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Effects of Conventional Flotation Frothers on the Population of Mesophilic Microorganisms in Different Cultures

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Abstract: Bioleaching is an environment-friendly and low-investment process for the extraction of metals from flotation concentrate. Surfactants such as collectors and frothers are widely used in the flotation process. These chemical reagents may have inhibitory effects on the activity of microorganisms through a bioleaching process; however, there is no report indicating influences of reagents on the activity of microorganisms in the mixed culture which is mostly used in the industry. In this investigation, influences of typical flotation frothers (methyl isobutyl carbinol and pine oil) in different concentrations (0.01, 0.10, and 1.00 g/L) were examined on activates of bacteria in the mesophilic mixed culture (*Acidithiobacillus ferrooxidans*, *Leptospirillum ferrooxidans*, and *Acidithiobacillus thiooxidans*). For comparison purposes, experiments were repeated by pure cultures of *Acidithiobacillus ferrooxidans* and *Leptospirillum ferrooxidans* in the same conditions. Results indicated that increasing the dosage of frothers has a negative correlation with bacteria activities while the mixed culture showed a lower sensitivity to the toxicity of these frothers in comparison with examined pure cultures. Outcomes showed the toxicity of Pine oil is lower than methyl isobutyl carbinol (MIBC). These results can be used for designing flotation separation procedures and to produce cleaner products for bio extraction of metals.

Keywords: flotation; bioleaching; frother; mixed culture; machine learning

1. Introduction

Pyrometallurgy and high-pressure leaching are two typical methods used for the extraction of metals from concentrates of flotation separation [1–3]. These methods have several disadvantages such as high investment and operation costs, environmental pollution (chemical reagents in the waste waters of hydrometallurgical plants and SO_2 gas generation from pyrometallurgical plants), high energy consumption in the pyrometallurgy processes, high technology requirements for pyro/hydro-metallurgy process, and finally special expertise for system operators [4,5]. Variations in the metal price have caused very intense competition among the high prestigious mining companies (Anglo American, BHP, Rio Tinto, Glencore, etc.) to revise their feasibility studies where the feasibility of mining projects has significantly depended on the project costs. Moreover, the problem of global warming and environmental pollution has led the mineral processing industry to focus on the use of low-cost, low-energy, and environmentally friendly methods [4,6–10]. Thus, several investigations have been focused on the operation and optimization of the bioleaching processes for the extraction of metals from low-grade deposits (by heap bioleaching [11,12]), waste (by columns [13,14]), and concentrates (by bioleaching tanks [13,15,16]) which considerably have lower costs and environment effects [11,16–24].

However, on one hand, few investigations studied the effects of flotation reagents on the bioleaching of sulfide flotation concentrates [25–32]. On the other hand, those studies (Table 1) are mainly focused on the cultures consisted one specific microorganism while in the industry, mixed microorganisms are mostly used for the bioleaching process [33]. Where using a mixed culture with different microorganisms can lead to the cooperative effects and bioleaching may show a higher efficiency than pure cultures [34–40].

Table 1. Investigations about the effects of flotation reagents on the bioprocess.

No.	Microorganism	Goal Metal	Process	Reagent	Description	Ref
1	*Acidithiobacillus ferrooxidans*	Copper	Bioleaching	Butyl amine	−45.0% (Cu-Recovery)	[41]
				Ethyl-xanthate	−36.7% (Cu-Recovery)	
				Isoamyl-xanthate	−20.0% (Cu-Recovery)	
				Butyl-xanthate	−11.7% (Cu-Recovery)	
2	*Acidithiobacillus ferrooxidans*	Copper	Bioleaching	Isopropyl-xanthate	−30.0% (Cu-Recovery)	[42]
		Iron (Pyrite)	Biooxidation		−50.0% (Fe-Oxidation)	
3	*Sulfolobus metallicus*	Copper	Bioleaching	Hostaflot X23	−14.0% (Cu-Recovery)	[43]
				Aero 3477	−34.0% (Cu-Recovery)	
				Flotanol C-7	−27.0% (Cu-Recovery)	
				Montanol 800	−30.0% (Cu-Recovery)	
4	*Leptospirillum ferrooxidans*	Iron (ferrous)	Biooxidation	Potassium amyl-xanthate	The inhibition effect of reagents (collector): NaEX > KAX > KIBX > KIPX > Aero3477 For frothers: MIBC > PO	[26]
				Potassium isobutyl-xanthate		
				Sodium ethyl-xanthate		
				Potassium isopropyl-xanthate		
				Dithiophosphate (Aero 3477)		
				Methyl isobutyl carbinol (MIBC)		
				Pine oil		
5	*Acidithiobacillus ferrooxidans*	Copper	Bioleaching	Isobutyl-xanthate	−53.0% (Cu-Recovery)	[29]
				Amyl-xanthate	−77.0% (Cu-Recovery)	
6	*Acidithiobacillus albertensis*	Sulfur	Biooxidation	Sodium isobutyl-xanthate	+ on the growth and S^0 oxidation	[31]
				Tween-80	+ on the growth and S^0 oxidation	
7	*Penicillium simplicissimum*	Cellulose	Decomposition	Tween-80	+11.60%	[44]
		Hemicellulose			+ 8.00%	

