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A Comparative Study on the Effect of Flotation Reagents on Growth and Iron Oxidation Activities of *Leptospirillum ferrooxidans* and *Acidithiobacillus ferrooxidans*

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Abstract: Recently, extraction of metals from different resources using a simple, efficient, and low-cost technique-known as bioleaching-has been widely considered, and has turned out to be an important global technology. *Leptospirillum ferrooxidans* and *Acidithiobacillus (Thiobacillus) ferrooxidans* are ubiquitous bacteria in the biomining industry. To date, the effects of commercial flotation reagents on the biooxidation activities of these bacteria have not been thoroughly studied. This investigation, by using various systematic measurement methods, studied the effects of various collectors and frothers (collectors: potassium amylxanthate, potassium isobutyl-xanthate, sodium ethylxanthate, potassium isopropylxanthate, and dithiophosphate; and frothers: pine oil and methyl isobutyl carbinol) on *L. ferrooxidans* and *A. ferrooxidans* activities. In general, results indicate that in the presence of these collectors and frothers, *L. ferrooxidans* is less sensitive than *T. ferrooxidans*. In addition, the inhibition effect of collectors on both bacteria is recommended in the following order: for the collectors, potassium isobutyl-xanthate > dithiophosphate > sodium ethylxanthate > potassium isobutyl-xanthate > potassium amylxanthate; and for the frothers, methyl isobutyl carbinol > pine oil. These results can be used for the optimization of biometallurgical processes or in the early stage of a process design for selection of flotation reagents.

Keywords: flotation; collector; frother; bioleaching; *Leptospirillum*; *Acidithiobacillus (Thiobacillus) ferrooxidans*

1. Introduction

Reagents (collectors, frothers, etc.) play essential roles during mineral separation by flotation. The main purposes for addition of reagents into the process are providing the highest selectivity (grade) and concentration results (recovery). They react with the surface of the target minerals and—based on their properties—change the hydrophobicity of mineral surfaces (i.e., collectors and depressants), or reduce the surface tension of pulp by providing a large air–water interface (i.e., frothers). The residual of these reagents remains on the surface of products (concentrate and tail). Generally, flotation products (typically concentrates) are subjected to pyro-, hydro-, or biometallurgy process for the metal extraction. Although the presence of these residual surfactants on flotation products has no essential effect on the pyrometallurgical extractions [1], they may have

significant impacts (positive or negative) on microbial activities involving in the biometallurgical metal extraction [2–5].

There are some investigations on the effect of flotation reagents in bioleaching technology that generally focused on their impacts over the metallurgical parameters (recovery and grade of products) [1,3,6–10], however, the fundamental effects of these chemicals on bacterial activities are not yet widely studied. It was reported that the recovery and grade in a bioleaching process of flotation products (in the presence of remaining surfactants) could be highly affected by type of reagents (their chemical compositions and structures), concentration of reagents, and type of microorganisms (various metabolisms, cellular membranes, strains, etc.) [1,3–8]. Among microorganisms, *Acidithiobacillus ferrooxidans* (*A. ferrooxidans*) was the first bacterium isolated from an acidic leaching environment for biomining, and it was used in many early bioleaching studies until *Leptospirillum ferrooxidans* (*L. ferrooxidans*) was found and it was reported that *L. ferrooxidans* can be the dominant iron-oxidizing bacteria in biomining processes [2,9,11–16]. The negative effect of different collectors and frothers on *A. ferrooxidans* activity was observed in various investigations [3,7]. Dehghan and Dianati (2015) reported the positive effect of potassium amylxanthate and potassium ethylxanthate on both *A. ferrooxidans* and *L. ferrooxidans*. The solubility of elemental sulfur as a passivation layer on the mineral surfaces was reported as a potential reason for such a positive effect [1].

In the early stage of designing a flotation process and selecting reagents, the major advances would be the metallurgical parameters of final products. A systematic investigation on the effects of commercial surfactants on microbial activities can be a key to: (1) better understand the mechanisms of reactions and (2) efficiently select reagents when, in the downstream, flotation products are going to be processed by bioleaching for extraction of metals. In this study, to extensively realize possible interactions of industrial surfactants on biooxidation, the influence of various conventional reagents for flotation of sulfides (collectors: potassium amylxanthate (KAX), potassium isobutyl-xanthate (KIBX), sodium ethylxanthate (NaEX), potassium isopropylxanthate (KIPX), and dithiophosphate (Aero3477); and frothers: pine oil (PO) and methyl isobutyl carbinol (MIBC)) on *L. ferrooxidans* and *A. ferrooxidans* activities were compared by fundamental measurements (pH, oxidation–reduction potential (ORP), dissolved oxygen (DO_2), microorganisms counting method, and iron ion (Fe_T (iron total), Fe^{2+}, and Fe^{3+}) analyses. The output of this work can be applied for the optimization and selection of bacteria and flotation reagents in mineral-processing plants.

2. Materials and Methods

Sixteen tests were designed (2 control tests without reagents, and 14 tests with the seven different reagents for the two bacterial species, *L. ferrooxidans* and *A. ferrooxidans*. Tests were performed in a 250 mL Erlenmeyer flask at 34 °C and agitated in an orbital incubator shaker (model: Wisecube) at 140 rpm. Glassware and pH electrodes were rinsed in 98% ethanol and distillated water to avoid contamination, and then they were dried [17].

2.1. Microorganism and Culture Conditions

Pure strains of *L. ferrooxidans* and *A. ferrooxidans* (prepared from the Center of Research and Development of the Sarcheshmeh Mine, Kerman, Iran) were separately cultured in 85 mL 9K medium containing 44.22 g/L $FeSO_4 \cdot 7H_2O$ as a source of energy (10.0 g/L sulfur was also added to the culture of *A. ferrooxidans*) [18]. The 9K medium—the growth medium for the bacteria—contained five different mineral salts ($(NH_4)_2SO_4$: 3.0 g/L, $MgSO_4 \cdot 7H_2O$: 0.5 g/L, K_2HPO_4: 0.5 g/L, KCL: 0.1 g/L, and $Ca(NO_3)_2 \cdot H_2O$: 0.01 g/L).

