Chapter 9 *Staphylococcus carnosus* study as an alternative bio-collector for metal minerals

Capítulo 9 Estudio de *Staphylococcus carnosus* como un bio-colector alternativos para minerales metálicos

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Abstract

Biotechnology has been explored as a potential low cost, environmentally benign alternative to many of the current mineral processing techniques. Recent investigations have shown that selected bacteria may also assist in the beneficiation of these minerals through bioflotation bioflocculation. Bioflotation represents an innovative in the minerals benefit process, where the bacteria are generally used as a collector avoiding the use of conventional reagents. The aim of this study was to evaluate the use of *Staphylococcus Carnosus* as bio-reagent in the flotation process of sulfides such as galena (PbS), pyrite (FeS_2) and chalcopyrite ($CuFeS_2$). To evaluate the bacterial influence on minerals floatability Hallimond flotation test was carried out. The absorption zeta potential and adhesion measurements were useed to determine the adhesion of the bacteria from each mineral. The assays were carried out with and without bacteria. The results showed that *S. Carnosus* has a hydrophobic behavior and different affinity grade to sulfides mineral substrates. This interaction allowed the bacteria to act as a collector. The biomodified sulfides show the following floatability in decreasing order: galena>chalcopyrite>pyrite. These differences point out the possibility of future application of *S. carnosus* in selective separation of sulfide minerals to depress the gangue type ores (pyrite among others).

Biotechnology, Bioflotation, Hydrophobic, Alternative, Processing

Resumen

La biotecnología ha sido explorada como una alternativa potencial de bajo costo y benigna para el medio ambiente a muchas de las técnicas actuales de procesamiento de minerales. Investigaciones recientes han demostrado que bacterias también pueden ayudar en el beneficio de estos minerales a través de la biofloculación de bioflotación. La bioflotación representa un proceso innovador en beneficio de los minerales. Donde las bacterias se utilizan generalmente como un colector evitando el uso de reactivos convencionales. El objetivo de este estudio fue evaluar el uso de *Staphylococcus Carnosus* como agente biológico en el proceso de flotación de sulfuros como galena (PbS), pirita (FeS_2) y calcopirita ($CuFeS_2$). Para evaluar la influencia de las bacterias en la flotabilidad de minerales, la prueba de flotación de Hallimond fue llevado a cabo. Las mediciones de absorción del potencial zeta y de adhesión se utilizaron para determinar la adhesión de las bacterias de cada mineral. Las experiencias se llevaron a cabo con y sin bacterias. Los resultados mostraron que la bacteria *S. Carnosus* tiene un comportamiento hidrofóbico y un grado de afinidad diferente a los sustratos minerales sulfurosos. Esta interacción permitió que las bacterias actuaran como colectoras. Los sulfuros biomodificados muestran la siguiente flotabilidad en orden decreciente: galena> calcopirita> pirita. Estas diferencias señalan la posibilidad de una futura aplicación de *S. carnosus* en la separación selectiva de minerales de sulfuro para deprimir los minerales de tipo ganga (pirita, entre otros).

Biotecnología, Bioflotación, Hidrofóbico, Alternativa, Procedimiento

9.1 Introduction

Today, Mexico continues to stand out as one of the world's largest producers of different minerals such as: copper, bismuth, fluorite, celestite, wollastonite, cadmium, molybdenum, lead, zinc, diatomite, barite, graphite, gypsum, gold and silver. Being the latter who occupies the first place of productive demand worldwide.

However, today mining companies are required to comply with newer and more stringent environmental legislation in order to ensure that exploration and mining activities have a reduced impact on the environment.

A very promising alternative with a lower environmental impact that requires less infrastructure and resources than traditional technologies is the biomining process.

Biomining comprises a series of microbiological processes that can be used for the extraction and recovery of metals from very low-grade minerals. And this process can be applied in three different areas of mining activity, such as: Bioflotation, Biolleaching and Biooxidation.

9.1.1. Bioflotation

The gradual depletion of high-grade deposits makes interesting the benefit of complex sulfides. Thus, it is important the development of better flotation schemes in order to beneficiate and process complex ores, which are represented by polymetallic sulfide associations. Along with the industrial necessity to develop new technologies for the processing of ores, the design of methods, which accomplish to strict environmental regulations is unavoidable. (Pecina *et al*., 2009).

In Mexico, the use of these biological reagents as bacteria and their manipulation for their selective adhesion on mineral surfaces are relatively important aspects for mineral beneficiation. Considering that there are serious concerns regarding the availability of these mineral elements in the future due to their low abundance, complexity or low-grade ores and difficult access, viable alternative methods are required.

Traditionally, the benefit of low-grade sulfides ores has been carried out through the conventional flotation process. Which employ highly selective inorganic modifiers, such as cyanides, sulfides and ferro-cyanides (Bradshaw *et al*., 1998). Despite the many advantages of flotation process, the hazardous chemicals used limits the development of this process and other minerals.

Nowadays the industry is trying to develop environmentally friendly technology for processing ores, different from traditional separation methods. Many previous attempts have been made to replace hazardous materials in the flotation processes, using environmentally friendly reagents, instead of harmful chemicals. The biotechnology has opened up possibilities for the utilization of microorganisms in mineral beneficiation as flotation collectors.

The use of microorganisms as a bio-collector could be an alternative in mineral flotation. This process offers various advantages such as, as lower operating costs in the processing of low grade ores, mineral selectivity in the processing of fine and ultrafine mineral particles and to the constant quest of reagents that attends the rigorous specifications for production of concentrates and stricter environmental legislation (Gericke and Govender, 2011; Lopez *et al*., 2015; Ramos-Escobedo *et al*., 2016).

