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School of Biological Sciences and Engineering

TITTLE: "EVALUATION OF SUITABILITY TO USE THE ACTIVITY OF SULFUR-REDUCING AND OXIDIZING BACTERIA IN A REACTORS AND ITS POSSIBLE APPLICATION IN THE TREATMENT OF SOLID SORBENTS USED IN THE SWEETENING OF NATURAL GAS IN ECUADOR".

Trabajo de integración curricular presentado como requisito para la obtención del título de Biólogo

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Dedication

I want to dedicate this work to my parents, who give me their unconditional support at all times besides being the pillars of my life, without their sacrifice none of this would have been possible. To my brother who has shown me that effort and sacrifice for an objective allow us to achieve great goals. To my puppies, kivito, rocky and mili who were/are the materialization of love. To my uncles, cousins, brother-in-law for their words of encouragement. All of them have always been there when I needed it most.

Marlon Xavier Chimarro Bermeo

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Quiero dedicar este trabajo a mis padres, quienes me brindan su apoyo incondicional en todo momento además de ser los pilares de mi vida, sin su sacrificio nada de esto hubiera sido posible. A mi hermano que me ha demostrado que el esfuerzo y el sacrificio por un objetivo nos permiten alcanzar grandes metas. A mis perritos, kivito, rocky y mili que fueron/son la materialización del amor. A mis tíos, primos, cuñados por sus palabras de aliento. Todos ellos siempre han estado ahí cuando más lo he necesitado.

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Marlon Xavier Chimarro Bermeo

Abstract

In the present literature review, It was evaluated the suitability of the application of sulfate-reducing bacteria (SRB) and sulfide-oxidizing bacteria (SOB) in a reactor with defined conditions of oxygen, temperature, pH and substrate in a desulfurization treatment for wasted solid sorbents manufactured for Natural Gas sweetening industry. The solid sorbents are manufactured with a mixture of black sands and clay sampled in different localities of the Ecuadorian coast region. The sands are characterized by its large content of metal oxides in minerals such as hematite, magnetite, and Ilmenite. These metal oxides in minerals are of special interest in the natural gas sweetening due to the reactions carried out between the metal oxides with the hydrogen sulfide present as a contaminant in sour natural gas. Such reactions fix the sulfur in the solid sorbents as metal sulfides. In this way, sour natural gas is converted to “sweet gas” and then can be marketed according to final standards and specifications of consumers. The analysis of wasted solid sorbents indicates the presence of several sulfur species besides minerals such as pyrite. The large content of sulfur in wasted solid sorbents makes them an ideal substrate for sulfur-bacteria (SRB & SOB) in order to convert the reduced sulfur forms to elementary sulfur, which can then precipitate. The sulfur collected in the reactor can then be used as fertilizer for soils besides other applications. It is proposed the sulfur collection of solid sorbents in reactors as a treatment that allow the reuse of them for sour natural gas sweetening.

Keywords; Sulfate-reducing bacteria (SRB), sulfide-oxidizing bacteria (SOB) pyrite, sulfates, sulfides, elementary sulfur.

Abstract

En la presente revisión de la literatura, se evaluó la idoneidad de la aplicación de bacterias reductoras de sulfato (SRB) y bacterias oxidantes de sulfuro (SOB) en un reactor con condiciones definidas de oxígeno, temperatura, pH y sustrato en un tratamiento de desulfuración para desechos sorbentes sólidos fabricados para la industria de endulzamiento de gas natural. Los sorbentes sólidos se fabrican con una mezcla de arenas negras y arcilla muestreadas en diferentes localidades de la región costera ecuatoriana. Las arenas se caracterizan por su gran contenido de óxidos metálicos en minerales como hematita, magnetita e ilmenita. Estos óxidos metálicos en minerales son de especial interés en el proceso de endulzamiento de gas natural debido a las reacciones llevadas a cabo entre los óxidos metálicos con el sulfuro de hidrógeno presente como contaminante en el gas natural ácido. Tales reacciones fijan el azufre en los sorbentes sólidos como sulfuros metálicos. De esta forma, el gas natural ácido se convierte en "gas dulce" y luego se puede comercializar de acuerdo con las normas y especificaciones finales de los consumidores. El análisis de los sorbentes sólidos desperdiciados indica la presencia de varias especies de azufre además de minerales como la pirita. El gran contenido de azufre en los sorbentes sólidos desperdiciados los convierte en un sustrato ideal para las bacterias de azufre (SRB y SOB) con el fin de convertir las formas reducidas de azufre en azufre elemental, que luego puede precipitar. El azufre recogido en el reactor se puede usar como fertilizante para suelos además de otras aplicaciones. Se propone la recolección de azufre de sorbentes sólidos en los reactores como un tratamiento biológico que permita su reutilización para el endulzamiento de gas natural agrio

Palabras clave: Bacterias reductoras de sulfato (SRB), pirita de bacterias oxidantes de sulfuro (SOB), sulfatos, sulfuros, azufre elemental.

TABLE OF CONTENT

INTRODUCTION AND JUSTIFICATION

Natural gas in Ecuador

The natural gas production goes hand in hand with the formation of crude oil in the interstices of very deep rocks in the earth. Its formation takes millions of years where the remains of plants and animals built up in thick layers on the earth's surface and ocean floors. The high pressure and heat change this carbon and hydrogen-rich material into coal, oil and natural sour gas. Natural gas is a non-renewable fossil fuel of great importance in Ecuador, its demand has grown due to the high electricity consumption by its growing population. It is classified as a clean and safe energy form with the environment. Natural gas once extracted contains a mix of hydrocarbons (C_nH_{2n+2}) such as Methane (CH_4), Ethane (C_2H_6), Propane (C_3H_8), Butane (C_4H_{10}) but Methane is mainly found in larger quantities (95%). Other compounds found in Natural Gas are contaminants such as, Carbon Dioxide (CO_2), Oxygen (O_2), Nitrogen (N_2), Hydrogen Sulfide (H_2S), Arsine, Ammonia, trace gases and metals.

As natural gas is not in its pure state, impurities must be removed before being transported and marketed. Natural gas is very useful due to its calorific value, such characteristic confers similar properties compared to other liquid fuels such as gasoline or diesel. It has a low cost and is commonly used in the manufacture of crystals, ceramics, goldsmiths, mechanics, metallurgy, food industry and domestic consumption.

In Ecuador, the company responsible for the exploration, exploitation, storage and marketing of natural gas and oil is Petroecuador. Currently other international companies such as Repsol and PDSVA have occupied the market to offer these resources. The points of production of Natural Gas in Ecuador are; the Emerald Refinery located in Esmeraldas, La Libertad Refinery located in Santa Elena and the Sushufindi Industrial Complex comprising two units; The Amazon Refinery and the Sushufindi Gas Plant. During the last decades, Ecuador has been obliged to address the growing demand for Natural Gas with the importation of this resource from neighboring countries due to its declining production. An activity that negatively affects the economy of the country.

Solid sorbents for sweetening of natural gas

The acidic gas has in its composition carbon dioxide and sulfur compounds higher than those admitted for transport and marketing. Pollutant reduction processes are called sweetening processes of Natural Gas. The sweetening processes are Natural Gas treatments with amines, potassium carbonate, physical solvents, membranes, non-regenerative beds, absorption, or redox reactions. The choice of treatment is made depending on the concentration of the pollutants as well as the type of pollutant of interest to be removed and, in the case of sulfurous compounds if sulfur recovery is required.

Solid sorbents are one of the alternatives to remove hydrogen sulfide with levels below 100 ppm. Oxides of iron, zinc, and copper are required for its manufacture. The removal of the contaminants occurs when the Hydrogen Sulfide reacts with the oxides (Fe, Cu, Ti etc.) of the solid sorbents, under specific conditions, the reaction produces iron sulfides. In this way, the sulfur is fixed in the solid sorbents and the Natural Gas stream decreases the concentration of the contaminant to levels allowed for its commercialization. Thus, the solid sorbents applied to the removal of hydrogen sulfide would complete the sweetening process of natural gas with very high pollutant loads.

The project "Ferruginous and Titaniferous Sands of Ecuador as Adsorbents of Acid Gases in the Hydrocarbons Industry", takes the advantages of the exploitation of the mineral resources of the Republic of Ecuador. The attention is focused on the ferruginous & titaniferous sands, called black sands, primarily composed by a mixture of oxides of iron and other elements such as Copper and Titanium. The black sands are located near river beds, generally in the littoral region of Ecuador where they have been formed through sedimentary processes in which the iron found in the surface of the sand grains is captured. The presence of high amounts of iron oxide gives it a black characteristic color of these sands. In addition to iron oxides, other minerals are present in the sands like: Magnetite (Fe_3O_4), Ilmenite ($FeTiO_3$), Hematite (Fe_2O_3), and Quartz (SiO_2).

Solid sorbents are manufactured by exerting mechanical compression on black sands with a binder material that keeps the sorbents stable. The result is solid sorbents, a flat plates of dark color that will be placed in parallel in an elongated cylindrical reactor through which the acidic Natural Gas will circulate, when leaving the reactor, sweet Natural Gas will have a smaller amount of contaminants.