2.2. Flotation Reagents

The pure reagents (collectors: KAX, KIBX, NaEX, KIPX and Aero3477; and PO and MIBC) were provided by the Mineral Processing Laboratory at the University of Tehran, Iran. To better compare

the effects of these reagents on bacterial activities and avoid the effect of reagent concentrations, a constant dosage of 0.01% w/v (the industrial dosage in the Sarcheshmeh plant) has been selected for the all surfactants.

2.3. Analytical Procedures

The effectiveness of reagents on bacterial activities was explored by the differences of test results to their control test (Δ variable = Value in the presence of reagent − Value of the control test; based on the day of measurements). The initial pH of culture was adjusted to 1.8 by H_2SO_4. The pH and ORP value were measured by pH/ORP analyzer (Mettler Toledo) on various days. The amount of dissolved oxygen (DO_2) in the media was determined by an oxygen meter (model JENWAY). The bacterial population (N) was counted by using a Neubauer lamp ($0.1 \times 1/400$ mm^2) (HBG, Giessen, Germany) and 100× magnification under a Zeiss biological microscope (Bacterial count per mL = N × 400 × 10^4) (Zeiss: Carl Zeiss AG, Oberkochen, Germany). The variation of Fe_T was monitored by an atomic adsorption spectrophotometer (AAS model: varian-20). The amount of Fe^{2+} was determined via titration by dichromate potassium (0.001 M) in the presence of H_2SO_4:H_3PO_4 solution (1:1) with diphenylamine as an indicator [19–21]. Fe^{3+} percentage was calculated by subtracting Fe^{2+} from Fe_T ($Fe_T = Fe^{2+} + Fe^{3+}$) [19]. The $\frac{Fe^{3+}}{Fe^{2+}}$ ratio was used to define the effects of reagents on *L. ferrooxidans* and *A. ferrooxidans* oxidizing activities.

3. Results and Discussion

3.1. Subsection

The deviation of pH from the control test (Δ_{pH}) in the presence of various collectors (Figure 1a–e) shows increases in the Δ_{pH} during the first 2 days of *L. ferrooxidans* and *A. ferrooxidans* activities in all tests ($+\Delta_{pH}$). These increases are higher in the presence of *L. ferrooxidans* than in *A. ferrooxidans* tests (except in the presence of KIBX and KIPX) (Figure 1c,d). The instability of these collectors when pH < 3 could be an explanation for these increases, where the pH may increase as a result of the H^+ consumption from the solution for the decomposition of the collectors in the early stage of experiments (Equations (1) and (2)) [7,22–24]. During the next 14 days, the Δ_{pH} decreased in all experiments. Reduction of Fe^{3+} to Fe^{2+} can lead to the decrease in pH values of the solution (Equation (3)) [1,25–29]. KIPX shows the highest pH reduction in the *A. ferrooxidans* test ($-\Delta_{pH}$) (Figure 1d).

$$RX^- + H^+ \rightarrow RXH \quad (1)$$

$$RXH \rightarrow ROH + CS_2 \quad (2)$$

$$Fe^{3+} + 6H_2O \rightarrow 2Fe(OH)_3 + 2H^+ \quad (3)$$

During the next 14 days, in the presence of all collectors the pH for the *L. ferrooxidans* test gradually decreased (KAX shows the highest rate of pH reduction (Figure 1b)). This phenomenon indicates that *L. ferrooxidans* bacteria, during 21 days, steadily adjusts to the test conditions ($-\Delta_{pH}$ at day 21 for Aero, KAX, and KIPX), while the Δ_{pH} for the *A. ferrooxidans* tests shows variations in different conditions. After 7 days, for the *A. ferrooxidans* tests in the presence of NaEX and KAX (higher than the control test; $+\Delta_{pH}$) and KIPX ($-\Delta_{pH}$), the Δ_{pH} increased (Figure 1a,b,d), whereas in the presence of KIBX and Aero, after 14 days the Δ_{pH} increased (Figure 1c,e). One of the main reasons for these differences between *L. ferrooxidans* and *A. ferrooxidans* can be attributed to the fact that, in contrast to *A. ferrooxidans*, *L. ferrooxidans* can oxidize Fe^{2+} even at pH below 1.2 [15,28,30]. Previous studies indicated that *L. ferrooxidans* is more resistant to low pH than *A. ferrooxidans* and can grow at a pH as low as 1.2 (the optimum pH range for the growth of *A. ferrooxidans* is 1.8–2.5) [15,31–33]. In general, the negative effect of reagents on *A. ferrooxidans* and *L. ferrooxidans* has the following order: NaEX > KAX > KIBX > Aero > KIPX and KIBX > NaEX > Aero > KIPX > KAX, respectively. For the frothers (Figure 1f,g),

in case of *A. ferrooxidans* the trend for both frothers is the same; after 7 days, the pH of the tests was lower than that of the control test ($-\Delta_{pH}$), after which it increases until day 21 when it significantly decreases. In the presence of *L. ferrooxidans*, the pH was continually higher than that of the control test ($+\Delta_{pH}$) during 21 days in the presence of both MIBC and PO. The inhibition order for both bacteria in the presence of two frothers shows the following order: MIBC > PO.

