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Study of the effects of conventional reagents for sulfide flotation on bio-oxidation activity of *Acidithiobacillus ferrooxidans*


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**ABSTRACT**

Bioleaching as a low cost and environment-friendly process could be a promising option for the enrichment of froth flotation products. Flotation reagents (collectors, frothers, etc.) are effective on the bacteria growth and oxidation activity; however, their impact has not been widely investigated. In this study, the effect of conventional reagents for sulfide flotation; collectors: potassium amylxanthate (KAX), potassium isobutyl-xanthate (KIBX), sodium ethyl-xanthate (NaEX), potassium isopropyl xanthate (KIPX) and Dithiophosphate (Aero3477), and frothers; pine oil (PO) and methyl isobutyl carbinol (MIBC) in various concentrations have been examined on *Acidithiobacillus ferrooxidans* activities. The results of this study demonstrate these flotation surfactants may have positive or negative influences on the bio-oxidation, based on their chemical compositions and/or concentrations. In general, the inhabitation effects of collectors would be increased in higher dosages and based on differences between results of various conditioning tests with the control test (without reagent) in different days, this effect could be considered by the following order: for 0.01 g/L: KAX > KIPX > KIBX > Aero3477 > NaEX, 0.1 g/L: NaEX > KIPX > KAX > KIBX > Aero3477, and 1 g/L: NaEX > KIPX > KIBX > KAX > Aero3477, and for frothers: MIBC > PO in all concentrates. These outputs potentially can be used for the selection of flotation surfactants when the flotation products are going to be further processed by bioleaching for the metallurgical extraction.

**KEYWORDS**

Bioleaching; Collector; Flotation; Frother; Thiobacillus; Toxicity

**Introduction**

Froth flotation is a widely used method for beneficiation and recovery of million tons of valuable minerals in the world. In metallurgical processes, flotation is the most important separation technique of the mineral enrichment (Deo and Natarajan, 1998; Pacholewska et al., 2008). The separation process involves many types of reagents. For instance, collectors which react with the surface of the target minerals and render them hydrophobic (the gangue associated minerals remain hydrophilic) for collection into the froth phase, and frother (usually nonionic molecules) which reduce the surface tension of pulp by providing a large air-water interface and create a stable froth for the floated minerals (Tuovinen, 1978; Okibe and Johnson, 2002; Chehreh Chelgani and Hart, 2014; Jafari et al., 2016).

Based on operational procedures and types of reagents, these chemicals may remain in the flotation products (slurry, mineral surfaces (tail and concentrate), etc.) (Tuovinen, 1978; Deo and Natarajan, 1998; Pacholewska et al., 2008). It was reported that the filtration process cannot remove all moisture of flotation products (concentrate or tail). For example, in the filter press, 20% moisture remains and in the rotary drum filter (RVDF), 10–65% moisture remains in the filter cake (Sutherland and Chase, 2011). There is extensive data on toxicity of these residual substances to be discharged from flotation operations (Smith, 1996). The presence of these toxic chemicals in mill tailings can lead to many environ-

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mental problems, such as destroying soil texture, polluting groundwater, and reducing ecological landscape, etc. (Liu et al., 2007). These chemical material have no significant effect on the downstream pyro-metallurgical processes (Dehghan and Dianati, 2015), whereas they can have a great impact on bioleaching process (Deo and Natarajan, 1998; Dong and Lin, 2012). Recently, bioleaching is considered as a promising option for the processing of low-grade ores, the enrichment of flotation concentrates, and the treatment of disposal tailings, because of its low cost and environment-friendly nature (Huerta et al., 1995; Dehghan and Dianati, 2015).

Bioleaching is the chemical and biological oxidation of iron and sulfur to convert an insoluble metal compound into a water-soluble form. Generally, bioleaching is involved with two mechanisms for the dissolution reactions: (1) the direct mechanism; as a result of physical contact between microorganisms and metal sulfides, metal sulfides oxidize to sulfate and metal ions by many enzymatically catalyzed steps, (2) the indirect mechanism (without contact); as a result of lixiviant generation by microorganism, ferrous iron (Fe$^{2+}$) oxidize to ferric iron (Fe$^{3+}$). Fe$^{3+}$ as an oxidizing agent (an electron carrier) contacts with mineral surfaces, reduces to Fe$^{2+}$ and oxidizes metal sulfides to sulfate and different types of sulfur compounds. In both mechanisms, the microorganism contributes to the process by generation of the oxidizing agent (Fe$^{3+}$), and by subsequent sulfur compound oxidizing generated from sulfide dissolution (Bosecker, 1997; Schippers and Sand, 1999; Stott et al., 2000; Rohwerder and Sand, 2003; Keeling et al., 2005; Brandl, 2008).

