3
Literature review

3.1.
Iron ores

Iron is the fourth most abundant element in the earth's crust. Their average percentage in it, is 5%, and occurs as a majority or minority constituent minerals in all classes. Over 400 minerals present iron in detectable amounts, with concentrations ranging from less than 1% to over 70% (Chemale and Takehara, 2013). In table 1 is indicated the composition of the principal iron ores.

Table 1 - Composition of the principal iron ores

Iron mineral	Chemical formula	Iron content (%)
Magnetite	Fe_3O_4	72.4
Hematite	Fe_2O_3	69.9
Goethite	$FeO(OH)$ or $Fe_2O_3.H_2O$	62.9
Limonite	$FeO(OH).\ nH_2O$ or $2Fe_2O_3.H_2O$	59.8
Pyrrhotite	$Fe_{1-x}S$ (x=0 to 0.2)	58.2-63.5
Siderite	$FeCO_3$	48.2
Pyrite	FeS_2	46.6
Ilmenite	$FeTiO_3$	36.8

Hematite, or Fe_2O_3, corresponds to 69.94% iron and 30.06% of oxygen. Its natural color goes from silver gray to black in some forms, and red to brown in earthy forms; sometimes presents an iridescent color when present in hydrated form. Its structure consists of layers of oxygen ions, spatially arranged in a

slightly distorted hexagonal arrangement, in layers of iron ions (Klein, 2002; Ramdhor, 1980; Deer *et al.*, 1981).

Hematite crystallizes in the hexagonal system. The crystals have a metallic shine steel blue, and earthy varieties are opaque. The transparency color is red with blood. The common massive ore is red hematite. The crystalline material with metallic luster is known as specular hematite and micaceous hematite, if the structure is lamellar (Klein, 2002; Ramdhor, 1980; Deer *et al.*, 1981).

Hematite is a mineral widely distributed in rocks of all ages and shapes, and occurs as the product of sublimation and volcanic activities such as contact metamorphic deposits and as accessory in igneous rocks. Generators are large deposits in Brazil, Australia, South Africa and North America (Santos, L.D., 2002).

The decreased grades of available iron-ore reserves have resulted in increased employment of beneficiation. Magnetic, electrostatic and gravity methods commonly used have the advantage of economy but recovery is generally low and decreases rapidly with decreasing particle size. As the depletion of high-grade hematite deposits continues and fine-grained taconites become of greater significance, the loss of fines will become unacceptable. Consequently, attention has been focused on concentration by flotation - a process used over many years for finely disseminated minerals of higher intrinsic value (Partridge and Smith, 1971).

3.2.
Conventional reagents in Iron ores Flotation

The most commonly used flotation reagents in iron ore flotation are collectors, depressants, frothers, activators, dispersants, flocculants and pH modifiers. For a better understanding of the importance of microorganisms application as bioreagents, it is introduced a brief summary of conventional iron ore reagents. Microorganisms have proven to have the same chemical functions and physical properties of the conventional reagents widely used in iron ore

flotation. Table 2 shows the principal reagents in Iron ore flotation and its function.

Also known as surfactants, or surface-active reagents, collectors generally consist of a hydrophilic functional group (s) and a hydrophobic hydrocarbon chain (s). Due to the dual character of its molecules, they can adsorb onto a mineral surface, making it hydrophobic and therefore, facilitating the attachment of air bubbles to the minerals to float them. Depending on the character of their hydrophilic functional groups, the collectors used in iron ore flotation can be classified into cationic and anionic collectors.

The cationic collectors are mainly represented by amines. These are derived of ammonia (NH_3), with one or more hydrogen atoms replaced by one or more hydrocarbon radicals. The amines are classified based on the number of hydrocarbon radicals attached to the nitrogen atom. They also can be classified into fatty amines, ether amines and condensates (Smith, 1998; Bulatovic 2007).

The most common anionic collectors used in iron ore flotation are the fatty acids (carboxylates, $R\text{-}COO^-$), sulfonates ($R\text{-}SO_3^-$), alkyl sulphates ($R\text{-}SO_4^-$) and hydroxamate ($R\text{-}CO\text{-}NH\text{-}O^-$), from which the fatty acids are the widely used in industrial operations.

Table 2 - Conventional reagents in Iron ore flotation

Reagent	Mineral	Function	Author
Amine	Hematite	Collector	(Iwasaki *et al.*, 1960).
Dodecylamine	Hematite	Collector	Shergold *et al.*, 1968 Shergold and Mellgren, 1977 Partridge and Smith, 1971
Sodium oleate	Hematite	Collector	Araujo *et al.*, 2005
Hydroxamates	Hematite	Collector	Fuernestau *et al.*, 1967; Fuernestau *et al.*, 1970),
Starch	Hematite ores	Depressor	Iwasaki, 1990; Araujo *et al.*, 2005, Ma, 2008
Amylopectin and/ or Amylose	Hematite	Depressor	Pinto *et al.*, 1992; Pavlovic and Brandão, 2003; Weissenborn, 1996
Sodium silicates	Iron ore silicate gangues	Dispersant	Yang *et al.*, 2008; Ma, 2011).
Propileneglycol	Hematite ores	Frother	Araujo *et al.*, 2005
Pine oil	Hematite ores	Frother	Araujo *et al.*, 2005

Starches and their derivates are the predominant depressants of iron oxide minerals in the reverse cationic/anionic flotation of hematite ore, with corn starch being the most widely used in the iron ore industry (Iwasaki, 1990; Araujo *et al.*, 2005, Ma, 2008). Starch is actually a mixture of two polymers, a linear polymer "amylose" and a branched polymer "amylopectin". In most of the studies on starches and their derivates in iron ore flotation, the polysaccharide is regarded as a single entity. The roles of amylose and amylopectin in iron ore flotation have received limited attention and there are still some disagreements in the literature.

Ultra-fine particles (<10 microns) which are part of iron ore suspensions cause problems at every stage of iron ore processing. The fine particles

heterogeneously coagulate with coarser particles and have to be fully dispersed prior to desliming. The dispersants used or proposed to be used in flotation include sodium silicate, polyacrylic acids and phosphoric and polyphosphorus acids. Sodium silicates are widely used in flotation industry due to its chemical properties, such as its buffering ability, surface charges, modifying ability, polymerizing capability and viscosity regulating ability (Yang *et al.*, 2008), and for being a dispersant of the silicate gangue minerals in the iron ore industry (Ma, 2011).

The use of specific frothers, i.e. alcohol and polypropylene glycol, is not a common plant practice. In reverse flotation of iron ores, the pH range stabilises the cationic and molecular species of the amine, conferring to it the property of collector to the cationic species and of frother to the molecular species. 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 (Araujo *et al.*, 2005).

3.3. Bioreagents in Iron ores flotation

3.3.1. Background

Biobeneficiation, by definition, refers to selective removal of undesirable mineral constituents from an ore through interaction with microorganisms, which act as surface modifiers, depressants, collectors or dispersing agents to enhance the separation of minerals by either flotation or flocculation (Smith *et al.*, 2006). Table 3 shows the brief review of the microorganisms used until the moment as beneficiation bioreagents.

The use of microorganisms in bioflotation processes was first studied by Solojenleen (1976) who announced the possibility of flotation of ores in the presence of sulphate reducing bacteria (SRB) (Hosseini *et al.*, 2005). Then, several works related to the area have been done. One great example is *Mycobacterium phlei* strain, which was used as flotation collector for hematite (Prakasan *et al.*, 2010; Botero *et al.*, 2007) and as a flotation depressant in the

anionic flotation of apatite and dolomite (Botero *et al.*, 2008). Zheng *et al.* (2001) also used *Mycobacterium phlei* and *Bacillus subtilis* for dolomite depression in anionic flotation.