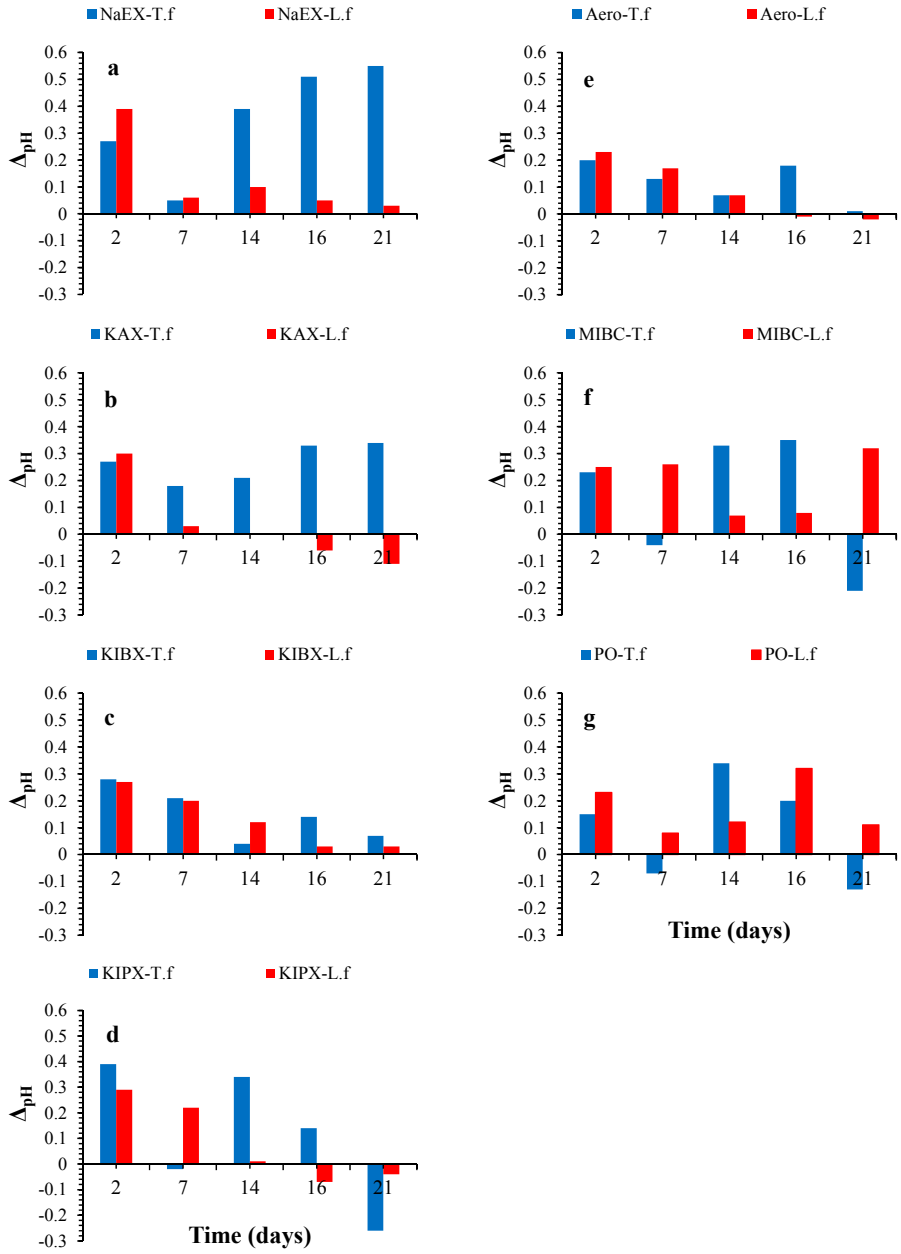


Figure 1. Effects of various flotation reagents on pH during *L. ferrooxidans* and *A. ferrooxidans* activities.

3.2. ORP Variations

The ORP analyses of all tests (Figure 2a–g) indicate that during the first 2 days there was low deviation in the ORP value from the control test (~Δ_{ORP} = 0). Within the next 19 days, as the reactions proceeded and activity of *L. ferrooxidans* increased, an increase in the ORP value is observed in the presence of all collectors ($+\Delta_{ORP}$) (except Aero), and Δ_{ORP} in *L. ferrooxidans* tests is continuously positive within 21 days. In the *A. ferrooxidans* tests, Δ_{ORP} is positive for KAX, KIBX, and Aero (Figure 2b,c,e), and it is constantly negative for KIPX and NaEX (Figure 2a,d). The ORP increases

can be explained by the biooxidation and regeneration of Fe^{3+} to Fe^{2+}, and the differences between bacteria performances can be interpreted by the fact that *L. ferrooxidans* has a lower maximum specific utilization rate than *A. ferrooxidans* at low ORP, and can intersect at a high ORP. In other words, *L. ferrooxidans* species have capability to withstand extreme conditions, such as a quite high ORP value [15]. Generally, the deviation of the ORP value from the control test (Δ_{ORP}) in the presence of collectors for *A. ferrooxidans* and *L. ferrooxidans* can be described in the following order: NaEX > KIPX > Aero > KAX > KIBX and Aero > KIPX > KIBX > NaEX > KAX, respectively. For both frothers, Δ_{ORP} is continuously negative for *L. ferrooxidans* tests during 21 days, and the PO in the *A. ferrooxidans* tests shows higher ORP than the control test ($+\Delta_{ORP}$) within the last 5 days of measurements (Figure 2f,g). Δ_{ORP} shows that MIBC has a higher inhibition effect than PO on both microorganisms.

