5
Results and discussion

The aim of this research was investigate the response of hematite flotation using a new bacterial strain, *Rhodococcus ruber*, in a modified Partridge-Smith flotation cell. Studies of adsorption, zeta potential, and FTIR measurements were carried out to determine the effectiveness of microorganism *Rhodococcus ruber* as a flotation bioreagent. The results are presented up next. Each experiment kept a ±10% of standard deviation, expressed in each graphic by a Y error bar.

5.1.
Mineral sample pre-treatment and characterization

The procedure for the obtaining of hematite sample until its classification for experimental assays is explained in Figure 18. It was necessary to performed grinding to reduce the size of the original hematite sample. After reducing the particle size from 1mm to 30µm approximately, remains of slime generated (particle size of slimes is smaller than 30 µm) are removed by wet sieving. Each fraction size range is represented in Table 9 (see Materials and Methods).

Hematite's high purity degree was confirmed by X –ray diffraction and by Energy dispersive X-ray spectroscopy (EDS).

X-Ray diffraction analysis (XRD) investigates crystalline material structure, including atomic arrangement, crystallite size and imperfections. Energy-dispersive X-ray spectroscopy (EDS, EDX, or XEDS), is an analytical technique used for the elemental analysis or chemical characterization of a sample. It relies on an interaction of some source of X-ray excitation and a sample. Its characterization capabilities are due in large part to the fundamental principle that each element has a unique atomic structure allowing unique set of peaks on its X-

ray spectrum (Goldstein, 2003). In theory, % weight of iron in hematite (Fe_2O_3) is 70%, and % oxygen is the 30% remain.

X-ray diffraction showed that hematite spectra match in a 99% with pattern hematite spectra, and EDS results showed that the weight % of iron is 68,724 and the % oxygen is 31,022, with remains of silicon (%0,144) and aluminium (%0,109). Images of the X-ray diffraction and EDS analyse is presented in the Appendix 9.1 and 9.2.

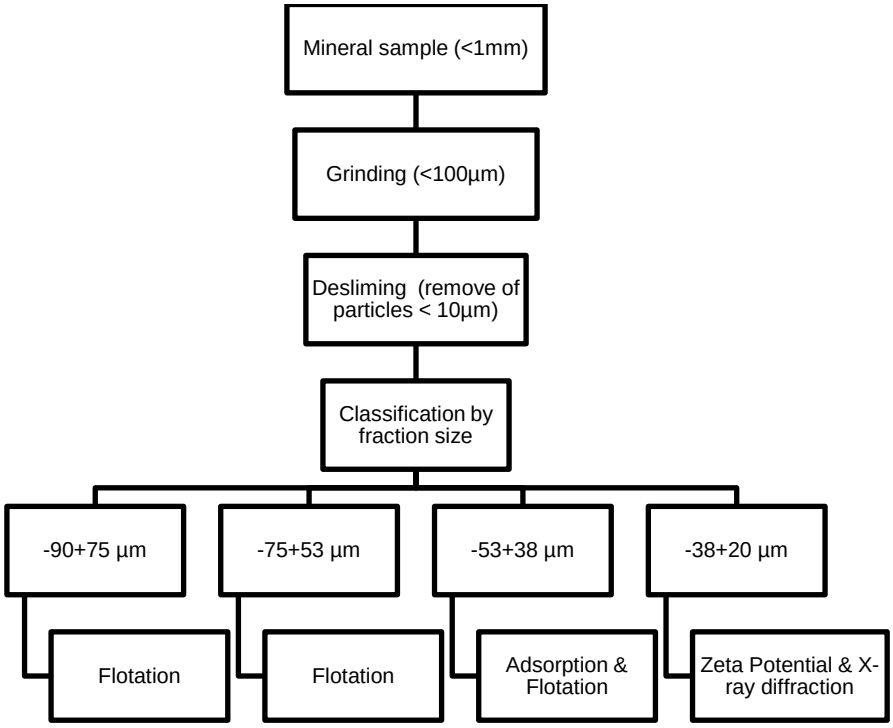

Figure 18 - Flowchart of hematite pre-treatment and characterization

As mentioned in 4.1, after grinding a considerable amount of ultrafine particles are liberated. First SEM studies showed that the presence of this particles represented interference with the normal behavior of hematite surface. Figure 19 shows how tiny particles of hematite seem to be attached electrostatically to major particles.

Therefore, a hematite sample of +38µm was passed through a Tyler mesh sieve #635 by wet screening for the removal of ultrafine particles. The new result can be seen in Figure 20. Compared to the previous image, cleaner hematite

particles were found. Consequently, it was decided to follow the same cleaning procedure for all the hematite samples used in the experiments.

The aim of the SEM study was encourage due to the uncertainty of the behaviour of hematite after electrokinetic measurements. By leaving the mineral surface free of ultrafine particles, it was achieved the expected hematite zeta potential profile.

Figure 19 – Scanning electron micrograph of hematite sample after grinding (no ultrafine particles removal) for particle size range -53+38µm (Magnification x200)

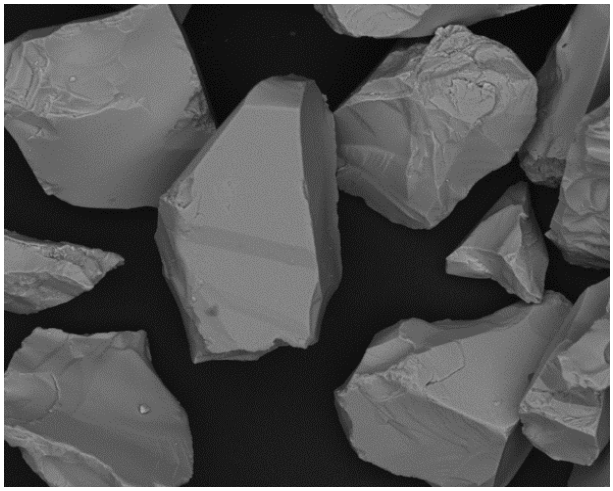

Figure 20 - Scanning electron micrograph of hematite sample after desliming for particle size range -53+38µm (Magnification x200)

5.2.
Bacterial growth and biomass characterization

After concrete the process of bacteria growth, washing, centrifugation and inactivation, a final cell concentrate is obtained. Aliquots of this concentrate were taken to carry on the different studies mentioned before.

Cell concentration was determined in grams of bacterial per liter of suspension. Such concentration was calculated using the optical density at spectrophotometer at a wavelength specific for bacteria (620 ηm). The data were obtained from the absorbance equipment, but using calibration curve (Figure21) - relationship between cell concentration and absorbance - it was possible to determine the concentration of cells present in a given suspension volume.

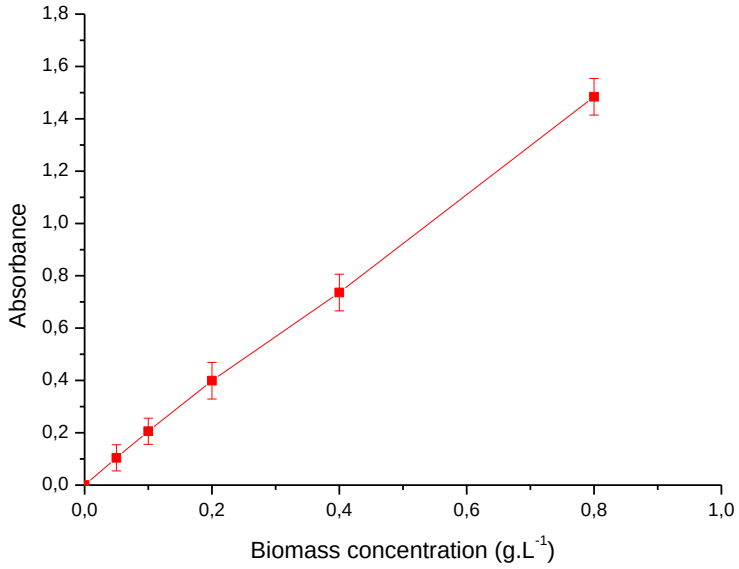

Figure 21 - Calibration curve of *Rhodococcus ruber* bacteria, relation between Biomass concentration ($g.L^{-1}$) and absorbance

For further studies, i.e. adsorption measurements and flotation studies, biomass concentration was related to the measurement of absorbance because it shows a more accurate correlation, as Merma (2012), Botero (2007) and Bueno (2008) showed in previous research using *R.opacus* as biomass.