Taking into account that the same kind of sandy material composition was employed to manufacture solid sorbents for the sweetening of sour natural gas in Venezuela, a similar composition of wasted solid sorbents can be assumed. According to Ricaurte (2009), the solid sorbents had a higher percentage of hydrogen sulfide removal compared with commercial brands. Fundamental elements analysis carried out in the pellets burned after reaction with the H_2S revealed that the worn pellets presented contents of iron (Fe), oxygen (or), silicon (Si), magnesium (Mg), aluminum, carbon (C) and sulfur (S) (Ricaurte, 2009). In addition, analysis of diffraction of x-rays identified the presence of minerals such as pyrite (FeS), iron sulfides (FeS_2), in addition to polymorphic and elementary sulfur (S^8, S^0). It is important to highlight the presence of these products in the surface layer of the pellets conforming the solid sorbents.

Notice that due to the compact structure of the resulting pellet, the reaction will occur only in the surface, leaving the core without reaction. Thus, the reactions taken from Marvin Ricaurte (2009) that can take place between the iron oxides and the hydrogen sulfide are the following:

$$Fe_3O_4 + 4H_2S \rightarrow 3FeS + 4\ H_2O + S \quad (1)$$

$$Fe_3O_4 + 6H_2S \rightarrow 3FeS_2 + 4\ H_2O + \ 2H_2 \quad (2)$$

$$Fe_2O_3 + 3H_2S \rightarrow 3Fe_2S_3 + 3\ H_2O \quad (3)$$

$$FeS + S \rightarrow FeS_2 \quad (4)$$

The consumption of H_2S once reacted with pellets is near to 70%. In addition, the sulfur content fixed in the pellet represents nearly 15.10% of its total mass according to Ricaurte (2008). Thus, this gives an idea of the absorption capacity of the pellets and the amount of sulfur compounds taking place in the pellets.

PROBLEM STATEMENT

During the last decades, Ecuador has gone through an extensive growth in the reliance on energy usage, mainly driven by the extensive population. Ecuador economy depends principally at the expense of oil producing and refining. One part of refined oil is used to support energetic matrix through fuel, heat homes, and supplying energy to the power industry. As an example, In Venezuela natural gas has an important weight (40%) in it is matrix of energy (Castro, 2011). Thus, Ecuador is one of the Andean countries that does not use properly this natural resource for a greater contribution to the energy matrix. Taking into account that the dependence of Ecuador is so deep with respect to oil and its derivatives, it is essential to take well advantage of natural gas resources to boost the contribution to the economy. In this way, the efforts should be directed to maximize the use of petroleum resource, as far as possible avoiding unnecessary expenses with purchases of foreign products for their treatment and promoting the use of the national raw material instead. The reuse of materials in turn also represents an important saving for the Ecuador State and reduces the amount of sorbents worn.

Once the Natural Gas sweetening process is finished, the sulfur-saturated solid sorbents have a different composition than the initial one. The presence of large quantities of sulfur compounds suggests a correct disposition of the residual material to avoid contamination problems as occurs in the case of acid mining spills, which are characterized by the reaction of metal sulfides with water and air under normal conditions. In this way, the use of oxidizing and reducing bacteria in sulfur compounds has not been evaluated so far in the solid sorbents used for the sweetening of natural gas. However, there are studies where the effectiveness of these microorganisms in the bioremediation of acid mining drains has been proven. This background gives us an indication of its possible applicability for the particular case of this work by having similar characteristics in the sulfur compounds generated in both processes. The particular case of acid mining drains will be explained in more detail in the following sections.

OBJECTIVES

General Objectives

- To propose a suitable way to reuse wasted solid sorbents saturated of sulfur after sweetening of Natural Gas in Ecuador.
- To search in literature information of bioremediation activities in acidic mining drainage contamination.
- To evaluate the suitability of sulfur-bacteria into a reactor with defined conditions for sulfur digestion present in wasted solid sorbents.

Specific objectives

- To search information of the metabolic pathways in sulfur bacteria found in acidic mining conditions. In addition to search in literature the bacteria types useful in biomining of metals and sulfur extraction.
- To study the performance and develop of microbes that grow under stress generated by contamination sulfur in natural conditions (SRB & SOB).
- To determine location sites for sampling of SRB & SOB.
- To search information of reactors previously used in bioremediation of acidic mining drainages.
- To evaluate the conditions needed for adequate growth of sulfur bacteria in the reactor for sulfur conversion.

METHODOLOGY

The methodology consisted of a literary review of the microorganisms used in biomining in the extraction of minerals utilizing sulfur metabolism and bacterial leaching reactions. These microorganisms have in their metabolism, the ability to use sulfur compounds as electron acceptors in the oxidation reaction of metal in the mineral. Sulfur bacteria can act under various temperature conditions and can be classified as oxidative and reducing. Residual solid sorbents of the natural gas sweetening process are material with large amounts of sulfur in its various forms. This residual material resulting from the sweetening of natural gas could be an excellent substrate for these bacteria. The following sections discuss the conditions under which these microorganisms have been cultured to assess their metabolic capacity to apply a desulfurizing treatment to the residual beds to be able to promote their reuse in the natural gas treatment processes in addition to having them in a safe and non-polluting way.

Microorganisms and the sulfur cycle

Sulfur is a bright yellow non-metallic element commonly found in nature and that can also be extracted from common minerals. Sulfur is one of the oldest inorganic nutrients on the planet. Like other inorganic compounds such as hydrogen, carbon dioxide and nitrogen, microorganisms use them for energy production and as building blocks of life. The sulfur-microorganisms can be classified in two groups: sulfate reducing (SRB) and sulfide oxidizing (SOB) bacteria (Kletzin, Urich, Müller, Bandeiras, & Gomes 2004; Muyzer & Stams 2008; Overmann 2000). The reducing sulfate bacterium uses a sulfate breathing process. In which, energy is obtained through organic compounds oxidation with the sulfur reduction (Barton & Fauque, 2009). Depending on the type of sulfur compound, these serve as electron acceptors in the production of hydrogen sulfide (Barton & Fauque, 2009). Oxidizing sulfur microorganisms are more flexible because they can be developed in aerobic and anaerobic environments. They can oxidize sulfur sulfide and convert it back into elemental sulfur or sulfate (Ghosh & Dam, 2009; Kletzin et al., 2004). Many invertebrate organisms develop in sulfur-rich environments and use it when developing endosymbiotic relationships with microorganisms that metabolize sulfur and thus avoid the toxicity of high levels of hydrogen sulfide in the ecosystem (Arndt, Gaill, & Felbeck, 2001; Dubilier et al., 2001; Duperron et al., 2011).

In sulfur cycle, sulfate-reducing bacteria (SRB) takes hydrogen sulfide from hydrothermal sources at the bottom of the ocean and allows the recycling of sulfur to transform it into organic matter in chemosynthetic processes. The isotopic evidence states that SRB evolved far before the oxygenic photosynthesis and cyanobacteria evolution (Baumgartner et al., 2006; Shen & Buick, 2004). Nowadays, sulfate-reducing bacteria exhibit a wide metabolic pathways to reduce sulfur species. Lastly, researchers found other cases where sulfate-reducing bacteria can reduce oxygen and nitrate in presence of oxygen. Later, after studying the SRB metabolism, bacteria had preference for some compounds compared to others, oxygen was clearly preferred as electron donor acceptor, then nitrite and sulfur compounds at least (Krekeler & Cypionka, 1995).

In this way, the first choice of SRB will always be for oxygen until it is depleted. However, preferred oxygen reactions might induce the formation of toxic compounds for a long time (Krekeler & Cypionka, 1995). In fact, a study reveals that commonly SRB converts sulfate to sulfur (S8, S0), but in oxic conditions, the bacteria transform the material in the opposite direction, from sulfur to sulfate (Krekeler & Cypionka, 1995). Qiu et al., (2009) used the SBR *Citrobacter* sp., to display sulfate to sulfide conversion in the presence of anoxic condition but in a media with oxygen, *Citrobacter* sp. strains grew rapidly, but they lacked sulfate-reducing activity. Therefore, the physicochemical compounds conforming the growth medium must be careful supervised due to the sensible response of the sulfate reducing activity in presence of oxygen in growth media.

Sulfur role in acid mine drains

Mining activities generate large amounts of corrosive and toxic waste. Mining extraction sites are surrounded by rock deposits, tailings ponds, and processed chemicals. These mining wastes are products that have no economic value and it is estimated that for every ton of minerals extracted a ton of waste is generated. The release of waste to the environment has strong negative long-term effects by altering the habitat of aquatic and terrestrial animals. Metals from mining waste are highly toxic to soil and aquatic systems. Metals are not degradable and are kept contaminating aquatic resources, representing a serious problem for public health. In the composition of the tailings, pyrite (Fe2S) is the most reactive and dominant compound as a sulfide mineral present in tailing wastes.