Acidithiobacillus ferrooxidans, commonly used in Mineral bioleaching, have been used as galena depressant in galena and sphalerite flotation (Patra and Natarajan, 2008). This bacterium has also been used in removing pyrite from mixtures of sulfide minerals (Subramanian *et al.*, 2003; Sharma, 2001). *A. ferrooxidans* have frequently been used as an excellent pyrite depressant in coal flotation (Chandraprabha and Natarajan, 2006).

Bacillus polymyxa was used in the separation of sulfide minerals (Santhiya *et al.*, 2001), iron ore flotation (Deo and Natarajan, 1999), and for pyrite separation from oxide gangue minerals (Somasundaran *et al.*, 2000).

Mesquita *et al.* (2003) used *Rhodococcus opacus* as hematite collector in hematite-quartz flotation. This bacterial strain was later used as a collector of calcite and magnesite by Botero *et al* (2007), and also for apatite-quartz system (Merma *et al.*, 2013).

3.3.2.Bacterial strains characteristics

Bacteria are single-celled organisms, prokaryotes, which can be found in isolation or in colonies. They are constituted by a micro-cell without cell nuclei with sizes ranging from 1 to 10 micrometers. As all the cells, they are bounded by a cell membrane of thickness ~ 70Å (plasma membrane), which consists of a double-layer lipid containing proteins inserted (Merma, 2012).

Bacteria can be classified using various criteria. One form of classification is according to the Gram stain (Figure 4). The difference in response to the Gram stain is related to the chemical and structural differences in the bacterial cell wall.

Gram positive bacteria are distinguished from Gram negative according to the ability to capture the Gram stain (procedure in which cells are fixed by heat treated successively with crystal violet dye and iodine and decolorized with ethanol or acetone). A gram negative bacterium has an outer membrane complex

involving its cell wall and excludes Gram stain, while Gram positive lack this membrane.

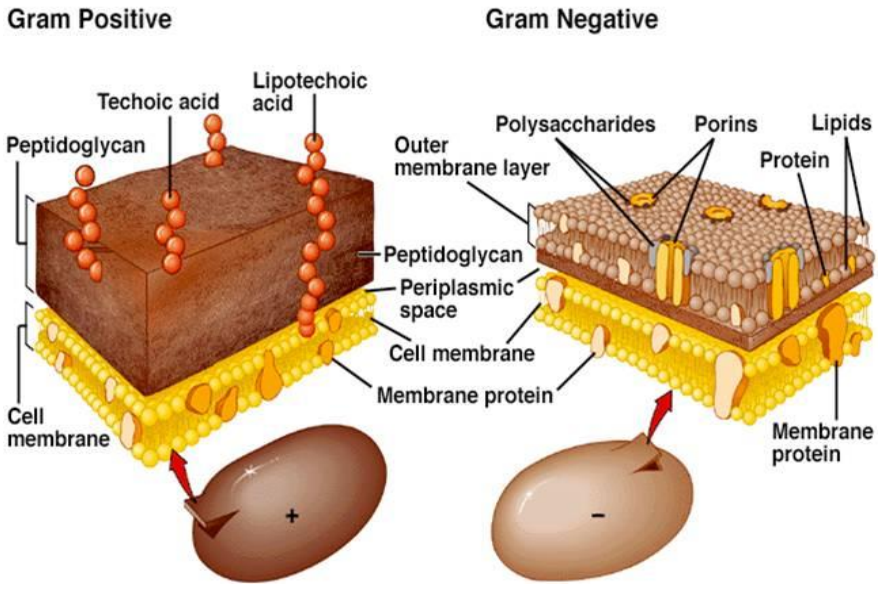

Figure 4 – Gram positive and gram negative bacteria cell wall (Wiley *et al.*, 2011)

Gram positive bacteria have a thick cell wall (~ 250Å) around its plasma membrane, whereas Gram negative bacteria have a thin cell wall (~ 30Å) covered by an outer membrane complex .

The cell walls of bacteria are constituted of polypeptide chains and of covalently linked polysaccharides, which form a molecule shaped as a bag that completely surrounds the cell. This frame is called peptidoglycan or murein. The surfaces of Gram positive bacteria are covered by teichoic acids, which are responsible of 50 % of its dry weight of their cell wall. The teichoic acids are polymers of glycerol or ribitol joined by phosphodiester bonds. The outer membranes of Gram negative bacteria are lipopolysaccharide complexes composed of proteins and phospholipids organized in a complex way.

The other way to distinguish bacteria is according to nutritional requirements. If the cell requires complex organic molecules of the type that would normally be provided by the destruction of other cells, is called heterotrophic. When the cell can use CO_2 from the air, reducing the need for

organic compounds, the cell is called autotrophic. If the energy required for CO_2 reduction is achieved via photosynthesis light, the cell is photoautotrophic. If the energy is provided by the oxidation of inorganic compounds such as Fe^{2+}, S, H_2, NH_3, etc, the cell is chemoautotrophic.

Thus, the composition of the cell wall determines the ability of adhesion of bacteria on various types of surfaces, apart from giving to the microorganisms a surface charge. These surface charge properties of microorganisms can be characterized by electrophoretic mobility measurements, represented by the zeta potential profile and the presence of the isoelectric point; this curve point indicates the cationic or anionic surface characteristics of the microorganism and is defined as the negative logarithm of the activity of the ions for which the total load on the shear plane is zero.

The isoelectric point (IEP) of the organism is determined by the presence of functional groups and the balance of anionic and cationic charges (Vilinska and Rao, 2008). Generally the trend is to be negative due to the predominance of the anionic groups on the cationic groups present in the cell wall (Van der Wal *et al.*, 1997) and the presence of peptidoglycan, abundant in amines and carboxyl groups. Other components that contribute to the negative charge are the teichoic acids, rich in phosphates. Therefore, the cells acquire charge by the ionization of functional groups on the surface of the cell wall, which implies that the surface charge depends on the pH of the solution (Rao and Subramanian, 2007; Natarajan, 2006).

The hydrophobicity of bacteria can be considered as the most important factor in the application of these microorganisms in mineral beneficiation. Depending on the gender and species, they may exhibit hydrophobic or hydrophilic character, that is, has amphipathic character. Furthermore, several authors (Botero *et al.*, 2007; Mesquita *et al.*, 2003) attributed the degree of hydrophobicity to the proportion of fatty groups regarding the hydrophilic functional groups present on the cell surface, and acid / basic character of the cell surface. It has stated that the presence of polysaccharides in the cell surface provides a hydrophilic behavior to the bacteria and minerals that interact with,

while the protein compounds provide a hydrophobic character (Deo & Natarajan, 1997).

The use of microorganisms and / or their derivatives as collectors or depressants in bioflotation is determined by the presence of various functional groups (phosphate, amines, amides, carboxyl groups) in the molecules that form the bacterial cell wall. Groups that provide the microorganism reagents similar to those used in conventional flotation of minerals (Dubel *et al.*, 1992; Zheng *et al.*, 2001; Hosseini *et al.*, 2005; Mesquita *et al.* 2003; Deo and Natarajan, 1997), so it is essential to know the characteristics of these micro-organisms composition and confirm the presence of these groups.

Generally, the cell wall of bacteria consists primarily of peptidoglycan and polymers, as well as extracellular polymeric substances (EPS), phosphoglycerides, phospholipids, proteins and organic acids such as mycolic acid.

Microorganisms and / or their metabolic products can modify both directly and indirectly mineral surface. While direct mechanism involves direct adhesion to mineral particles of microbial cells, the indirect mechanism refers to products of metabolism or soluble fractions of the cell which act as surface active reagents. Both interactions lead to changes in the mineral surface rendering it hydrophilic or hydrophobic by the presence of nonpolar and polar groups on the cell wall or the metabolic products and thus be applied to the flocculation and flotation of minerals (Rao and Subramanian, 2007; Vilinska and Rao, 2008).

Through the years, research on Biobeneficiation has grown, gathering important conclusions about the behavior of the system microorganism-mineral. Table 3 presents a brief resume of the function of these bacterial strains, working as collectors, depressants and/or frothers.