Table 1. *Cont.*

No.	Microorganism	Goal Metal	Process	Reagent	Description	Ref
8	*Acidithiobacillus ferrooxidans*	Iron (ferrous)	Biooxidation	Potassium amyl-xanthate	The inhibition effect of reagents (collector): KAX > KIPX > KIBX > Aero3477 > NaEX For frothers: MIBC > PO	[32]
				Potassium isobutyl-xanthate		
				Sodium ethyl-xanthate		
				Potassium isopropyl-xanthate		
				Dithiophosphate (Aero 3477)		
				MIBC		
				Pine oil		
9	*Ferroplasma*	Iron (ferrous)	Biooxidation	Sodium ethyl xanthate	All reagents have a negative effect on the biooxidation of iron.	[45]
10	*Acidithiobacillus ferrooxidans*			Sodium (alkyl) dithiocarbamate		
11	*Leptospirillum*			Xanthate (Mix)		
				Sodium ethyl-xanthate		
				Sodium n-propyl xanthate		
				Sodium isobutyl xanthate		
				Potassium amyl xanthate		
				Potassium n-butyl xanthate		
				Sodium (alkyl) dithiocarbamate		
				Sodium (alkyl) dithiocarbamate and sodium di-(alkyl) dithiophosphate		
				Isopropylthionocarbamate		
				Dithiophosphate(mixture)		
				Sodium 2-mercaptobenzthiazole		

This study investigated influences of two typical flotation frothers pine oil (PO) and methyl isobutyl carbinol (MIBC) on a population (microorganisms count) of a traditional mixed mesophilic microorganisms culture (*Acidithiobacillus ferrooxidans*, *Leptospirillum ferrooxidans*, and *Acidithiobacillus thiooxidans*). This is because there is a direct relationship between bioleaching rate (recovery of valuable metals from ores) and the population of microorganisms [46]. Three different concentrations of frothers were examined (0.01, 0.10, and 1.00 g/L). For comparison purposes, outcomes were compared with results of the same conditionings on pure cultures of *Acidithiobacillus ferrooxidans* and *Leptospirillum ferrooxidans.* Various parameters were measured in the control tests: pH, ORP (oxidation-reduction potential), total iron (Fe^T) in the solution, and DO (dissolved oxygen in the media). Mutual Information (MI) assisted by Pearson correlation was used to explore the relationship among these measured variables and select the most important parameters for further assessments. Outputs of this investigation can be used for mineral processing plants which flotation separation is their main beneficiation method to design ambient conditions. This method helps to produce cleaner products by a leaching tank. The most efficient route for processing of flotation concentrate is a leaching tank where it can process high-grade feeds and it has a high process recovery [15], for the downstream processes and environment.

2. Materials and Methods

2.1. Bacterial Strain and Growth Conditions

Pure strains of *Acidithiobacillus ferrooxidans* (T.f), *Leptospirillum ferrooxidans* (L.f), and *Acidithiobacillus thiooxidans* (T.t) which have different oxidation abilities (Table 2) were obtained from the research and development center of Sarcheshmeh mine, Kerman, Iran. Microorganism strains were cultivated in the environment presented in Table 3. 5 cc of each pure culture was selected to build the mixed culture.

Table 2. Oxidation ability of microorganisms.

Microorganism	$S^0 \rightarrow SO_4$	$Fe^{II} \rightarrow Fe^{III}$
Acidithiobacillus ferrooxidans (T.f)	√	√
Leptospirillum ferrooxidans (L.f)	-	√
Acidithiobacillus thiooxidans (T.f)	√	-

Table 3. Cultivating environment parameters.

		9K Culture							
$(NH_4)_2SO_4$	$MgSO_4 \cdot 7H_2O$	K_2HPO_4	KCL	Ca $(NO_3)_2 \cdot H_2O$	pH [1]	$FeSO_4 \cdot 7H_2O$	S^0	Incubation Temperature	Rotation Speed
3.00 g/L	0.50 g/L	0.50 g/L	1.00 g/L	0.01 g/L	1.80	44.22 g/L	10.00 g/L	34.00 °C	140 rpm

[1] pH adjusted by 98% acid sulfuric.