Bacterial growth assays are of great importance due to the definition of nutritional conditions under which the microorganism can successfully grow.

Research developed by Borges (2011) showed that under same laboratory conditions, and varying the culture medium, the best results were found for Tryptone Soy Broth (TSB). Therefore, once the culture medium is specified, bacteria growth assay were performed.

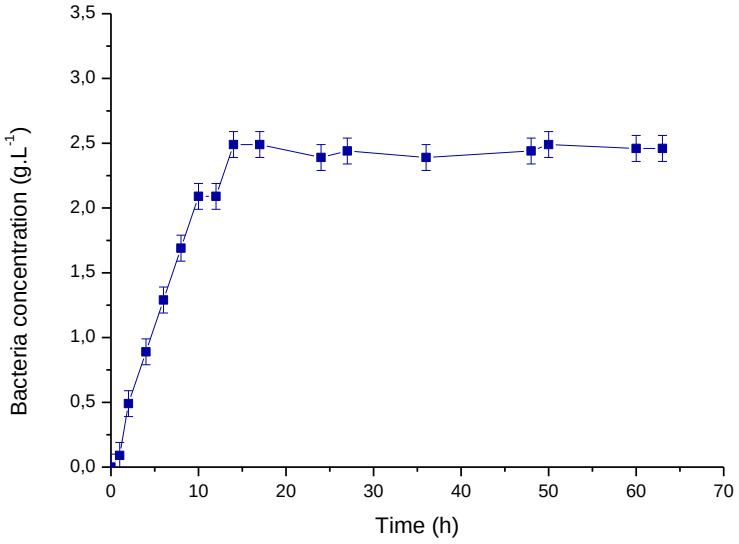

Figure 22 – Bacteria growth curve in function of time

It was found that *Rhodococcus ruber* reached the maximum growth phase in the first fourteen hours, as it can be seen in Figure 22. Borges (2011) found out that the maximum growth phase was achieved in the first twelve hours. The differences between both results are possibly due to the origin of culture medium (different suppliers), laboratory procedure, or bacteria cells inoculation.

As mentioned in 4.2.2, for *Rhodococcus ruber* cell counting, it was taken samples of 0.1mL from 8 dilutions of biomass suspension concentrate (see Table 10), and measured at Neubauer chamber coupled in an Optic microscope. Results were set in Figure 23, relating the number of cells with absorbance measurement, and with the bacteria cells concentration.

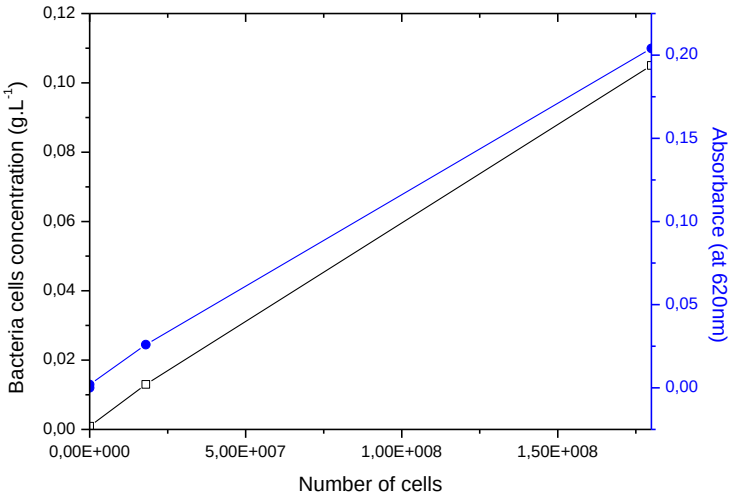

Figure 23 - Relation between the number of cells with the bacteria concentration, and with absorbance

Because it could not be possible to achieved a better correlation for higher values of biomass concentration (cell populations are higher as concentration increases), it was extrapolated the linear regression of the number of cells versus bacterial cells concentration, obtaining the values presented in Table 11.

Table 11 - Correlation between biomass concentration and number of cells

Biomass concentration ($g.L^{-1}$)	Number of cells
0.15	2.5E+008
0.30	5.0E+008
0.45	7,5E+008
0.60	1E+009
0.75	1,25E+009

5.3.
Zeta potential studies

Zeta potential (ZP) measurements were carried out for the evaluation of a possible variation of the electrokinetic properties of hematite after interaction with *Rhodococcus ruber* cells.

Figure 24 shows the behavior of *Rhodococcus ruber* under the effect of different concentrations of NaCl. Sodium chloride was chosen as an indifferent electrolyte, according to previous studies (Merma, 2012; Botero, 2007). The effect of pH variation offered by the values of the potential suggests that H^+ and OH^- ions are the determiners of potential in this system (Hunter, 1981; Mesquita, 2001). The cells are negatively charge for the pH range above IEP, with a diminution in the potential magnitude when the electrolyte concentration was raised from 10^{-4}M to 10^{-2}M. The diminution of the zeta potential profile is associated to the compression effect of the double electric layer, and to the accumulation of contra-ions, caused by the raise of the ionic force of the medium (Hunter, 1981; Lykema, 1983).

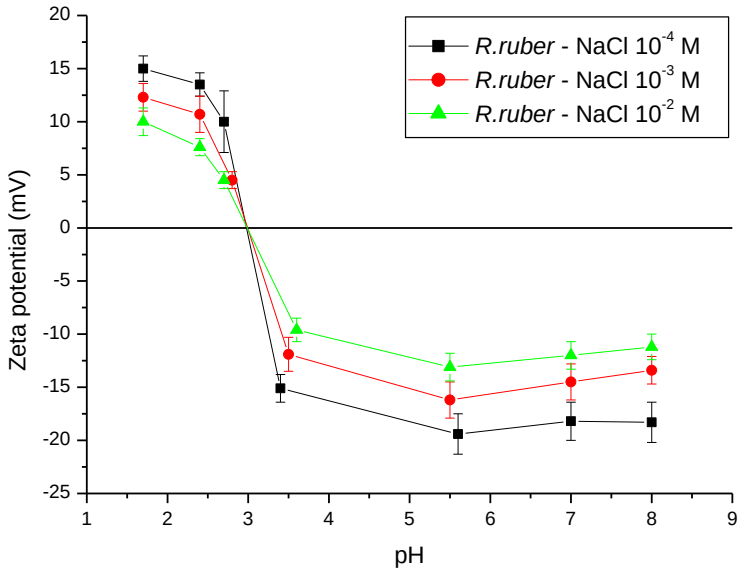

Figure 24 - Zeta potential of *Rhodococcus ruber* (different NaCl concentrations as background electrolyte; biomass concentration 0,20 $g.L^{-1}$)

The profile and the location of the isoelectric point (IEP) at around pH 3 go in concordance with the results presented in a previous study of *Rhodococcus ruber* as a metal removal biomass (Borges, 2011). According to Merma *et al.* (2013), different ZP profiles can be due mostly, to bacteria growing conditions, because both strains could come from different culture medium. The values of ZP demonstrate the stability of cells suspension for an indifferent electrolyte 10^{-3}M at -15mV on the alkaline range whilst on acid range, where the stability is reduced after 2 minutes of settling. At low values of pH, bacteria cells started to agglomerate and settle.

