Approaches to predetermine the variables affecting the efficiency for the design of mining effluents and acidic drainage treatment systems

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Abstract

Research using the metabolic activities of sulfate-reducing anaerobic bacteria to remove metals and sulfate neutralizing the acidity has found widely varying efficiencies in active or passive systems. Factors such as the particular composition of each effluent, available carbon source, temperature, pH, redox potential (Eh) and residence time influence the efficiency. Analyzing the complexity of the prior variables, being furthermore dependent between them, we carried out laboratory tests plans to elucidate how these variables could limit the efficiency and mainly, on which variables one could act to improve and sustain the efficiency in each particular process.

The assays were carried out by mixing three solutions in different proportions. Solution I contains sulfate and mineral salts. Solution II: nutrients compounds coming from some waste and solution III: the metal to be precipitated. The initial pH and Eh are measured. Flasks are filled, closed and film-sealed to ensure a anaerobic medium. The metal sulfide formation is detected by a strong black precipitate. When the flask is opened final pH and Eh are measured and samples are taken for chemical analysis. The microbial development is checked by microscopic observation and microphotography.

The results show it is possible to profit by the enormous diversity and versatility of microorganisms in nature and a wide range of residues can be used as carbon source. Even in assays containing Hg^{++}, microbial growth was obtained and HgS was precipitated.

It is proposed equivalent laboratory tests plans carried out before to design a process, could allow to predetermine for each effluent and each carbon source, the necessary quantitative relation between them and the pH-Eh range which should be kept in the inner of each system at a particular range of temperature. It seems the residence time should be taken as the adjusting variable for maintaining the other values previously determined.

Key words: sulphate reduction, anaerobic bacteria, conditions, efficiency.

Introduction

Bioremediation methods have done remarkable progress to mitigate AMD (Acid Mine Drainage). The metabolic activities of sulphate-reducing bacteria (SRB) capable of producing H_2S (biological reaction) and precipitating metals sulphides (chemical reaction) are being utilized in different active or passive systems (Piramid 2003).

Nevertheless such systems, even at pilot or full scale, have found widely varying efficiencies (Karathanasis et al 2010). Design guidelines use sizing *"rules of thumb"* based on AMD loading at design flows (Zipper et al 2010). A framework for research leading to experimentation must begin with quantified observations.

Several variables impact the efficacy including $SO_4^=$ concentration, temperature, effluent or AMD particular composition, residence time, pH, redox potential (Eh), and carbon source. Factors such as particle size of the organic matter, the labile : recalcitrant C ratio and the availability of N and P are also important. The question is how to manage the complexity of all the prior variables, being furthermore dependent between them, in such a way to reach and maintain in each particular system conditions for SRB development and metals sulphides precipitation conducting the system with a high efficiency and performance.

Some considerations about the prior variables and the manner they influence the microbial activity deserve to be considered. The true agents making possible such transformations are microorganisms, living beings with a high specific surface and sensibility to the physic-chemical conditions of the micro-environment around their membrane cell. So, they have a very high capacity to give a response (positive or negative) to each external stimulus, even when they don`t know nothing about the system size, the effluent rate or the residence time. These variables are our problem to give them what they require.

About the microbial nutritional demands, SRB use organic carbon molecules as methanol, ethanol and lactate (Posgate 1984). However, these substrates are expensive and can make this technology prohibitive. Nevertheless, the enormous diversity and versatility of microorganisms in nature make possible the development of different SRB populations taking their energy source, carbon and nitrogen sources from a great diversity of organic labile compounds contained in available organic materials such as agricultural and food processing wastes. For field implementation, identification and selection of locally available organic material is preferred because they are less expensive. Each organic material or residue has a particular labile: recalcitrant organic compounds rate. We present laboratory tests plans to evaluate the viability of using a particular organic material for reducing $SO_4^=$ in a particular effluent, and in the positive case, to quantify in the first instance the required relations between both.