Bioflotation can be used as an alternative to the conventional process and consists of the selective separation of commercial minerals (Cu, Pb, etc.) from the gangue (pyrite, etc.) through microorganisms interactions (Deo and Natarajan., 1997). Where the adhesion is governed by physicochemical interactions (most likely electrostatic interactions) (Botero *et al*., 2008, de Mesquita *et al*., 2003); Dwyer *et al*., 2012; Merma *et al*., 2013).

Generally, the three mechanisms used during biomodifaction of minerals surface are: a) the adhesion of hydrophilic bacteria to the mineral substrate; b) the oxidation that directly or indirectly, generates the biomodifaction of sulfur; and c) adsorption or chemical reaction with metabolic products on the substrate (Rao *et al*., 2010).

Some microorganisms such as Acidithiobacillus ferrooxidans, Leptospirillum ferrooxidans, Polymyxa Paenibacillus, Acidithiobacillus thiooxidans, among others (Ramos Escobedo *et al*., 2012, Nagaoka *et al*., 1999, Subrahmalannian *et al*., 2003, Pecina *et al*., 2009, 2010; Santhiya *et al*., 2001a, 2001b, 2001c, 2002) were reported for sulfidic ores benefit.

In the case of bioflotation of sulfides minerals, the most common minerals and some non-metallic minerals systems employed the bacteria Acidithiobacillus ferrooxidans and Acidithiobacillus thiooxidans, reaching a successfully separation of sphalerite (ZnS)-galena (PbS). Sphalerite without promoter/collector is selectively floated from lead ores, especially as galena is oxidized (PbS to PbSO4), highlighting the importance of parallel single bacterial adhesion (Santhiya *et al*., 2001). While for pyrite and arsenopyrite systems, using xanthate as a collector emphasizes that Acidithiobacillus ferrooxidans acts as a pyrite depressant.

This reaction is established because bacteria have greater affinity for iron-rich substrates, showing a descending effectiveness of the next minerals: pyrite>galena≈millerite> molibdenite≈calcocite. The tests were performed at pH 2 without collector (Nagaoka *et al*., 1999). The depressant effect is interpreted in terms of surface free energy decrease that weakens contact with the Xanthate. Moreover, Vilinska, (2007) mention that bacteria, achieve superficial chemical changes and could be manipulated for specific purposes in flotation, depending on the concentration and composition of medium culture, as well as bacteria adaptation to a mineral type.

Possible interaction mechanisms based on the interaction of carbohydrate metabolite/galena to acid / base model are set based using xanthate as a collector. (Subrahmanian *et al*., 2003). The depressant effect is interpreted according to the decrease in surface free energy diminishing the contact with the xanthate. The advantage of bioflotation is that bacteria can have the same functions as conventional reagents and provides specificity for different type of minerals. The aim of this study was to evaluate the use of S. carnosus as froth flotation reagent for sulfide minerals (galena (PbS), pyrite (FeS_2), chalcopyrite ($CuFeS_2$)).

9.1.2. *Staphylococcus carnosus* description

9.1.2.1. Characteristics and taxonomy

Staphylococcus carnosus is a unicellular non-pathogenic, Gram-positive bacteria. It comes of the *Staphylococacceae* family. Schleifer and Fischer (1982) describe *Staphylococcus carnosus* morphologically as cocci from 0.5 to 1.5 μm in diameter, presented mainly in pairs or individually. *Staphylococcus carnosus* is a facultative aerobic microorganism may develop NaOH concentrations more than 15%, with the ability to reduce nitrates and produce acetoin. In addition, these micro-organism has polysaccharides, carboxylic acids, and lipid groups (Ramos-Escobedo *et al.*, 2016). The peptidoglycan represents the major component of the cell wall (50-80 wt%) (Madigan *et al*., 2004)

Exist very few studies on the use of *S. carnosus* as a biocollector in the mineral processing research.). The aim the purpose of this investigation is to evaluate the *S. carnosus* floatability in systems of sulfide minerals of commercial interest (chalcopyrite and galena) and gangue type (pyrite, etc.) under similar conditions used in chemical flotation. The importance of this investigation is to find an alternative flotation bio-reagent applied to sulfidic ores.