While flotation reagents change the surface properties of minerals, these surfactants can affect the bioleaching mechanism (the bacteria growth and oxidation activity) (Dong and Lin, 2012). Although there are numerous studies on bioleaching process, only a few systematic investigations on the potential effects of flotation reagents on iron and sulfur bio-oxidation by bacteria have been reported to date. The results of those studies in various conditions indicated that changes on mineral surface properties (as a result of reaction with the flotation surfactants) may improve or limit bacterial activities through the bioleaching process (Dong and Lin, 2012; Dehghan and Dianati, 2015).

Testing on Acidithiobacillus ferrooxidans (At. ferrooxidans) in the presence of different reagents with various dosages including 8-hydroxyquinoline (150 and 400 mg/L), Tween 80 (10, 50, 100, and 1000 mg/L), Triton X100 (10, 50, 100, and 1000 mg/L) or Tergitol-7 (2.5, 12.5, and 250 mg/L) indicated no positive effect on the bioleaching of sulfide metals in the presence of 0.002% yeast extract (Puhakka and Tuovinen, 1987). While in another study, it was reported that the oxidation ability of At. ferrooxidans considerably decreased in the presence of Tween 20, 40, 60, and 80. It was demonstrated that the bacterial activity, the surface tension of the medium, and the rate of substrate oxidation at saturation decreased as the concentration of these reagents increased (Torma et al., 1976). In contrast, the addition of Tween 80 and sodium oleate to the solid medium during the growth of Aspergillus fícuum (fungi) showed an increase in the production of the enzyme (phytase production), while Triton X-100 had a negative effect on this process (Ebune et al., 1995). In agreement with these influences on fungi, the effects of nonionic surfactants (Tween 80, Tween 20, and Triton X-100) on Nectria catalinensis (fungi, Ascomycetes) indicated that on the 20th day of growth, and in response to higher concentrations of Tween 80; extracellular proteins cell membrane permeability rises. Tween 20 and Triton X-100 can increase cellobiase yield, but they were inhibited growth and cellulolytic enzyme production (Pardo, 1996). In addition, the effects of Tween 80 and rhamnolipid (as an anionic reagent) on Penicillium simplicissimum (fungi) activity during solid-state fermentation were studied and the results indicated that the enzyme activities of amylase, CMCase, and xylanase increased by the presence of Tween 80 and rhamnolipid (they increased the fungal biomass slightly); however, they had a negative effect on the protease production (Zeng et al., 2006).

Zhang et al. (2008) demonstrated that at certain concentrations ($10^{-8}$ g/L), Tween 80 and sodium isobutyl-xanthate (NAIBX) could enhance the growth and sulfur-oxidizing activities of At. ferrooxidans, but at higher concentrations, they limit
the growth and are even harmful (Zhang et al., 2008).

The toxicity of various flotation surfactants (collectors: N-dodecyl mercaptan (DOM), potassium amylxanthate (KAX), potassium ethylxanthate (KE), sodium butylxanthate (NABX), sodium isopropylxanthate (NAIPX), sodium amylxanthate (NAX), primary amine acetate; frother: DOWFROTH 250, pine oil, 1,1,3-triethoxybutane (TEB), and modifying agents: carboxymethylcellulose (CMC)) to iron-oxidizing of \textit{At. ferrooxidans} had been examined by (Tuovinen, 1978). The wide variation of inhibition during ferrous-iron and thiosulfate oxidation by the different reagents suggested that their toxicity was dependent on their chemical composition, and the formation of intermediates. DOWFROTH 250 and NABX showed the least toxicity among all reagents (Tuovinen, 1978). Loon and Madgwick (1995) tested the effect of isopropyl-, isobutyl-, ethyl-, and amylxanthate on Cu²⁺ production during \textit{At. ferrooxidans} leaching of chalcopyrite. The results indicated that these chemicals inhibited the bacterial growth and decreased the formation of soluble copper and iron. Isopropyl xanthate showed relatively low toxicity during the process. In another study, it was shown that the \textit{At. ferrooxidans} activity for bioleaching of pyrite and chalcopyrite can affect by NAIPX; the collector has inhibited cell growth and adsorbed at the bacteria-solution interface (Loon and Madgwick, 1995). Moreover, Deo and Natarajan (1998) reported that the bacteria (\textit{Bacillus polymyxa}) efficiently stripped collectors (dodecyl amine, diamine, isopropyl xanthate, and sodium oleate) from mineral surfaces through bioremediation. They found that bacteria can utilize the collectors to satisfy their carbon and nitrogen requirements fully (Deo and Natarajan, 1998).