The IEP of hematite was found at pH 4,8 approximately. According to literature, the isoelectric point of hematite variates between a pH range of 4,8-6,4 (Smith and Mishra, 1991; Dubel and Smith, 1992; Yang *et al.*, 2007; Deo and Natarajan, 1997; Mesquita *et al.*, 2003; Yang *et al.*, 2013; Sarvamangala and Natarajan, 2011). These results can be seen in Figure 25.

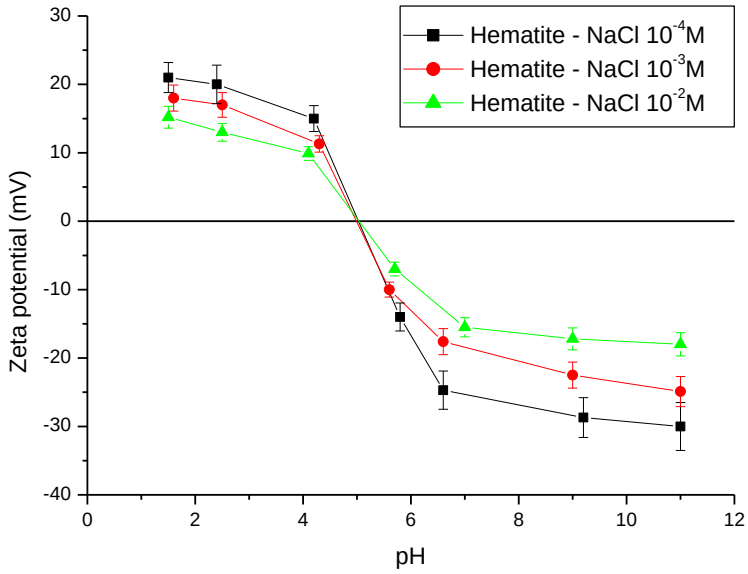

Figure 25 - Zeta potential of hematite (different NaCl concentrations as background electrolyte; biomass concentration 0,20 $g.L^{-1}$)

The result of the interaction of hematite and *Rhodococcus ruber* cells can be seen in figure 26 as a modification of ZP profile of hematite. The electrokinetic behavior of hematite changed completely and adopted the ZP profile of *R.ruber*; this can be a result of cells interaction at the hematite surface (Vilinska & Rao, Dubel *et al.*, Raichur *et al.*, Faharat *et al.*, Botero *et al.*, Mesquita *et al.*, Deo *et al.*, Subramanian *et al.*, e Chandaprabha & Natarajan).

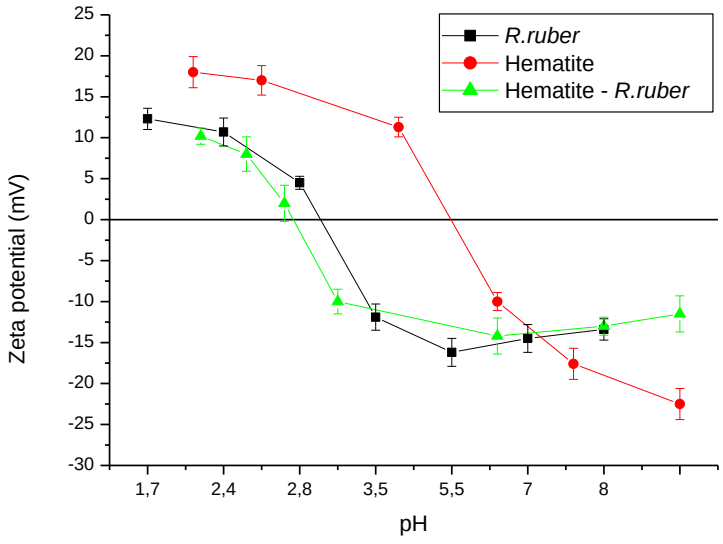

Figure 26 – Zeta potential of hematite before and after *Rhodococcus ruber* interaction (NaCl 10-^{3}M as background electrolyte; biomass concentration 0,20 g.L^{-1})

Bacterial cell surface can be charged due to the presence of functional groups present on the cell wall constituents. Different in ZP profiles of the same bacterial strains are justified in the fact that the cell wall composition is related to the culture medium and in the growth time of bacterial cells that could present or not differentiated characteristics (Mesquita et al, 2003; Merma et al, 2013).

Studies by Van der Wal *et al.* (1997) have shown that anionic groups dominate over cationic groups. This seems to be a general phenomenon and it is in agreement with the observation that most bacterial cells have isoelectric points below pH 4 (Van der Wal *et al.*, 1997), as observed in this works.

According to previous researches, in acid medium, the surface of the bacteria and of the mineral have a contrary charge thus taking place at this point of a possible electrostatic interaction between both surfaces and forming a biofilm onto the mineral surface and therefore the value of the zeta potential of the mineral surface is close to the value of the zeta potential of the bacteria (Hirajima *et al.*, 2012 e Faharat *et al.*, 2009).

5.4. Adsorption studies

Studies of bacterial adhesion provide important information about the affinity of the microorganism towards the mineral surface. Sharma and Hanumantha (2002) reported that this affinity can be presented due to the interaction between the functional groups of the bacteria cell wall and the mineral surface. Deo and Natarajan (1997) and Faharat *et al.* (2009) expressed their adsorption/adhesion tests respect of the number of bacterial cells adsorbed onto hematite surface. Moreover, it was Mesquita (2001) who performed bacterial cells adhesion studies expressed in the milligrams of *Rhodococcus ruber* adhered per gram of hematite (mg *R.ruber*. g^{-1} hematite), represented by Q, or adsorption capacity of bacterial cells onto hematite surface. Botero *et al.* (2007) also performed adhesion experiments in similarity to Mesquita for the mineral system *R.opacus*– calcite, magnesite, and barite.

The -53+38µm size fraction of hematite was used for adsorption studies because it was the size fraction of higher surface area among the other fractions used for flotation experiments. Contact time, biomass concentration and pH solution were evaluated to determine the adsorption capacity of *Rhodococcus ruber* among the surface of hematite.

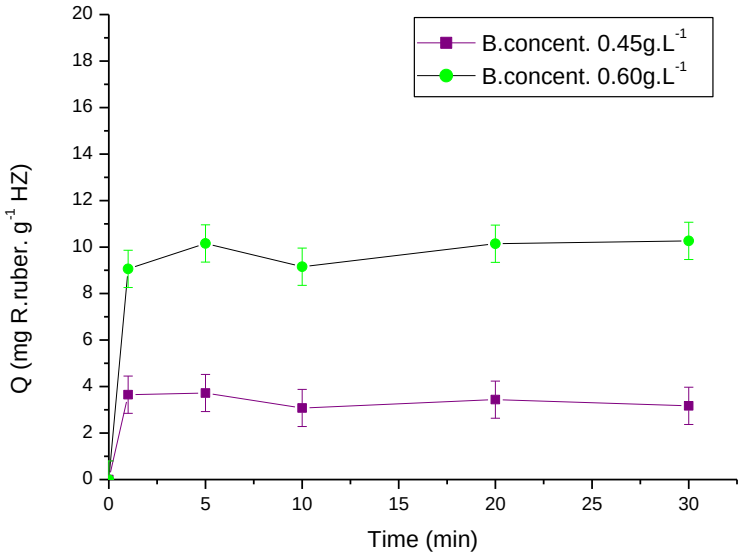

Figure 27 - Adsorption measurements for *Rhodococcus ruber* adsorb onto hematite surface in function of the conditioning time (biomass concentration: 0,45 g.L^{-1}and 0,60 g.L^{-1}; particle size range: -53+38 µm)

Rhodococcus ruber adsorption onto hematite surface was studied, first, on function of conditioning time (Figure 27). For concentrations 0,45 g.L^{-1} and 0,60 g.L^{-1}, the adhesion time remained constant in the first five minutes. Therefore, the optimum adhesion time considered for later studies was five minutes, not depending of the biomass concentration.