Figure 9.1 Growth curve of *S. carnosus* bacteria

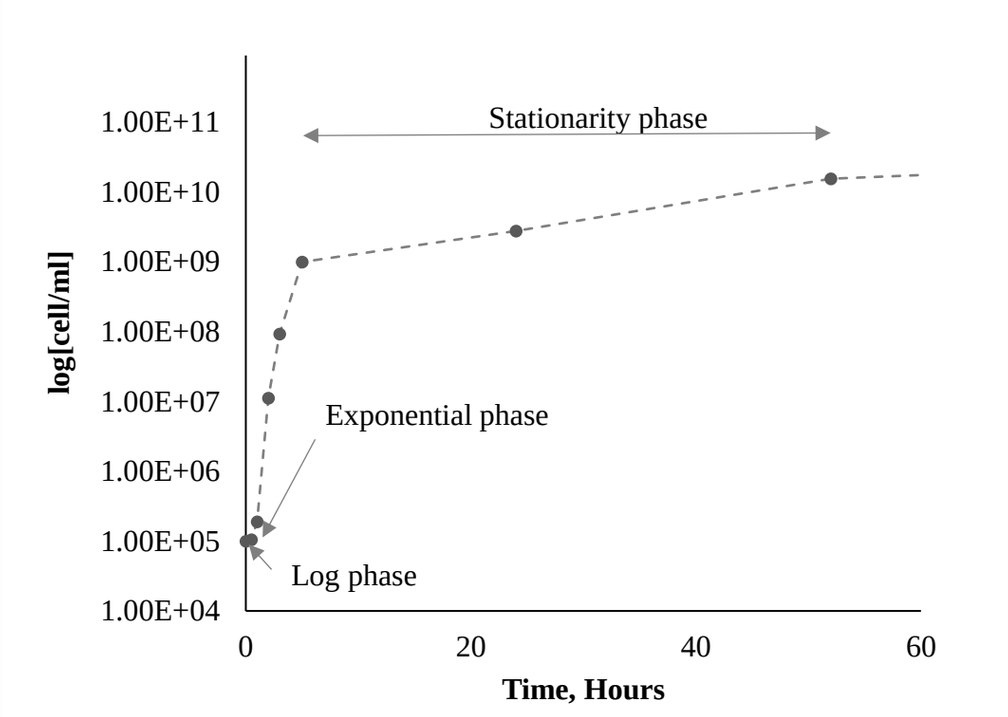

Figure 9.2 Hydrophobicity of the bacteria as a function of pH

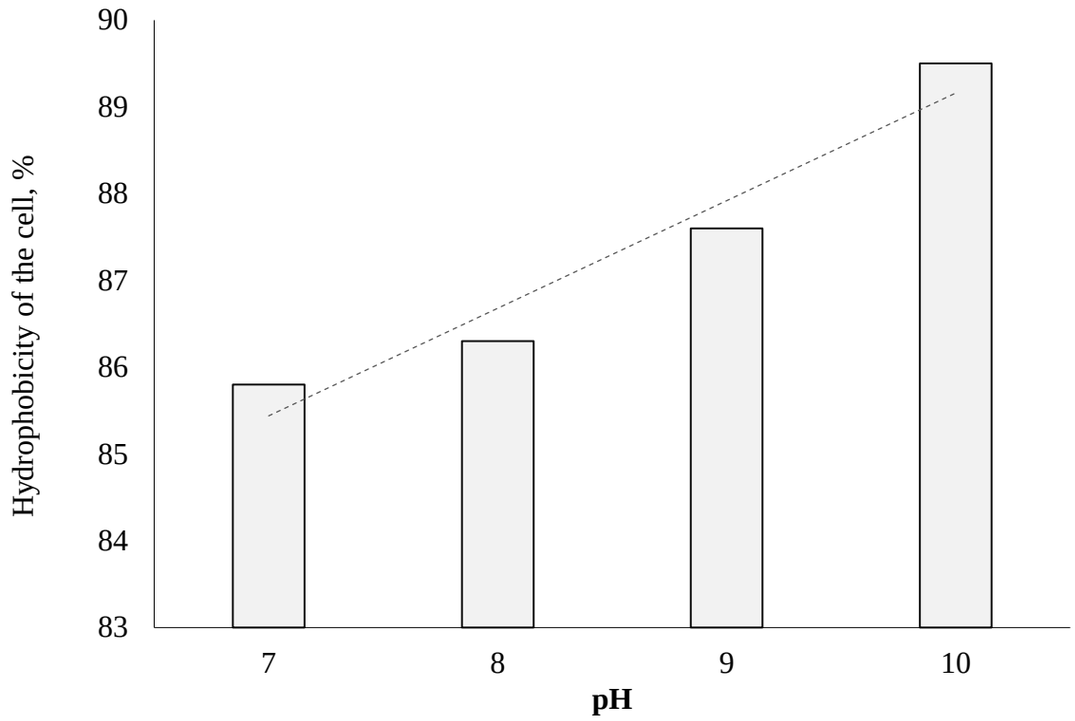

Figure 9.3 Effect of the culture period the bacteria in the floatability of the chalcopyrite with fresh bacteria (culture medium) and washed bacteria (without culture media)

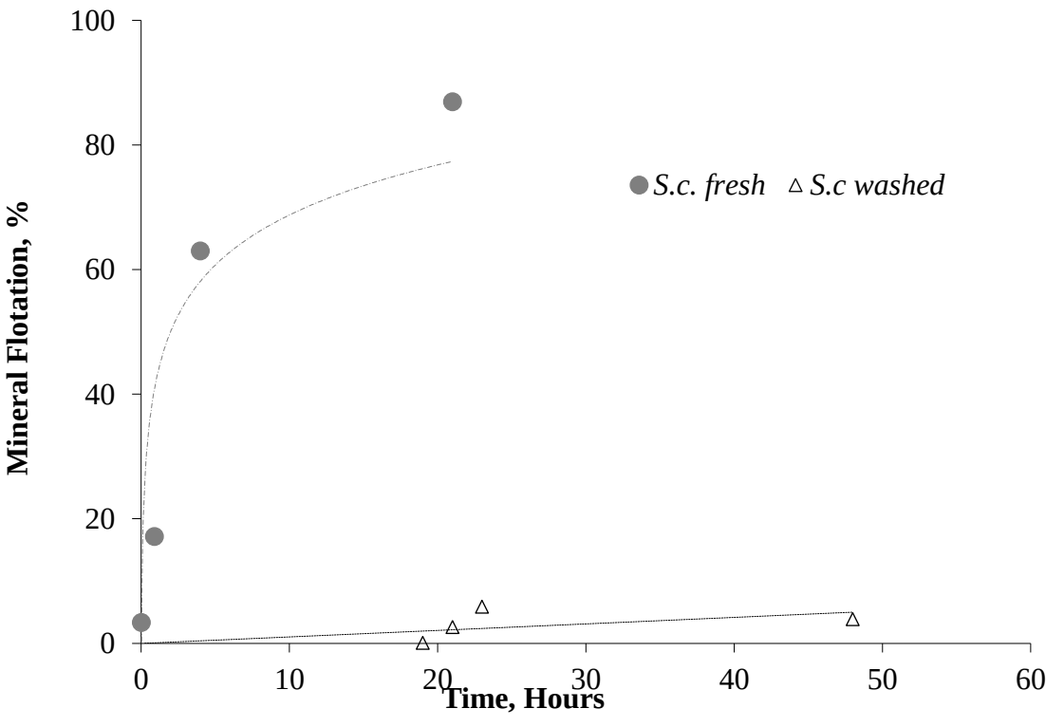

Figure 9.4 Effect of the floatability of *S. carnosus* + $CuFeS_2$ bacteria with culture medium and without culture medium at pH 9