Okibe and Johnson (2002) conducted an extensive study on the toxicity of a wide range of flotation reagents (xanthates, carbamates, dithiophosphates, a mercaptobenzthiazole, and a frother) to five moderately thermophilic and acidophilic bacteria (\textit{Leptospirillum ferrooxidans} (L. ferrooxidans), \textit{Acidimicrobium ferrooxidans}, \textit{At. ferrooxidanscaldus}, and \textit{Sulfobacillus metallicus} (S.m)) and one archaeon (\textit{Ferroplasma} (F)). The results showed a wide variation in the rate of toxicities for the various reagents and the sensitivities of the microorganisms. Generally, the dithiophosphates and the mercaptobenzothiol were the most toxic reagents, and the \textit{L ferrooxidans} and \textit{F} isolates were the most sensitive microorganisms. In another investigation, the effect of chemicals (collectors: Hostaflot X23, KAX, Aero 3477; frother: Flotanol C-7, Montanol 800) used in the flotation separation was tested on the S.m activity. The positive effect of KAX by increasing the leaching rate and the negative effect of frother (Flotanol C-7) by decreasing the chalcopyrite leaching rate was observed (Okibe and Johnson, 2002). Pacholewska et al. (2008) studied the impact of amyl- and ethyl-xanthate, Coroflot (frother), Selkol 1981 (activator), and the industrial solution CuSO₄ (modifying reagent) on the metabolic activity of \textit{Acidithiobacillus thiooxidans} (\textit{A. thiooxidans}) and reported that xanthates were influenced only to a small extent on the bacterial populations and their activities. The frother also did not show a significant effect, whereas the activator and modifying reagent revealed highly toxic effect and led to the inactivation of bacteria metabolism (Pacholewska et al., 2008). Dong and Lin (2012) investigated the effect of ethyl-, isopropyl-, butyl-, isoamyl-xanthate, and butylaminel (typical chalcopyrite flotation collectors) on the \textit{At. ferrooxidans} activity and demonstrated that all these surfactants can depress the copper extraction by the bacteria. The order of the inhibition effects on the bioleaching process was: butyl-, isopropyl-, isoamyl-, ethyl-xanthate, and butylamine (Dong and Lin, 2012). Moreover, the effects of KEX and KAX on the activity of \textit{At. ferrooxidans}, \textit{A. thiooxidans}, and \textit{L. ferrooxidans}, and also their mixed culture on the bioleaching of zinc sulfide showed that the presence of these reagents had positive effects on the growth of mesophilic bacteria, and subsequently increased the zinc dissolution rate under bioleaching process. Results indicated that the effects of KEX and KAX on bacteria activity were highly dependent on the slurry pH, and as a result, pH should be precisely controlled (Delghan and Dianati, 2015).

These studies have demonstrated that the bioleaching process in the presence of flotation surfactants was mostly sensitive to the type of
reagents (chemical compositions), the concentration of reagents, type of microorganism, and pH adjustment. In this work, to study the effect of some of these factors, such as the pH, concentration, and various types of reagents on *At. ferrooxidans* activity, the influence of conventional collectors (NaEX, KIPX, KIBX, KAX, and dithiophosphate (Aero3477)), and frothers (pine oil (PO) and methyl isobutyl carbinol (MIBC)) on bio-oxidation of iron and sulfur by *At. ferrooxidans* in various conditions are investigated through various analyses (pH, oxidation–reduction potential (ORP), dissolved oxygen (DO), microorganisms counting method, iron ion (Fe$^{3+}$ (iron total), Fe$^{2+}$, and Fe$^{3+}$ measurements). To understand the effect of each reagent in various days, their results were compared with the control test (without reagent). The results of this investigation can be used to better realize the reaction mechanisms of bacteria when flotation surfactants are subjected to the bioleaching process.

**Materials and methods**

**Bacterial strain and growth conditions**

A pure strain of *Acidithiobacillus ferrooxidans* (*At. ferrooxidans*) was obtained from the research and development center of Sarcheshmeh mine, Kerman, Iran. *At. ferrooxidans* was cultivated in a 9K medium containing five different mineral salts ((NH$_4$)$_2$SO$_4$: 3g/L, MgSO$_4$.7H$_2$O: 0.5g/L, K$_2$HPO$_4$: 0.5g/L, KCL: 1g/L, and Ca (NO$_3$)$_2$.H$_2$O: 0.01g/L). The initial pH of the media was adjusted to 1.8 with H$_2$SO$_4$. As a source of energy 44.22 g/L FeSO$_4$.7H$_2$O and 10 g/L sulfur were added to the media. Incubation was performed at 34°C in an incubator shaker having the rotation speed of 140 rpm.