Table 3 - Bacteria used on Mineral biobeneficiation

Bioreagent	Mineral system	Function	Author
M.phlei	Hematite	Collector	Dubel *et al.*, 1992
	Apatite-Dolomite	Collector	Zheng *et al.*, 1997
	Hematite fines	Collector and flocculant	Yang *et al.*, 2007
	Coal fines	Collector and flocculant	Raichur *et al.*, 1996
B.subtilis	Coal fines	Flocculant	Vijayalakshmi & Raichur, 2003
A.ferroxidans	Coal	Pyrite depressor	Amini *et al.*, 2009
Pseudomonas aeruginosa[1]	Coal	Frother	Fazzelipoor *et al.*, 2009
T.ferroxidans	Pyrite-Chalcopyrite	Pyrite depressor	Hosseini *et al.*, 2005
L.ferroxidans	Pyrite-Chalcopyrite	Chalcopyrite depressor	Vilinska & Rao, 2008
E.coli	Quartz	Collector	Faharat *et al.*, 2008
R.opacus	Calcite-Magnesite	Collector	Botero *et al.*, 2007
	Hematite-Quartz	Collector	Mesquita *et al.*, 2003
	Apatite-Quartz	Collector	Merma *et al.*, 2013
P.polymyxa	Hematite, corundum, calcite, quartz.	Quartz collector	Deo & Natarajan, 1997, 2001
	Pyrite-Esfarelite	Pyrite depressor	Patra & Natarajan, 2004

[1] It did not work as surface modifier but as a surfactant.

3.3.2.1.Microorganisms as biosurfactans

Microbial surfactants or biosurfactans are considered nowadays as potential substitutes of synthetic chemicals from petrochemical origin (Khoshdast and Sam, 2011). The main advantages of biosurfactants over their chemical counterparts are their lower toxicity, better environmental compatibility, biodegradability, and effectiveness in a wide range of temperatures and pH. Last, but not least, their production by renewable resources provides further impetus for serious consideration of biological surfactants as possible alternatives of the commonly used industrial chemicals. The achievements of the microbial production of biosurfactants and various aspects concerning their potential commercial application regarding bioremediation of soils polluted with heavy metals are well documented and summarized in a number of reviews (Cohen and Exerowa, 2007).

Among the various species of biosurfactants much work has been done on rhamnose containing microbial surfactants produced by *Pseudomonas aeruginosa* strains. These ubiquitous environmental bacteria cells can be isolated from many different habitats including water, soil and plants. These Rhamnolipids produced by *P. aeruginosa* microorganisms are usually as a mixture of two or four species. They differ by the length of hydrophobic chains (from C_8 to C_{12}) some of which are unsaturated with one double bond. Numerous articles have considered the process of microbial cultivation of rhamnolipid type biosurfactants on different substrates and their properties in terms of film thickness and wettability (Khoshdast and Sam, 2011).

The first investigation on using these biosurfactants in flotation of coal and minerals was done by Fazaelipoor *et al.* (2009). They studied frothing characteristics and flotation applicability of rhamnolipid type biosurfactants as frother.

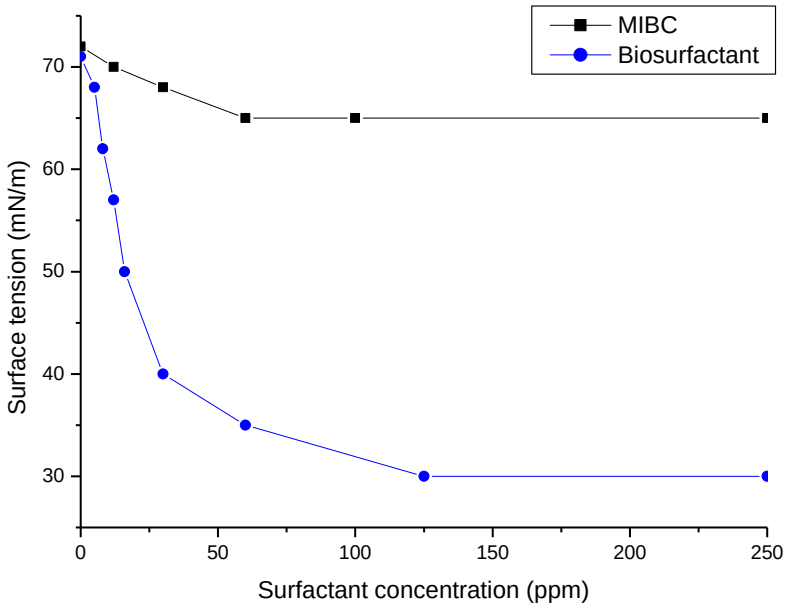

Figure 5 - Comparison of the biosurfactant and MIBC in reducing surface tension of distilled water (Khoshdast and Sam, 2011)

These biosurfactants showed better surface-activity and static frothability, i.e. frother height and half-life, in comparison with MIBC. Figure 5 compares surface tension values from the biosurfactant and MIBC. The results indicate the better ability of the biosurfactant to reduce surface tension of water compared to MIBC. The height of froth was measured as a function of aeration rate at two different surfactant concentrations. The biosurfactant produced higher froth heights compared to MIBC.

There might be two responsible reasons for these results. First, rhamnolipid biosurfactant has a much higher molecular weight (>438 g/mol) compared to MIBC and this higher molecular weight leads to a more viscous, and hence more stable, froth layer. Second, there is only one –OH group in MIBC and this group will interact with water molecules to form an oriented monolayer at the surface; but in biosurfactant, there are several oxygenated units in the molecular chain and each unit can interact with water molecules through hydrogen bonding, causing the molecules to tend to lie flat at the surface, possibly increasing the viscosity of the froth.

The results indicate that at the lower concentration MIBC produces more stable froth, while at higher concentration the reverse is true. It seems that at lower concentration the interfacial adsorption density of biosurfactant is less than MIBC due to greater structural and atomic dimensions. At higher concentration the adsorption density of biosurfactant increases by multi-layer adsorption mechanism because of free oxygen and multiple –OH groups.

Coal flotation tests using rhamnolipid biosurfactants as the sole frother of the process yielded products of 72 - 79% recovery with 10 - 15,5 % ash content supporting 55 - 57,5 % efficiency. These results seem to be promising to introduce rhamnolipid type biosurfactants as a new “biofrother”.

3.3.2.2. Electrokinetic properties of the bioreagents

It is essential to understand the mechanisms and resulting consequences of microbe–mineral interactions before the utility of the microorganism in the processing of minerals could be established. Since mineral beneficiation processes depend on the surface chemical properties of the minerals, any changes in the surface chemistry brought about by biotreatment is of significance (Natarajan and Deo, 2001).

The adhesion or adsorption of microorganisms to mineral surface depends on solution conditions (pH and ionic strength) and surface properties of both mineral and microorganism (zeta potential and hydrophobicity) (Hirajima *et al.*,2012) The solution conditions determine activation of functional groups present leading to a greater or lesser adhesion to mineral surface.

Examples of these statements are for the study of the systems of quartz-*E.coli* (Faharat *et al.*, 2008), coal-*M.phlei* (Raichur *et al.*, 1996), hematite-*M.phlei* (*Dubel et al.*, 1992), where the authors found that under acidic conditions of pH values, zeta potential measurements after interaction with the bacteria were close to the values of the zeta potential of the bacteria, which proves the adhesion by the formation of a biofilm the surface of the mineral.

An interesting case study is the use of *L.ferrooxidands* in sulphide minerals (Vilinska and Rao, 2008). Zeta potential studies showed that the bacteria isoelectric point was at pH 3,3. It was stated that the positive values of zeta potential at low pH were due to protonation of ammonia groups, and the increase in negative charge beyond pH 3, 3 is caused by the dissociation of anionic functional groups. Therefore, it was stated that cells could be adsorbed on the mineral surface through electrostatic interactions as well as interactions with chemical functional groups.