2.2. Flotation Reagents

Flotation frothers (MIBC and PO) were prepared in the mineral processing laboratory at the University of Tehran, Iran. A wide range of their concentrations which are common in the various flotation plants (0.01, 0.10, and 1.00 g/L) was used and their influences were explored by different analyses.

2.3. Experimental Procedure

Twenty-one experiments, nine tests for each frother (three different dosages and three different cultures) and one control test (without frother) for each culture, were conducted. To do experiments, microorganisms were cultivated in a 9K medium containing five different mineral salts ($(NH_4)_2SO_4$: 3 g/L, $MgSO_4 \cdot 7H_2O$: 0.5 g/L, K_2HPO_4: 0.5 g/L, KCl: 1 g/L, and Ca $(NO_3)_2 \cdot H_2O$: 0.01 g/L). The initial pH of the media was adjusted to 1.8 with H_2SO_4. As a source of energy 44.22 g/L $FeSO_4 \cdot 7H_2O$ 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. Effects of MIBC and PO on microorganisms count and their activities in the various cultures are assessed by measuring different parameters (pH, ORP, Fe^T, and DO) (Table 4). All the cultures were monitored and the mentioned parameters were measured for 21 days. To save time and cost, mutual information and Pearson correlation were used for the feature selection (FS). FS indicates the relationship among parameters and can be used to rank them.

Table 4. Methods for measuring of various parameters.

Parameter	Definition
pH ORP	The pH and ORP value of tests were measured by pH-ORP analyzer (Mettler Toledo).
DO	An oxygen-meter (Model JENWEY) was used to measure the amount of dissolved oxygen in the media.
Fe^T	The amounts of Fe^T were determined by atomic adsorption Spectro-photometer (AAS).
Count	The bacterial number (growth) was determined by using a Neubauer lam and 100 × magnification under a Zeiss biological microscope (Bacterial count per mL = N × 400 × 10^4), it could be indirect evidence of cell activity and cannot capture the non-culturable cells.

2.4. Feature Selection

Feature or variable selection is used to select the most effective variables on specific responses. It assists to optimize the number of variables which typically have to be measured during a process, reduce the number of parameters, and to save cost and time. In other words, collinearity may lead to measuring various parameters that show the same concept [47–49]. Therefore, FS was used through the value of measured parameters (pH, ORP, Fe^T, and DO) in the control tests to find the most effective parameters on microorganisms count (MC). The selected parameters were used as indicative factors for further assessments.

2.4.1. Pearson Correlation

Pearson correlation (r) categorizes the magnitude and value of the linear relationship between two variables. "r" statistically determines the strength of a correlation and donates negative values ($-1 \leq r < 0$) when by increasing one variable another one decreases and positive values ($0 < r \leq 1$) when they have the same orientation. "r" close to 0 means there is no relationship [50,51]. Pearson correlation was used to explore linear correlations between the measured parameters (pH, ORP, Fe^T, and DO) in the control tests through 21 days of monitoring.

2.4.2. Mutual Information

Mutual information (MI) is a unique method which can determine both the linear and nonlinear correlation between variables. MI between two variables (x;y) is non-negative and is defined as:

$$\mathrm{MI(x;y)} = \sum_{y \in R} \sum_{x \in S} p(x,\ y) \log_2 \frac{p\ (x,\ y)}{p(x)p(y)}, \tag{1}$$

where $p(x)$ and $p(y)$ are probability density functions and $p(x,y)$ means the joint probability of a given stimulus.

3. Results

3.1. Control Test

Exploring MC in three different cultures and in the absence of frothers (Figure 1a) shows that the MC is increasing during 21 day activities, and the $\frac{\text{MC after 21 days}}{\textit{Initial MC}}$ ratio for the mixed culture is higher than T.f and L.f cultures ($\frac{6.4}{0.66}$ vs. $\frac{6.8}{0.78}$ and $\frac{3.2}{0.76}$, respectively). These results indicate that the MC grows faster in the mixed culture than two other cultures and/or bacteria may have higher activities in the mixed culture. Figure 1b shows that the $\frac{\text{ORP after 21 days}}{\textit{Initial ORP}}$ ratio is higher for the mixed culture than T.f and L.f cultures ($\frac{680}{335}$ vs. $\frac{652}{408}$ and $\frac{675}{376}$, respectively). The mixed culture has the lowest DO and Fe^T while T.f culture has the lowest pH through assessments (Figure 1c–e). These results show correlations among these measured variables (pH, ORP, Fe^T, and DO). Statistical analyses were used to do variable importance measurement (VIM) and to select the most representative parameters for further evaluations.