**Flotation reagents**

The pure conventional collectors (NaEX, KIPX, KIBX, KAX, and Aero3477), and frothers (PO and MIBC) for the flotation of sulfides were prepared from the mineral processing laboratory at the University of Tehran, Iran. To better understand the effects of these chemical surfactants on *At. ferrooxidans* activity, a wide range of their concentrations (0.01, 0.1, and 1 g/L) is investigated.

**Analyses procedure**

For seven reagents in the three different concentrations, 21 tests were designed. A control test without the mentioned reagents was also conducted. To have an accurate evaluation, various analyses and comparisons were considered in the
same days. The effectiveness of various reagents on bio-oxidation was explored by comparison of various test results to the control test. The pH and ORP value of tests were measured by pH and ORP (Ag/AgCl reference electrode) analyzer (Mettler Toledo, Columbus, OH). The ORP measurement directly analyzed the oxidizing and reducing agents during the bacteria activity in the media. The presence of an oxidizing agent, such as oxygen increases the ORP value, while the presence of a reducing agent such as substrate or carbon- and hydrogen-containing compounds decreases the ORP value (Lombardi and Garcia, 2002). An oxygen meter (Model JENWEY) was used to measure the amount of DO in the media. In addition, the Fe\(^{3+}\)/Fe\(^{2+}\) ratio was used to define the effects of reagents on At. ferrooxidans oxidation action. The amount of Fe\(^T\) was determined by atomic absorption spectrophotometer (AAS model: varian-20, Agilent Technologies, Santa Clara, CA). The amount of Fe\(^{2+}\) was measured via titration by dichromate potassium (0.001 M). Fe\(^{3+}\) percentage was calculated by subtracting Fe\(^{2+}\) from Fe\(^T\) (Fe\(^T\)=Fe\(^{2+}\)+Fe\(^{3+}\)). The bacterial number (growth) was determined by using a Neubauer lamp (0.1 x 1/400 mm\(^2\)) (HBG, Giessen, Germany) and 100X magnification under a Zeiss biological microscope (Bacterial count per mL = N x 400 x 10\(^4\)) (Zeiss: Carl Zeiss AG, Oberkochen, Germany).

Results and discussions

pH variations

The variations of pH were measured during the At. ferrooxidans activity in the presence of different collectors (dosages: 0.01, 0.1, and 1 g/L), and also in their absence (the control test) (Figure 1). Results show an initial increase in the pH through the first 2 d in the presence of xanthates. Xanthates are unstable in acidic solutions, thus they hydrolyze and form unstable xanthic acids, followed by decomposition into alcohol and CS\(_2\) (Equations (1) and (2)). This instability would increase when pH is below 3 (Iwasaki and Cooke, 1959; Pomianowski and Leja, 1963; Tuovinen, 1978; Jones and Woodcock, 1983; Jafari et al., 2016). Moreover, the pH increase may occur when the H\(^+\) from the solution is consumed for the xanthate decompositions in the initial days. The bio-oxidation of Fe\(^{2+}\) to Fe\(^{3+}\) through the process at the early stages could be another reason for such an increase in pH profiles (Equation (3)) (Dehghan and Dianati, 2015). This increase was not observed for the samples with Aero3477 (as a collector), and also in the presence of frothers (PO and MIBC). This phenomenon can possibly be explained by the fact that there is only one –OH group in MIBC and PO for the H\(^+\) consumption (Khoshdast and Sam, 2011; Jafari et al., 2017a, 2017b), and Aero3477 is partially a stable compound in the acidic conditions. The reaction of Aero3477 with a weak acid is rather slow, and it can react with sulfuric acid based on Equation (4) (Bulatovic, 2007):

\[
RX^- + H^+ \rightarrow RXH 
\]

\[
R - O - C(OH)_{\text{ NH}} \rightarrow R - OH + CS_2 
\]

\[
2Fe^{2+} + 2H^+ + 1/2 O_2 \rightarrow 2Fe^{3+} + H_2O 
\]

\[
2(RO)_2P(\text{H})/\text{P} + 2H_2SO_4 \rightarrow (RO)_2PS_2S_2P(RO)_2 + H_2O + H_2SO_3 
\]
inhibited the bacterial activity (Torma et al., 1976; Tuovinen, 1978; Jafari et al., 2016). Therefore, an increase in the concentration of these active reagents inversely affects the oxidizing mechanisms and the pH reduction rate. Moreover, it was reported that in low dosages, surfactants can modify the surface properties, improve the contact between the bacteria and the energy substrate sulfur particles, and increase bio-productions (Zhang et al., 2008; Jafari et al., 2017a).