The pyrite exhibited an IEP at pH 7, 5 which changed to pH 5 after interaction with the *L. ferrooxidans*. The zeta potential of pyrite decreased in the entire pH range, getting closer to the profile of the zeta potential of bacterial cells, indicating adsorption to the cell surface of pyrite. However, the highest values of zeta potential values between pH 3 and 7 illustrated that the pyrite surface is not completely covered or the presence of iron ions on the surface due to bacterial oxidation of pyrite. The IEP of chalcopyrite presented a value of 6, 5 and moved to 3 after interaction with bacteria above pH 6, the zeta potential of chalcopyrite was similar to the zeta potential of pure chalcopyrite. The authors claimed a preferred chalcopyrite in cell adhesion, but since the surface area of chalcopyrite is almost twice that of pyrite, it is assumed that contains higher amount of surface imperfections than pyrite and therefore higher adsorption of cells onto chalcopyrite.

R. opacus was also used in the flotation of calcite – magnesite. It was found a higher affinity of the bacteria for calcite than magnesite which is seen in greater displacement of the IEP of magnesite (Botero *et al.*, 2008). The changing values of the potential profile were attributed to the presence of protein compounds excreted by the bacteria that interact with the surface of the mineral fact which is also quoted by Chandaprabha & Natarajan (2006). These authors also worked with the pyrite-chalcopyrite system using the bacterial strain *A. thiooxidans,* where a great displacement of the isoelectric point of the bacteria adhered to pyrite was found. According to the, the interaction of bacteria with the mineral samples and the changing surface properties of the mineral also affect the surface properties of the bacteria.

Although there has reviewed and discussed several works on the direct use of microorganisms in flotation, no attention has been made to the use of microbial products in flotation. The adaptation of the bacteria to the mineral substrate affects secreted metabolic products, which can generate higher amount of polysaccharides or proteins. This can be seen in the work of Pakudone & Natarajan (2011), where the authors adapted *S. cerevisiae* cells to the presence of quartz, and the secreted product were higher amounts of protein. And due to these proteins, the isoelectric point of quartz changed from 2 to 3, 2 after interaction with such adapted cells, due to the presence of amine groups on proteins (Merma, 2012).

Therefore, the electrokinetic behavior of the minerals after interaction with the bacterial cells and / or metabolic products, confirm that these bioreagents exhibit a degree of selectivity for various minerals.

3.3.3. Previous studies in Iron ores Biobeneficiation

It is essential to understand the mechanisms and resulting consequences of microbe–mineral interactions before the utility of the microorganism in the mineral processing could be established. Since mineral beneficiation processes such as flotation and flocculation depends on the surface chemical properties of the minerals, any changes in the surface chemistry brought about by biotreatment would be of significance (Sarvamangala *et al.*, 2011).

The application of microorganism for Iron ore beneficiation started in the last decade of 21st century, being the very first microorganism in application *Mycobacterium phlei*, for bioflotation of hematite fines.

Table 4 - Bacteria used in Iron ores biobeneficiation

Bacterial strain	System	Function	Author (s)
Mycobacterium phlei	Hematite	Collector	Dubel *et al.*, 1992
	Hematite fines	Collector and flocculant	Yang *et al.*, 2007
Escherichia coli	Hematite, quartz corundum	Collector	Faharat *et al.*, 2009
Bacillus polymyxa or Paenibacillus polymyxa	Hematite, corundum, calcite, quartz	Quartz collector	Deo& Natarajan, 1997; Deo *et al.*, 2001
Bacillus subtilis	Hematite, quartz, calcite, corundum	Collector	Sarvamangala *et al.*, 2001
Rhodococcus opacus	Hematite-Quartz	Collector	Mesquita *et al*, 2003
Rhodococcus erythropolis	Hematite, quartz, apatite, kaolinite	Collector	Yang *et al.*, 2013

3.3.3.1. *Mycobacterium phlei*

M. phlei is a nonpathogenic microorganism easily found in nature, and has been applied in Biobeneficiation for minerals such as hematite (Dubel *et al.*, 1992) and Yang *et al.* (2007), pyrite and spharelite (Jia *et al.*, 1996), and coal (Raichur *et al.*, 1996; Jia *et al.*, 2011).

Zeta potential measurements carried out by Dubel *et al.* (1992) showed the zeta potential of *M. phlei* and hematite as a function of pH. The zeta potential of *M. phlei* is much more negative than hematite`s and its isoelectric point (IEP) is at about pH 2,5 while hematite is at about pH 5,5.

Since electrophoretic mobility measurements indicated that the hematite is much less negatively charged than *M. phlei* and that the organism has a hydrophobic surface, it was not surprising that it functioned as a collector for

hematite. The greater flotation at pH 2,5 may be due to greater adhesion of uncharged hydrophobic bacteria to highly charged hematite particles (Dubel *et al.*, 1992; Yang *et al.*, 2007).

Infrared spectrum (IR) was used to analyze the hematite surface functional groups as they were modified by the adsorbed microbial material. Results showed the main vibration peak is from hematite's own functional group of Fe-0. The wave number 970 cm^{-1} is the Si-0 stretching vibration peak because of the presence of quartz on the hematite surface (Yang *et al.*, 2007).

The hematite absorbed by *M. phlei* appeared as many new adsorption peaks, such as at wave number 500 cm^{-1} appears substituted aromatic compound groups; at wave number 720-750 cm^{-1}appear -$(CH_2)_n$-groups; at wave number l000 and 2600 cm^{-1}appear carboxyl groups; at wave number 1400 and 2950 cm^{-1} appear –CH_2(-CH_3); at wave number 1600 cm^{-1} appears carbonyl groups; at wave number 1800 cm^{-1}appears aromatic hydrocarbon groups. The six functional groups were not found in the hematite; therefore they were part of *M.phlei* functional groups. In conclusion, it was proved that on the hematite surface occurred *M. phlei* adsorption, and there are six adsorption functional groups.

As flotation studies of hematite and *M.phlei* strain, it was found that hematite surface had different dissociations at different pH values and behaved in different surface capabilities.

Figure 6 compares the results obtained by Dubel *et al.* (1992) and Yang *et al.* (2007), for the effect of bacteria concentration on hematite floatability. Both experiments were performed in a Hallimond tube for a particle size range of +53-20 µm, at pH 7 and with a flotation time of 3 minutes.

For Yang´s results, the floatability profile reached the higher value (80%) for a *M. phlei* concentration of 18 ppm whilst Yang`s achieved a floatability value slyly higher than 40%, for a bacteria concentration of 130 ppm.

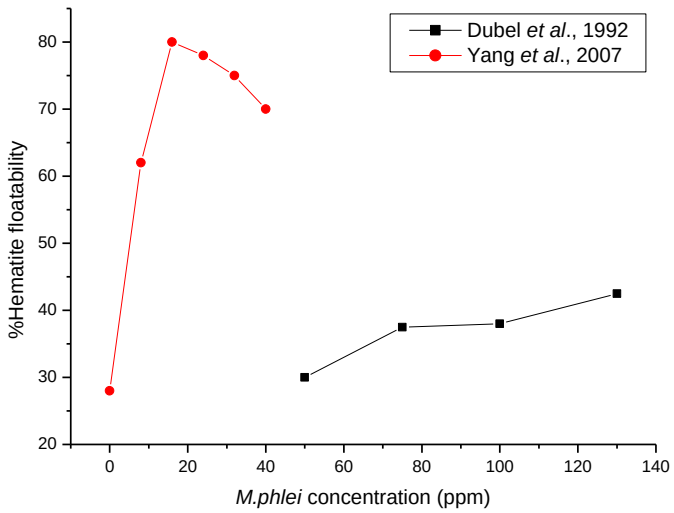

Figure 6 - Hematite flotation in a Hallimond tube using Mycobacterium phlei, as function of bacteria concentration (pH 7) (Dubel *et al.*, 1992, and Yang *et al.*, 2007)

Figure 7 shows the comparison made between the results of Yang and Dubel for the effect of pH on hematite floatability. According to Yang *et al.* (2007) the best pH range was found at about 5-7. Dubel *et al.*, 1992 found out that the greatest floatability (78%) was at about pH 2,5, and dropped to about 50 % near pH 4. The recovery then remained essentially constant as a function of pH to a pH value more basic than pH 10.