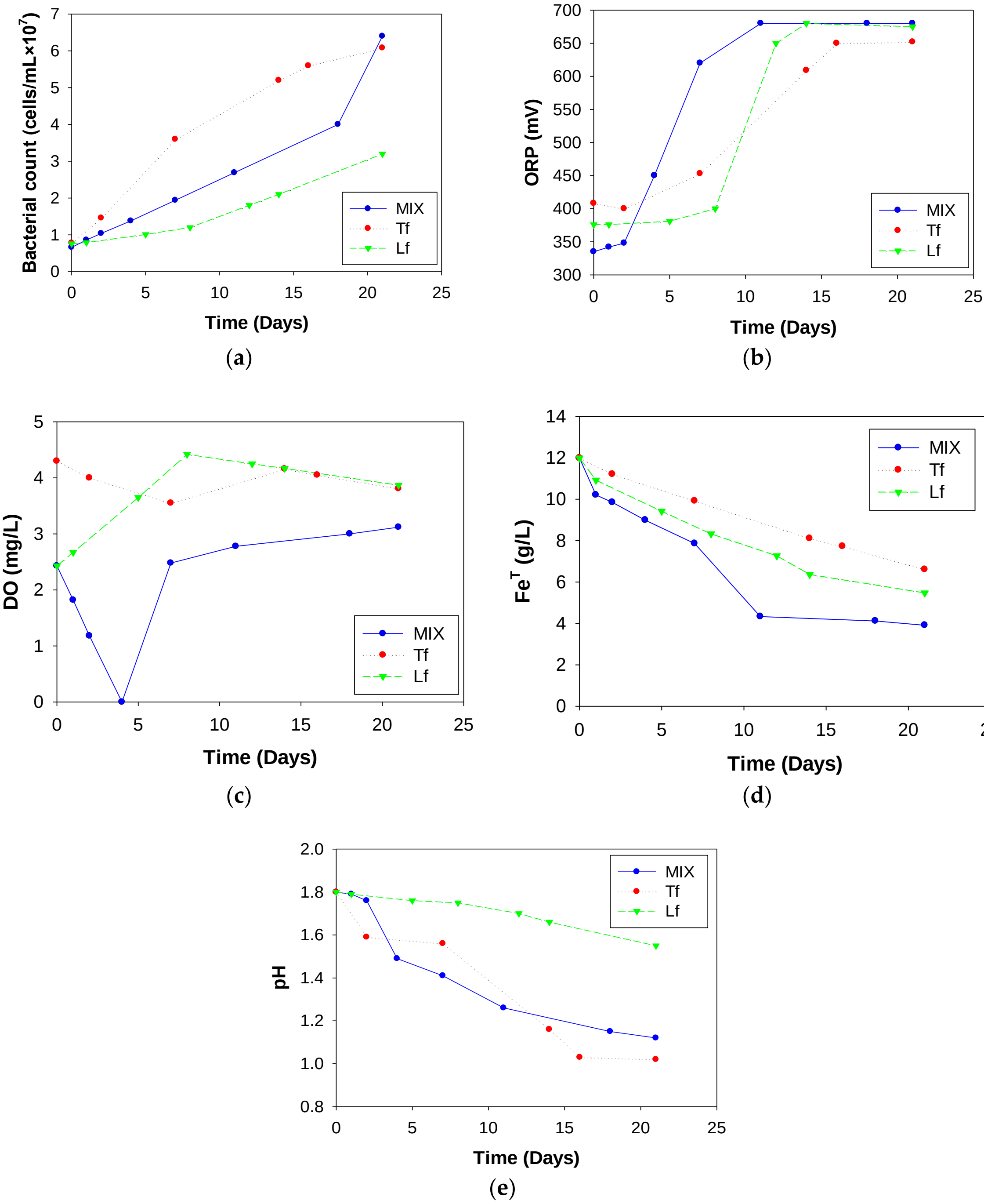


Figure 1. Exploring variation of process parameters in three different cultures for control tests during 21 days monitoring. (**a**) Bacterial count; (**b**) ORP; (**c**) DO; (**d**) Fe^T; (**e**) pH.

Feature Selection

Exploring linear relationships by Pearson correlation through various measured parameters in three different cultures over 21 days indicate that the pH and Fe^T have the highest negative "r" value with MC (Figure 2) while DO shows an insignificant correlation. In other words, when pH and Fe^T decrease, MC increases (Figure 2). Moreover, Pearson correlation shows a high relationship between ORP and Fe^T. MI was used to explore nonlinear relationships between parameters and rank them based on their importance. If the MI was close to 1, it means there is a high correlation between X and Y, and a value close to 0 means there is no relationship. MI can be used to rank variables based on their effectiveness on a dependent variable and rank independent variables based on their importance (VIM) [52]. In this study, MI was used to rank the measured parameters (pH, ORP, Fe^T, and DO) in the control tests and rank them based on their effects that may receive from the MC value (VIM). Using VIM by MI and Pearson correlation together provides a direct determination to decide whether to add an additional variable for assessments or not. MI results (Figure 3) illustrate that pH and Fe^T receive the highest effectiveness from MC among all measured parameters. Thus, these two parameters are selected to study the effect of the conventional flotation frothers (MIBC and PO) on the different cultures.