\[
2\text{Fe}^{3+} + 6\text{H}_2\text{O} \rightarrow 2\text{Fe(OH)}_3 + 6\text{H}^+ \quad (5)
\]

\[
\text{S}^0 + 6\text{H}_2\text{O} + \frac{3}{2}\text{O}_2 \rightarrow 2\text{SO}_4^{2-} + 2\text{H}^+ \quad (6)
\]

The final pH (after 21 d) for all tests with 0.01 g/L reagent concentrations is approximately the same as the control test (pH 1.02) (Figure 1(a)). Results (Figure 1(b,c)) indicate that NaEX (in high concentration: 0.1 and 1 g/L), compared with other reagents, has the highest pH deviation from the control test on the day 21th (pH 1.61 vs. 1.02). KIBX is dissociated less than other xanthates. Aero3477 relatively showed (Figure 1) the smallest deviation (in all concentrations) from the control test among other collectors. In general, for collectors, the pH deviation from the control test is decreased in the following order: for 0.01 g/L: KAX > KIPX > NaEX > Aero3477 > KIBX, 0.1 g/L: NaEX > KAX > KIBX > Aero3477 > KIPX, and 1 g/L: NaEX > KIPX > KIBX > KAX > Aero3477. This order can be explained by the fact that the rate of dissociation for xanthates in an acidic solution depends on the length of the hydrocarbon chain (longer radicals are dissociated slower than xanthates with shorter hydrocarbon chains) (Bulatovic, 2007). For the frothers after 21 d, pH for MIBC tests is lower than the control test in the 0.01 and 0.1 g/L reagent concentrations, whereas in the highest concentrations (1 g/L) the pH deviation from the control test for PO is smaller than MIBC (Figure 1(c)).

**ORP variations**

The ORP analyses of samples (Figure 2) indicate that during the first 2 d there was a small increase in the ORP value for all test conditions (in the control test slightly decreased from 408 to 400 mV). As the reactions proceeded and activity of *At. ferrooxidans* are increased, within the next 5 d a sharp increase in the ORP value was observed in some samples, except for KIPX in the 0.01 g/L, KIPX and NaEX in the 0.1 g/L, and KIBX and NaEX in the 1 g/L. This trend can explain by the bio-oxidation stages; bacterial oxidation and regeneration of Fe$^{3+}$ to Fe$^{2+}$ (Jafari et al., 2016). These results indicate that the high concentration of surfactants can limit the growth and oxidizing activities of *At. ferrooxidans*.

After 16 d, the ORP value for the all samples with 0.01 g/L collectors is higher than the control.
test (ORP: 600 mV), and using this concentration the ORP value for all collectors is increased to ~650–700 mV after 21 d (Figure 2(a)). In 0.1 g/L concentrations, the ORP trends for KAX, KIBX, and Aero3477 show a significant growth to over ~660 mV after 7 d (the control test is gradually increased), and KIPX and NaEX do not show any changes. These values steadily rose to over 680 mV after 21 d (the control test 652 mV), and for NaEX and KIPX, the ORP value and remained around ~400 mV (Figure 2(b)). The ORP value for samples with the highest concentration approximately showed the same trend as the 0.1 g/L concentrations, the ORP of NaEX and KIPX remained around 680 mV after 21 d (Figure 2(c)).

For both frothers, in 0.01 g/L, the ORP value is sharply increased from ~400 to ~670 mV after 7 d, and then the value is gradually increased to around 690 mV until day 21 (close to the value of the control test: 650 mV) (Figure 2(a)). In 0.1 g/L concentrations (Figure 2(b)), the ORP value does not grow until day 14, and then, on day 16, it is significantly increased to 597 and 687 mV for MIBC and PO, respectively. In 1 g/L concentrations (Figure 2(c)); the OPR value for both frothers hits the maximum after 14 d (<670 mV), and then it is decreased to ~400 mV on day 21.