Pyrite has the characteristic of returning in extremely acidic conditions when it comes into contact with water through the production of sulfuric acid. Sulfuric acid eventually dissolves other metals contained in mining waste, resulting in a low pH and the presence of soluble sulfates, iron, aluminum, and transition metals. A condition is known as acidic mine drainage (AMD). In fact, minerals with a sulfur content ranging from 1 to 5% are more likely to react in environmental conditions with water and air to generate acidic drain conditions (Tiwary, 2001). In mining extraction, the large quantities of waste rock produced are characterized by having a large content of sulfides and very low carbonate content (Shannon, 2004).

Since the acidic conditions generated by the contact of mining waste with water in normal conditions are similar to the conditions that would be obtained if solid sorbents were exposed to water due to the similar composition of pyrite and sulfur compounds, the reactions that take place when mining waste and water come into contact are important to get an idea of the reactions that will take part in the reactor when the spent waste solid sorbents enters as a substrate into the reactor. Equation 5 show the main components of the reaction (Muñoz & Karina, 2012).

$$\text{Sulfide ores} + \text{rain water} + \text{air} \rightarrow \text{low pH} + \text{metals \& sulfates} + \text{precipitates} \quad (5)$$

Considering pyrite as a principal source of sulfur in the wasted solid sorbents, the chemical reactions that take place when pyrite get in touch with water to generate acidic conditions will be the following (Taylor, 1996):

$$4FeS_{2(s)} + 14O_2 + 4H_2O \rightarrow 4Fe^{2+} + 8H^+ + 8SO_4^{-2} \quad (6)$$

$$4Fe^{2+} + 8H^+ + O_2 \rightarrow 4Fe^{3+} + 2H_2O \quad (7)$$

$$4Fe^{3+} + 12H_2O \rightarrow 4Fe(OH)_{3(s)} + 12H^+ \quad (8)$$

The reactions are explained in 3 steps according to Pope et al., (2010):

- Oxidation of sulfide ore (FeS_2) to sulfate ion (SO_4^{-2}).
- Oxidation of ferrous ion (Fe^{2+}) to ferric ion (Fe^{3+}).
- Hydrolysis of ferric ion (Fe^{3+}) that precipitates as iron hydroxide ($Fe(OH)_3$) at pH of 2-3.

As pyrite is in large quantities in wasted sorbents, the reactions that take place when mining wastes get in contact with water and air are similar to the reactions that would happen if wasted solid sorbents get in contact with water. In equation 6, H^+ (proton ions) and SO_4^{-2} (sulfate ions) raise the acidity and concentration of dissolved solids in water (Gray, 1997). The metals dissolved like Fe^{3+} (ferric ion) flows downstream and precipitate (Kimball et al., 2009); affecting aquatic and terrestrial ecosystems with high levels of toxic compounds (Mayes et al., 2009). Ferric oxide presence also cause danger, higher concentrations can kill fish and affect terribly aquatic life (Gray, 1998). Fe^{3+} can oxidize sulfate again in presence of hydrogen sulfides according equation 9 (Sheoran et al., 2011) allowing more acidic conditions intensified by additional sulfate ions, ferric ions (Tovar & Salomé, 2010). Furthermore, the situation get worse when all typical metals like zinc, copper, arsenic are dissolved and released through water effluents to the environment.

$$FeS_2 + 14Fe^{3+} + H_2O \rightarrow 12Fe^{3+} + 2SO_4^{2+} + 16H^+ \qquad (9)$$

In addition, many bacterial strains that colonize rock formations in mines in acidic conditions accelerate the formation of AMD (Escobar et al., 2010). A widely studied bacteria involved in the intensification of AMD is Acidithiobacillus ferrooxidans and Leptospirillum ferrooxidans (Escobar et al., 2010) that in a pH range of 2-4 increase the rate of oxidation by short times at least (De la Torre et al., 2011). AMD generates low pH and higher concentration of sulfates and metals, especially ferric iron ions (Johnson & Hallberg, 2005). Under AMD conditions, the medium typically has a pH less than 3 and a sulfate concentration of 3000 mg/L. Such conditions may vary depending in geochemistry, rock permeability, area affected, and by variations from mine to mine. The remediation and mitigation of AMD are challenging (Sheoran et al., 2011).

Degenerative reactions in wasted solid sorbents

Bearing the environmental context of AMD in mind, if the consumed solid sorbents are not disposed in a correct way and they are let free in normal conditions, the generation of acidic conditions similar to AMD are possible. The difference lies in that AMD is inevitable hard to avoid (Kuhn, 2011). The by-products of poorly wasted solid sorbents disposed are mainly three: acidity, metal precipitation, and sedimentation (Gray, 1997). Acidic conditions in the environment, neutralize the buffering capacity of bicarbonate in river ecosystems by lowering pH. The release of heavy metals directly affects aquatic species due to their toxicity. Sedimentation alters the turbidity that harms aquatic life making water less useful and increasing water treatment costs. Furthermore, acidic conditions generated by inadequate treatment of calcined sorbents can affect the soil and the surface and ground water quality (Gray, 1997). There exist regions of Ecuador where mining is the main economic activity: the monitoring of toxic contaminants (Prodeminca, 1998) revealed high concentration of heavy metals such as arsenic, copper, zinc, and, lead in toxic quantities for aquatic life (Tovar & Salomé, 2010). The accumulation of toxic compounds, especially heavy metals, blocks vegetation establishment in banks of rivers. (Guerra & Zaldumbide, 2010). It is important to highlight that the impacts generated in ecosystems have been caused by small and medium mining activity. The application of sorbents in sour gas sweetening process results a challenge because it is the first time that sorbent treatment is evaluated in the oil sector.

The sweetening of natural gas using solid sorbents based on metal oxides will be implemented at the Natural Gas extraction points in Ecuador. The Amistad field, located in the Gulf of Guayaquil, is an important point for the extraction of natural gas. This area represent a great risk due to the high level of humidity generated by the high rate of precipitation (Kuhn, 2011). As stated by Sacher (Sacher, 2011), the risk of the area comes from the acidic conditions produced by the moisture. The hostile conditions cause economical lost and several health affections in population (Sacher, 2011).

Preventive measures for waste sorbents

In order to prevent the formation of acidic conditions, sulfide ores must to be kept in sulfur reduced forms. An alternative preventive measure is to avoid sulfide's contact with oxygen and/or water (Gray, 1997). One way to avoid its contact implies the use of soil and water covers (Jordanov et al., 2007). The wasted solid sorbents can be covered with a waterproof layer which can be a geomembrane and gradually revegetated (Terrambiente, 2007). Another preventive treatment includes the removal of bacterial strains in solid sorbents by the application of chemicals such as sodium benzoate avoids acceleration of sulfide oxidation (Bravo & Alejandro, 2007). It is previously recommended evaluation of preventive measures such as long term stability and sustainability. The application of chemicals to kill bacteria is effective but implies repeated applications of the biocide chemicals in the solid sorbents, which is not environmental friendly (Johnson & Hallberg, 2005). In this sense, it is necessary to assess the preventive treatment in terms of stability and sustainability before application. As is remarkable, more research is needed to establish more complex pollution prevention measures to be applied to treat the solid sorbents.

Acidic contamination by oxygen and water can be avoided storing the sorbents in sterile conditions. The sterile conditions are obtained through a drainage system flowing water to the rivers. This system has other advantages, it prevents leaks and can redirect polluted wastewater to a treatment system (Terrambiente, 2007). Such preventive measures are detailed by Younger and Wolkersdorfer (2000) in order to avoid contamination to surrounding streams.

Remediation of acidic conditions

Once a site has been contaminated, it’s fundamental to evaluate the strategies to manage the acid throwing out. The treatment options go in the hand of characterization and evaluation of the site discharges including receiving watersheds surrounding the site (Sheoran et al., 2011). The evaluation of the movement of water flux has to follow an assessment methodology (Sheoran et al., 2011) bearing hydrochemical and hydrogeological approaches in dry and wet weather conditions (Pope et al., 2010). Also, measurements related to water flow and water quality has to be followed for temporal changes understanding (Sheoran et al., 2011). The study of the water effluents by characterization and monitoring will determine if pollution management measures are enough to accomplish the Ecuadorian Environmental legislation. If water effluents collected in drainage systems are in a higher proportions of contaminants or toxic heavy metals, then bioremediation is a suitable treatment alternative.

Microorganisms in mining process

In this environment, low carbon concentrations make an electron donor indispensable to improve bacterial activity. Sulfur oxidizing bacteria can use inorganic carbon sources, while sulfate-reducing bacteria need an organic carbon source and an electron donor (Johnson & Hallberg, 2005). The bacteria that are part of the degradation processes of the mineral matrix for the extraction of metals of commercial interest took on a special importance and commercial value due to their characteristic of accelerating the reaction of the processes. Among this group of bacteria are bioleaching and biooxidants microorganisms. Biooxidation processes require acidophilic bacteria of metal sulfides, mainly iron and/or sulfur compounds.

These organisms belong to groups of mesophilic and thermophilic bacteria (Brierley & Brierley, 2013). The bacteria that are part of the degradation processes of the mineral matrix for the extraction of metals of commercial interest took on a special importance and commercial value due to their characteristic of accelerating the reaction of the processes. Among this group of bacteria are bioleaching agents and biooxidants. Biooxidation processes require acidophilic bacteria of metal sulfides, to oxidize mainly iron and/or sulfur compounds.