The metabolism of living beings does not escape the laws of physics and chemistry. A bacterial cell acts as a "carrier" of electrons across the respiratory chain proteins or cytochrome system from the organic molecules to be oxidized to $SO_4^=$ to be reduced. Sulphate can operate like final acceptor of electrons and protons. The electrons transport is possible and is facilitated in the measure for a certain pH, the Eh is such that the products of the transformation are stable, while the substrates or original compounds are unstable, but different acceptors can compete with $SO_4^=$ for such position. It has been shown different species of SRB have alternative acceptors. So, $SO_4^=$ has competition with other electron acceptors. Independently of SBR, different microbial communities can develop with the same organic compounds but with different electron acceptors depending on the Eh at certain pH (Wall and Krumholz 2006)

Graphs have been constructed (Ehrlich 1995) showing the limits of pH-Eh zones in which some groups of bacteria of interest in bioremediation can develop. SRB develop at negative Eh. On the basis of thermodynamic principles it is predicted that different acceptors would be preferentially used according to the following succession: O_2, Mn(IV), Fe(III), NO_3^-, U(VI), $SO_4^=$, S° or CO_2.The subject is that as each acceptor is reduced, Eh decreases generating conditions to consume the following acceptor and so successively. Taking into account the labile organic compounds of each particular organic material and the chemical composition of each particular effluent affect the Eh, one question is which is the Eh required for SRB development reducing efficiently $SO_4^=$ to SH_2 in each particular system. The chemical reaction between H_2S and metals ions to precipitate metals sulphides is also dependant upon Eh-pH (Pourbaix 1966) and in general is feasible or fostered in a range of negative Eh.

Laboratory tests plans have been carried out in principle to obtain quantified answers to the prior questions and to propose equivalent assays as a kind of protocol to obtain quantified information necessary for the design and control of each particular system.

Methods

The synthetic media were prepared by mixing the three following solutions:

Solution I, containing sulphate and elements necessary for life, normally present in mining effluents: $NaSO_4$: 2.0 g, KCl: 0.5 g, $MgCl_2.6H_2O$: 2 g, KH_2PO_4: 0.2 g, $CaCl_2.2H_2O$: 0.2 g, K_2HPO_4: 0.2 g, $NaHCO_3$: 1.7 g, tap water: 1 litter. When using milk whey as the organic nutrient, it was necessary to add 15 g of NaCl to Solution I. In some cases, solution I was prepared in two forms and assays were done in parallel : with ammonia as a source of nitrogen by adding 1.0 g of NH_4Cl to solution I or without the addition of a nitrogen source in solution I.

Solution II, nutrients compounds coming from some solid and liquid wastes. Liquid wastes as milk whey, are directly the Solution II and may be used varying the relation V:V respect to Solution I. For example, 1, 2, 3, 4 and 5 ml of whey for each 100 ml of Solution I. Solid wastes as the wine industry wastes: flock, refuse of grapes, stock of grapes and dry leaves coming from two types of trees were used. When working with solid wastes and in the order to liberate the maximum contained labile compounds, they are triturated, mixed with water in different relations V:W, as it is indicated in each particular case and kept at 20-25°C during 48 hours before to separate the solution by filtration. Each one of the separated solutions may be used as Solution II. Obviously, as the relation water volume to residue weight decreases, the labile organic compounds concentration in the solution increases. Solutions II coming from a certain solid residue are added to Solution I in different relations V:V, for example 1, 2, 3, 4 or 5 ml for each 100 ml of Solution I.

Solution III, is a solution of the metal to be precipitated as sulphide and used as a marker. For example, $FeSO_4.7H_2O$: 5 g in 100 ml of SO_4H_2 0.5 % V:V when using the precipitation of the black iron sulphide as a marker or a solution of $HgCl_2$ 0.1 M, to analyze the sulfate-reducing anaerobic bacteria development and HgS

precipitation. Solution III is added in different relations V:V respect to Solution I, for example 0.5, 1, 2, 3, 4 and 5 ml of Solution III for each 100 ml of Solution I.