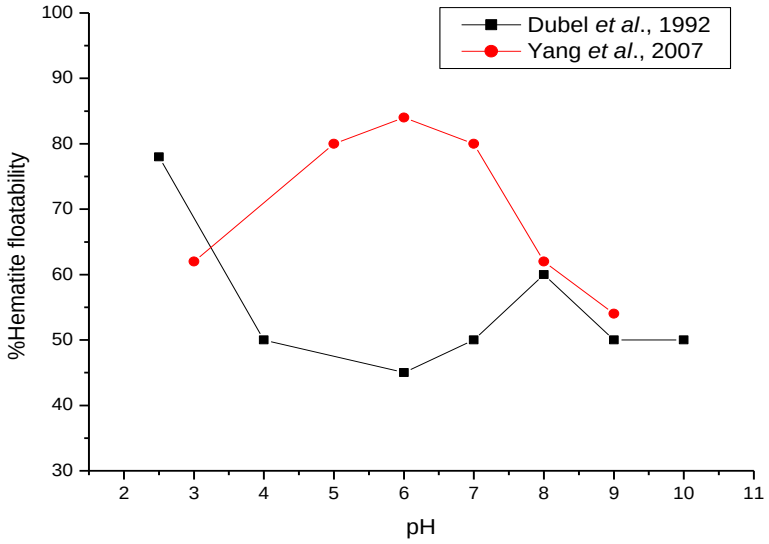

Figure 7 - Flotation recovery of hematite as function of pH (75 ppm) (Dubel *et al.*, 1992), and as a function of pH (24 ppm) (Yang *et al.*, 2007)

3.3.3.2. *Paenibacillus polymyxa*

Bacillus polymyxa is a Gram-positive, neutrophilic, periflagellated heterotrophic bacterium indigenously associated with several oxide mineral deposits (Deo and Natarajan, 1997).

Bacillus polymyxa or *Paenibacillus polymyxa* have been used as bioreagents for mineral systems quartz-corundum-calcite and hematite (Deo and Natarajan, 1997), hematite-corundum and quartz (Deo *et al.*, 2001), sulphide minerals (Sharma *et al.*, 2000), for removal of calcium and iron from bauxite ore (Anand *et al.*, 1996), and more recently it was used for coal cleaning of sulphur and ash (El-Midany and Abdel-Khalek, 2014).

Change in the surface properties of minerals were first investigated by Deo and Natarajan (1997), measuring the zeta potential of quartz, corundum, calcite and hematite before and after microbial treatment as well as the cells of *Bacillus polymyxa* treated. The isoelectric point of bacterial cells was observed to lie at a pH of about 1,5 to 1,7. However, after interaction with the above minerals, the zeta potentials of the cells were observed to shift in a less negative direction. The IEP of the cells shifted to a higher pH value of about 2,8 - 3,0 after interaction with corundum and calcite.

Before interacting with the bacterial cells, the IEPs of corundum, hematite and quartz corresponded to pH values of 7,5, 5,8 and 1,7, respectively, while the calcite mineral used in this study exhibited negative surface charge throughout the pH ranges of 5,5 to 11. Zeta potentials for calcite could not be measured at acidic pH values lower than 5,5 due to excessive dissolution of the mineral. After bacterial interaction, the surface properties of all the minerals were found to be significantly altered as attested to by shifts in their IEP values. While the zeta potentials of corundum, hematite and calcite shifted in a more negative direction, those of quartz changed towards a less negative direction after bacterial interaction, indicating that the nature of surface chemical change on quartz resulting from biotreatment could be quite different from that on hematite, corundum and calcite.

Table 5 - Zeta potential profiles for quartz, corundum and hematite before and after interaction with *B.polymyxa* cells (Deo and Natarajan, 2001)

Mineral	IEP before interaction	IEP after interaction
Quartz	1,75	4
Corundum	7,5	< 2
Hematite	5,8	< 2

Deo *et al.* (2001) measured the zeta potential of hematite, corundum and quartz before and after interaction with *P. polymyxa*. Table 5 shows the isoelectric point of these minerals before and after interaction with *P.polymyxa*. The isoelectric point of bacterial cells was observed to lie at a pH of about 1,5 to 1,7. Hematite, corundum and quartz exhibited isoelectric point IEP at pH 5,8, 7,5 and 1,75, respectively and it can be seen that interaction with cells alone in the absence of growth medium and metabolite, caused significant changes in the case of all three minerals.

Deo and Natarajan (1997) also studied the different spectrums of hematite, quartz, calcite and corundum after interaction with *P.polymyxa.* New peaks that have appeared on the difference spectrum are due to the interaction of cells with minerals. Table 6 summarizes the different infrared spectrum of quartz, hematite, corundum and calcite after interaction with bacterial cells.

While the 1640 cm^{-1} peak is attributed to the presence of carboxylate anion, another peak at 1460 cm^{-1} is that of CH_2 rocking and OH bending modes or the C-OH group of frees polysaccharides. The peak at 1118,97 cm^{-1} is due to C-OH. The peak at 1041 cm^{-1} is of primary alcoholic group of CH_2OH and another peak at 674 cm^{-1} may be due to CH_2 rocking vibrations. On the basis of FTIR spectra, it is clear that chemical interaction as well as hydrogen bonding occur during the interaction of various minerals with bacterial cells.

The authors concluded from the FTIR spectra that proteins are specifically adsorbed on quartz, while polysaccharides are adsorbed on hematite and

corundum. The mode of interaction of bacteria with quartz minerals is thus very different from that with hematite and corundum.

Deo and Natarajan (1997) and Deo *et al.* (2001) also showed the effect of pH on the adsorption of bacterial cells on hematite, corundum, calcite and quartz, and for the mineral system conformed by hematite, corundum and quartz. The authors found out that the cell adsorption is high from pH 4 to 7 and then it decreases in the case of hematite and corundum. However, the adsorption of cells on quartz is relatively low under all pH conditions. The marked decrease in cell adsorption on hematite was observed above pH 9. Although the adsorption of bacterial cells decreased on hematite and corundum in the alkaline pH range, a good number of cells still adsorbed suggesting that other non-electrostatic forces such as chemical interaction, hydrogen bonding and/or hydrophobic interaction also play an important role in the adsorption processes.

Table 6 - IR spectrum of quartz, hematite, corundum and calcite after bacterial cells interaction (Deo and Natarajan, 1997)

Mineral	Prominent band peaks
Quartz	3450 cm^{-1} – Stretching vibrations of the hydroxyl groups (combination of OH and NH bands).
	2930 cm^{-1} CH stretching modes indicating the presence of alkyl (CH_3, CH_2, CH) groups.
	1600 cm^{-1} – NH bending of secondary amide, -CONH group.
	1050 – 1100 cm^{-1} – Asymmetric stretching of phosphate groups.
	600- 800 cm^{-1} – NH wagging vibrations.
	300 – 600 cm^{-1} – CH_2 rocking vibrations.
Hematite, corundum and calcite	3400 cm^{-1} – OH stretching and –C-OH- and –CH_2OH groups due to polysaccharides
	300 – 600 cm^{-1} – CH_2 rocking vibrations.
	900 cm^{-1} – CH_2OH groups
	700 cm^{-1} – COO- bending of the carboxyl group

3.3.3.3.*Escherichia coli*

As mentioned lines above, hematite surface gained hydrophobic properties after being treated with *Mycobacterium phlei* cells (Dubel *et al.*, 1992; Yang *et al.*, 2007). However, hematite gained hydrophilic properties after interaction with *Bacillus polymyxa* (Deo and Natarajan, 1997; Deo *et al.*, 2001).