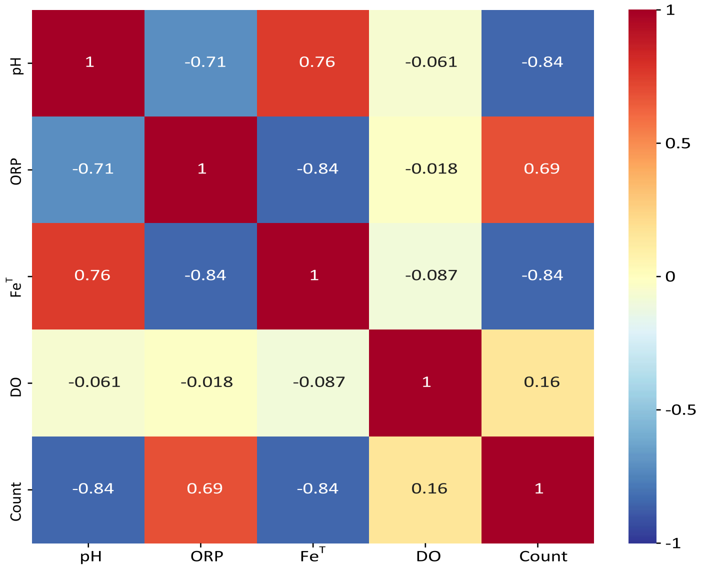


Figure 2. Pearson correlations between the measured parameters in the control tests.

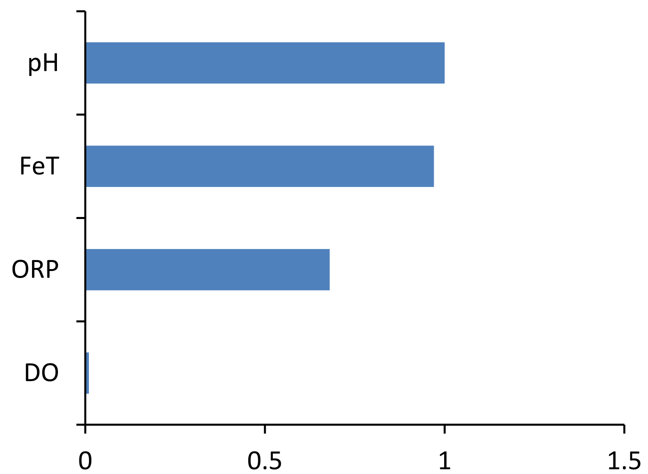


Figure 3. Ranking effectiveness of the measured parameters on microorganism population by Mutual Information.

3.2. Frothers

3.2.1. Population of Microorganisms

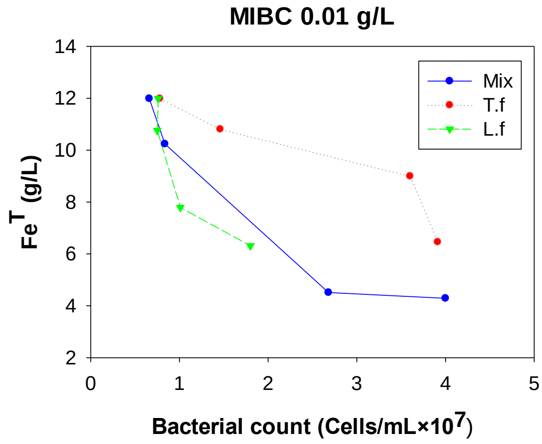
A comparison between the population ratio of microorganisms ($\frac{\text{MC after 21 days}}{\textit{Initial MC}}$) in the absence (control tests) with the presence of frothers indicates (Table 5) that MIBC and PO reduce the MC during the process. In other words, generally by increasing the frother dosages the MC is decreased. This decrease in the highest examined dosage (1 g/L) was considerably higher than the other dosages while in the case of L.f and mixed culture the population is even lower than the initial day (MC ratio < 1) (Table 5). In the presence of frothers and their different dosages, the MC ratio has the following order: T.f > mixed > L.f. In general, the MC ratio is higher in the presence of PO compering with MIBC.

Table 5. The $\frac{\text{MC after 21 days}}{\textit{Initial MC}}$ ratio in various conditions.