Once each medium was prepared with the corresponding composition, the pH was adjusted to the indicated values for each case (pH in), also measuring the initial Eh. Flasks were filled, closed and film-sealed to ensure an anaerobic medium. The assays were incubated at ambient temperature (20-25 °C). A positive development is determined by the formation of a strong black precipitate like the shown in Figure 1, associated to the development of a microbial culture determined by microscopic observations and microphotography´s . At the time to open each assay, immediately and in the same flask the final pH and Eh were measured with a micro-meter Fisher, model Accumet 50, connected with the corresponding electrodes. A sample of the solution is taken, filtered and dilutes 1:10 in SO_4H_2 solution 0.5 % (V:V) to stop any other reaction and the metal used as tracer is analyzed by atomic absorption.

Results

Solutions II coming from different organic wastes are of very different colors suggesting the great differences between the organic compounds contained in each one. To obtain the specific enrichment microbial cultures for each organic waste, assays were carried out by duplication in flasks of 250 ml. To ensure not obtaining false negatives by defect of nutrients, each culture media was prepared with the corresponding organic solution obtained with the maximum possible concentration, that is, with the minimum relation water volume to waste weight in each case. For flock and refuse of grapes such relation is 1:1. For stock of grapes the relation is 3:1. Due to the dense liquor obtained from dray leaves and the water absorbed by them, the relation was 20:1 and 10:1. In all the cases each solution II, including milk whey, was added in the quantity of 5 ml of solution II for each 100 ml of Solution I.

For each culture media prepared with each waste, assays were carried out in parallel with ammonia nitrogen (+ NH_4^+) and without ammonia nitrogen (- NH_4^+).

Table 1 shows the obtaining of enrichment cultures (+: positive; -: negative) selected by their capacity of develop using the organic compounds contained in each waste or residue.

Table 1 *Enrichment cultures (+: positive; - : negative) selected by their capacity of development from the organic compounds contained in each waste.*

Organic waste	**+ NH_4^+**	**- NH_4^+**
Flock	+	+
Refuse of grapes	-	-
Stock of grapes	+	+
Milk whey	-	-
Dry leaves	+	+

Many variations were introduced in the solution I composition to obtain positive bacterial development utilizing the organic compounds coming from refuse

(seeds) of grapes, but it was not possible at any condition. It suggests that most of the organic compounds contained in the seeds of grapes are not soluble or diffusible to the liquid phase. It is also suggested by the transparency of solution II obtained from refuse of grapes.

Table 2 associates the sulphate reducing bacteria development to the concentration of the organic compounds given by the relation water volume to waste weight for the wine industry wastes: flock, refuse of grapes and stock of grapes. For all the assays it was added 3,5 ml of the corresponding solution II by each 100 ml of solution I, with and without the addition of ammonia nitrogen. It is also possible to obtain only one concentrated solution II for each waste and carry out the assays varying the volume of solution II added to each assay. For example: 1, 2, 3, 4 and 5 ml of Solution II by each 100 ml of Solution I.

Table 2 *Microbial development and formation of a strong black precipitate (+ : positive; - : negative) in function of the nutrients concentration given by the relation volume of water to waste weight for wine industry wastes, with and without the addition of metal sulfide*

Organic waste	Water Volume	Solution I	
	Waste Weight	+ NH4+	- NH4+
Flock	1:1	+	+
	2:1	+	+
	3:1	+	+
	4:1	-	-
	5:1	-	-
Refuse of grapes	1:1	-	-
	2:1	-	-
	3:1	-	-
	4:1	-	-
	5:1	-	-
Stock of grapes	3:1	+	+
	4:1	+	+
	5:1	-	-

Microscopic observations and microphotography´s of floating solutions samples taken from the assays of Table II shown the microbial development is in accordance with the formation of the black precipitate. In the microscopic observations of tests solutions with metallic precipitation negative (-), microbial development was not observed. It is expectable due to the medium was not sterilized, but it was not observed the typically dense microbial development as the observed in assays with positive black precipitate formation carried out with the organic compounds obtained from flock and stock of grapes respectively .