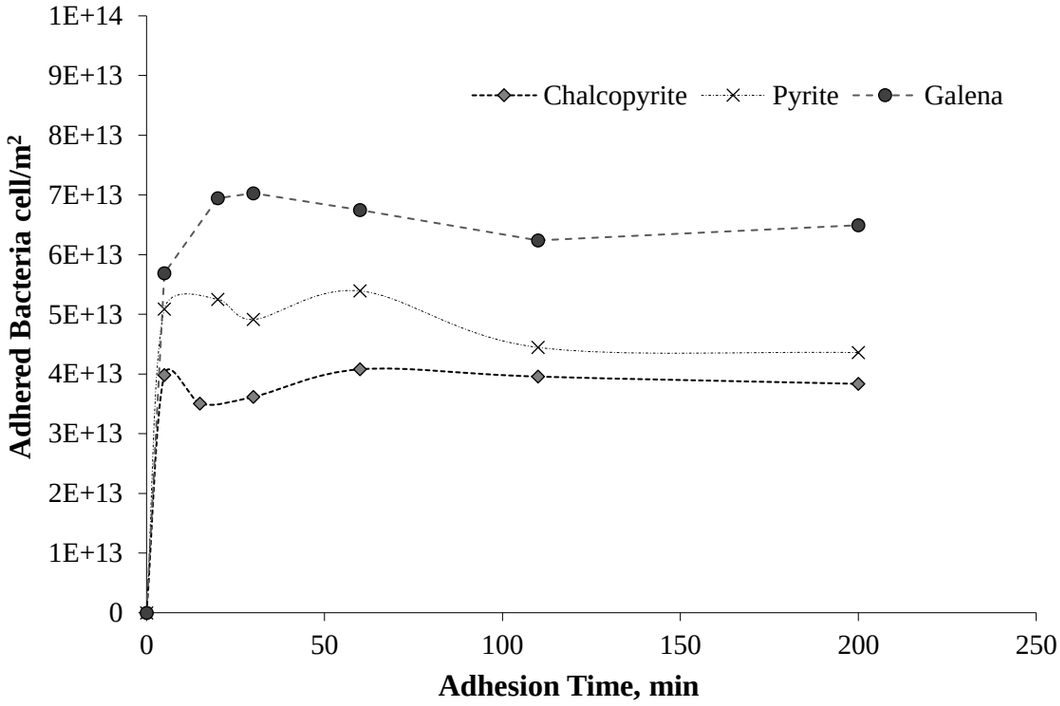

Figure 9.5 Adhesion isotherms of *S. carnosus* bacteria with minerals of interest at pH 9

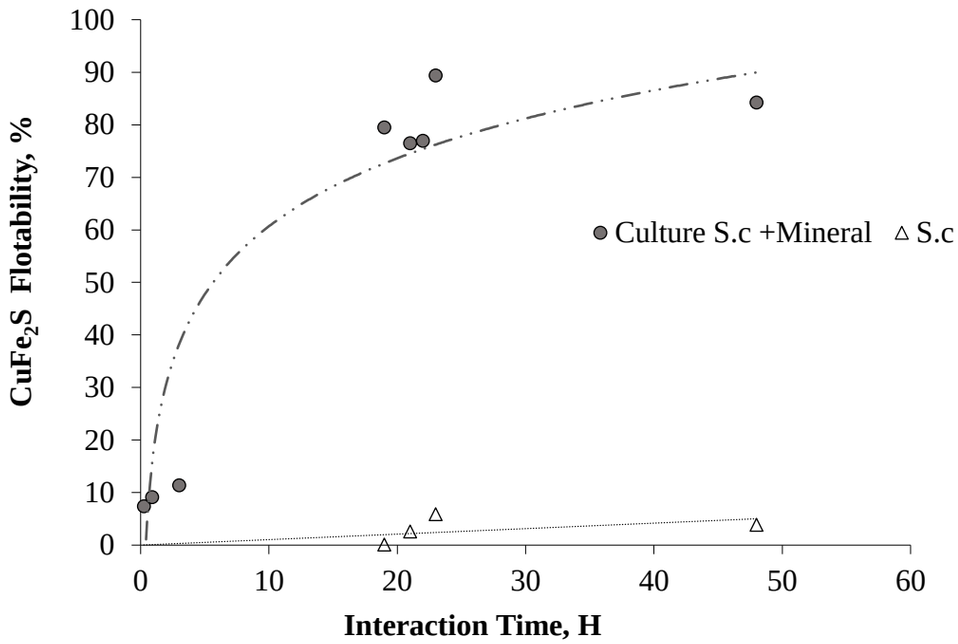

Figure 9.6 Zeta potential of *S. carnosus* bacteria (control) depending on: a) pH and b) Time (H)