In order to evaluate the effect of *Rhodococcus ruber* biomass concentration was selected the values of pH where it was presented a better adsorption of the bacterial cells onto the hematite surface.

Rhodococcus ruber cells adhered onto hematite particles as a function of pH values was also studied and is presented in Figure 28. The adhered quantity of microorganism is high at the acid pH range, especially at pH around 3. The highest quantity of *Rhodococcus ruber* biomass per gram of hematite was around at a concentration of bacterial cells of 0,60 g.L^{-1}, as previously found by Mesquita *et al.* (2003), for the hematite-*R-opacus* system. For a higher concentration (0,75 g.L^{-1}), the quantity adsorbed remained the same as 0,60 g.L^{-1}. These results reaffirmed the previous studies of the influence of biomass concentration in the adsorption capacity of *Rhodococcus ruber* cells.

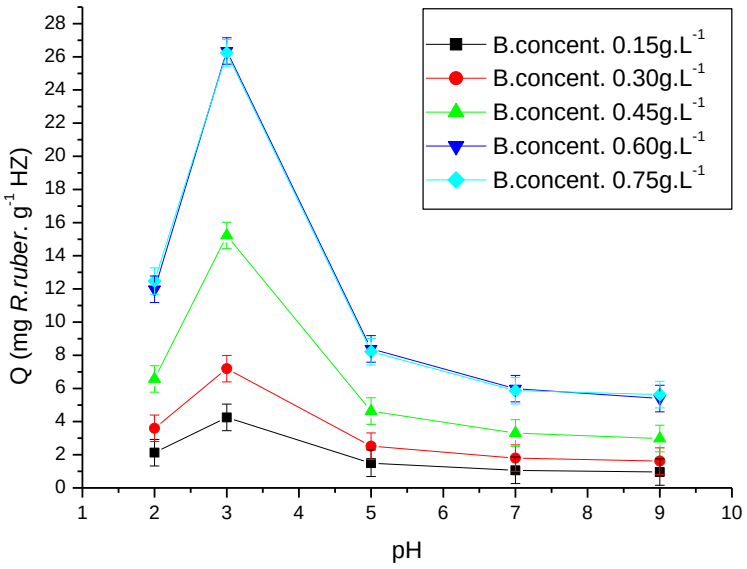

Figure 28 - Adsorption measurements for *Rhodococcus ruber* adsorb onto hematite surface in function of pH (conditioning time: 5min; particle size range: -53+38 µm)

Figure 30 also shows that at higher biomass concentration, higher is its capacity of adsorption, from concentration 0,15 g. L^{-1} to 0,60 g.L^{-1}. From that value, adsorption capacity of *Rhodococcus ruber* remains constant for higher concentrations.

Deo and Natarajan (1997) found out a marked decrease in cell adsorption of *P.polymyxa* on hematite above pH 9. A high adsorption at low pH may be due to the fact that in the acidic pH range electrostatic forces of attraction between the negative bacterial cell surfaces and positive hematite surface is significant. At pH values higher than the isoelectric point, both cell surfaces and the hematite surface is negatively charged resulting in electrostatic repulsion between the mineral and bacterial cells.

Mesquita *et al.* (2003) studied the effect of pH for the adhesion of *R. opacus* cells onto hematite and quartz particles. Even though the bacterial cells tended to adhere on both mineral samples, the adhesion was higher on hematite than on quartz, for which almost negligible adhesion was observed above pH 5,5.

5.5.
FTIR studies

Figure 29-31 present the infrared spectra for hematite before and after interaction with *Rhodococcus ruber* strain, as well for the *Rhodococcus ruber* strain itself.

The Infrared spectra for *Rhodococcus ruber* biomass showed similar characteristic peaks of other microorganisms, especially of the *Rhodococcus* family. Figure 29 presents the FTIR spectra of *R.ruber*. The results of spectrogram are compared with the analysis of the literature. Thus, the spectrogram is shown the range characteristic of fatty acids between 3001 and 2359,52 cm^{-1} assigned to groups of CH, CH_2, CH_3, a range of between 1588,12 and proteins 1476,26 cm^{-1} indicated by groups NH, NH_2, NH_3, and the region of polysaccharides between 1184,09 and 905,43cm^{-1}, characterized by functional groups COOH and CONH. On the basis of this analysis and with previous references that these groups are characteristic of bacteria (Garip *et al.*, 2009) can be stated that they are present in the composition of *Rhodococcus ruber* wall, as they also were found in *R.opacus* cell wall (Merma, 2012; Botero, 2007; Bueno, 2008).

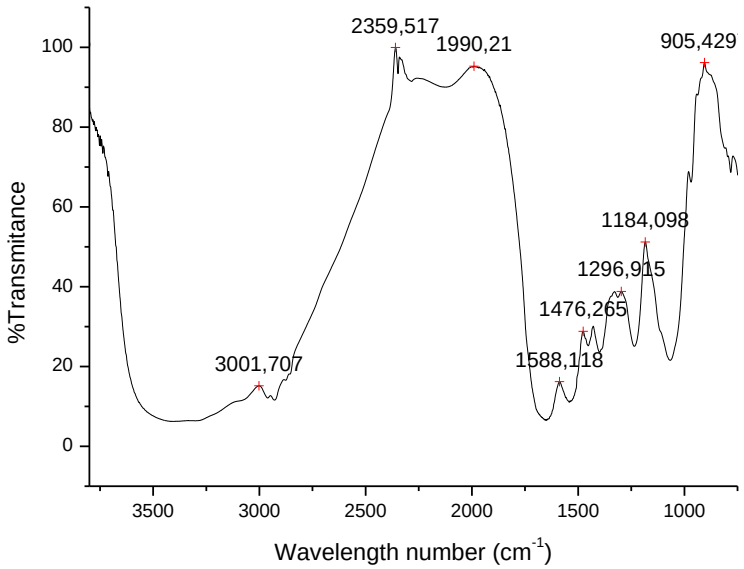

Figure 29 – FTIR spectra for *Rhodococcus ruber* biomass and principal %transmittance peaks

Figure 30 shows that before interaction, hematite surfaces contained hydroxyl groups (hydrogen bonded OH^{-1}) as well as metal to oxygen stretching vibrations. According to Deo & Natarajan (1997), hematite present the following groups: 300-600 cm^{-1} CH_2 rocking vibrations, 900 cm^{-1} CH_2OH groups, 700 cm^{-1} COO- bending of the carboxyl group. Moreover, Yang *et al.* (2007) classified the wave number 970 cm^{-1} of the Si-0 vibration peak and the Fe-0 vibration peak.