Different variations were introduced in solution I composition to obtain positive bacterial development utilizing the organic compounds contained in milk whey, but all of them gave negative results. Taking into account that the development and activity of some anaerobic sulphate reducing bacteria depends on the medium salinity, requiring a relative salinity to develop (Posgate 1984; Jorgensen and Bak

1991). For such cases Jorgensen proposed the addition of 1.5 % V:V of NaCl. For such reason 15 g of NaCl was added to solution I and assays were carried out with and without the addition of a nitrogen source. In both cases positive development of sulphate reducing bacteria was obtained in 24-48 hours associated to the typical strong black precipitate. Microscopic observations showed in both cases only one bacterial morphology, a vibrio. Such morphology associated to the requirement of a saline medium suggests that is a microorganism like Desulfovibrio salexigenes. The addition or not of ammonia didn't introduce significant differences about the development of mix cultures selected by their capacity to grow utilizing the specific compounds coming from each waste.

Negative results in treatment systems using the sulphate reducing bacteria activity have been related to the possible toxic effect of the metals involved in the effluent. So, an important highly toxic ion as Hg^{++} was used as the tracer metal to analyze the possibility of obtaining enrichment cultures development selected by their capacity of sulphate reduction using the specific organic compounds coming from flock and milk whey, but furthermore being resistant to mercury at a Hg^{++} concentration 5×10^{-4} M.

The assays with solution II coming from flock (water Volume : flock Weight = 1 : 1) were prepared by adding 3 ml of this solution II, by each 97 ml of Solution I. Solution III constituted by a solution 0.1 M of HgCl was added in the relation of 0.5 ml by each 100 ml of the mixed solutions I and II. The assays with milk whey were prepared in the same way but using the saline solution II (15 g of NaCl per liter of solution II. Once prepared the final medium for each test, pH was adjusted to around 7.0. Only in this case the assays were inoculated with 100 micro liters of a stagnant water sample to amplify the diversity of the original microbial community from which the specific search microbial culture could develop. Once closed and sealed the assays were incubated at 20 °C. After 2-3 days the began the formation of the black precipitate for both organic wastes used meaning there are in nature sulphate reducing bacteria resistant to Hg^{++}. Others organic wastes have not yet been proved to precipitate Hg^{++}.

Table 3 presents the results of five assays conducted using organic milk whey compounds as nutrients, 3% (V:V), and varying the quantity of Fe^{++} added, therefore changing the initial Eh. It grows from the above-mentioned table that to a potential around or below – 190, both microbial development of sulphate-reducing anaerobic bacteria and precipitation of iron sulphide are possible. It shows that both phenomena, biological and chemical, are Eh dependent.

To analyze the sulphate reducing bacteria development utilizing the organic compounds contained in dry leaves, it was used the dry leaves coming from two trees: dry leaves of the blackberry tree and dry leaves of plane tree, being both the main trees in San Juan city. Two relations water volume to dry leaves weight were tested, 20:1 and 10:1. In the first instance it was added 5 ml of each one of these solutions II, by each 100 ml of solutions I. Different variations were introduced in solution I composition including the use of tap water as solution I. All the assays resulted in positive development and the formation of the black precipitate as it is shown in figure 2, indicating dry leaves contain suitable compounds as carbon source, but also nitrogen compounds and other elements necessary for the

microbial development. Assays in the same conditions with the organic compounds coming from dry leaves of both trees, gave the same results. It shows dry leaves contain all the nutrients required for sulphate reducing bacteria development. Furthermore, the trees foliation is abundant and renovated each year being an inexhaustible source of bacterial nutrients.