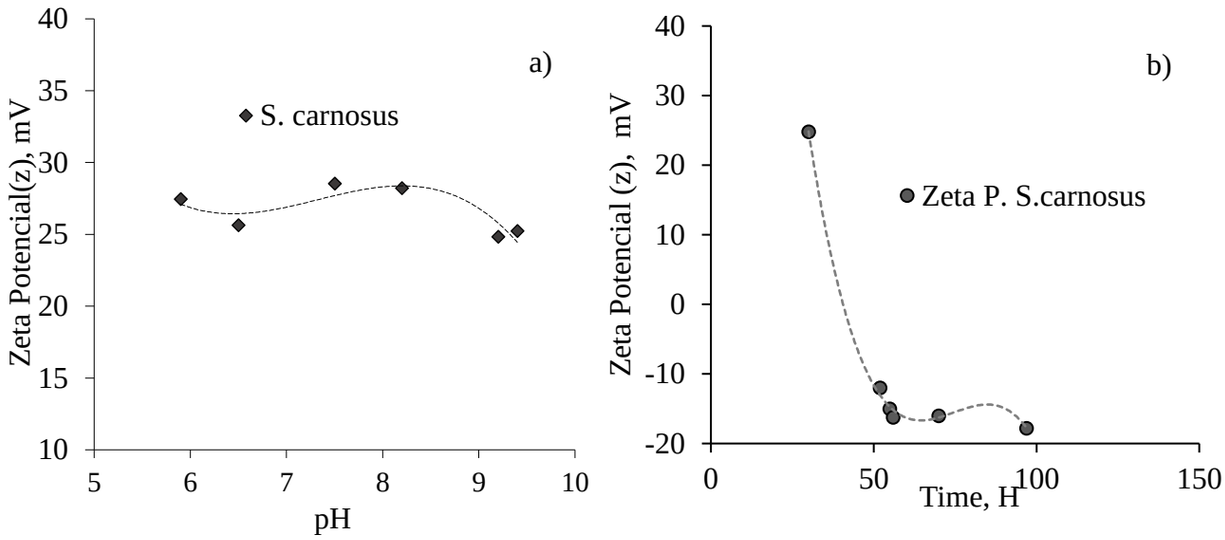

Figure 9.7 Zeta potential of S. *carnosus* bacteria with the mineral of interest. a) Before the interaction and b) after the interaction

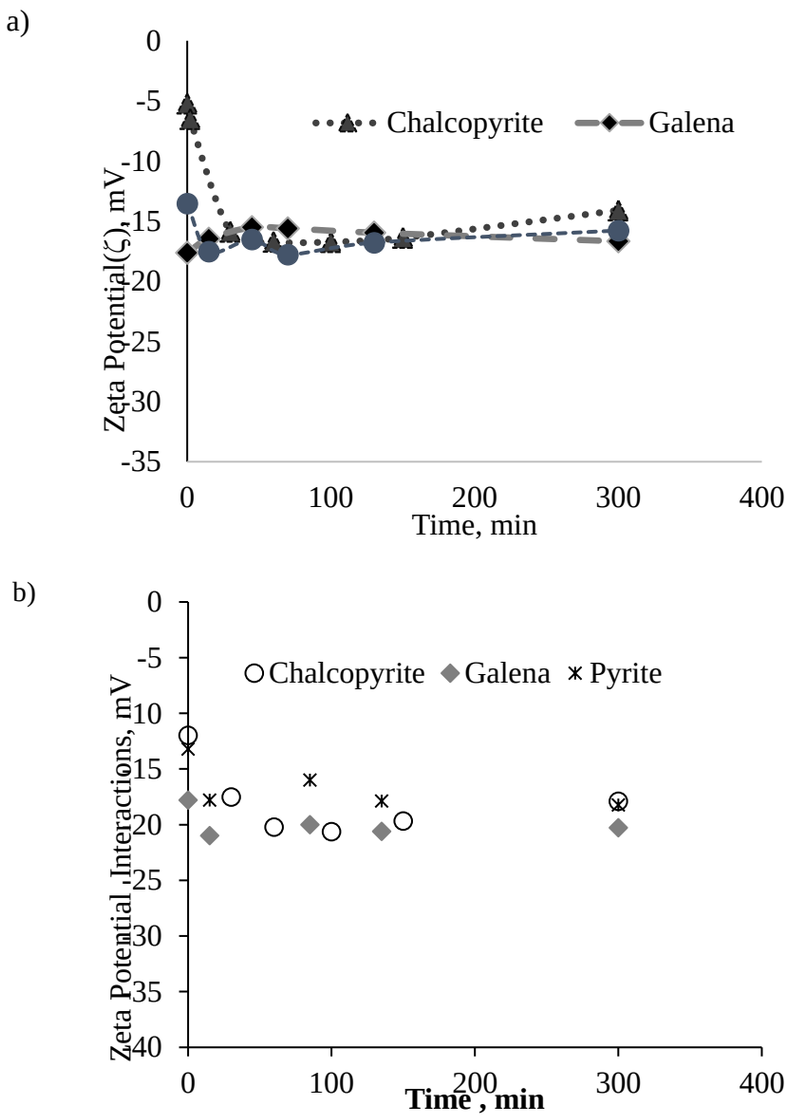

Figure 9.8 Floatability of the natural mineral at pH 9. Minerals conditioned in a 1 x 10^{-3} M solution of $NaNO_3$ at pH 9, in the absence of bacteria

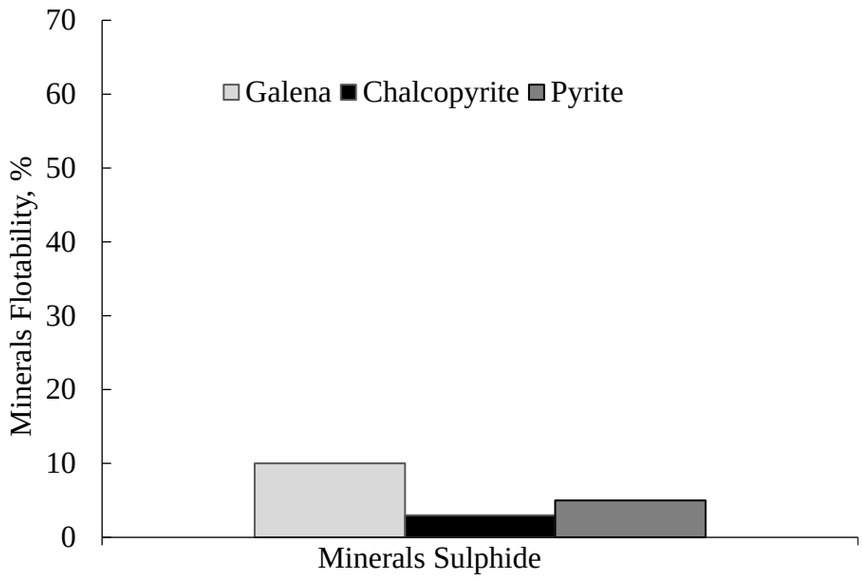

Figure 9.9 Floatability of bio-modified minerals. After interaction with *S. carnosus* bacteria at a pH of 9