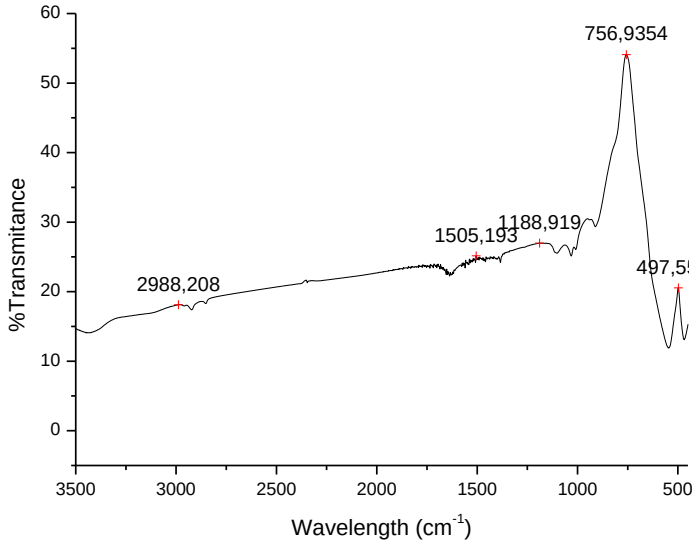

Figure 30 – FTIR spectra of the hematite sample and principal %transmittance peaks

The difference spectrum of cells adsorbed onto hematite is given in Figure 31. New peaks that have appeared on the difference spectrum are due to the interaction of cells with minerals. Bands for COO^- bending of carboxylic group at 1668,15 cm^{-1} was observed, as the 1640 cm^{-1} peak is attributed to the presence of carboxylate anion. The peak at 758, 8639 cm^{-1} may be due to CH_2 rocking vibrations.

Yang *et al.* (2007) found out that the hematite absorbed by *M. phlei* appeared as six functional groups not characteristic of bacteria: aromatic compound groups, - $(CH_2)_n$-groups, carboxyl groups, CH_2 ($-CH_3$), carbonyl groups, aromatic hydrocarbon groups.

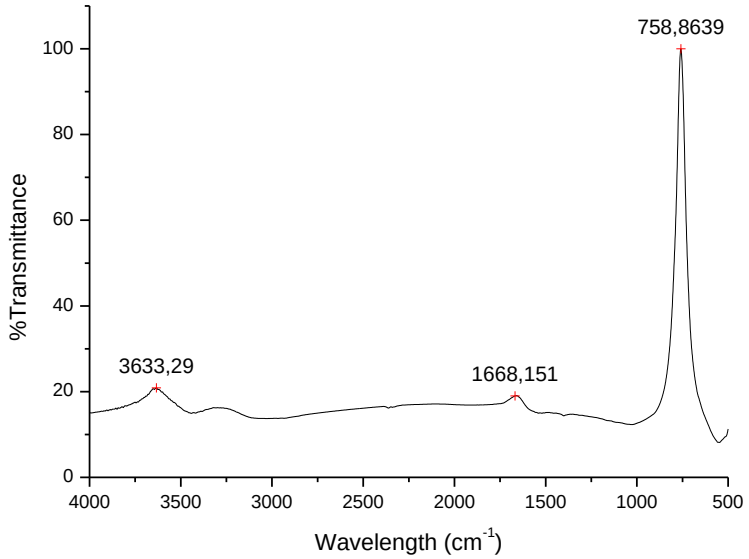

Figure 31 - FTIR spectra of hematite after interaction with *Rhodococcus ruber* biomass and principal %transmittance peaks

On the basis of the peaks showed in the FTIR spectra, it is clear that the main presence of carboxylic groups instead of other organic groups. For the system hematite- *R.erythropolis*, Yang *et al.* (2013) demonstrated that adsorption occurs mainly by chemical adsorption, including chemical interactions of carboxylic group and phosphate group with hematite surface, and hydrophobic association among hydrophobic hematite particles. Therefore, in this case, possibly chemical interaction occurred after interaction of hematite with *Rhodococcus ruber* due to the presence of carboxylic groups.

Since the surface groups on *Rhodococcus ruber* are similar to the groups for fatty acids, it is possible to use this strain as a collector for hematite separation from hematite ores.

5.6. Flotation results

Microflotation studies were carried out in a modified Partridge-Smith cell. As mentioned before, biobeneficiation experiments used have been carried out in a modified Hallimond tube for almost all the researchers involved in the area.

Often, floatability tests are made on the liberated mineral particles, with the aim of assessing collectors and regulators as to determine an optimum pH for flotation. According to Wills (2006), in the Hallimond tube technique, dynamic conditions prevail. The mineral particles are held on a support of sintered glass inside the tube containing the distilled water and the collector under test. Air bubbles are introduced through the sinter and any hydrophobic mineral particles are lifted by the bubbles, which burst at the water surface, allowing the particles to fall into the collecting tube.

Microflotation experiments end up being effective by treating a small weighed sample of pure mineral, or a mixture of pure minerals (i.e. hematite and quartz, as Mesquita (2001)), the weight collected in the tube can be related to the floatability. The Hallimond tube has the advantage of eliminating costly as saying. However, as frothers are not used in the test, it is doubtful whether the method truly simulates industrial flotation (Wills, 2006).

Partridge and Smith (1971) studied the behavior of the floatability for the system hematite-dodecylamine-starch. As hematite presents a high bulk density (5.3), no conventional laboratory flotation testing method was considered completely suitable, so a new type of cell was designed and constructed (Figure 33).

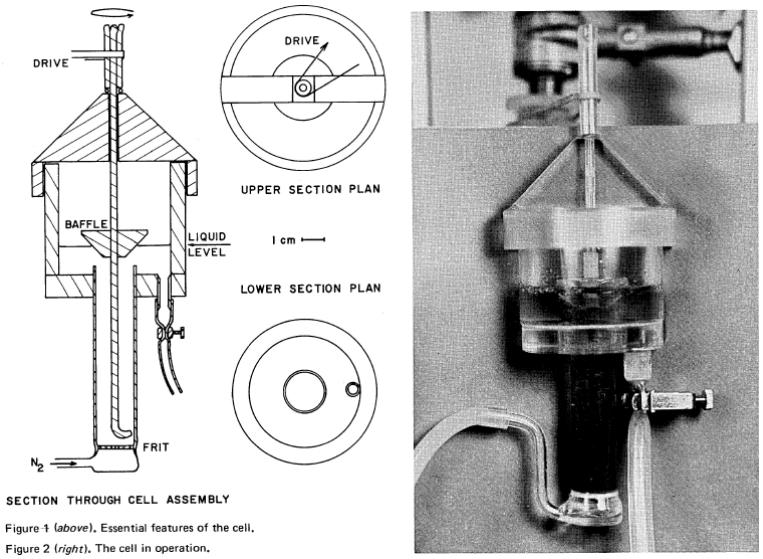

Figure 32 - Partridge-Smith flotation cell used for the flotation of the hematite-dodecylamine-starch system (Partridge-Smith, 1971)

Microflotation tests were also performed in a modified Partridge-Smith cell with natural samples of pyrite, pyrrhotite and arsenopyrite by Peres *et al.* (1993). The effect of pH on recovery and rest potential was investigated and then the influence of the rest potential on recovery, at pH 7. Selectivity between arsenopyrite and pyrrhotite is difficult, but the possibility of separation between pyrite and the other sulphides was not achieved.

For the flotation experiments using *Rhodococcus ruber* strain as bioreagent, hematite particles were initially contained in a column of the test solution closed at the bottom by a glass frit of fine 40 µm pore size. The bed was maintained in a gently moving suspension by the rotating magnetic stirrer and a controlled flow of air was passed through the porous base. The volume of the cell was of 0,23L.