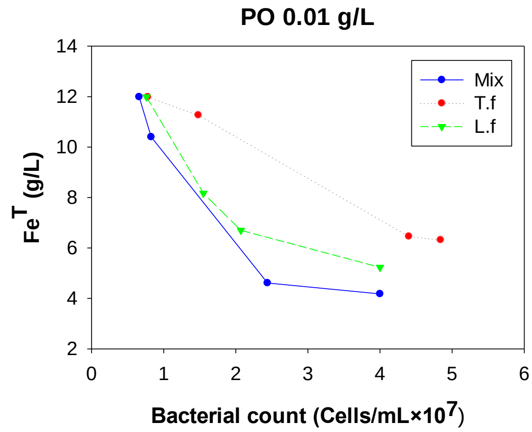
			MIBC (g/L)	
Culture	Control	0.01	0.1	1
T.f	7.8	5.4	6.3	3.1
L.f	4.2	2.4	2.1	0.5
Mixed	9.7	5.9	1.2	0.6
			PO (g/L)	
Culture	Control	0.01	0.1	1
T.f	7.8	6.2	7.6	3.9
L.f	4.2	5.3	3.7	0.8
Mixed	9.7	6	8.5	1.8

3.2.2. Fe Total

Figure 4 shows the negative relationship between Fe^T and MC for three different cultures in all tests where by increasing bacteria population the Fe^T is decreasing. In general, by increasing the dosages of frothers, by stopping the growth MC, the amount of Fe^T in the solution remains high through the process (Figure 4). Furthermore, these results illustrate that, after 21-day measurement, the amount of Fe^T in the solution for T.f culture is higher than two other cultures since there is a moderate slope of reduction between MC and Fe^T for T.f culture in all experiments. The mixed culture generally shows the highest Fe^T reduction in the solution compared with two other cultures and the Fe^T reduction ratio during the process has the following order: mixed > T.f > L.f. The Fe^T in the solution is approximately lower in the presence of MC.



(**a**)



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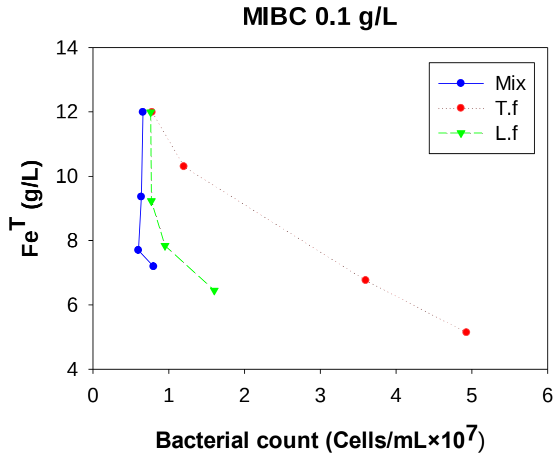

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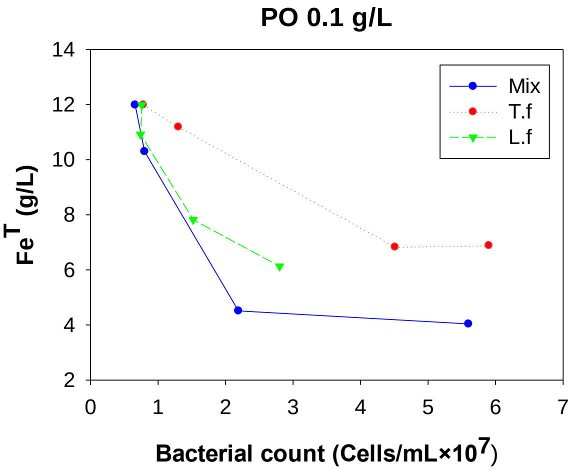

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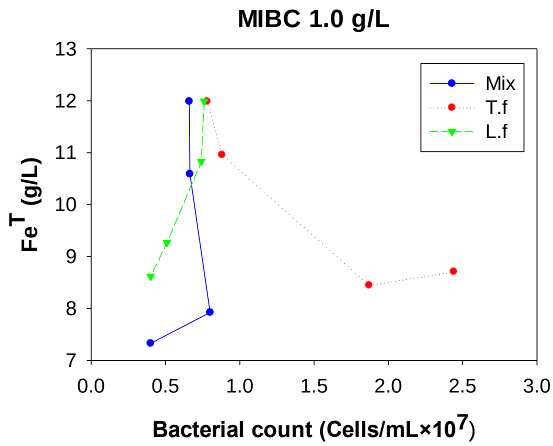