The growth of the ORP is higher when the quantity of bacteria in the leaching system is higher. Decreases in the ORP value during the process indicated the activity of bacteria in solutions is slowing down. These results are in good agreement with pH results which have shown that KIBX and Aero3477 (in different dosages) the smallest deviation from the control test (Figure 1). Generally, the deviation of ORP value from the control test can be described in the following order: for 0.01 g/L: NaEX > KIPX > KIBX > Aero3477 > KAX, 0.1 g/L: NaEX > KIPX > Aero3477 > KAX > KIBX, and 1 g/L: NaEX > KIPX > KIBX > KAX > Aero3477). Between the frothers, in 1 g/L the ORP deviation from the control test for PO is smaller than MIBC, whereas for the 0.1 g/L only NaEX and KAX increased. The DO value for all tests with 0.01 and 1 g/L collectors increased (the control test decreased), whereas for the 0.1 g/L only NaEX and KAX increased. The DO value for all tests is higher than the control test, except in the 0.1 g/L which NaEX and KAX are higher only. It was reported that O2 levels below 1–2 mg/L may adversely affect the oxidizing activity of bacteria (Deveci et al., 2003). Moreover, increases in the DO value in the various tests demonstrated the inhibitory effect of collectors that lead to the limited availability (i.e., transfer) of oxygen (Hulme and Stranks, 1970; Torma et al., 1976; Dopson et al., 2006). Therefore, through the process, the DO deviation from the control test in the presence of collectors can have the following order: for 0.01 g/L: KAX > KIBX > NaEX > KIPX > Aero3477, 0.1 g/L: NaEX > KAX > KIPX > KIBX > Aero3477, and 1 g/L KAX > NaEX > KIBX > Aero3477 > KIPX).

After 7 d, MIBC in all dosages indicated a diverse trend compared with the control test (MIBC increased while PO is decreased). PO showed higher deviation from the control test
than MIBC in all concentrations (especially at 0.1 g/L the DO deviation for PO test is 2.58 mg/L (Figure 3(b)). After 14 d, the DO value of both frothers is increased, but the value for PO is still lower than the DO of control test. On day 21, both frothers show higher DO than the control test especially at 1 g/L concentrations. These results are in good agreement with another investigation that reported frothers hinder oxygen transfer (Dopson et al., 2006).

**Population of At. ferrooxidans**

Results of bacterial growth by microscopic counting (Figure 4) indicated that during 21 d of process, the population of cells in all tests increased. According to these results, the organic surfactants inhabited bacterial growth in the solution. These inhabitations effectively depended on the reagent concentrations; by increasing the dosages, the population of cells in all tests decreased. Aero3477

![Figure 3. DO variation in the different condition during 21 d of assessment.](image1)

![Figure 4. Bacterial cell number variation in the different condition during 16 d of assessment.](image2)
at 0.01 g/L (Figure 4(a)) was the only exception which showed higher cell population than the control test. In various concentrations, results revealed that KIPX and NaEX have the lowest growth during the bacterial activity. After 7 d, in the 0.01 g/L, KAX and KIBX have the same growth as the control points (Jafari et al., 2017b). In general, the negative effect of various collectors on the cell population has the following order: for 0.01 g/L: NaEX > KIPX > KAX > KIBX > Aero3477, 0.1 g/L: NaEX > KIPX > KIBX > KAX > Aero3477, and 1 g/L: NaEX > KIPX > KIBX > KAX > Aero3477).

Both frothers in 0.01 g/L have the similar trends such as the trend of control test (Figure 4(a)). Moreover, results indicated that both frother limited bacterial growth. The PO tests in different concentrations indicated a higher number of bacterial cells than MIBC tests.

**Fe variations**

Results of FeT measurement (Figure 5) illustrate that in the solution for all tests, FeT is decreased during the bacterial activities. These FeT reductions can be as a result of precipitation jarosite and other ferric oxides and hydroxides (Equations (7–9)). Results show that after a sharp decrease from the initial amount of iron (8 d), the system reaches a steady state and the gradual conversion of ferric iron into jarosite can be observed. Moreover, in various collector concentrations approximately the rate of FeT reduction for all tests is the same, except for NaEX which in comparison with the potassium xanthates demonstrate the highest FeT deviation from the control test during the process (especially in 0.01 g/L). Potassium is the most favorable cation for jarosite formation (Dutrizac, 1983). The easier formation of potassium jarosite compared with the jarosite of sodium can explain this higher deviation (Gahan et al., 2009). Weston et al. (1994) reported the negative effect of sodium jarosite on the bio-oxidation process. In higher dosages of collectors (0.1–1 g/L) the rate of jarosite formation is relatively increased (Figure 5(b,c)) (Weston et al., 1994). On the other hand, 9K medium contains abundant of NH4+ and K+ so the precipitation of KFe3SO42(OH)6 and NH4Fe3SO42(OH)6 accrued in the solution (Wang et al., 2013). Both frothers had almost the same trends.

