sahinkaya et al. (2007a)

20	8 and 65 controlled	24	Acetate	0.5-0.7	1	0.5-0.7	-	Sahinkaya et al. (2007b)
30-40	30 controlled	16.8-24	Mixture of VFA (COD)	2.5-5.2	1.5-7.3	0.67-1.67	-	Celis-Garcia et al. (2007)
50	18-26 (room temperature)	24-48	Ethanol/lactate (COD)	2.5	1.5-3	0.8-1.67	320 Fe 220 Zn 20 Cd	Gallegos-Garcia et al. (2008)
25	25±3 (room temperature)	48	Ethanol/lactate (COD)	0.5-1	0.83-1.66	0.6	-	Celis et al. (2008)
25	Room temperature	9-24	Lactate (COD)	5-13.33 1-2.67	1-2.67	5 1	5-27 Zn, Cu, Pb, Cd	Villa-Gomez et al. (2011)

Legends to figures

Figure 1 – Schematic representation of a CSTR (A), a PBR (B), a GLR (C), a UASB reactor (D), an immersed membrane bioreactor (IMBR) (E) and extractive membrane bioreactor (EMBR) (F)

Figure 2 – Schematic representation of an up-flow FBR (A) and a down-flow FBR (B)

Figure 1

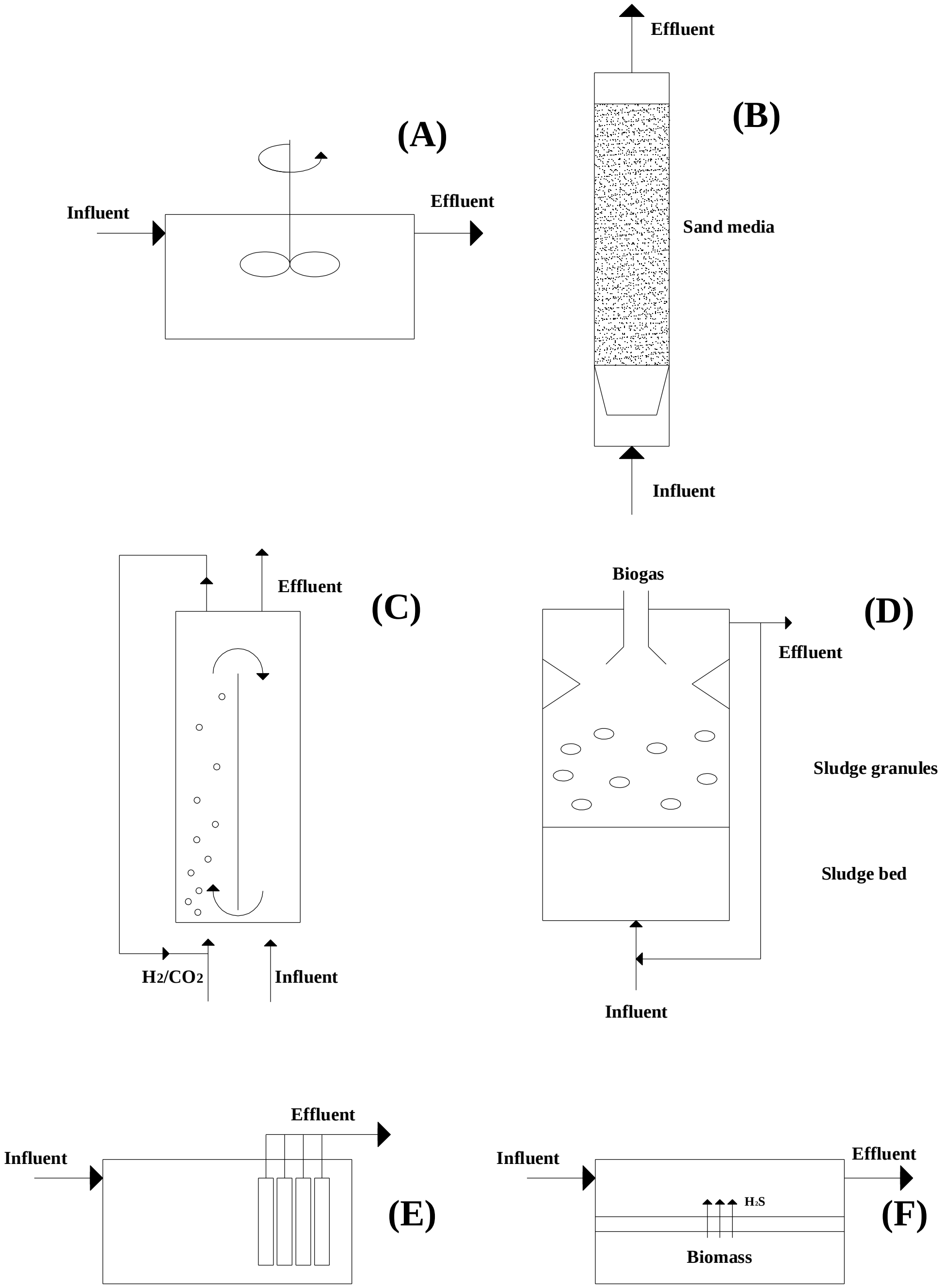
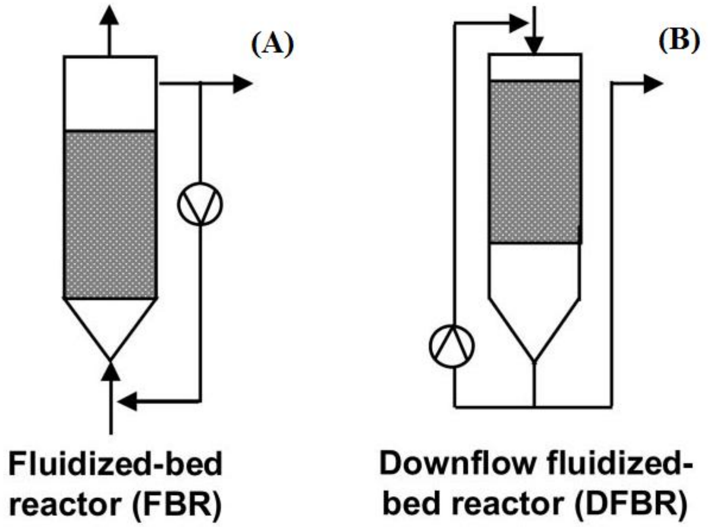

(A)
Influent
Effluent
(B)
Effluent
Sand media
Influent
(C)
Effluent
H2/CO2
Influent
(D)
Biogas
Effluent
Sludge granules
Sludge bed
Influent
(E)
Effluent
Influent
(F)
Influent
Effluent
H2S
Biomass

Figure 2


(A)
(B)
Fluidized-bed reactor (FBR)
Downflow fluidized-bed reactor (DFBR)