Particles exhibiting hydrophobic surface properties became attached to the bubbles. Depending upon the properties of the solution the bubbles either formed froth or burst. Floated particles were retained in both cases, either in the froth or on the annular floor of the upper section, and were collected at the end of the test by washing through the side tube. The gas flow rate was standardized at 15 $mL.min^{-1}$ at atmospheric pressure. Flotation was continued for 5 minutes after the first appearance of bubbles.

Rhodococcus ruber frother was formed in 2 minutes of air released at the Partridge-Smith flotation cell. The bigger amount of bubbles is formed at a value of pH of 3. A lower amount of bubbles was observed at pH 4 and 5, decreasing until its complete depression at pH range from 6 to 11. In resume, *Rhodococcus ruber* cell suspension achieves a higher froth formation from a pH range of 3-5, producing the highest amount at the pH of its isoelectric point (pH 3).

At this pH, the bacteria begin to coagulate forming cell flocs, because it has zero charge on its surface. If we consider that a bubble air has an IEP around 2.5, a greater interaction of the bacteria cells with the surface of air bubbles could occur, since the electrostatic repulsion between the bubbles and the cells of the bacteria would be reduced (Merma, 2012; Okada, 1990).

The flotation studies were done for different particle sizes: -90 + 75 µm, -75+53 µm, -53+38 µm. The solution was conditioned with NaCl 10^{-3}M, at a pH of 5, at 25ºC, and under constant agitation to keep the particles suspended.

Before flotation experiments were performed, blank assays were made to know how much of the initial mineral sample was carried just by air bubbles (no addition of biomass or frother), expressed as %floatability, or how much was burst to the froth by agitation, expressed as %entrainment. Table 12 presents these previous results.

Table 12 - %Entrainment and %floatability for each particle size range

Particle size range	%entrainment	%floatability
(-90+75 µm)	5	8
(-75+53 µm)	3	9
(-53+38 µm)	2	10

As particle size decreases, the percentage of entrainment also decreases, while % floatability achieved higher values.

5.6.1.Effect of biomass concentration on mineral floatability

Biomass concentration was varied in order to find an optimal concentration of *Rhodococcus ruber* cells for the performance of further flotation studies. It was found that the highest hematite floatability corresponded for a concentration value of 0,60 $g.L^{-1}$, in concordance with previous results of zeta potential and adsorption studies (Figure 33).

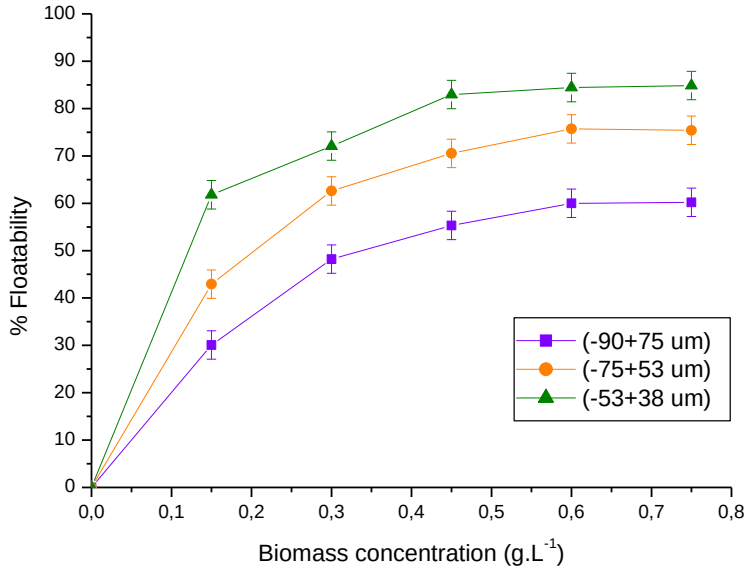

Figure 33 - Hematite microflotation as function of biomass concentration (particle size range: 90-75µm, 75-53 µm and 53-38µm; flotation time: 5min; pH 3)

According to Dubel *et al.* (1992), hematite flotation in a Hallimond tube for a particle size range of +53-20 µm, at pH 7 and with a flotation time of 3 minutes, the floatability of hematite increases as *M. phlei* concentration does.

The effect of *P. polymyxa* onto hematite was studied by Deo and Natarajan (2001), and they showed that the flotation recovery of hematite was unaffected by increasing the cell number. At a cell density of $1,5\times10^{9}$ $cells.mL^{-1}$, only 9% of the hematite sample was recovered in the float fraction.

5.6.2.Effect of pH on mineral floatability

In the flotation process, an important variable, perhaps the most important, is pH of the suspension. This statement is supported by the fact that mineral surface or the bacterial cell wall is activated through dissolution or hydrolysis reactions.

For this stage of the work, floatability studies were performed at a constant particle size range and biomass concentration 0,15 $g.L^{-1}$, and variating the pH values, from 2 to 9. The solution containing hematite and *Rhodococcus ruber* biomass was conditioned with NaCl 10^{-3}M, at 25ºC and carried out for 5 minutes.

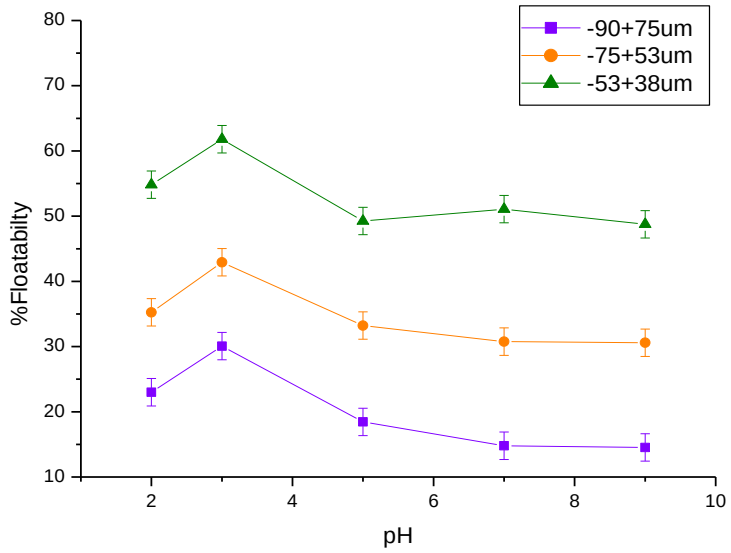

Figure 34 – Hematite microflotation as function of pH (particle size range: 90-75µm, 75-53 µm and 53-38µm; flotation time: 5 min; biomass concentration: 0,15 $g.L^{-1}$)

As it can be seen in figure 34, for all the size ranges, their highest floatability values are at a pH around 3, pretty much similar to the pH of the IEP of *R.ruber*. This result goes in concordance with was found out lines above, and in similarity with Merma *et al.* (2013), Mesquita *et al.* (2003) and Botero *et al.* (2007).Also, as seen in Figure 47, higher floatability values are achieved for the smallest particle size range, -53+38 µm. This was expected because smaller particles are easier to be attached to *Rhodococcus ruber* biomass and less heavy to be carried by gas microbubbles.

These results are in accordance with experiments results previously presented (zeta potential, contact angle measurements and adsorption experiments), where the different response of interaction between *R. ruber* cells with hematite, mainly for pH values above 3, was observed. Despite the high influence of pH, the strong affinity between hematite and microbial cells is clearly evident.