These bacteria can be classified according to the temperature ranges in which they can grow: mesophiles grow up to 40 ° C, thermophiles between 40 ° C and 55 ° C and extreme thermophiles in the range of 55 ° C to 80 ° C. When organisms are under these temperature conditions and oxidize iron and/or sulfur, protons and iron ions 3+ (Fe^{3+}) are produced, which in the medium function as attack agents for metal sulfides, a degradation process called non-contact mechanism of mineral matrix degradation.

Proton and ion production keeps the pH low. Under aerobic conditions, the bacteria oxidize the sulfur intermediates to sulfuric acid and protons dissolved in the medium, for example, the oxidation reaction of the elemental sulfur to sulfuric acid, this reaction is only biologically possible under bioleaching conditions.

The bioleaching extraction methods can be classified in two, contact or direct methods and non-contact or indirect methods. The direct bioleaching method is the transfer of electrons directly with the membrane of the microorganism attached to the mineral matrix, for this, the bacteria secrete extracellular polysaccharides to be fixed to the mineral matrix and to oxidize iron and sulfur compounds. Indirect methods use iron protons 3+ as oxidizing agents for metal sulfides. These are produced by the oxidation of iron 2+ (Fe^{2+}) by the action of oxidative bacteria. In natural systems, both bioleaching mechanisms can work together to be called a cooperative method (see figure 1).

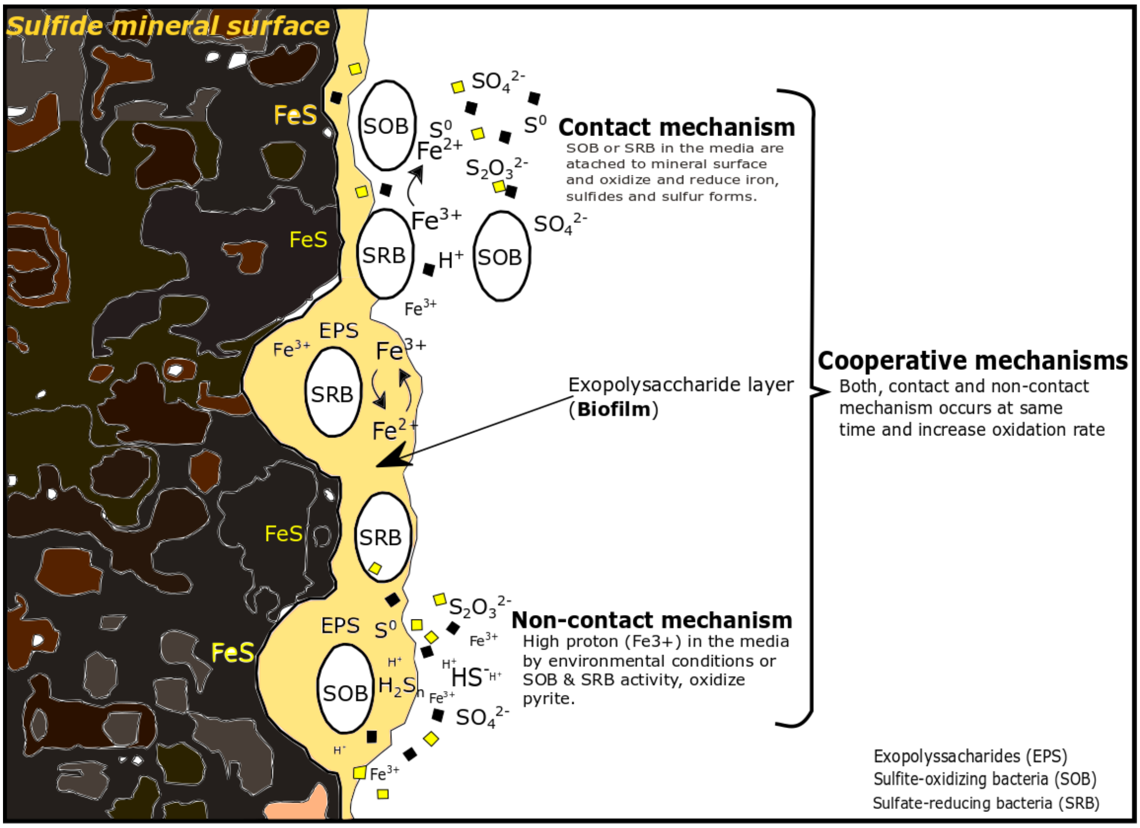


Figure 1. Bacterial mechanisms in mineral bio-leaching. Sulfide mineral oxidation is improved when combined mechanisms of bioleaching, contact and non-contact mechanisms work simultaneously to improve pyrite (FeS) oxidation in a biofilm layer formed in the surface of the mineral.

Acidic bioremediation

Bioremediation is considered an economically and environmentally friendly option compared to traditional techniques. There are previous works in which reducing bacteria-sulfate have been used in the treatment of acid drains in reactors. These consist of the elimination of metals and sulfates with the possibility of their reuse. In these works, the efficiency of the system depends on the consortium of bacteria used in the reactor, carbon source and configuration of the reactor (Aguinaga, McMahon, White, Dean, & Pittman, 2018). The fundamental basis of microbial remediation is to maximize the ability of microorganisms to neutralize acidic conditions and immobilize metals (Johnson & Hallberg, 2005).

Sulfate-reducing bacteria (SRB) in passive treatments

Bioremediation alternatives are efficient and economical ways to reduce pollution without waste generation (Kalin, Fyson, & Wheeler, 2006). For acidic drainage, recommended treatments include neutralization mechanisms with lime (Younger, 2000). Nevertheless, lime treatment does not resolve the toxicity generated by higher concentrations of toxic heavy metals (Kalin et al., 2006), it is expensive and generates unstable sludge (Foucher et al., 2001). Other alternatives include the usage of sulfate-reducing bacteria (SRB) considered a passive treatment, environmentally friendly and self-renewable (Foucher et al., 2001; Kalin et al., 2006). Bacteria use substrates available in soil as energy source in microbial metabolic pathways. Bacteria treatment application in soil is called bioaugmentation. This process requires constant monitoring of bacteria and has low cost of implementation (Doshi, 2006). Moreover, bioaugmentation is directly applicable to natural ecosystems (Younger, 2000).

Bioremediation with SRBs is based on providing a mixture of salts and organic compounds available locally. The mixture of salts is essential to optimize the sulfate and metal removal processes (K. Kefeni, Msagati, & Mamba, 2017). The addition of carbonates and organic matter under acidic conditions allows the reduction of the pH of the medium that affects the growth of the SRB. The reactions that take place at the remediation sites with BSR produce hydrogen sulfide from sulfates and the formation of hydrogen carbonates from organic matter. The hydrogen sulfide formed reacts with the metal ions to produce insoluble metal sulfide precipitates, in this way the metals are removed by sulfide precipitation (K. Kefeni et al., 2017). The importance of SRB and SOB lies in the fact that these processes seem to have a great potential reducing sulfur saturated sorbents generated by sour natural gas sweetening in Ecuador.

Activity of sulfate-reducing with sulfur species

Sulfate-reducing bacteria (SRB) correspond to a very broad group of highly versatile metabolism microorganisms. Different varieties of SRBs are distributed in diverse environments in nature, with evidence of growth over wide temperature ranges (4 - 92°C), and with wide variations in pH (2.3 - 10.6) (Liu et al., 2018). This highlights the fact that the inhibition of SRBs does not depend on temperature and pH settings. Some strains are strict anaerobic microorganisms like *Desulfovibrio* and *Desulfotomaculum* (Lyew & Sheppard, 1997), they grow at a pH of 2 in acidic conditions (Muyzer & Stams, 2008). SRB achieve alkalinization by rising low pH of water in acidic drainages through reduction of sulfate and soluble toxic metal concentration (Tebo & Ya Obraztsova, 2006).

The lack of oxygen in the systems allows SRB to catalyze sulfate reduction (Muyzer & Stams, 2008) by the use of sulfate as electron-donor acceptor. Sulfate reduction catalysis oxidizes organic carbon substrate and delivering by-product carbonate and hydrogen sulfide as stated in equation 10 (Doshi, 2006).

$$2CH_2O + SO_4^{2-} \rightarrow 12HCO_3^- + H_2S \quad (10)$$

The carbonate production contributes to alkalinity and acid neutralization (Lindsay et al., 2011). The reaction between hydrogen sulfide with dissolved metals generates insoluble sulfides (Johnson & Hallberg, 2005) as stated in equation 11 (Doshi, 2006):

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

In this way, hydrogen sulfide would allow the removal of metals like Fe^{2+} (iron). The precipitation of the metal sulfide will decrease metal concentration in acidic drainage (Lindsay et al., 2011).