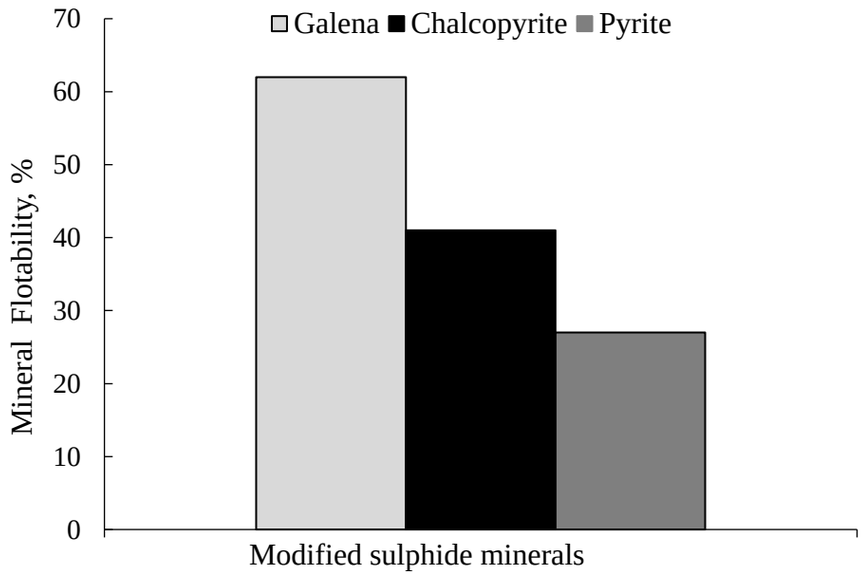

Table 9.1 Chemical analysis of minerals

Minerals	Formula	Cu%	Zn%	Fe%	S%	Pb%	Purity %
Chalcopyrite	$CuFeS_2$	31.24	0.22	32.58	30.45	0.01	90
Galena(+)	PbS	0.009	0.157	0.135	12.39	73.01	85
Pyrite(+)	FeS_2	0.002	0.004	40.42	47.439	0.006	88
(+) Associated gangue silicates.							

Table 9.2 Surface area of the mineral fraction used

Minerals	Surface area, m^2/g
Chalcopyrite	0.0109
Pyrite	0.00874
Galena	0.00673

9.2 Methodology

9.2.1 Reagents and minerals

Three pure mineral samples of chalcopyrite ($CuFeS_2$), pyrite (FeS_2) were obtained from Santa Eulalia, Chihuahua, while the galena (PbS) was attained from the Plomosas Mine at Chihuahua. The chemical compositions of the chalcopyrite, pyrite and galena used during the microflotation tests are presented in Table 9.1. Other chemical reagents used for this test were distilled water was used to prepare the solutions and n-hexadecane was used as foaming agent.

9.2.2 Microorganism

The bacteria used was *Staphylococcus carnosus* ATCC No. 51365 strain. Bacteria was cultured in tryptone soy broth with yeast extract (ATCC Medium No 1887). The culture media consisted of 30 g tryptone and 3 g yeast, which were diluted in 1 L of distilled water and the pH was adjusted to 7. The medium was autoclaved (Model No 25 x All American Autoclave) for 15 min. After annealing the culture, broth was inoculated with the lyophilized bacterium *Staphylococcus carnosus* (ATCC 51365) and then incubated for 24 h at 37 ° C. (Ramos-Escobedo *et al.*, 2016).

9.2.3 Growth Kinetics

The kinetics of bacteria growth was conducted by counting the microorganisms in the culture medium at different times in a period between 0 to 60 h, using the Neubauer chamber (Neubauer Reichert) and phase contrast microscopy (Axioskop 40, ZEISS). Cell counting was performed in 14.5 μl of sample.

9.2.4 Preparation of minerals

The crystals were crushed in porcelain mortar and then wet sieved using -100 + 200 mesh, -200 + 400, and -400. The fraction between -100 +200 mesh was separated for flotation test. A sample of 5 g of each mineral (-400 mesh) was analyzed by atomic absorption spectroscopy (AAS) to determine its chemical composition (Table 9.1).

9.2.5 Bacterial adhesion

For bacterial adhesion test, 1 g of each mineral was collected in 50 ml of fresh bacterial culture, over a period of 5 to 1440 min. The initial concentration of bacteria at time t was determined by direct counting in a Neubauer chamber. The amount of attached bacteria was determined by Eq. (1):

$$B_{Ad} = \frac{(B_0 - B)V}{wA_m} \tag{1}$$

Where: B_{Ad} is adhering bacteria (cell/m^2); B_0 and B are the concentration of free bacteria at zero time and t, respectively (cell/ml); V is the volume of sample in ml; w is the exact weight of mineral in grams, A_m is the surface area determined by Coulter counter mineral (Table 9.2).

9.2.6 Zeta potential

Prior to the measurement of zeta potential, microorganisms were washed to eliminate the culture medium used in culture of bacteria. To accomplish this, a solution of $NaNO_3$ at pH 7 was prepared and the sample was centrifuged for 5 min at 2,000 rpm, the supernatant was pulled, and the precipitate was washed with the appropriate solution (pH 6, 7, 8, 9). Then, the samples were shaken in a Vortex to re-suspend the bacteria, which were placed in test tubes and refrigerated. After that, samples of 20 ml of the microorganisms in suspension were analyzed for zeta potential in the equipment Zetaphoremeter IV (CAD Instruments). The effect of the mineral-bacterium interaction was determined by contacting 5 g of each mineral (38 μm) in 250 ml of fresh culture. At a given time (0-1440 min), a sample of 3 ml was centrifuged at 1000 rpm for 5 min to remove bacteria and ore. Subsequently, they were separately suspended in 30 ml of 10^{-3} M $NaNO_3$ at pH 9 and its zeta potential was measured.