Figure 1 *Photography showing the strong black precipitate that is produced associated to sulphate-reducing bacteria development.*

Figure 2 *Photography showing a series of tests varying different parameters and utilyzing the labile organic compounds contained in dry leaves.*

Assays were carried out using as solution II, the liquor obtained from dry leaves of blackberry tree in a relation water volume to waste weight water 20:1. The volume added by each 100 ml of solution I was variable: 0.5, 1.0, 2.0, 3.0, 4.0, 5.0 and 7.0. Solution I composition was only $MgSO_4.7H_2O$, 0.5 g per liter of tap water. Solution III was added in the proportion of 1 ml by each 100 ml of Solution I. Sulphate reducing bacteria development associated to the formation of the black precipitate was obtained in the assays with the addition of 4.0, 5.0 and 7.0 ml of solution II, being negative with less nutrients concentration.

Table 3 *Microbial development and precipitation of iron sulphide by anaerobic sulphate reducing bacteria depending on the initial Eh using the organic compounds of milk whey as nutrients*

Test N°	Sol. III (Fe++) % (V:V)	Initial pH	Initial Eh (mV)	Final pH	Microbial development	Iron sulphide Precipitation
I	0.5	7.580	- 135	7.582	-	-
II	1	7.558	-189.2	7.803	+	-
III	1.33	7.443	- 219.5	8.722	+	+
IV	1.66	7.537	- 237.5	8.855	+	+
V	2	7.437	- 255.9	8.750	+	+

Discussion

To carry out this type of evaluation methods testing the organic labile compounds coming from some solid waste or residue, but once they diffuse and are separated from the solid waste, allows to evaluate conditions for sulphate reducing bacteria development and metals sulphide precipitation independently of the others metals removal mechanisms such as sorption to the residue mass. Also allows to visualize the metals sulphide precipitation in a more clear manner. Not any organic waste or residue is suitable for sulphate reducing bacteria development. Between the organic material we tested refuse of grapes, which didn't allow to obtain at least one positive development at any of a wide range of tested conditions, indicating most of the organic compounds contained in the seeds of grapes are not soluble or diffusible to the liquid phase.

Milk whey seems not to be an organic waste that may be used in a wide range of conditions. The microbial culture capable of growing from the milk whey compounds requires a saline medium. So, it is like only the effluents containing a relative salinity given specifically by NaCl, could be treated using milk whey. The assays carried out with flock, stock of grapes and dry leaves have shown to be adequate sources of organic compounds for sulphate reducing bacteria. Dry leaves have the advantage of being widely available and an inexhaustible source of nutrients renewed every year.

Conclusions

To use the general proposed method for analyzing a particular effluent and a particular organic waste, solutions I and III should be replaced by an effluent sample and solution II of organic labile compounds should be obtained as it was explained from the particular waste. The assays should be incubated at a temperature representing the middle temperature or the temperature in winter and in summer of the place were the effluent should be treated. To carry out this type of test plans must allow to get answers and quantified information about:

- Are the particular waste organic labile compounds suitable for sulphate reducing bacteria development or some group of this type of bacteria?
- Is it possible to obtain a bacteria strain or bacterial community capable of growth from the organic waste labile compounds, but furthermore with capacity of adapting to the particular physic-chemical conditions of the effluent (as the metals involved)?

- Which are in the first instance the quantified relation between labile compounds or waste weight to neutralize the effluent and precipitate the metals contained in the effluent?
- Which is the particular double pH-Eh to be reached to developed sulphate reducing bacteria precipitating the particular metals involved as metals sulphides?

Answers to the prior questions should allow beginning an active or passive system design based on quantified observations which are particular for each effluent, each organic waste and the ambient temperature of each particular place.

In general, in the developed and applied active treatment systems (continuous anaerobic bioreactors), as organic source compounds such as methanol have been used, with the corresponding associated costs. We are proposing to use the liquors containing the labile organic compounds coming from wastes or residues without economical value.

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