Escherichia coli were found to act as a flotation collector for quartz under acidic conditions (Faharat *et al.*, 2009). In this case, the *E.coli* strain was genetically modified to express silica-inducing protein (*sip*), believed to facilitate adhesion and modify mineral surface properties.

Figure 8 shows the zeta potential of microbial cells, quartz, hematite, and corundum as a function of the pH. The microbe has positive charges in the acidic

region and negative charges in the alkaline region; its IEP is at pH 4,5. The zeta potential curve for corundum shows that the mineral surface is positively charged in the acidic and alkaline regions, and the IEP is around pH 8,0.The zeta potential curve of hematite is similar to that of the cells, and its IEP of hematite is around pH 4,0, IEP for quartz has been reported to be approximately pH 2,0. These results are in agreement with previously reported data (Deo and Natarajan, 1997; Deo *et al.*, 2001; Faharat *et al.*, 2009; Mesquita *et al.*, 2003; Deo *et al.*, 2001; Merma *et al.*, 2013).

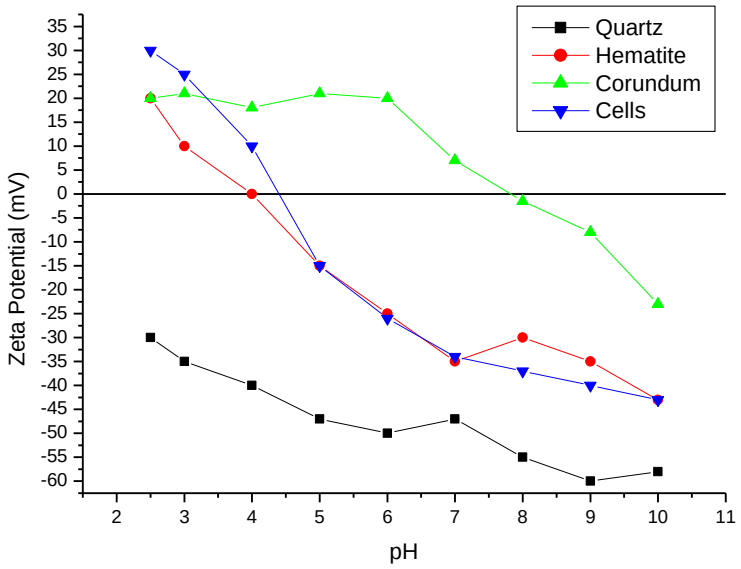

Figure 8 - Zeta potential curves of minerals and cells as function of pH (Faharat *et al.*, 2009) (Faharat *et al.*, 2009)

After the treatment with *E.coli* cells ($2{,}2 \times 10^{8}$ cells.mL^{-1}, 10 min), Faharat *et al.* (2009) experiments showed that for hematite, no significant changes occurred in the zeta potential profile of it after interaction with *E.coli* cells. It could be possible that microbial cells and hematite particles have similar surface potentials, indicating that even when adsorption could happen, changes in their surface potential was not noted. Due to the similarity in the surface potentials, the generated repulsion forces hindered the adsorption of *E.coli* cells to hematite surface, and the number of adsorbed cells was insufficient to change the surface potential of hematite.

The adhesion of *Escherichia coli* onto quartz, hematite and corundum was also investigated by Faharat *et al.* (2009). Further, the number of cells adsorbed onto quartz was higher than that on corundum and hematite. After conditioning, the number of cells adsorbed onto quartz, corundum, and hematite was 14.0×10^9, 8.0×10^9, and 3.3×10^9 cells/m^2, respectively.

The effect of pH on the number of cells adsorbed onto quartz, hematite, and corundum minerals is shown in Figure 9. These experiments were carried out at the same conditions mentioned before, but with a conditioning time of 10 min. In the case of hematite, the number of adsorbed cells was low at all pH values except for the region close to IEP of the mineral.

According to Faharat, it is possible to float biotreated quartz at pH <4,3 with a recovery of 58% under these experimental conditions. The flotation recovery of biotreated corundum was lower than that of quartz. Maximum recovery was obtained in the pH range 5,0–8,0, which is the same range in which the total interaction energy is a negative and high adsorption density is achieved. Biotreated hematite did not float at any pH value, and its flotation recovery was less than 10%.

It was also studied the effect of the bacterial concentration on the flotation recovery of biotreated minerals. 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. It was clear that the number of cells adsorbed onto quartz is much higher than that adsorbed onto hematite or corundum (Faharat *et al.*, 2009)

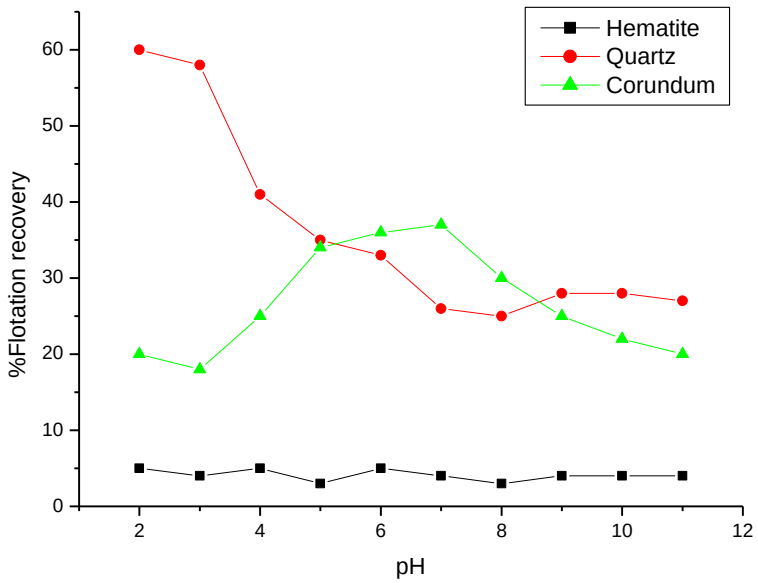

Figure 9 – Floatability of biotreated minerals as a function of the pH (cell concentration $2.2x10^{8}$ cells.mL^{-1}) (Faharat *et al.*, 2009)

3.3.3.4.*Bacillus subtilis*

A system of hematite along with quartz, corundum and calcite was chosen to separate hematite from the rest of the oxide gangue minerals. Sarvamangala *et al.* (2011) used *Bacillus subtilis* for the separation of hematite from the other oxide gangue minerals. Selective separation of hematite from quartz, calcite and corundum was studied through microbial induced flotation and flocculation.

B. subtilis is a Gram-positive neutrophilic periflagellated aerobic, catalase-positive capsulated bacteria commonly found in soil. Typical scanning electron micrographs depicting adhesion of bacterial cells onto quartz, calcite, corundum and hematite are illustrated in Figure 10. As could be readily seen, profuse and significant adhesion of bacterial cells could be seen on hematite, compared to other oxide minerals. Bacterial cells thus exhibited higher surface affinity towards hematite when compared to the other minerals (Sarvamangala *et al.*, 2011).

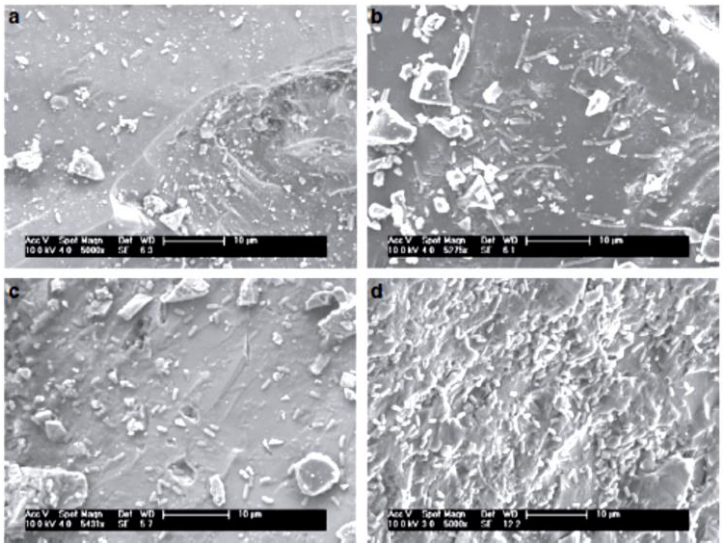

Figure 10 - SEM micrographs of *B.subtilis* adsorbed onto (a) quartz, (b) calcite, (c) corundum, (d) hematite (Sarvamangala *et al.*, 2011).