Sulfate-reducing bacteria (SRB)

In order to estimate the factors influencing the performance of sulfate-reducing bacteria, it is evaluated the components affecting their growth and activity. Such components include substrates, influent pH and temperature, metal toxicity and microenvironments. This factors affect the growth rate of bacteria in the reactor and avoid normal functioning of the metabolism with the sulfur forms. As specific conditions in the reactor are needed for a successful product recovery, in the majority of the cases conditions of pH and temperature with consistent influent composition allow to the bacteria to have all substrates to metabolize sulfur compounds. Thus, the parameters need to be in permanent control.

Substrates

SRB metabolism require an available organic carbon source of small molecular weight like lactate, glucose or methanol (Muyzer & Stams, 2008). Substrate is the most limiting factor for well SRB performance (see eq. 10). In acidic drainages, the concentration of organic carbon is under 10ml/L (Neculita et al., 2007). Under these conditions, Johnson & Hallberg (Johnson & Hallberg, 2005) recommended to add recalcitrant and biodegradables like sawsand and manure to keep the long term carbon source in the medium. The microorganisms present in recalcitrant material degrade complex compounds to small carbon chain (Muyzer & Stams, 2008). The depletion of the carbon source will affect directly the performance of SRB, this fact add importance to the selection of an appropriate carbon source (Zagury et al,, 2006).

In express tests of a reactor designed to evaluate the reducing activity of SRBs depending on the carbon source, it was evident that the efficiency of the bacteria depends on the carbon source that is subministered to the reactor. Dairy, chicken manure, and sawdust were evaluated as carbon sources for an experimental reactor and the removal of heavy metals and sulfates resulted in 79%, 64%, and 50% respectively after 35 days of treatment in the reactor. Also as an additional result, the metals removed were Cd, Cu, Fe, Mn, Ni, and Zn (Zhang & Wang, 2014).

Influent pH

Exist several sulfate-reducing bacterial strains that can grow under a wide pH range. Kilborn (Kilborn, 1996) found acidophilic *Thiomonas* spp. sensitive to acidity (Johnson & Hallberg, 2005) that is inhibited at pH below than 5.5. Andrade (2010) reported that the majority of SRB exhibit an optimal performance at pH range from 5 to 8. Tsukamoto & Miller (1999) reported high efficiency of SRB at pH 2,5 to 3. McCullough (2008) advertised that pH under 5 did not inhibit the sulfate reduction carried out by sulfate bacteria. On the other hand, there are studies that suggest that a neutral pH is required for heterotrophic microorganisms to degrade larger substrates for SRB to thrive (Kilborn, 1996). In such scenario, a pH higher than 5.5 is required to keep SRB growth and activity (Kilborn, 1996). It could be considered the sum of sodium hydroxide in the pretreatment to rise the pH because of its solubility (Doshi, 2006). The addition of limestone is also recommended (Gusek, 2002). The recommendations are important to be taken into account in the design of a passive treatment using SRB.

Temperature

Sulfate-reducing bacteria can stand at temperatures ranges from -5°C to 75°C (Neculita et al., 2007). The temperature does not inhibit SRB performance once they have adapted and acclimatized to such conditions (Tsukamoto & Miller, 1999). However, mean temperatures have shown to produce high-performance activity by SRB (McCullough et al., 2008). Temperatures ranges of 20°C-28°C enhanced sulfate reduction rates (McCullough et al., 2008). Even in low-temperature conditions, the bioreactor design can be considered to be constructed deep into the soil to keep a mean temperature (Doshi, 2006).

Metal toxicity

An inhibition in SRB efficiency by metal toxicity can happen depending on the metal concentration in the medium (Utgikar et al., 2002). According to Doshi (2006), a concentration of 20 mg/L of cadmium and nickel, 25 mg/L of zinc, 60 mg/L of chromium and 65 mg/L of lead repressed SRB by inhibiting sulfate reduction in an anaerobic bioreactor (Doshi, 2006). So, metal concentration has to be lower than toxic levels for SRB to address a successful operation (Neculita et al., 2007). It is necessary to take metal toxicity measurements before applying SRB treatment. Other factors influencing SRB activity include precipitation of metal sulfides which are responsible of clogging (Kalin et al., 2006). Addressing the removal of metal sulfides constitutes a preventive measure before metal sulfides inhibit SRB performance (Utgikar et al., 2002).

Sulfide-oxidizing bacteria (SOB)

SOB has important metabolic process in sulfur transformation. Sulfur conversion systems are dominant in marine environments where the biofilms made by protobacterial and photobacterial microorganisms reacts with metals (especially iron) in H_2S fluxes. This systems produce greater amounts of sulfates, which limit the dissimilatory cycle of sulfur. Sulfur is oxidized by aerobic litotrophic microorganisms. In addition, some heterotrophic microorganisms, including bacteria and archaea, have the ability to oxidize reduced sulfur compounds (Meyer, 1976). The biological activity of microorganisms allow oxidation until the formation of sulfuric acid. The justification for the oxidation reaction of reduced sulfur compounds is given by the need to regenerate protons consumed in the first instance (Murr, Torma, & Brierley, 1978). These types of reactions are important in the treatment of contamination by sulfur compounds, corrosion and mining (He et al., 2011).

Appropriate chemolithotrophic sulfur-oxidizing bacteria species of interest for sulfur conversion are *Thiobacillus, Sulfolobus, Thermothrix, Beggiatoa* and *Thiothrix*. Two groups are taxonomically distinguish: short rods of the *Thiobacillus* genus and long filamentous bacteria of the *Beggiatoa* and *Thiothrix* genus. Chemolithothrophic can be classified according to the energy source. Obligate chemolithothrophic sulfur-oxidizing bacteria uses carbon jointly with inorganic sulfur forms to obtain energy (species of *Thiobacillus* and *Thiomicrospira*), facultative chemolithothrophic sulfur-oxidizing bacteria uses CO2 as energy source material and organic carbon sources from which they extract energy of bonds (some species of *Paracoccus denitrificans* and several species of *Thiobacillus*, *Thermotrix* and *Thiobacillus*). Other bacterial groups are classified as strictly aerobic whereas other as facultatively anaerobic, *Thiothrix nivea* and *T. micro-aerophila* are tolerant to low aerobic conditions (Syed et al., 2006).

Sulfur oxidizing bacteria are classified as chemolithotrophs because they use as energy source inorganic compounds like reduced forms of sulfur compounds. They get energy from the oxidation of electron donors and by fixing the CO2 in the Calvin cycle. Most sulfide oxidizing bacteria use oxygen as the final electron acceptor while in some species, such as *Thiobacillus denitrificans*, can use nitrate ($NO3^-$) under anaerobic conditions as a final electron acceptor (He et al., 2011). The most common sulfur compounds used as an energy source are H_2S, S^0, and $S_2O_3^{-2}$. In such reactions, the final products are sulfates (P. N. Lens & Kuenen, 2001). The following reactions take place in sulfur oxidation (Ampuero & Elena, 2013):

$$H_2S + 2O_2 \rightarrow SO_4^{2-} + 2H^+ \quad (12)$$

$$HS^- + \frac{1}{2}O_2 + H^+ \rightarrow S^0 + H_2O \quad (13)$$

$$S^0 + H_2O + \frac{1}{2}O_2 \rightarrow SO_4^{2-} + H_2O \quad (14)$$

$$S_2O_3^{2-} + H_2O + 2O_2 \rightarrow 2SO_4^{2-} + 2H_2O \quad (15)$$

Substrate

Sulfide-oxidizing bacteria can take whereas inorganic reduced sulfur forms like hydrogen sulfide, sulfides, sulfites or elemental sulfur as well as some organic sulfur forms as substrate (Cattaneo et al., 2003). Carbon supply in the influent is used as material source for cell wall structures. Other species of sulfur-oxidizing bacteria allow the sulfur oxidation in presence of nitrates, nitrites (oxidized forms of nitrogen) as electron acceptors and then reduce reduced forms of sulfur from polluted sources. The presence of organic substrate as carbon source increase performance of denitrification and desulfurization rates (Cardoso et al., 2006).

Temperature

The dissociated forms of sulfur become toxic for bacterial activity when a maintained pK_a value is reached when linked factors as sulfide concentration, the pH value and temperature are established (McCartney & Oleszkiewicz, 1991). Gram negative bacteria has a wide range of temperature specifications for growth requirements. Chemolithotrophic oxidizing bacteria from mesophilic or thermophilic conditions can thrive best at temperature range of 4-90°C (Tang et al., 2009). *Thermothrix azorenzis* has a higher optimal temperature score (75-86°C) (Odintsova et al., 1996). Other species like *T. novellus, Pseudomonas acidovorans, Pseudomonas putida*, *Beggiatoa* spp. and *T.*

thioparus are known as mixotrophic sulfur-oxidizing bacteria for carrying inorganic and organic sulfur oxidation activity (Oyarzún et al., 2003).