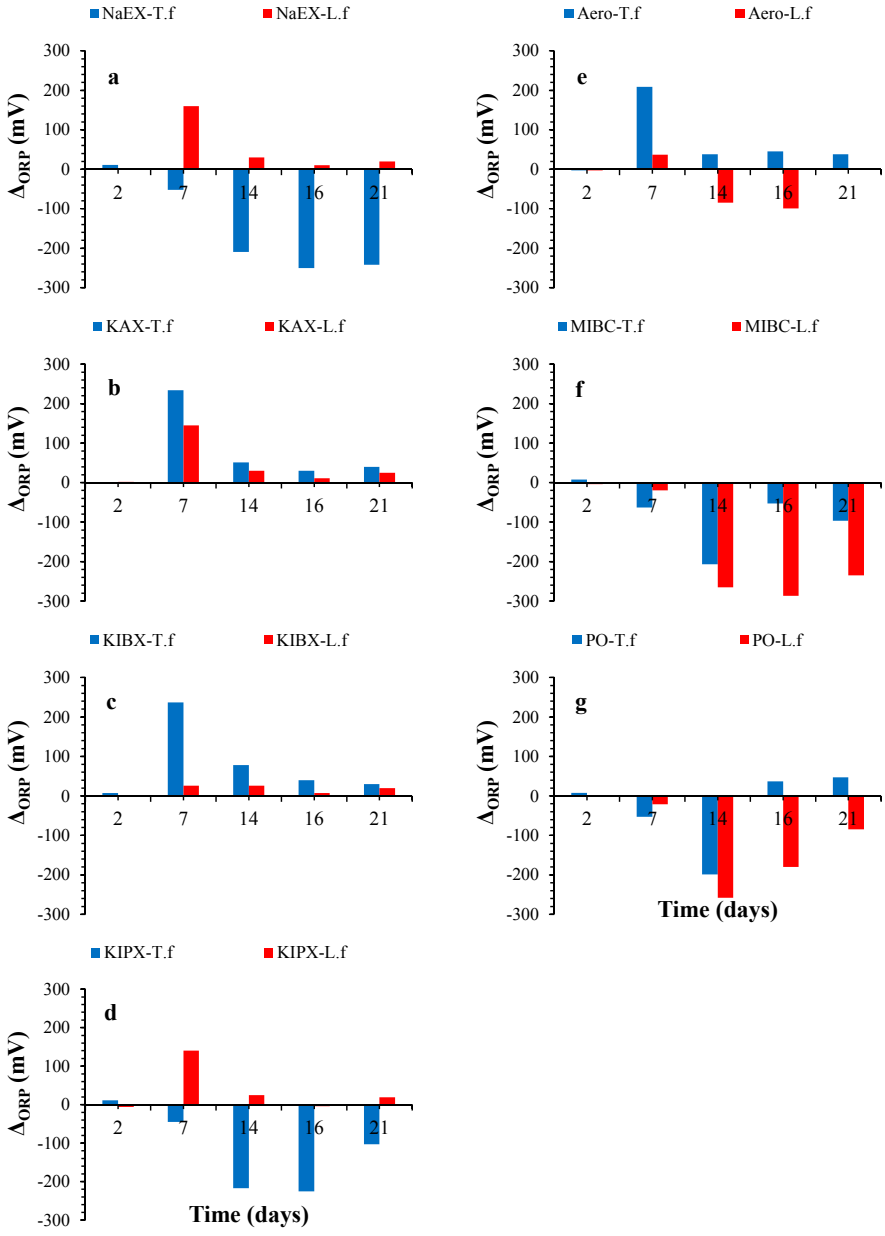
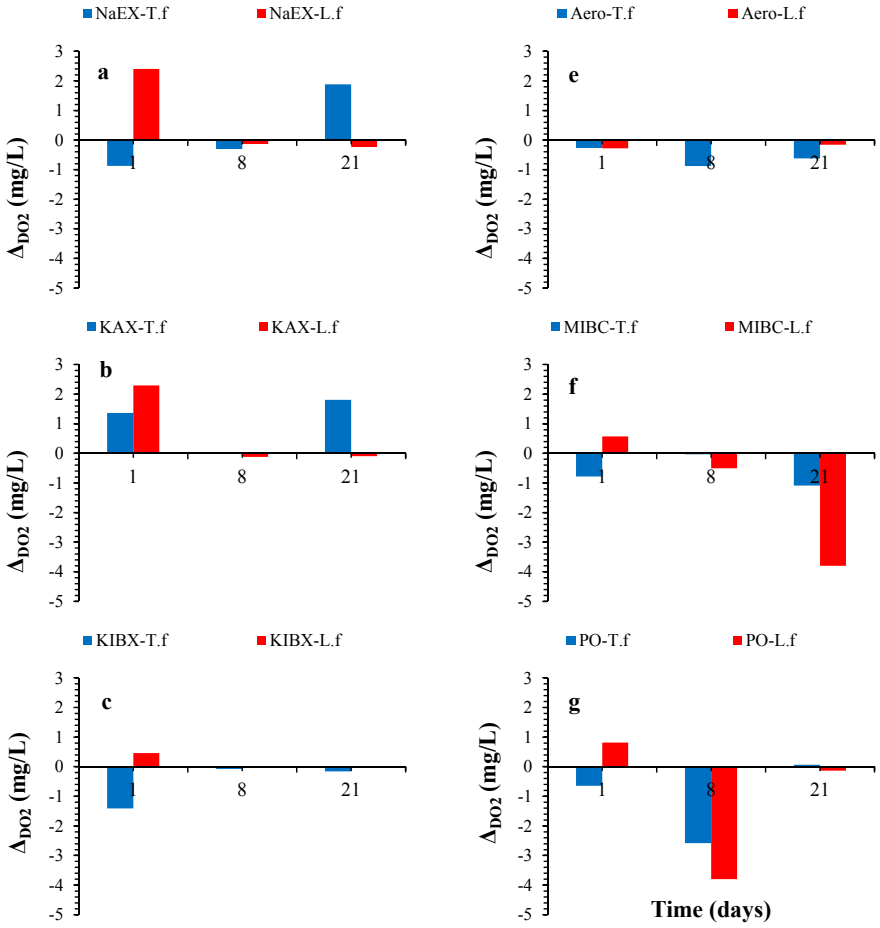


Figure 2. Effects of various flotation reagents on ORP during *L. ferrooxidans* and *T. ferrooxidans* activities.