From the SEM micrographs, adsorption density of cells of *B. subtilis* 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.

Adsorption behavior of bacteria on the above minerals was also studied at a lower pH of about 4–5. At this pH also the cell adsorption density was the highest on hematite compared to corundum. Bacterial adsorption was found to be sensitive to pH especially with respect to hematite and corundum. With an increase in pH hematite and corundum surfaces were rendered more and more negative. From a mineral beneficiation point, a solution of pH of about 6,8–7,2 appears more beneficial for separation of hematite from either corundum or quartz since bacterial cell coverage on these minerals was found to be sufficiently different from each other.

Flotation behavior of hematite and quartz, calcite and corundum was studied before and after interaction with bacterial cell free extract and bacterial cells. Flotation recovery of quartz was about 92% on interaction with bacterial cells, for

the recovery of calcite and corundum the percentage flotation recoveries were about 74% on interaction with bacterial cells. *B. subtilis* confers surface hydrophobicity on quartz, calcite and corundum, while similar biotreatment renders hematite more and more hydrophilic.

3.3.3.5. *Rhodococcus opacus*

Rhodococcus opacus, non-pathogenic hydrophobic bacteria was investigated as a flotation reagent for the hematite–quartz system (Mesquita *et al.*, 2003). Later, *R.opacus* was used for the mineral system barite-calcite-magnesite (Botero *et al.*, 2007), and for apatite-quartz (Merma *et al.*, 2013).

Figure 11 shows the zeta potential of *R. opacus* cells, hematite and hematite after interaction with *R.opacus*, as a function of pH.

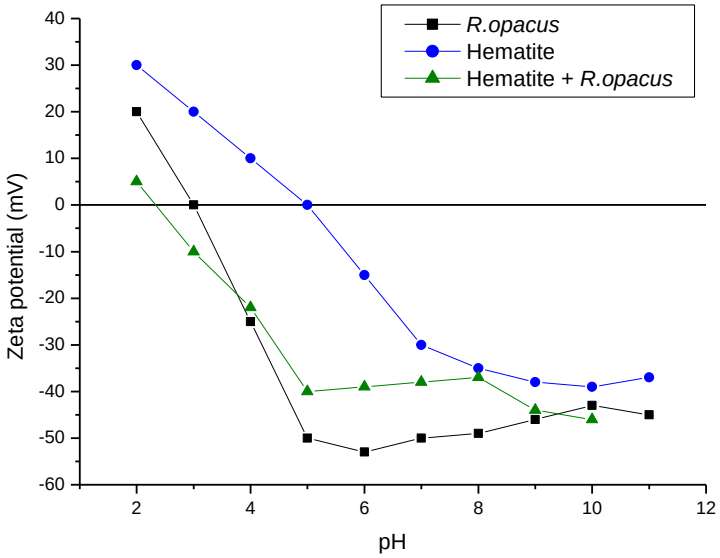

Figure 11 - Zeta potential for hematite before and after the interaction with a cellular suspension of *R. opacus*; 0,1 mM NaCl (Mesquita *et al.*, 2003)

The surface of the *R. opacus* cells was negatively charged over a wide range of pH, namely, 3,5–14, with the isoelectric point (IEP) corresponding to a pH of

about 3,2. The IEP for the mineral samples corresponded to pH values of approximately 2.0 for quartz and 5,1 for hematite. For hematite in the pH range of 3,2–5,5, and there was a displacement in the pH values corresponding to the isoelectric points, shifting from 5,1 to 2,6 in the case of hematite.

The quantity of *R. opacus* cells adhered onto hematite and quartz particles as a function of pH values is presented in Figure 12. The adhered quantity of microorganism is high at the acid pH range. 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.

Figure 13 shows the good floatability of the hematite at about 90%, at pH value around 4, and about 72% for pH values between 6 and 12, when a cellular concentration of 600 ppm was used.

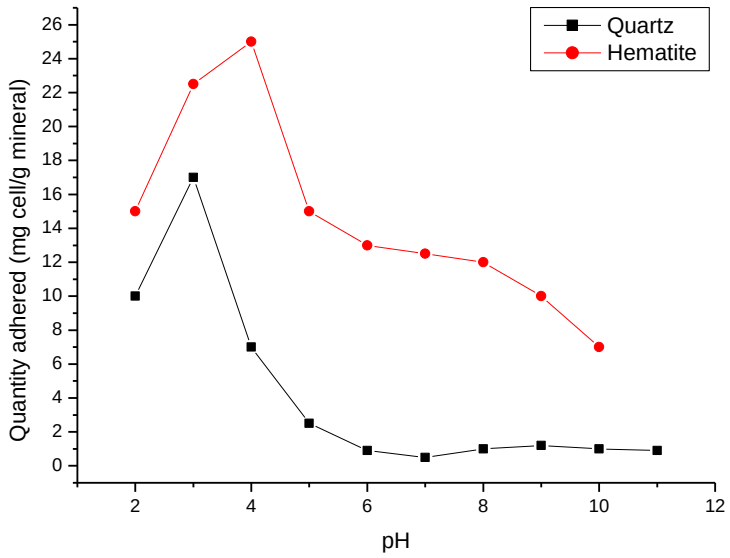

Figure 12 - The adhesion of *R. opacus* cells on hematite and quartz as a function of pH; 0,1mM NaCl (Mesquita *et al.*, 2003)

It was also demonstrate that the best floatability of quartz occurred for pH value around 3. The floatability of the quartz is, however, considerably smaller than that of hematite (about 42%). Practically no floatability for other pH values was observed.

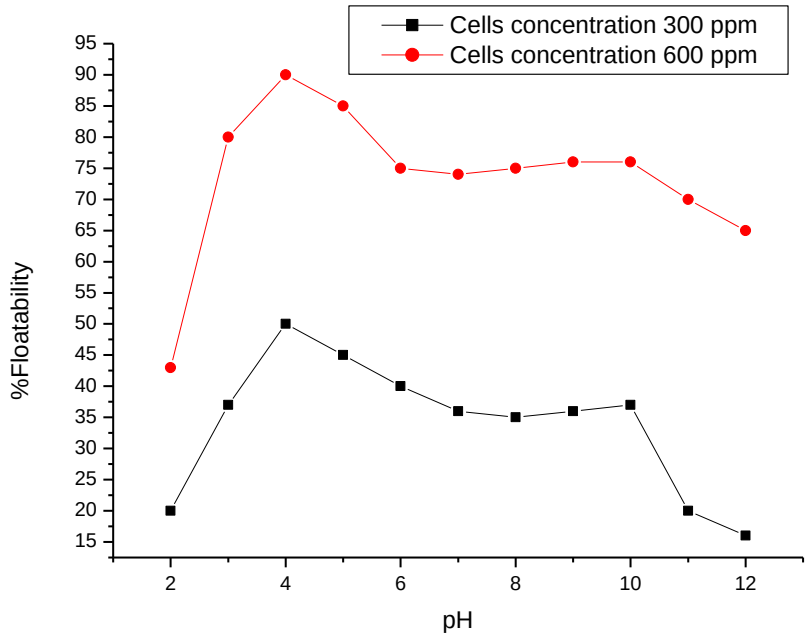

Figure 13 - Floatability of hematite as a function of *R.opacus* cells concentration and pH (Mesquita *et al.*, 2003)

3.3.3.6.*Rhodococcus erythropolis*

R. erythropolis, a nonpathogenic microorganism is found widely in nature, has been widely used for bioremediation of oil contaminated water and soil. The flocculation–flotation functions of *R. erythropolis* for processing refractory hematite ores that are prevalent in ore re- serves in China were investigated by Yang *et al.* (2013). The four main minerals that are present in hematite ores (hematite, quartz, kaolinite and apatite) were tested to determine feasibility of using *R.erythropolis* as a collector for separating hematite from hematite ores.