Metal toxicity

Sulfides may repress microbial activity when sulfide species are in high concentration (Moosa & Harrison, 2006). In fact, the inhibition is achieved when undissociated sulfide species reached a concentration of 2.5 kg/m^3 (Moosa & Harrison, 2006). In batch continuing monitoring, the sulfide-oxidizing activity was inhibited as toxicity by sulfide concentration reaches 100 mg l^{-1} (Wiemann et al., 1998). Toxicity of sulfide in the medium affect the quaternary structure of functional microbial enzymes by in a process of the diffusion of undissociated sulfide forms through the cell wall, forming sulfur/sulfide cross-links between polypeptides (Chen et al., 2010). In this way, the cross-links affect the coenzyme structures and other sulfide and sulfate proteins involved in sulfur metabolism (Chen et al., 2010).

The sulfide inhibitory concentration depends on the bacterial specie, the concentration in medium scores in the ranges of 100 – 800 mg/L of dissociated sulfide and 50-400 mg/L of undissociated H_2S (Parkin et al., 1990), and when the sulfide in the medium deals with bacterial biofilms, microorganisms can tolerate much higher sulfide concentrations (Lens et al., 1998). The sulfide concentration interrupt normal functioning of several anaerobic species, most sensitive include methanogens and acidogenic bacteria are least affected (O'Flaherty et al., 1998).

Influent pH

Experiments were set on chemostat varying the concentration of sulfide species, the inhibition of sulfide-oxidizing bacteria was reached across the range of 6 to 7.5 pH affecting the growth & death tares considerably (Moosa & Harrison, 2006). Successful bacterial growth is achieved when pH according to the temperature is fixed in 1-9 pH range (Tang et al., 2009). Several *Thiobacillus* spp. were studied at different pH to set an optimal measure for exponential growth (T. *thiooxidans* 2.0-3.5 pH and *Thiobacillus ferrooxidans* 1.3-4.5 pH) but experiments showed *thiooxidans* was able to grow at extreme acidic conditions (pH 1). Neutrophilic bacteria such as *T. denitrificans* and *T. novellus* use a neutral pH of 6 to 8. T. *thioparus* can perform oxidation of both sulfides and thiosulfates in pH of 5 to 9 (Vlasceanu et al., 1997).

Locations for sulfur bacteria sampling

The genera of bacteria of interest are also used in bioremediation of soils and activated sludge from biological wastewater treatments. Bacteria with these properties can be taken, for example from hot springs sources. In Pichincha, there is a tourist complex called "El Cachaco", a spa that oscillates at temperatures of 23-27 C with an ambient temperature of 20 C with temperatures of 2 C at night. Bacteria was detected and some molds and yeasts. Mesophilic aerobic *Staphylococcus*, coliform bacteria, and molds. The 82% of bacteria were gram negative, prevailing *Aeromonas,* while 18% were Gram positive, prevailing the genus *Staphylococcus*. Molds such as *Aspergillus, Penicillium* and *Rhizopus* were also identified (Reina & Xavier, 2017). Other study done in the "Termas Guapante" in the waste water of the water shelf and the predominance of bacterial genus such as *Bacillus subtilis, Enterobacter cloacae, Citrobacter amalonaticus, Alcaligenes faecalis* and others like *Escherichia coli, Leminorella grimontii*, *Afipia clevelandensis*, *Rahnella aquatilis,* and *Staphylococcus aureus* (Zela & Alejandra, 2015). In these studies a prevalence of Gram-negative bacteria is found according to other bacterial analyzes in sulfur oxidation slopes of other authors in Ecuador (Almeida & Vanessa, 2015; Andrade & Vanesa, 2015; Armas & Santiago, 2015; Bonifaz & Patricia, 2015; Luzuriaga & Elizabeth, 2015; Peñafiel & Javier, 2015; Yungán &

Leonidas, 2015; Zela & Alejandra, 2015). Each point of study in the thermal baths are different locations with different composition of metals and a typical and different bacterial population (Rosa Jorge et al., 2000). Table 1 summarize bacteria sampled from "La Merced" hot springs in Pichincha province, the information was taken from Zela & Alejandra, (2015).

Table 1.

Bacterial genus in "La Merced" hot springs and features

Genus	**Cell wall type**	**Metabolism**	**Optimal temperature**	**Other features**
Acinetobacter	Gram Negative	Aerobic	33°C-35°C	Mobile, no spores.
Aeromonas	Gram Negative	Facultative anaerobic	22°C-28°C	Rounded, no spores, fermenter.
Brevundimonas	Gram Negative	Aerobic	30°C-37°C	Straight, no spore, mobile.
Budvicia	Gram Negative	Facultative anaerobic	23°C-27°C	No mobile, no spores, encapsulated.
Citrobacter	Gram Negative	Aerobic	33°C-37°C	Mobile, lactose fermenter.
Pseudomonas	Gram Negative	Aerobic	30°C-37°C	No spores, variable mobility, slightly curved.

Xenorhabdus	Gram Negative	Facultative anaerobic	28°C	Mobile, live in symbiosis with nematodes.
Bacillus	Gram Positive	Aerobic/anaerobic	35°C	Nitrite reducer, fermenter or oxidizer

Thermophilic maths isolated from “La Merced” Hot spring. Citrobacter is an Enterobacteriaceae commonly found in sludge of agricultural wastewater treatments, remove sulfate.

Other points of interest for sampling bacteria in acidic environments is searching in mining extraction points such as the Mirador project in Ecuador. In the mining sector of Tundayme, native acidophilic bacteria of interest were isolated in oxidation processes of metal sulfides where their oxidative capacity of sulfur. Microorganisms have a limited activity when the substrate is limited, so it is necessary to maintain a system with new sulfur medium to keep them growing. The production of iron and sulfur in each experiment suggests a higher production of sulfur than iron, with a ratio of 6/1 respectively (Jiménez & Vicente, 2015). Table 2 shows the conditions of bacteria isolated from the Tundayme mining sector; information partially extracted from Jiménez & Vicente, (2015).

Table 2.

Sulfite-oxidizing bacteria from Tundayme mine Zamora Chinchipe.

Genus	**Environmental conditions**		**Maximum growth and yield conditions**	
	Temperature	**Ph**	**Temperature**	**Ph**

***Acidithiobacillus* (sample7)**	24°C-30°C	2.4-6.5	30°C	1.8
***Acidithiobacillus* (sample3)**	24°C-30°C	2.4-6.5	30°C	6.5
***Acidithiobacillus* (sample1)**	24°C-30°C	1.8-6.5	30°C	2.4
***Acidithiobacillus* (sample9)**	24°C-30°C	1.8-6.5	30°C	6.5
***Leptospirillum* (sample11)**	24°C-30°C	1.2-6.5	30°C	1.2
***Leptospirillum* (sample5)**	24°C-30°C	1.2-6.5	30°C	6.5

The table shows the laboratory conditions for bacteria of leaching interest. The activity of the bacteria was evaluated in each sample and the conditions to get the maximum yield.

SRB & SOB in heterogeneous environments

SRB are commonly found in nature in soil sediments living in consortiums (Lyew & Sheppard, 1997). Heterotrophic bacteria help SRB through fermentative process to provide the products to grow and metabolize sulfur (Zagury et al., 2006). In the field, SRB and heterogeneous microorganisms usually tend to aggregate in areas which offer physical protection (Lyew & Sheppard, 1997), where they form a conductive microenvironment for their survival (Lyew & Sheppard, 1997). Such microenvironments enhance their tolerance to oxygen, heavy metals and acidic mediums (Doshi, 2006).

The products obtained by anaerobic degradation are complex organic compounds such as carboxylic acids, amino acids, alcohols, sugars and aromatic compounds that are oxidized by sulfate-reducing bacteria. To oxidize these compounds, bacteria use the Calvin-Benson-Bassham cycle to obtain available cell carbon. All products are not oxidized by reducing bacteria, some compounds do not oxidize, such as Acetyl-CoA. On the other hand, the growth of reducing bacteria has not been achieved using organic compounds such as proteins, cellulose, fats, nucleic acids or starch, SRBs depend on other organisms to degrade these compounds (Liu et al., 2018). The metabolism of reducing bacteria uses ATP as a source of energy to reduce sulfate to sulfite and sulfite to sulfide (Barton & Fauque, 2009).

The activity of sulfate-reducing bacteria generates hydrogen sulfide among other compounds. Hydrogen sulfide can be toxic for SRB, specially depending to its concentration, ranging from 477 to 617 mg/L of H_2S (Neculita et al., 2007). It could be considered that once hydrogen sulfide is obtained, it can be converted to elemental sulfur by activity of sulfide-oxidizing bacteria (SOB) in another reactor (Muyzer & Stams, 2008). As SRB and SOB can be found coexisting in nature. Wastewater treatments are not the exception, there is evidence of both SRB and SOB bacterial communities present in biofilms. Both bacterial communities showed an internal sulfur cycle that allows their developing in the biofilm as a result of the high organic load input and low oxygen dissolved (Okabe et al., 2005). Van den Ende et al., (1997) experiments with *Desulfovibrio desulfuricans* (SRB) and *Thiobacillus thioparus* (SOB), which were obtained in mixed cultures using a chemostat as limiting compounds. Then, they utilized lactate and oxygen once the medium contained sulfate excess. Both organisms developed a syntrophic interaction in which *D. desulfuricans* produced the H_2S and CO_2 necessary for the growth of *T. thioparus* with the production of S^0. However, the rapid cycling of sulfide, S^0 and thiosulfate did not permit the recovery of S^0. The usage of a fluidized loop reactor for the biological treatment of sulfide-rich synthetic wastewater scored a recovery of 95% S0 using sodium sulfide in the influent. In the fluidized loop reactor, the inoculation was made with an isolate of *Thiobacillus denitrificants* spp. employing the LDPE plastic with china clay (carrier material) (Krishnakumar et al., 2005). Thus, Van den Ende et al., (1997) demonstrated that both SRB & SOB are capable of developing a biofilm on a support material.