\[
\text{Fe}^{2+} + 2\text{H}_2\text{O} \rightarrow \text{FeOOH} \downarrow + 3\text{H}^+ \tag{7}
\]

\[
\text{Fe}^{3+} + 3\text{H}_2\text{O} \rightarrow \text{Fe(OH)}_3 \downarrow + 3\text{H}^+ \tag{8}
\]

\[
3\text{Fe}^{3+} + \text{M}^+ + 2\text{HSO}_4^- + 6\text{H}_2\text{O} \rightarrow \text{MFe}_3\text{SO}_4(\text{OH})_6 \downarrow + 8\text{H}^+ \tag{9}
\]

[where: \(\text{M = K, N, NH}_4, \ldots\)]
The behavior of microorganisms is significantly dependent on the $\frac{Fe^{3+}}{Fe^{2+}}$ ratio. During bioleaching process, the main role of bacteria is to maintain this ratio high. Based on the results (Figure 6) the $\frac{Fe^{3+}}{Fe^{2+}}$ ratio in all tests gradually increased. KAX (0.01–0.1 g/L) showed the highest ratio (higher than the control test), and KIPX indicated the lowest ratio among the collectors through the At. ferrooxidans activity (lower than the control test). These increases can be as a result of the growth of the cells, which more $Fe^{2+}$ ions are bio-oxidized into $Fe^{3+}$ ions. During the bio-oxidation, the solution for the At. ferrooxidans gradually becomes deteriorated; therefore, to overcome this shortcoming bacteria increase their products, such as increasing the $\frac{Fe^{3+}}{Fe^{2+}}$ ratio (Yu et al., 2013; Jafari et al., 2017a). By an increase in the collector dosages, the rate of reduction of $Fe^{3+}$ to $Fe^{2+}$ is decreased, which results in the negative effect of high collector concentration on bacterial activities. In general, the negative effect of various collectors on the $\frac{Fe^{3+}}{Fe^{2+}}$ ratio is shown in the following order: for 0.01 g/L: Aero3477 > KIPX > KIBX > NaEX > KAX, 0.1 g/L: NaEX > KIPX > Aero3477 > KIBX > KAX, and 1 g/L NaEX > KIPX > KIBX > KAX > Aero3477. In the presence of the frothers in various concentrations, the ratio slowly decreases. The rate of this decrease based on various concentration is for 0.01 g/L: PO > MIBC, 0.1 g/L: MIBC > PO, and 1 g/L MIBC > PO. By increasing the frother dosages, the rate of this decrease accelerated and as a result the $\frac{Fe^{3+}}{Fe^{2+}}$ ratio is significantly dropped (negative effect of higher concentrations).

There is a good correlation between various analyses with the Fe analysis results. According to the results presented in the previous sections, it can be concluded that in the presence of various reagents, the pH increased for all tests when $Fe^{2+}$ oxidized completely within the first 2 d (Figure 1) (Song et al., 2014). Then pH subsequently decreased because of $Fe^{3+}$ hydrolysis into jarosite and other ferric oxides and hydroxides. When the system is reached a steady state, the slow conversion of ferric iron into jarosite (Figure 5) leads to the gradual pH reduction after 14 d (Figure 1) (Song et al., 2014). Moreover, the ORP values (Figure 2) and the $\frac{Fe^{3+}}{Fe^{2+}}$ ratio results (Figure 6) are in agreement with the fact that the increase of the ORP is precisely dependent on the presence of ferrous-oxidizing microorganisms which enable the regeneration of $Fe^{3+}$ (the ORP increased due to a higher $\frac{Fe^{3+}}{Fe^{2+}}$ ratio) (Romano et al., 2001). During the first 7 d, DO sharply decreased (Figure 3), oxygen ions are consumed (Equation (3)), and accordingly more $Fe^{2+}$ ions are bio-oxidized into $Fe^{3+}$ ions (the $\frac{Fe^{3+}}{Fe^{2+}}$ ratio is increased, Figure 6). This also can be as a result of jarosite and other ferric oxides and hydroxides precipitation (Figure 5). According to the results

![Figure 6. $\frac{Fe^{3+}}{Fe^{2+}}$ Variation in the different condition during 21 d of assessment.](image-url)
oxidizing bacteria, the obvious that in a higher population of ferrous-positive or negative influences on the bio-oxidation. (structures) and concentrations may have some reagents based on their chemical compositionsperation, it can be demonstrated that the flotation increased the concentration, the rate of ferrous-oxidation was sensitive to the type of reagents (chemical work, to be understood the mechanism of flotation bio-oxidation of \textit{At. ferrooxidans}, and frothers (PO and MIBC) for sulfide flotation reagents when the products will be subjected to the bioleaching for further processing.