3.3. DO_2 Variation

Oxygen species in the culture play a critical role for the bacteria to complete their cycle of respiration [34,35]. Within the initial day, Δ_{DO_2} for the *L. ferrooxidans* tests in the presence of NaEX, KAX, and KIBX is positive, and for Aero and KIPX it is negative. Δ_{DO_2} gradually decreases to be less than or close to the control test at day 21 for all collectors (Figure 3a–e). For *A. ferrooxidans* tests, Δ_{DO_2} is negative in all collectors (except KAX), and during next 7 days the value reaches the control tests in all collectors (except Aero). During the measurement, until day 21, DO_2 values remain lower than the DO_2 value of the control test ($-\Delta_{DO_2}$) for *A. ferrooxidans* tests except for NaEX and KAX, which show the highest positive deviations (Figure 3a,b). The presence of unstable collectors in the low pH culture can limit the availability (i.e., transfer) of oxygen (xanthates are unstable in acidic solutions, and their instabilities increase by decreasing the pH [7,22–24]). These reactions' inhibitory effect increase the DO_2 value [36–38]. Thus, a decrease in the DO_2 value shows a positive effect of reagents on activities of microorganisms. During the process, the DO_2 deviation from the control test in the presence of collectors had the following order for *A. ferrooxidans* and *L. ferrooxidans*: KIPX > KIBX > Aero > NaEX > KAX and KIPX > Aero > NaEX > KAX > KIBX, respectively. For both frothers and in the presence of both microorganisms, after the first day, Δ_{DO_2} is constantly negative. These negative values are the highest in the presence of MIBC at day 21 and of PO at day 8 for both bacteria. Results indicate that PO is more inhibitive than MIBC on the transfer of oxygen into the culture for *A. ferrooxidans*; and for *L. ferrooxidans*, MIBC > PO (Figure 3f–g).



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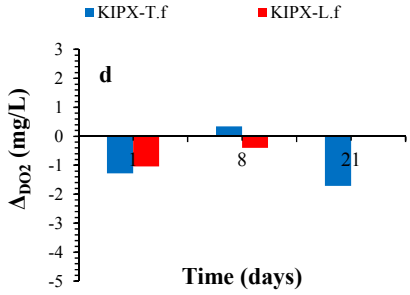
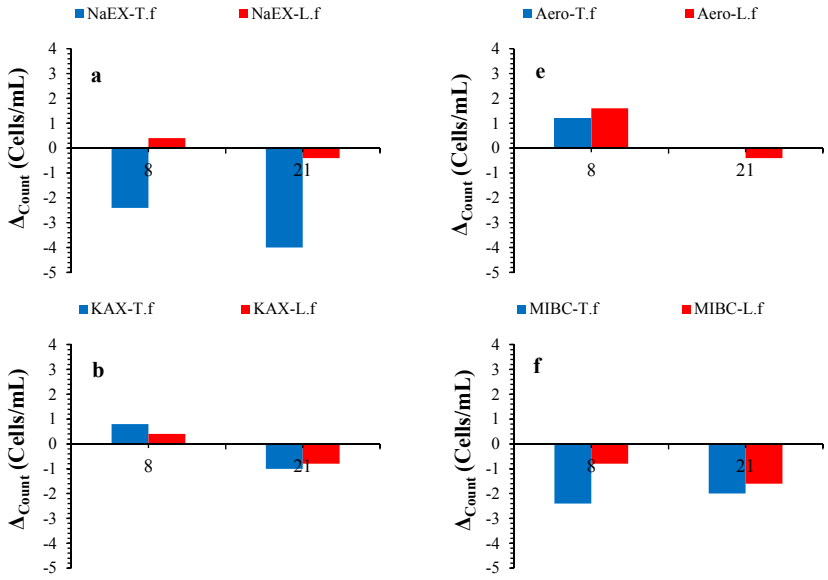


Figure 3. Effects of various flotation reagents on dissolved oxygen during *L. ferrooxidans* and *T. ferrooxidans* activities.

3.4. Microorganism Population

During the first 8 days of the process, the *L. ferrooxidans* population is constantly higher than the control test ($+\Delta_{Count}$) in the presence of all collectors (Figure 4a–e), while for *A. ferrooxidans*, Δ_{Count} is positive just in the presence of Aero and KAX (Figure 4b,e). Within next days, for all collectors the population of both bacteria progressively decreases to less than the control test ($-\Delta_{Count}$). For both *L. ferrooxidans* and *A. ferrooxidans*, NaEX and KIPX show the highest deviation ($-\Delta_{Count}$) from the control test (Figure 4a,d), which is in good agreement with the ORP results (Figure 2a,d). In general, results show that the inhibition effect of collectors is higher on *A. ferrooxidans* cell numbers than on the *L. ferrooxidans* population (in agreement with pH result (Figure 1a–e)). These results can explain the observed dominance of *L. ferrooxidans* over *A. ferrooxidans* during the process; although it was reported that at 30 °C, the growth rate of *L. ferrooxidans* is slower—it is about half of *A. ferrooxidans* [15] (in this study and in the control test after 21 days, 3.2×10^7 vs. 5.6×10^7 cells/mL for *L. ferrooxidans* and *A. ferrooxidans*, respectively)—the growth of *L. ferrooxidans* is favored compared to *A. ferrooxidans* in an environment with a pH lower than 1.2 [15,33,39]. In general, the negative effect of collectors on *A. ferrooxidans* and *L. ferrooxidans* cell numbers has the following order: NaEX > KIPX > KIBX > KAX > Aero and KIPX > KAX > NaEX > Aero > KIBX, respectively. For both frothers, the Δ_{Count} is negative in the presence of microorganisms (Figure 4f,g). This negative effect in the presence of MIBC is higher than PO.