Some studies reported a wide floatable pH range, well below the IEP of hematite. Strong flotation of hematite from pH 3 to 11 was reported, using $C_{18}H_{37}NH_4Cl$ as a collector (Iwasaki *et al.*, 1960). The IEP of the hematite sample was reported to be pH 6.7. Along the same line, it was found that hematite could be floated using dodecylamine as collector over the pH range from 0.8 to 2.0 (Shergold *et al.*, 1968), from pH 3-10 (Shergold and Mellgren, 1977), and from pH 2-12 (Partridge and Smith, 1971). Therefore, it seems that the mechanism of electrostatic adsorption stabilized by hydrophobic interactions alone cannot satisfactorily explain the floatability of hematite well below its IEP.

Yang *et al.*, 2007 found out that the surface characteristics of *M. phlei* are different at different pH values. Therefore, the pH in flotation should affect *M. phlei* directly on the function of hematite.

Bacillus polymyxa rendered quartz surfaces more hydrophobic while simultaneously conferring hematite, corundum and calcite surfaces enhanced hydrophilicity. According to Faharat *et al.* (2009) it is possible to float biotreated quartz at pH <4.3 with a recovery of 58% under these experimental conditions. Biotreated hematite did not float at any pH value, and its flotation recovery was less than 10%.

Mesquita *et al.* (2003) showed that a good floatability value was achieved for hematite at about 90%, at pH values around 4, and about 72% for pH values between 6 and 12, when a cellular concentration of 600 $mg.L^{-1}$ was used. It was

also demonstrate that the best floatability of quartz occurs for pH values around 3, being the floatability of the quartz smaller than that of hematite (about 42%).

Hematite recovery increased rapidly with the increasing of pulp pH from 3 to 6, and then decreased rapidly at above pH 6, as Yang *et al.* (2013) showed after the interaction with *R.erythropolis*. Hematite recovery was optimal at a pulp pH 5– 6,5. It was concluded that for the system hematite-*R.erythropolis*, besides physical adsorption (electrostatic interactions), chemical adsorption (hydrogen bonding, chemical interaction forces) would be involved in the bacterial–mineral interface (Van Loosdrecht *et al.*, 1989), resulting in a hydrophobic surface and a high floatability (about 70%) for pH values above 7,0.

5.6.3. Use of Flotanol as froth enhancer

As mentioned in the Literature review, some microorganisms currently used as bioreagents can also behave as biofrothers. The property of reduce surface tension of water was conferred naturally to these microorganisms due to the presence of amides in their cell walls, which could make them behave as frothers.

According to Araujo *et al.* (2005) despite the existence of records of the use of specific frothers (alcohol and polypropylene glycol) in the reverse flotation of iron ores in the USA (Houot, 1983), their use is not a common plant practice. Flotation is being performed in a pH range that stabilises both, the cationic and the molecular species of the amine, rendering possible that the cationic species act as collector and the molecular species act as frother. The partial replacement of amine by a specific frother is under investigation, at laboratory scale (both single mineral flotation and ore flotation).

Microflotation test results performed by them with some commercial frothers, as the synthetic polyglycol-type frothers, replacing about 10% of the total amine dosage, increased both recovery and selectivity in several tests. Pine oil frothers also performed well when replacing amines (Silva, 2004).

Soares (2012) characterized frothers with different hydrocarbon chains and investigated their influence on the surface of hematite, using the frothers Flotanol

M (75mg.L-1), Montanol 100 and Montanol 800, which were solubilized with the collector Flotigam EDA.

Flotation experiments using flotanol as frother followed the same experimental procedure as mentioned in 4.6. The mineral was conditioned with the biomass suspension inside the Partridge-Smith cell under constant stirring with a magnetic agitator for 5 minutes in aqueous medium using the indifferent electrolyte NaCl 10^{-3} M, and a concentration of flotanol of 75mg.L^{-1}. The value of the concentration of flotanol was taken from the literature and chosen for evaluation in the hematite-*Rhodococcus ruber* system after previous blank tests.

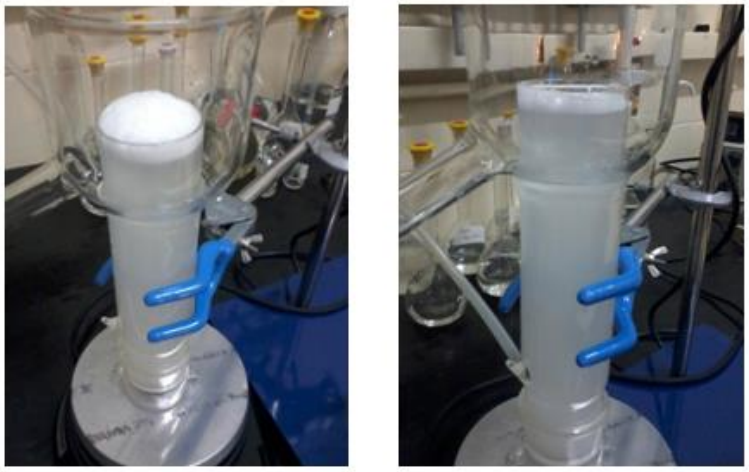

Figure 35 - Images of blank floatability experiments: (a) biomass+flotanol, (b) only biomass (biomass concentration: 0,60 g.L^{-1}, pH 3).

Figure 36 – Hematite bioflotation using *Rhodococcus ruber* biomass in a Partridge-Smith flotation cell (pH 3; biomass concentration: 0,15 $g.L^{-1}$; using flotanol).

Figure 35 shows the images of blank floatability experiments, with no hematite particles present in the flotation device. As can be seen there is a significant difference between both images. Image 35a shows the mixture of *Rhodococcus ruber* biomass (0,60$g.L^{-1}$) with flotanol (0,75 $g.L^{-1}$), and the high of the froth formed is taller than the froth formed only by the biomass (Figure 35b). This behavior is supported by the fact the *Rhodococcus ruber* strain only form a stable froth at a pH value of 3. Therefore, even when this strain works effectively as a biocollector of hematite, it strongly depends on the pH of pulp solution and of concentration.

Figure 36 exhibits the mineralized froth for hematite bioflotation using *Rhodococcus ruber* biomass, with pH around 3 and biomass concentration 0,15 $g.L^{-1}$, using flotanol.

Furthermore, flotation experiments were carried out using flotanol mixture with biomass suspension inside the Partridge-Smith cell in aqueous medium using the indifferent electrolyte NaCl 10^{-3} M, under same experiment conditions.

The effect of flotanol in function of pH and biomass is shown in Figures 37 and 38. In both figures the particle size range is represented by numbers 1 (-90+75µm), 2 (-75+53µm) and 3(-53+38µm).

Results of hematite bioflotation demonstrated the affinity of *Rhodococcus ruber* for hematite working successfully as a collector, especially for pH 3 and for a biomass concentration of 0,60 $g.L^{-1}$, . When adding 75$mg.L^{-1}$ of flotanol to the aqueous medium, flotation results varied, as Figures 37 and 38 shows.