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### References


### Table 1. The order of negative effects of flotation reagents on bio-oxidation of \textit{At. ferrooxidans} activity.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Concentrate (g/L)</th>
<th>Collectors</th>
<th>Frothers</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>0.01</td>
<td>KAX &gt; KIPX &gt; NaEX &gt; Aero3477 &gt; KIBX</td>
<td>PO &gt; MIBC</td>
</tr>
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<td></td>
<td>0.1</td>
<td>NaEX &gt; KAX &gt; KIBX &gt; Aero3477 &gt; KIPX</td>
<td>PO &gt; MIBC</td>
</tr>
<tr>
<td></td>
<td>1</td>
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<td>MIBC &gt; PO</td>
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<td>ORP</td>
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<td>MIBC &gt; PO</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>NaEX &gt; KIPX &gt; Aero3477 &gt; KAX &gt; KIBX</td>
<td>MIBC &gt; PO</td>
</tr>
<tr>
<td></td>
<td>1</td>
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<td>PO &gt; MIBC</td>
</tr>
<tr>
<td>DO2</td>
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<td>MIBC &gt; PO</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>NaEX &gt; KAX &gt; KIPX &gt; KIBX &gt; Aero3477</td>
<td>MIBC &gt; PO</td>
</tr>
<tr>
<td>Count</td>
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<td>MIBC &gt; PO</td>
</tr>
<tr>
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<td>0.1</td>
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<td>MIBC &gt; PO</td>
</tr>
<tr>
<td></td>
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<td>MIBC &gt; PO</td>
</tr>
<tr>
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<td>MIBC &gt; PO</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
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<td>MIBC &gt; PO</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>KAX &gt; Aero3477 &gt; KIBX &gt; NaEX &gt; KIPX</td>
<td>PO &gt; MIBC</td>
</tr>
<tr>
<td>Fe$^{3+}$/Fe$^{2+}$</td>
<td>0.01</td>
<td>Aero3477 &gt; KIPX &gt; KIBX &gt; NaEX &gt; KAX</td>
<td>PO &gt; MIBC</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
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<tr>
<td></td>
<td>1</td>
<td>NaEX &gt; KIPX &gt; KIBX &gt; KAX &gt; Aero3477</td>
<td>MIBC &gt; PO</td>
</tr>
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</table>

**Conclusion**

The inhabitation effects of flotation reagents on the bio-oxidation were not investigated extensively. In this work, to better understand the mechanism of flotation reagents on bacterial activity, the influences of conventional collectors (NaEX, KIPX, KIBX, KAX, Aero3477), and frothers (PO and MIBC) for sulfide flotation on bio-oxidation of \textit{At. ferrooxidans} in various conditions have been investigated by systematic analyses (pH, ORP, DO, microorganisms counting method, and the Fe$^{3+}$/Fe$^{2+}$ ratio). Results indicated that the bio-oxidation was sensitive to the type of reagents (chemical compositions), and their concentrations. Mostly by increasing the concentration, the rate of ferrous-oxidizing bacteria was decreased. Results demonstrated that potassium iron oxides are formed more than the sodium ones; therefore, NaEX (in comparison with potassium xanthates) has a higher deviation in various analyses from the control test during the process. According to the results, the order of negative effects for collectors can be arranged as: for 0.01 g/L: KAX > KIPX > KIBX > Aero3477 > NaEX, 0.1 g/L: NaEX > KIPX > KAX > KIBX > Aero3477, and 1 g/L: NaEX > KIPX > KIBX > KAX > Aero3477, and in all concentrates, for frothers MIBC > PO. These results potentially can be used for the selection of sulfide flotation reagents when the products will be subjected to the bioleaching for further processing.

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( Figures 4 and 6 ), both bacterial number and the Fe$^{3+}$/Fe$^{2+}$ ratio are increased during the process. It is obvious that in a higher population of ferrous-oxidizing bacteria, the Fe$^{3+}$/Fe$^{2+}$ ratio is increased.

Taking all above-mentioned results into consideration, it can be demonstrated that the flotation reagents based on their chemical compositions (structures) and concentrations may have some positive or negative influences on the bio-oxidation. Based on the results of various analyses ( Table 1 ), in general, the following order can be recommended for the inhabitation effect of collectors: for 0.01 g/L: KAX > KIPX > KIBX > Aero3477 > NaEX, 0.1 g/L: NaEX > KIPX > KAX > KIBX > Aero3477, and 1 g/L: NaEX > KIPX > KIBX > KAX > Aero3477. Moreover, results ( Table 1 ) show that PO would contribute to less toxicity than MIBC for the bacterial activates in all concentrates.