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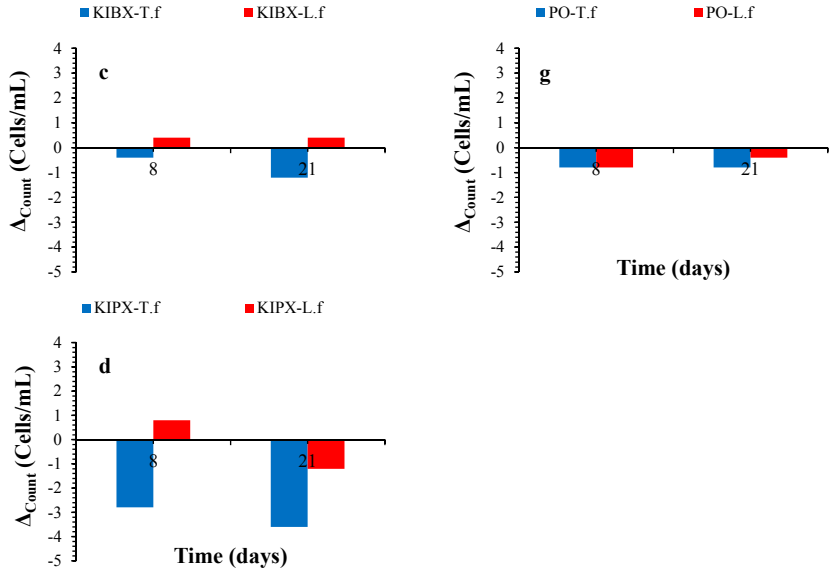


Figure 4. Effects of various flotation reagents on bacterial population during *L. ferrooxidans* and *T. ferrooxidans* activities.

3.5. Fe Variation

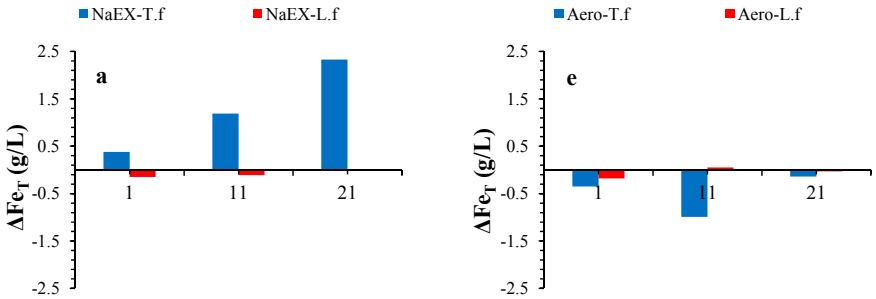
Results of Fe_T measurement for *L. ferrooxidans* tests within 21 days of the procedure (Figure 5a–e) show that Δ_{Fe_T} is continuously negative, except in the presence of KIBX, which gradually increases until the last day of measurements (Figure 5c). During the process, the Fe_T value for *L. ferrooxidans* tests in the presence of all collectors is approximately around the value of control test ($\Delta_{Fe_T} \simeq 0$). In *A. ferrooxidans* tests, during interactions, Δ_{Fe_T} is negative for KAX, KIBX, and Aero (Figure 5b,c,e), and it is constantly positive for KIPX and NaEX (Figure 5a,d). These results are in good agreement with the ORP and microorganism counting results (Figures 2 and 4). Lower Fe_T than the control test ($-\Delta_{Fe_T}$) in the presence of collectors can be described by their inhibition effects, as they lead to the precipitation of jarosite and other ferric oxides and hydroxides (Equations (4)–(6)). Jarosite precipitation is perceived as an unwanted interaction [40]. On the other hands, the positive value of Δ_{Fe_T} may be explained by the positive effect of reagents on the biooxidation activities, which can decrease the precipitation of iron into the medium.

$$Fe^{3+} + 2H_2O \rightarrow FeOOH \downarrow +3H^+ \quad (4)$$

$$Fe^{3+} + 3H_2O \rightarrow Fe(OH)_3 \downarrow +3H^+ \quad (5)$$

$$3Fe^{3+} + M^+ + 2HSO_4^- + 6H_2O \rightarrow MFe_3(SO_4)_2(OH)_6 \downarrow +8H^+ \quad (6)$$

where M can be K, Na, H_3O, and NH_4.



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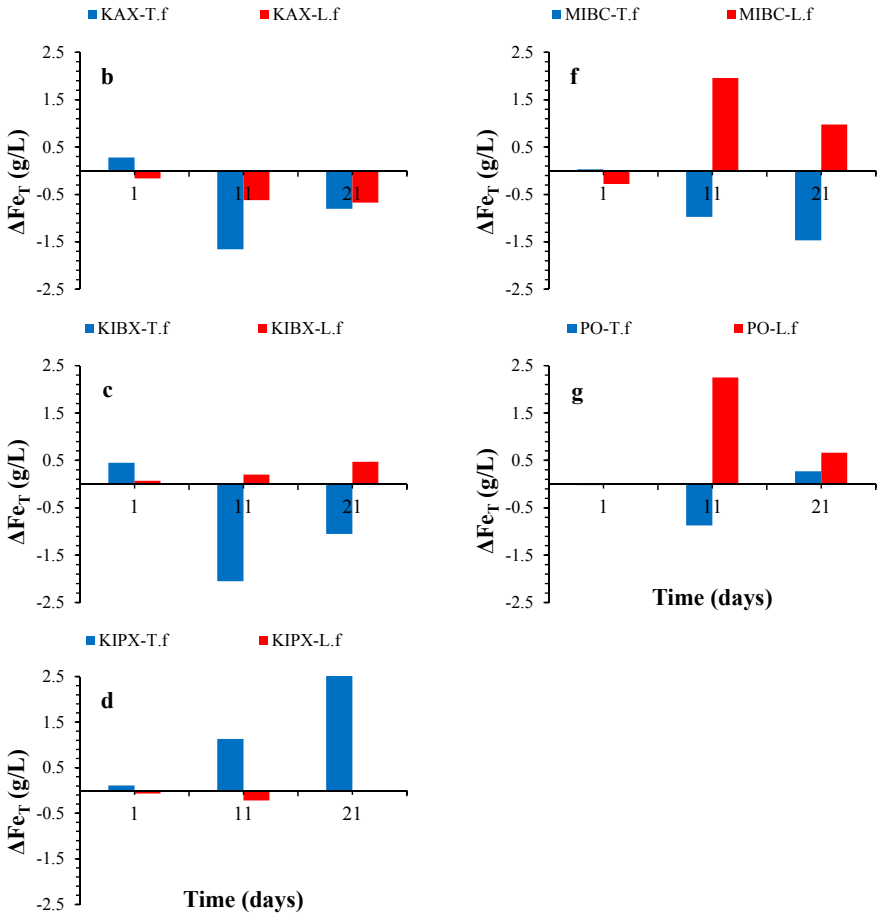