9.2.7 M.A.T.H. (Microbial adhesion to hydrocarbons)

Hydrophobicity was measured by MATH, for which, 1 ml of suspended bacteria and 0.16 ml of solvent n-hexadecane, were mixed in a vortex for 20, 45, 60 and 90 seconds. Tests were performed at pH 7. The concentration of bacteria was recorded as a function of conditioning time by counting microorganisms in the aqueous phase using a Neubauer chamber. (Ramos-Escobedo *et al.*, 2016).

9.2.8 Microflotation

Microflotation tests were performed using a Partridge microcell (Partridge and Smith, 1972), which is superimposed on a magnetic stirring grid. For this purpose, the sample was conditioned before floatation, using a 25 ml beaker with 25 ml of sample (culture medium with bacteria in suspension) at different pH values (6, 7, 8, 9) and 1 g of the mineral of interest, (chalcopyrite, pyrite, galena and). The mixture was conditioned for 24 h at 160 rpm.

During flotation, the mixture bacteria / mineral was poured in the flotation cell, which was regulated at a nitrogen pressure of 60 psi and flow rate of 60 ml/min. After solution was added to the respective pH to the sample to reach the volume optimal for flotation. Flotation was carried out for two min. The not floated and floated minerals, were filtered, dried and floatability was calculated according to Eq. (2)

$$\%\ Mineral\ flotability = \frac{C}{A}\ \text{X}\ 100 \qquad (2)$$

Where: A is the weight fed in grams of ore and C is the weight in grams of concentrated ore (float).

9.3 Results and discussion

9.3.1 Growth phases and kinetic of *S. carnosus*

The kinetics of *S. carnosus* growth is presented in Figure 9.1, which shows the bacterial concentration versus incubation time. This figure shows how the exponential phase starts at 2 h and ends at about 6 h. According to the results, it was determined that the optimal incubation time for the bacterium was about 20 h, as the bacteria concentration remained practically constant for larges times.

The concentration of the bacteria was determined using the optical density method in a UV-1800 Shimadzu spectrophotometer, at a wavelength of 620 nm. Each of the experimental tests was done in triplicate.

In the Figure 9.2 can be observed and the concentration of the bacterium of *Staphylococcus carnosus* with respect to pH.

9.3.2 Hydrophobicity of *S. carnosus*

The hydrophobicity of bacteria was determined by affinity of *S. carnosus* to n-hexadecane (nonpolar solvent) (Ramos-Escobedo *et al., 2016).* The results are presented in Figure 9.2, showing a high percentage of bacteria removal from aqueous phase, obtaining values from 87.5 to 89.5%, noting a slight increase as more alkaline is the pH. This indicates that the surface of *S. carnosus* has hydrophobic characteristics, which are accentuated in alkaline conditions, property suitable for conventional flotation of sulfides minerals.

Key features of great importance for using *S. carnosus* in this research are the hydrophobic characteristics of bacteria, which are necessary for flotation. Therefore, it may function as a collector in sulfides systems, resulted by the adherence of bacteria to the ore, hydrophobic properties. On this aspect, Langwaldt and Kalapudas, 2007 reported the application of *S. carnosus* in the nickel concentration of shales by flotation. No report has documented the use of *S. carnosus* in sulfides systems (Thewes *et al., 2014).*

9.3.3 Effect of *S. carnosus* in the preparation time of bacteria and in the presence of a hydrophobic medium

In Figure 9.3 the test results for chalcopyrite floatability are presented, respect to subsequent washout observed using fresh bacteria (wash and application of bacteria) and washed bacteria after 48 h (bacteria stale). Results indicate that fresh bacteria remains hydrophobic causing chalcopyrite recoveries up to 80% after an interaction period of 10 h. Ore floated with washed bacteria after 48 h is not significant, which shows that the surface of the bacteria undergoes changes that decrease the hydrophobicity due to the separation of the cells from the medium, i.e. absence of nutrients generated by means culture in which the microorganism was cultured.

The floatability of the chalcopyrite generates information on the hydrophobic effect of *S. carnosus,* an increase in the mineral floatability can be interpreted as optimizing the hydrophobic effect of the mineral-bacterium interaction.

The change in the hydrophobic surface of *S. carnosus* is unknown. Moreover, whereas in an industrial system conditioning chemical collectors is relatively fast (minutes order), the results with *S. carnosus* indicate that biomodification of the mineral surface requires a very long period to generate optimal conditions flotation.

9.3.4 Effect of culture on the floatability of minerals

In Figure 9.4, the culture medium effect on the biomodification and the percentage of chalcopyrite floatability is presented. The results of the curve sharper were performed using bacteria suspensions in culture medium. The percentage of chalcopyrite floatability increased, reaching 86% with the culture medium. Less favorable results were observed using washed bacteria, preserved in solution at pH 9, which significantly reduces its ability to float chalcopyrite, that in the absence of nutrients during experimentation. Based on these results, it was determined carrying out the experimentation using bacterial culture, since the results show that the bacteria retain their hydrophobicity and achieve longer adhesion to mineral generating good floatability.

9.3.5 Characterization of the adhesion of *S. carnosus*

The rate of bacterial adhesion of *S. carnosus* on various sulfide ores were determined by adhesion isotherms as shown in Figure 9.5. The adhesion of *S. carnosus* is almost completed in the first 5 min of the bacteria-mineral interaction by chalcopyrite and pyrite. While in the case of galena the adhesion of the bacteria is reached after of 20 min. Adhesion speed can be represented as *galena >> pyrite> chalcopyrite*

The results suggest that the adhesion of the bacteria is favored in the galena mineral. Because the galena has more adhesion bacterias at pH alkaline (Patra *et al.*, 2008). Followed by a lower adhesion to iron containing minerals ($CuFeS_2$, FeS, FeS_2). The results indicate that *S. carnosus* exhibits different rates of adhesion sulfide ores, which may be used to generate a selective adhesion during the separation of mineral mixtures in future work.