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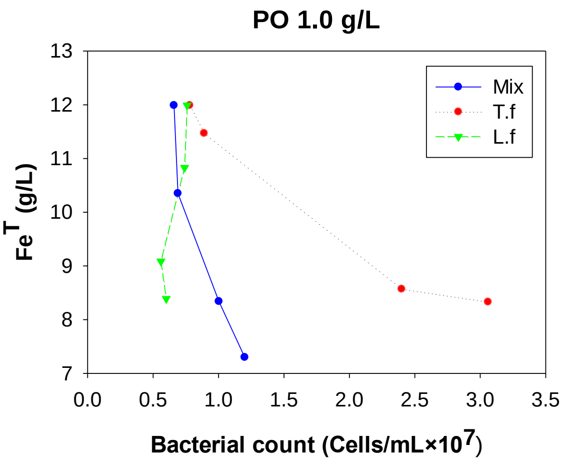
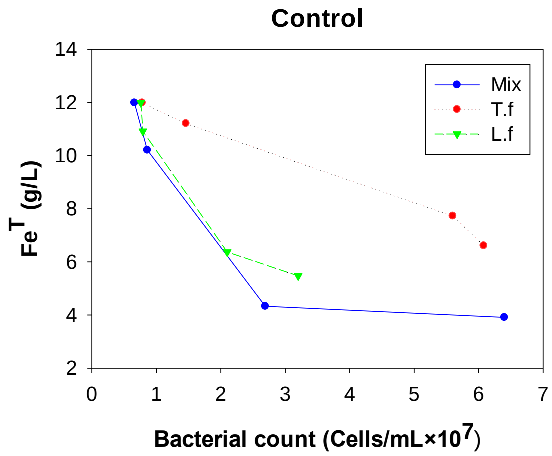


(**f**)

Figure 4. *Cont.*

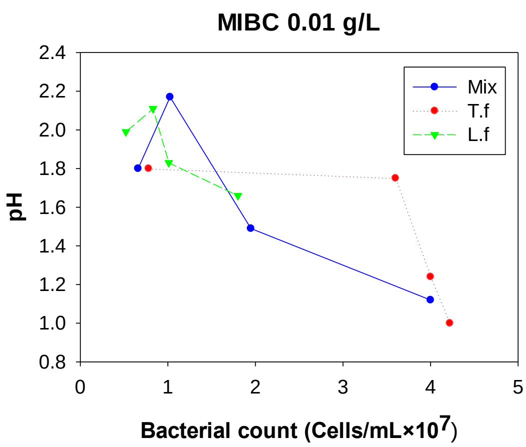


(g)

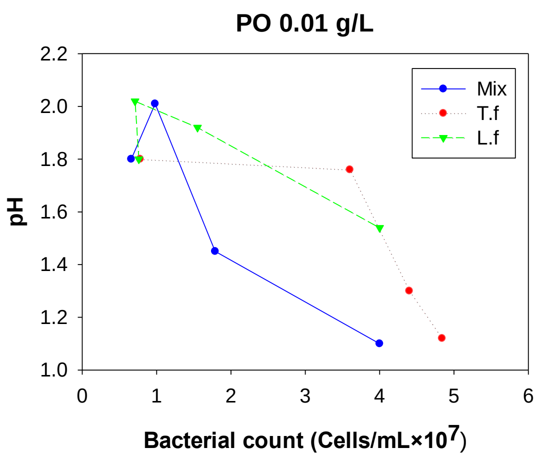
Figure 4. Relationship between population of bacteria and Fe total in different conditions. (**a**) 0.01 g/L MIBC; (**b**) 0.01 g/L PO; (**c**) 0.1 g/L MIBC; (**d**) 0.1 g/L PO; (**e**) 1 g/L MIBC; (**f**) 1 g/L PO; (**g**) Control test.

3.2.3. pH

Figure 5 illustrates the negative relationship between pH and MC for three different cultures in all conditions where by increasing bacteria population (MC) the pH value is decreasing. In other words, by increasing MC and as a result of their activities, the pH value is reducing. In general, the rate of pH reduction is decreased by increasing the dosages of frothers (Figure 5). These results indicate that L.f has the highest and T.f has the lowest pH value during the process monitoring (L.f > mixed > T.f). In the presence of PO, the pH reduction is moderately continuous for all cultures while in the presence of MIBC (above 0.01 g/L), the pH reduction is only detectable for the T.f culture (Figure 5).



(a)



(b)