Figure 5. Effects of various flotation reagents on Fe_T during *L. ferrooxidans* and *A. ferrooxidans* activities.

The $\frac{Fe^{3+}}{Fe^{2+}}$ ratio results for *L. ferrooxidans* tests in the existence of all collectors (Figure 6a–e) show that this ratio gradually increases during the process ($+\Delta\frac{Fe^{3+}}{Fe^{2+}}$), except in the presence of KAX, which remains less than the control test ($-\Delta\frac{Fe^{3+}}{Fe^{2+}}$) (Figure 6b). In contrast to *L. ferrooxidans*, this ratio for *A. ferrooxidans* tests in the presence of all collectors steadily decreases to less than the control test ($-\Delta\frac{Fe^{3+}}{Fe^{2+}}$) throughout the 21-day interactions (except for KAX, whose $\Delta\frac{Fe^{3+}}{Fe^{2+}}$ is positive) (Figure 6b). In the presence of the both frothers, $\Delta\frac{Fe^{3+}}{Fe^{2+}}$ was negative for both bacteria during first 11 days. For *L. ferrooxidans*, the $\Delta\frac{Fe^{3+}}{Fe^{2+}}$ increases after day 11 and becomes positive. The rate of this increase is higher for MIBC than PO (Figure 6f,g). The following orders show the approximate inhibition effect of various collectors on the $\frac{Fe^{3+}}{Fe^{2+}}$ ratio for *A. ferrooxidans* and *L. ferrooxidans*: NaEX > KIPX > Aero > KIBX > KAX and KAX > Aero > KIBX > KIPX > NaEX, respectively. For the frothers, it is MIBC > PO for *A. ferrooxidans*, and PO > MIBC for *L. ferrooxidans*. The growth of bacterial population increases the biooxidation of Fe^{2+} into Fe^{3+} ions (the increase of $\frac{Fe^{3+}}{Fe^{2+}}$ ratio). Interactions in the solution (biooxidation, dissolution of reagents, etc.) deteriorate the culture step-by-step for both *L. ferrooxidans* and *A. ferrooxidans*. An increase in the bacterial products, such as the $\frac{Fe^{3+}}{Fe^{2+}}$ ratio, can improve this deterioration [41]. It was reported that *L. ferrooxidans* is resistant to a 500 mM Fe^{3+} concentration, whereas the growth of *A. ferrooxidans* is limited above a 36 mM Fe^{3+} dosage [42].