Engineered consortiums

To improve the effectiveness of the system in the reactor, genetic engineering can be used in sulfate-reducing bacteria. Engineering in a consortium of bacteria can allow the cultivation of microbes that are abundant in acid mining and that have not been cultivated. This could elucidate their functional roles with sulfur (Brune & Bayer, 2012). Microbial consortia play a fundamental role allowing survival of microorganisms in hostile environments. In environmental problems, they have been successfully applied to facilitate the complex interactions between organisms that can withstand the acidic conditions of mining (Keller & Surette, 2006).

The organisms participate in syntrophic degradation of compound complexes that allow completing the metabolic reactions to obtain energy in a joint work of both organisms (J. Zhou et al., 2011). Sulfate removal can be improved by combining the properties of these organisms to interact with metals. These organisms are a source of genetic information that can be used to modify microbes. These organisms also possess resistance genes that improve interaction with sulfates in acidic environments. The mechanisms of bioleaching of metals and the adaptations of oxidizing iron and sulfur bacteria to acidic environments can be explored in a consortium of bacteria designed to enhance their activity in acidic environments. In natural consortiums, the oxidizing iron and sulfur bacteria are symbiotic, potentially mutualistic and synergistic, synergistic relationships are very important to balance the metabolism of sulfur and iron. Even so, the function of the consortium is not fully understood (Latorre et al., 2016).

For the edition of the consortium of bacteria, one must first start by addressing the signaling between cells and communication. Communication allows dividing work between individuals or populations to carry out complex degradation tasks. The exchange of information is carried out with the exchange of peptides (gram-positive bacteria) or acyl-homoserine lactone (acyl-HSL) signaling molecules (gram-negative bacteria). Extracellular polysaccharides in the models of *At. ferrooxidans* states that communication is essential for biofilm formation, essential for contact bioleaching processes. These microorganisms respond to compounds such as acyl-homoserine lactones (AHLs), used as a part of auto-inducer1 (AI-1) type in the quorum-sensing system (Keller & Surette, 2006). It might be possible to increase expression of AHL to enhance the organism's attachment to mineral particles to improve the leaching process in *At. ferrooxidans* through synthetic biology (Brune & Bayer, 2012).

Bioreactors

The fluidized biofilm bed reactor has several applications in wastewater treatment, utilized support media for microbial growth and retention. In the most important features highlights the biofilm formed in the support media (small-size particles). Active microorganisms and the large surface area available for sulfur conversion in the liquid in plants that are usually of small size. Fluidized beds offer easy operation and stability as well as greater operation efficiency of the activated sludge with less time and space (less cost), see figure 2. The large concentration of microorganisms in the FBBR allow the removal of several variables such as biological oxygen demand (BOD), chemical oxygen demand (COD), nitrogen (N), sulfur (S), etc.

The process consist of a flow of substrate in the influent through a packed support bed at a flow rate sufficient to bring motion to the system. As the flow of influent goes through the down flow fluidized bed reactor, the growing microbial concentration in the biofilm consume biodegradable waste material. The microbes in the support material may include aerobic, facultative or anaerobic typically found in bio-trickling filters. The predominating microbial specie depend strongly in the type of waste contaminant to be removed, and environmental conditions established in the reactor (Brosilow et al., 1997).

In an aerated fluidized bed biofilm reactor, the air is injected directly through an internal tube to promote oxygenation and mixing. Air pumping allow liquid circulation and therefore effluent recirculation can be omitted. In addition biofilm can be removed by gas effervescence of support media collision.

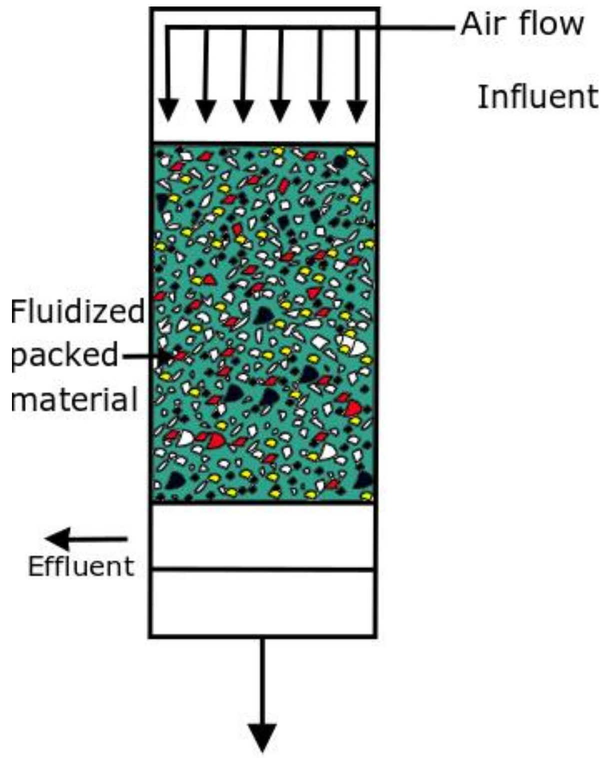


Figure 2. Down flow fluidized bed reactor (DFBR). The influent flow is directed down, it must pass through the packed biofilm material and the effluent comes outside without waste contaminants.

In the context of the wasted solid sorbents, the sorbents will become the substrate of the down flow fluidized bed reactor (DFFBR). A DFFBR can combine two antagonist respiratory processes, sulfate reduction and sulfide oxidation. The efficiency achieved in such reactor evaluated with a sulfur wastewater reports 72-77% of sulfur removal efficiency (Celis-García et al., 2008) when an electron donor such as lactate (Doshi, 2006) and two electron acceptors (sulfate and oxygen) were supplied as substrate into the reactor. Thus, the feasibility of the joining process depends on the rate of sulfate conversion and the percentage of sulfide converted to elemental sulfur.

Initially, sorbents with high sulfur charge supply the sulfur in the influent by the addition of hydrogen peroxide (H_2O_2) in a container mixed with water to make iron sulfide to precipitate in sulfates, sulfides, and, sulfuric acid (Yin et al., 2018). In this way, the sulfur-laden influent (together with carbon, nitrogen, oxygen, etc.) enters the reactor as substrate. Microorganisms attached to the support surface after 3 weeks will reach the highest concentration and allow biofilm formation (Xu et al., 2012). The biofilm with sulfate reducing and sulfide oxidizing bacteria developed rapidly over the plastic support (Celis-García et al., 2008, p.). Bacterial activity allow the recovery of yellow sediments similar in color and texture to S^0 from the reactor. Several SRBs were shown to tolerate the presence of oxygen (X. Zhou et al., 2011), and when carbon source is available, the elementary sulfur can be transformed again into sulfide (Islam et al., 2018). In fact, the recovery of elementary sulfur is related to the amount of oxygen dissolved in the influent.

The oxygen in the influent is measured as the oxygen demand (OD), which plays as a controlling factor. Higher OD in the influents resulted in reduced elemental sulfur recovery (Islam et al., 2018). In addition, mineral silicates, porous glass beads and polyethylene have been used as carriers in fluidized bed reactors (Nagpal et al., 2000, p.). Polyurethane foam, vegetal carbon, lava rock and alginate beads, among others, have also been used as supports (Silva et al., 2006). Carbon source is needed for successful sulfur conversion, effluents from industrial plants with high biochemical oxygen demand can be used for this purpose. This suggests a treatment for carbon removal in industrial wastewater effluents along with the sulfur transformation.