The surface of *R. erythropolis* was negatively charged between pH 2 and 10. The isoelectric points (IEPs) corresponded to pH values of approximately pH 5,2 for hematite, pH 3,0 for quartz, pH 3,3 for kaolin and pH 3,0 for apatite. Microbe-mineral interactions resulted in significant surface charge changes on mineral surfaces. The isoelectric points for hematite, quartz, apatite and kaolin after interaction with *R. erythropolis* are shown in Table 7. After interaction, the IEP of hematite increased from pH 5,2 to pH 5,6. The IEP of quartz, kaolin and apatite

did not appear again. This indicated that adsorption of *R. erythropolis* cells on mineral surfaces was conferred by bacterial and mineral surface properties. The changes in surface properties of four pure minerals could contribute to flotation separation of hematite from hematite ores using *R. erythropolis* as a collector.

Table 7 - Zeta potential of hematite, quartz, apatite and kaolin after interaction with *R.erythropolis* (Yang *et al.*, 2013)

Mineral	IEP *R.erythropolis*	IEP after interaction with bacteria
Quartz	< 2	2,4
Apatite	< 2	2,7
Hematite	< 2	5,6
Kaolinite	< 2	2,9

FTIR spectra for *R.erythropolis* are shown in Figure 14. Therefore, it is possible to use this strain as a collector for hematite separation from hematite ores, according to Yang *et al.* (2013).

After interaction with *R. erythropolis*, hematite surface contained six groups from *R. erythropolis* (the band at 3398,52 cm^{-1} can be assigned to stretching vibrations of the hydroxyl groups (a combination of OH and NH bands). The surface groups on *R.erythropolis* are similar to the groups for fatty acids).

The displacement of these groups along different directions occurred on hematite surface. Before interaction, hematite surfaces contained hydroxyl groups (hydrogen bonded OH) as well as metal to oxygen stretching vibrations. However, the spectra obtained after interaction with *R. erythropolis* contained new surface species. These results demonstrated that adsorption of *R. erythropolis* on hematite surface occurs mainly by chemical adsorption, including chemical interactions of carboxylic group and phosphate group with hematite surface, and hydrophobic association among hydrophobic hematite particles. The chemical adsorption makes hematite surface hydrophobic and form hydrophobic floccules of hematite particles that enhance floatability and separation of hematite in hematite ores.

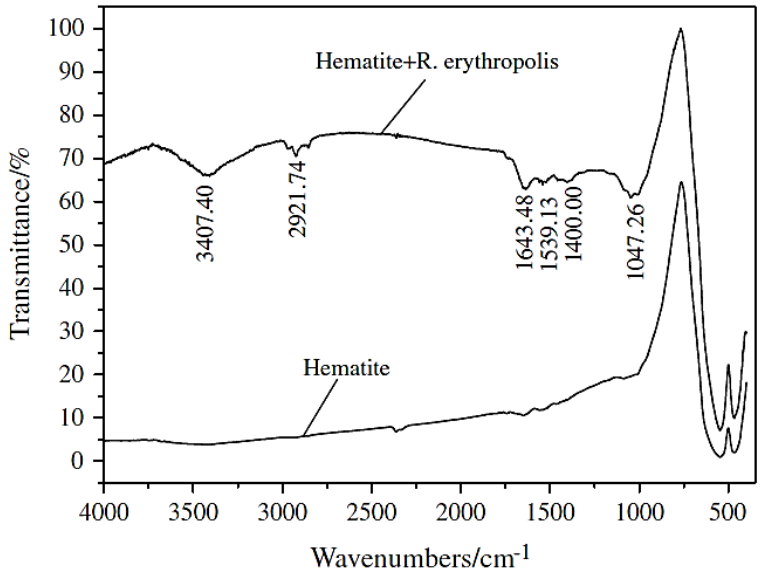

Figure 14 - Infrared spectra for hematite before and after interaction with *R.erythropolis* (Yang *et al.*, 2013)

The effects of pulp pH on flotation recovery of hematite, quartz, kaolin and apatite are shown in Table 8. Hematite recovery increased rapidly with the increasing of pulp pH from 3 to 6, and then decreased rapidly at above pH 6. These data showed that the affinity of *R. erythropolis* is much stronger for hematite than for three other minerals. Hematite recovery was optimal at pulp pH 5– 6,5. The recovery of hematite was 86,85% at a pulp pH of 6.

The effects of *R. erythropolis* concentration on flotation recovery of hematite, quartz, kaolinite and apatite are also shown in Table 8. The hematite recovery increased rapidly as concentration of *R. erythropolis* increased from 0 to 75 $mg.L^{-1}$, but leveled off at above this value. The recoveries of other three minerals increased slightly with increasing concentrations of *R. erythropolis*. At a concentration of 75 $mg.L^{-1}$, the recovery of hematite was 89,67%.

Table 8 – Higher values of flotation recovery for the mineral system using *R.erythropolis*

Mineral	Mineral recovery at pH around 6 (%)	Mineral recovery at 75 mg.L^{-1} *R.erythropolis* (%)
Hematite	86,85	89,67
Quartz	25,00	22,5
Apatite	32,50	33,5
Kaolinite	32,50	26,00

To examine possibility of separation for hematite using *R. erythropolis* as a collector, a mineral mixture of hematite–gangue (weight ratio 1:1) was processed by one-stage flotation under optimal conditions of a pulp pH of 6 and a *R. erythropolis* concentration of 75 mg.L^{-1}. It was concluded that it is possible to efficiently separate hematite from hematite–gangue mixtures through flotation using *R. erythropolis* as collector for hematite. The flotation of *R. erythropolis* was selective for collecting hematite over three other minerals. Therefore, *R. erythropolis* worked as a collector for hematite (Yang *et al.*, 2013).

The adsorption of *R. erythropolis* onto hematite led to formation of hydrophobic agglomerates of micro-fine hematite particles. SEM images of adsorption of *R. erythropolis* onto hematite surfaces showed 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.

3.4. *Rhodococcus ruber* as a potential bioreagent

The cell genre *Rhodococcus* was established by Goodfellow and Anderson, in 1977, whom recognized others as *R.rhodochrous, R.bronchialis, R. coprophilus, R.corallinus, R.equi, R.erythropolis, R.ruber, R, rubropertinctus, R.opacus* and *R.terrae.* The morphology of the *Rhodococcus* genre is basically of rod cells (Borges, 2011).

The first studies of the genre *Rhodococcus* as biosurfactant, is related to 1990, according to Singer *et al.* (1990) (Bicca *et al.*, 1999). Later, a further study

of the production of biosurfactant by hydrocarbon degrading using *Rhodococcus ruber* and *Rhodococcus erythropolis* strains was carried out in Brazil by Bicca *et al.* (1999). This process was based upon the ability of biosurfactants to reduce surface tension, blocking the formation of hydrogen bridges and certain hydrophobic and hydrophilic interactions. One of the strains showed to be a potential producer, attaining 63% of emulsifying index for a Diesel-water binary system.

Another research, carried out by Kuyukina and Ivshina (2010), showed the effective and environmentally safe biosurfactant produced by *R. ruber*. The biosurfactant produced possesses significant advantages over synthetic surfactants, particularly low toxicity and ready biodegradability, stable activity under extreme conditions (e.g. high or low temperature and pH, high salinity), a wide range of functional characteristics and the possibility to be produced from relatively cheap or renewable raw materials (e.g. hydrocarbon mixtures, vegetable oils). *Rhodococcus ruber* was recently used in the removal of Co (II) and Ni (II) present in aqueous solutions (Borges, 2011).