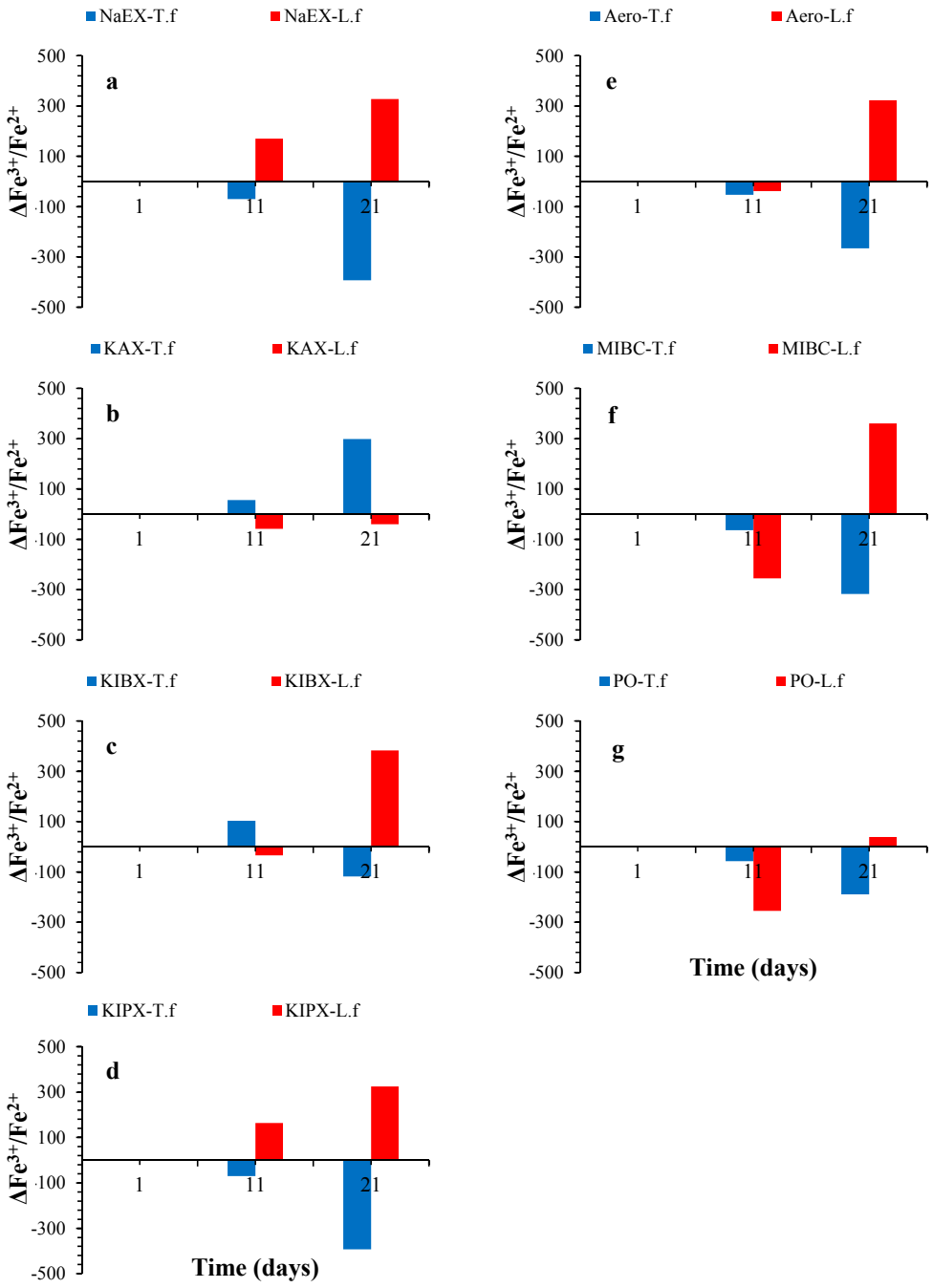

Figure 6. Effects of various flotation reagents on $\frac{Fe^{3+}}{Fe^{2+}}$ ratio during *L. ferrooxidans* and *A. ferrooxidans* activities.

In other words, Fe^{2+} oxidation by *L. ferrooxidans* is significantly less sensitive to end-product inhibition by Fe^{3+} (38 mM) than *A. ferrooxidans* (2.5 mM), and *L. ferrooxidans* is able to oxidize Fe^{2+} into Fe^{3+} ions even at low oxygen concentrations [43]. Therefore, when the quantity of Fe^{3+} in solution is high, *L. ferrooxidans* will have a significant selective advantage over *A. ferrooxidans* [15]. In the initial days of the process, the $\frac{Fe^{3+}}{Fe^{2+}}$ ratio is low, which enables *A. ferrooxidans* to grow a large numbers of cells (before conditions become more favorable for *L. ferrooxidans*). Nevertheless, *L. ferrooxidans* has a higher attraction to Fe^{2+} and is less sensitive to inhibition by Fe^{3+} on prolonged aeration, which likely makes *L. ferrooxidans* dominate [44].

Taking all abovementioned results into consideration, it can be demonstrated that the flotation reagents—based on the type of microorganisms in the process—may have positive or negative effects on the biooxidation. Results show organic compounds such as flotation reagents have stronger inhibition effects on biooxidation activities of *A. ferrooxidans* versus *L. ferrooxidans* (KIBX approximately shows positive effects on *L. ferrooxidans* activities). In general, based on various analyses, the negative effects of collectors on *A. ferrooxidans* and *L. ferrooxidans* have the following orders: NaEX > KIPX > Aero > KIBX > KAX and KIPX > KAX > NaEX > Aero > KIBX, respectively. Moreover, the possible inhibition of these reagents shown by various measurements (Table 1) can be deduced to be in the following order for the both bacteria: KIPX > Aero > NaEX > KIBX > KAX. Results (Table 1) indicate that MIBC would contribute to higher toxicity than that of PO for the bacterial activities in 0.01% w/v dosage of these frothers.

Table 1. The order of negative effects of flotation reagents on biooxidation of both *Acidithiobacillus ferrooxidans* and *Leptospirillum ferrooxidans* activities.

Parameters	Collectors	Frothers
pH	NaEX > KIBX > KAX > Aero > KIPX	MIBC > PO
ORP	KIPX> Aero > NaEX > KIBX > KAX	MIBC > PO
DO_2	KIPX > Aero > KIBX > NaEX > KAX	MIBC $\geq$ PO
Count	KIPX > NaEX > KAX > KIBX > Aero	MIBC > PO
Fe_T	KAX > KIPX > KIBX > Aero > NaEX	MIBC $\geq$ PO
$\frac{Fe^{3+}}{Fe^{2+}}$	Aero > KIPX > NaEX $\geq$ KAX > KIBX	MIBC $\geq$ PO

ORP: oxidation–reduction potential; DO_2: dissolved oxygen; Fe_T: total iron.

4. Conclusions

L. ferrooxidans and *A. ferrooxidans* are typical iron-oxidizing bacteria under highly acidic conditions. To extensively compare the influence of various conventional flotation reagents on biooxidation, bacterial activities of these microorganisms were studied in the presence of different collectors and frothers with the same concentration (0.01% w/v) by fundamental measurement techniques. Results indicated that, generally, the tested reagents have negative effect on the bacterial activities. These negative effects were stronger on biooxidation of *A. ferrooxidans* versus *L. ferrooxidans*. In general, the negative effects of collectors on both bacteria can be deduced to be in the following order: KIPX > Aero > NaEX > KIBX > KAX. For the frothers, MIBC was more toxic than PO. Results demonstrated the dominance of *L. ferrooxidans* over *A. ferrooxidans* during the growth and bacterial activities in the presence of various chemical reagents. These results can be used for selection of reagents in the initial stage of designing flotation separation methods.

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