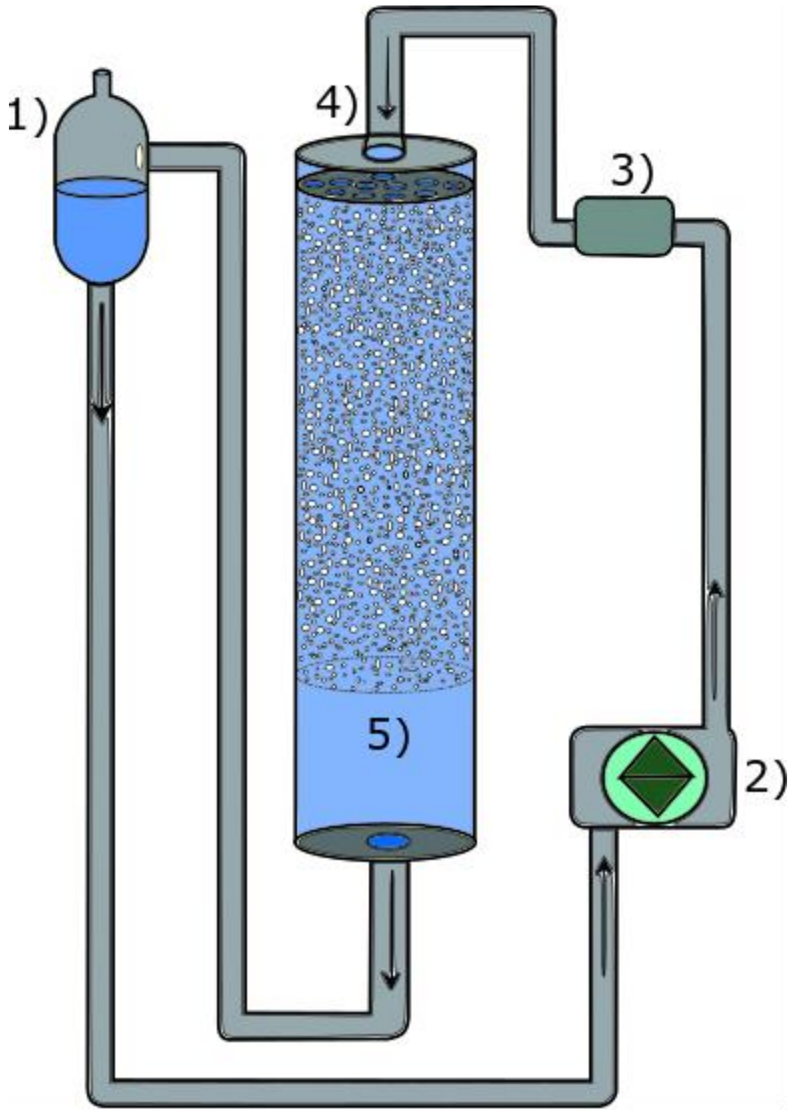


Figure 3. Scheme of the Down flow bed biofilm reactor (DFFBR). 1) Influent substrate which usually contains a carbon source, oxygen demand, etc. 2) Control of parameters such as oxygen supply, nitrogen and carbon income, etc. 3) Sampling point. 4) Down flow of the influent through the packed bed. 5) Effluent without contaminants.

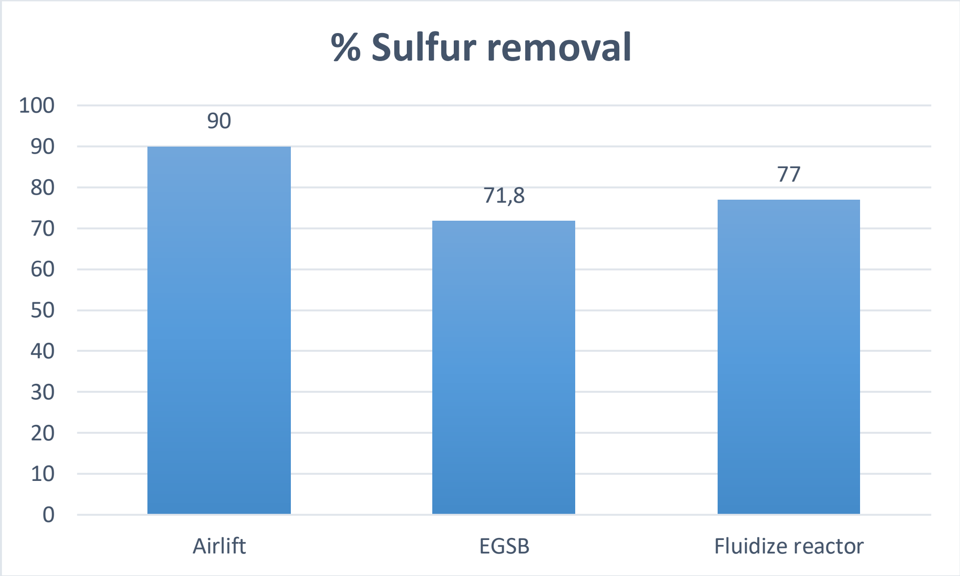
Other bioreactors are also used for biological treatment with elemental sulfur as a target. In airlift bioreactors, sulfur recovery efficiency reaches up to 95% (Abdel-Monaem, et al., 2014) using oxygen as limiting factor. A 100% sulfur recovery was achieved at 8.0–8.5 pH (Abdel-Monaem Zytoon et al., 2014). The conditions are set for autotrophic SOB and heterotrophic sulfide oxidizing bacteria (SOB) but the carbon supply is the drawback of such process (Abdel-Monaem Zytoon et al., 2014). The efficiency rates is airlift systems vary depending on the amount of sulfur availability and the oxygen demand (OD) applied. Lower end-product recovery at higher oxygen demand of 2ml/L (Abdel-Monaem Zytoon et al., 2014). The control of oxygen as limiting factor in bioreactor fed with liquid sulfide solutions is achieve by controlling the air dose to the bioreactor medium. In the other hand, at oxygen-limited conditions there was a slight increase of sulfur recovery as the pH was increased.

An aeration tank and an EGSB reactor were used to evaluate the performance of both SOB & SRB cultivated on in the EGSB. The characteristics of the reactor are a working volume of 4 l (height of 120 cm and an internal diameter of 50 mm). The temperature was maintained at 30 ± 1°C. To control the dissolved oxygen in the system, a separated 5-l vessel was used as the aeration tank. The influent was fed at the bottom of the reactor using a peristaltic pump. The pH and dissolved oxygen (DO) concentration were monitored, with the latter being controlled by adjusting the aeration flow rate. The continuous tests revealed that concomitant sulfate reduction and sulfide oxidation occurred in a single reactor. The DO presents an effective controlling factor to manipulate the performance of the SRB + SOB system, ranging 81.5–98.3% reduction of fed sulfate and 37.2–71.8% of produced sulfide to S0 at DO= 0.02–0.26 mg l-1. Restated, such reactor has a comparable sulfate removal rate as reported (27–93%) (Lenset al., 2000) but a higher S0 conversion rate than that in literature (Celis-Garcia et al., 2008).

RESULTS

According to the experiments previously carried out with the sulfurous bacteria, a reactor must preserve aerobic and anaerobic conditions for the correct functioning of the oxidative and reducing bacteria respectively. In this way, an important factor for its correct performance is through the control of dissolved oxygen in the medium. The temperature, pH and reactor design factors are adjustable factors according to the information collected. This is due to the various conditions that these microorganisms can withstand to metabolize sulfur however there are parameters under which they reach quite high removal rates.

According to the sulfur removal percentages reached in the reactors, the highest corresponds to the Airlift reactor, with 91% removal compared to the other two reactors with a value close to 70%. For the choice of the reactor of interest, it should be taken into account that the influent used in the experiments was the same. The synthetic feed influents in the Airlift reactor were sulfides, very similar to the composition of the influents used in the other reactors. It should be borne in mind that in the spent solid sorbents not only sulfides will be found, but several forms of sulfur such as sulfates and sulfides. Therefore, the performance of the reactors with an influent similar to sorbents should be evaluated. In addition, according to the processes of bacterial leaching, during the sulfur conversion, it is also possible to precipitate metals such as iron.



Plot 1. Performance of several reactors utilized in desulfuration experiments. The following graph shows the maximal performance of sulfur achieved in reactors that were considering the sulfur activity of SRB and SOB.

CONCLUSIONS AND RECOMMENDATIONS

- Studies show the effectiveness of sulfate-reducing and sulfite-oxidizing bacteria when they are placed under specific conditions for their growth. Many factors that influence its effectiveness depend on factors such as the type of carbon source in addition to the reactor operating conditions.
- The microorganisms present in the mining activity areas of Ecuador are the perfect candidates, to begin with, pilot tests to evaluate the conversion of sulfur fixed in spent beds, likewise, bacteria found in hot springs should be taken into account for these purposes.
- The reactors can be adjusted to maintain the necessary conditions so that both microorganisms (SRB & SOB) can grow in the reactor. The reactor that seems to be the most suitable for this purpose is the Down flow fluidized bed reactor DFFBR.

- The use of microorganism for removal of sulfur wasted solid sorbents is an effective tool to reduce the sulfur load of the solid sorbents. However, In addition to sulfur removal activity, the extraction of iron (Fe) from the solid sorbents is a by-product of the treatment of desulfuration.
- The oxygen demand presents a promising controlling factor for SRB & SOB performance in the reactor. Then, the use of SRB and SOB in a reactor is recommended as a suitable way to treat the wasted sorbents to reuse them repeatedly for gas sweetening.
- Wasted solid sorbents could be used as a support material for the growth of sulfur-occluding bacteria due to the spherical characteristics of its design. In such case, the support material for bacteria growth can be replaced and avoided.

ABBREVIATIONS

SRB	Sulfate-reducing bacteria
SOB	Sulfate-oxidizing bacteria
BOD	Biological oxygen demand
COD	Chemical oxygen demand
AMD	Acidic Mining Drainage
RSH	Mercaptans
OD	Oxygen demand
DFFBR	Down flow fluidized bed reactor
AHLs	ACYL HOMOSERINE LACTONES

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