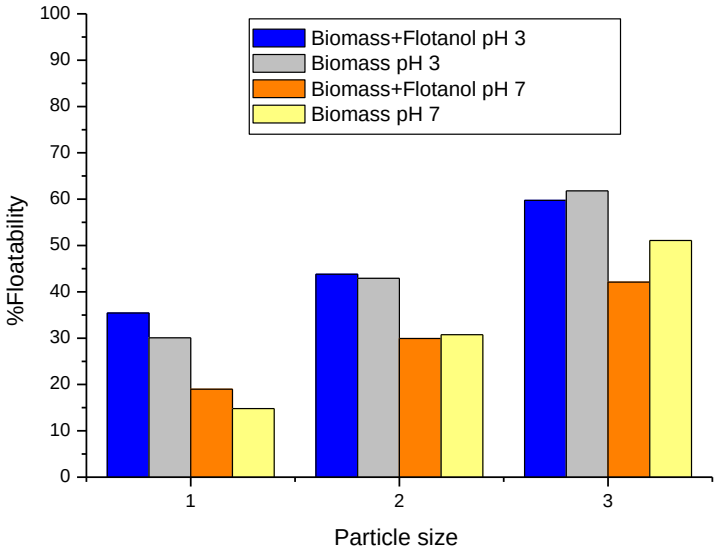

Figure 37 - Effect of Flotanol in the bioflotation of hematite with *Rhodococcus ruber* in function of pH and particle size (biomass concentration 0,15 $g.L^{-1}$)

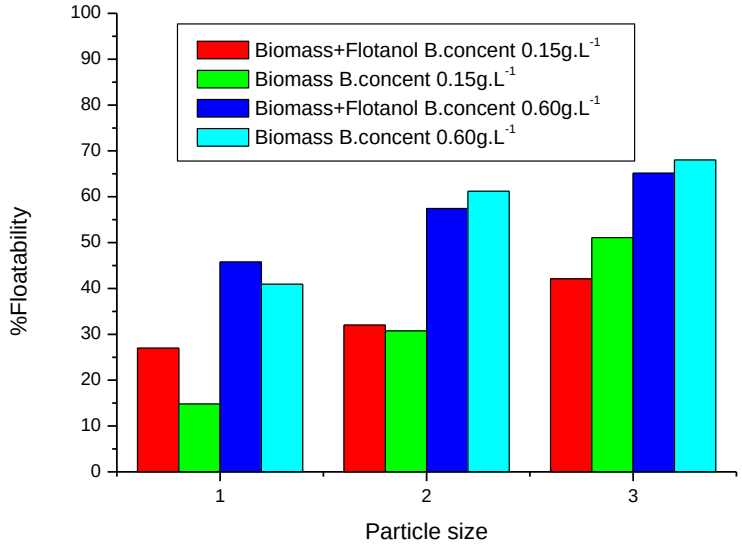

Figure 38 - Effect of Flotanol in the bioflotation of hematite with *Rhodococcus ruber* in function of biomass concentration and particle size

For smaller particles size (-53+38µm), and using the best conditions for bioflotation of hematite (pH 3 and 0,60 $g.L^{-1}$), the floatability of hematite with *Rhodococcus ruber* increases while floatability with *Rhodococcus ruber* and flotanol presents a marked influence in hematite floatability by achieving upper values than the other size ranges. Therefore, use a conventional frother as flotanol can be effective for minor particle size.

Mesquita (2001), Botero *et al.* (2007) and Merma *et al.* (2013) showed that *R.opacus* strain can work not also as a selective collector but as a biofrother. More recently Merma (2012) studied the behaviour of *R.opacus* as water surface tension (70 $mN.m^{-1}$) reducer. The greatest reduction in surface tension was with 0,15 $g.L^{-1}$ biomass. Froth was higher in between pH 3 and 7, with surface tension values between 54 $mN.m^{-1}$and 56 $mN.m^{-1}$. The adaptation of the bacteria quartz caused an increase in tension surface of pH values 3, 5 and 7 and a reduction in alkaline medium.

5.7. Scanning electron microscopy (SEM) analysis

Figures 39-41 shows the results of SEM of hematite particles after interaction with *Rhodococcus ruber* biomass.

Figure 39 – Scanning electron micrograph of hematite clean sample after interaction with *Rhodococcus ruber* biomass, for particle size range -53+38µm (Magnification x120)

The difference between them is respect of the lent increment of the equipment. The micrographs revealed the presence of *Rhodococcus ruber* rod cells adhere onto hematite surface (particle size fraction 53-38 um). As it can be seen from both figures, *Rhodococcus ruber* cells tend to gather at the moment of adhesion onto the mineral surface, forming a bond colony of cells.

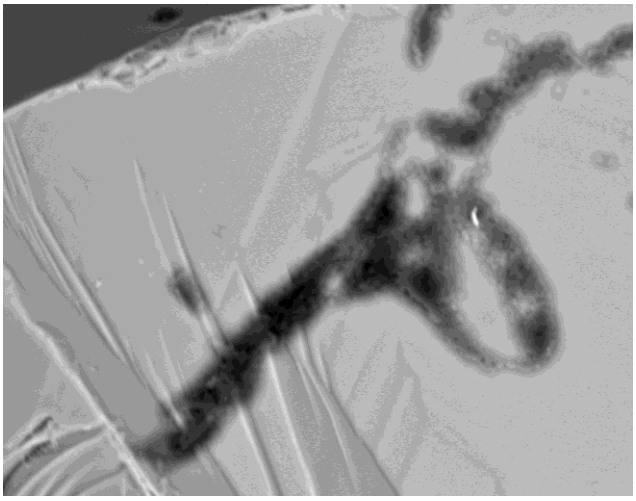

Figure 40 - Scanning electron micrograph of hematite clean sample after interaction with *Rhodococcus ruber* biomass, for particle size range -53+38µm (Magnification x3000)

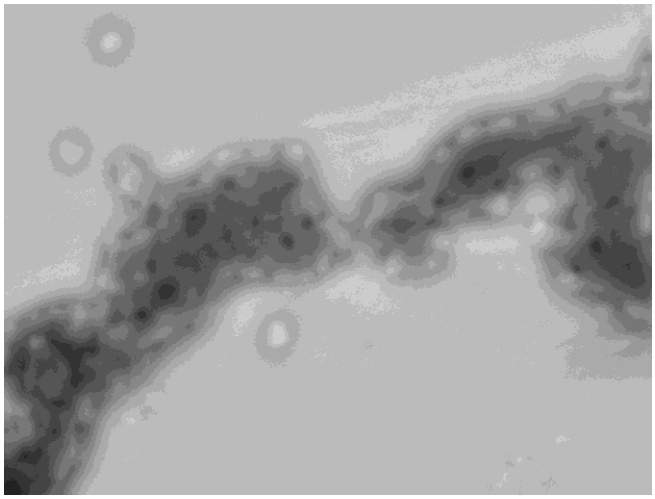

Figure 41 - Scanning electron micrograph of hematite clean sample after interaction with *Rhodococcus ruber* biomass, for particle size range -53+38µm (Magnification x10000)

For the *B. subtilis* cells adsorption onto hematite, adsorption density of cells was found to be significantly higher on hematite compared to that on corundum, calcite, and quartz respectively. Compared to quartz, adsorption density of bacterial cells was almost ten times higher on hematite surfaces. Adsorption density of bacterial cells on calcite was two times higher than that on quartz. Among the above minerals, quartz exhibited the least surface adsorption of *B. subtilis* followed by calcite (Sarvamangala *et al.*, 2011).

Mesquita *et al.* (2003) showed that scanning electron micrographs of quartz and hematite particles were obtained of the best microflotation test results, at pH 4.5 for hematite and pH3.5 for quartz. SEM examination of the interacted mineral surfaces revealed the presence of *R. opacus* cells adhered on the mineral surfaces. Even though the bacterial cells tended to adhere on both mineral surfaces, for hematite particles, more density of cells adhered is pronounced.

Moreover, Yang *et al.* (2013) found out in the SEM images of adsorption of *R. erythropolis* onto hematite surfaces, that the bacteria on hematite surface were attracted to each other. The individual hematite particles were transformed into agglomerates of hematite through bridging of the bacteria.