9.3.6 *S. carnosus* and biomodified minerals Zeta potential

Figure 9.6 shows the results of zeta potential of the bacterium *S. carnosus* varying the pH. It shows a positive charge along pH values evaluated. The utility of measuring the zeta potential is primarily determining the surface charge of both controls (bacteria and mineral) as that of bioengineered mineral (mineral + bacteria), their behavior explains the interaction mechanisms between the mineral-bacteria. Some interesting aspects are evident due to the evolution of the zeta potential with *S. carnosus.* Where for the case of fresh bacterium, the bacterium ζ exhibits a positive charge independent of pH (Fig. 9.6 (a)). In the case of separate culture medium and suspended in electrolyte solution (10^{-3}M $NaNO_3$) and adjusted to pH 9 with NaOH, changes to a negative value were observed (Fig. 9.6 (b)). The reason for this result is due to the bacterial wall of *S. carnosus*, which consists of peptidoglycan and polysaccharide, and the strain reducing action by nitrate (Schleifer, 1982) is highlighted. Liu et al, 2000; mentioned that the absorption of polysaccharides on the surface of mineral can happen by some beneficial mechanisms such as hydrogen bonding, or chemical complexation.

In the case of the polysaccharides, these commonly establish equilibrium between cyclic and linear forms; therefore, due to the bacteria conditions with the electrolyte ($NaNO_3$), and the susceptibility of the aldehyde group of polysaccharide to chemical interaction with ions (NO^{3-}). This could promote the release of H^+ ions (Wade, 2004), affecting the modification of the bacterial surface with the consequent change in the value of the zeta potential.

Moreover, the bacteria with negative charge is less hydrophobic than fresh cells or cells in their culture medium stored under refrigeration, indicating that the separation of the bacteria from the medium and resuspension of the cells in the electrolyte generates an alteration on the surface characteristics of *S. carnosus*.

The results of biomodified minerals (Figure 9.7) reveal changes in both surfaces, mineral and bacterial. It shows that the zeta potential of mineral evaluated is modified to the value generated by the ζ of bacteria, indicating that the surface is covered with *S. carnosus*. In addition, the zeta potential of the modified bacteria to negative values could be due to the modification described above. The authors consider the possibility that the ions released from minerals contribute to the modification of the zeta potential of the bacteria

The correlation of the results suggests that in the initial stage of modification with *S. carnosus* could be characterized by a physical process due to the attractive forces generated between the positive bacterial cell and the negative charge of mineral substrate. Subsequently loading the bacterium, modifies the complex to negative values, however, the release of the bacteria is observed, indicating the adherence of *S. carnosus* to ore. See Figure 9.7.

9.3.7 Effect of Biomodification floatability mineral sulfides

Figure 9.8 shows the results of the natural floatability of mineral conditioned at pH 9 in the absence of bacteria. The results indicate a lower floatability than 10% of the sulfides evaluated (pyrite, chalcopyrite and galena). The hydrophilicity of sulfide ores relates to the precipitation of metal hydroxides in the mineral surface (Senior and Trahar, 1991).

The effect of biomodification mineral sulfides by conditioning with *S. carnosus* are shown in Figure 9. The results indicate that floatability of all minerals evaluated increases due to bio-modification, corroborating the hydrophobicity of *S. carnosus.*

The floatability of minerals in descending order is as follows:

galena >> chalcopyrite> pyrite>

The highest floatability is obtained by the biomodified galena (61.17%), followed by that reported for the chalcopyrite (41.2%) and 27% for the pyrite. It should be noted that faster floatability of chalcopyrite and galena are desirable because they are valuable minerals. Instead, the floatability of the pyrite is not desirable because they are gangue mineral type (unmarketable).

Galena has the highest adhesion of bacteria (see isotherms results) which causes a significant increase in the floatability of the mineral. No correlation was found with the results of the floatability of the other minerals (chalcopyrite and pyrite) and adhesion isotherms. Because the hydrophobicity of the biomodified minerals is influenced by the alteration of the bacterial surface itself species from the mineral with which it interacts, as demonstrated in tests zeta potential, in these cases, the hydrolysis of iron species (Fe (II)) of iron ore (pyrite) counteracts the hydrophobicity conferred by bacterial cells.

9.4 Acknowledgement

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9.5 Conclusions

From the results, it can be concluded that *S. carnosus* has a hydrophobic effect on minerals, resulting on its adherence on surface minerals and acting as a natural collector. The bio-modification generates different degrees of minerals hydrophobicity, which indicates the possibility to control the bio-collector for selective separation of minerals. The magnitude of the floatability of minerals showed the descending order as follows:

galena >> chalcopyrite> pyrite

The sulfides mineral bio-modification mechanism was evaluated in the presence of *S. carnosus* and the results shows a physical mechanism, due to the electrostatic attraction generated between the bacterial surface charge or positive zeta mineral powder and negative surface charge (negative zeta potential).

It is concluded that the usage of this strain are promising for environmentally friendly bioreagent, thus being applicable for the eco-friendly development of the bioflotation of sulfide minerals.

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9.7 Nomenclature

A	Weight fed mineral in grams
C	Weight of the mineral concentrate in grams
B_{Ad}	Number of bacteria Adhered cells /m^2
B_0	Concentration of free bacterial at zero time cells/m^2
B	Concentration of free bacterial at time t in cells/m^2
V	Sample volume, mL
w	Ore weight, g
A_m	Specific surface area of the mineral, g/m^2
t	Time, h
h	hour
g	grams
wt%	percentage in weight
min	minutes

Greek symbols

ζ	Zeta Potential

9.8 References

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