Figure 5. *Cont.*

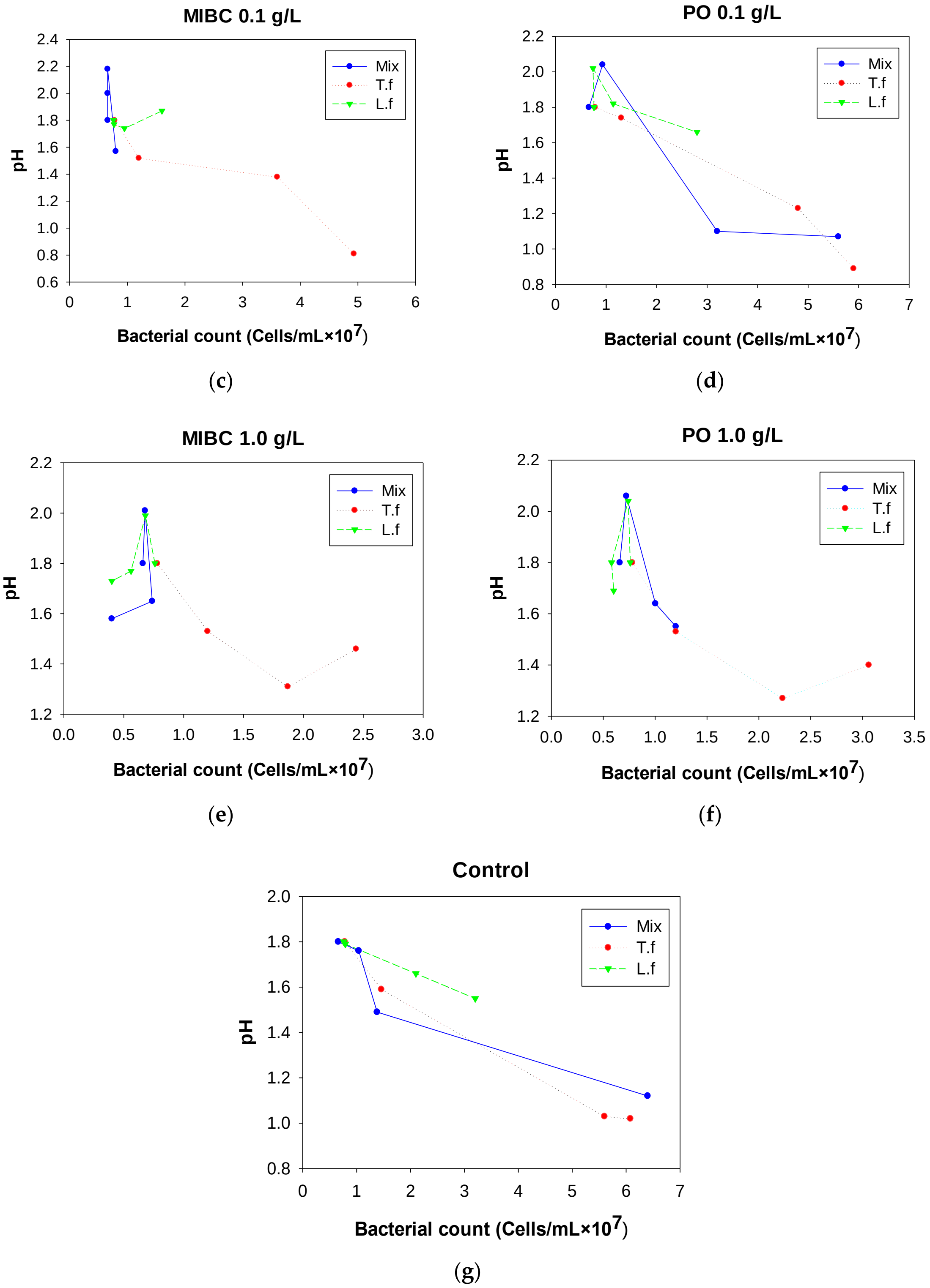


Figure 5. Relationship between population of bacteria and pH in different conditions. (**a**) 0.01 g/L MIBC; (**b**) 0.01 g/L PO; (**c**) 0.1 g/L MIBC; (**d**) 0.1 g/L PO; (**e**) 1 g/L MIBC; (**f**) 1 g/L PO; (**g**) Control test.

4. Discussion

Oxidizing metal sulfides to sulfate via contact between bacteria and mineral (direct) and oxidizing Fe^{2+} to Fe^{3+} or/and S^o to SO_4 (without contact: indirect) are the main mechanisms of metal extraction in the bioleaching process [53–59]. Moreover, it was well understood that oxidation of Fe^{2+} to Fe^{3+}

during bioprocess decreases pH values and Fe^T in the solution (precipitation of iron as jarosite and other iron oxides/hydroxides: Equations (2)–(4)) [60–62]. These phenomena lead to both direct and indirect bioleaching mechanisms [63–69]. Moreover, increasing bacteria population (MC) plays a fundamental role in the bioleaching process, mostly affecting pH and Fe^T. Thus, there should be negative correlations between MC-pH, and MC-Fe^T during bio-activities (Figures 2 and 3). On the other hand, there should be also a positive relationship between pH and Fe^T (Figure 2). Akinci et al. demonstrated that the rate of pH reduction during bioleaching in different bacterial cultures have a decreasing order as follows: *A. thiooxidans* > mixed culture > *A. ferooxidans* [70] that supports outcomes presented in Table 5.

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

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

$$3Fe^{3+} + M^+ + 2HSO_4^- + 6H_2O \rightarrow MFe_3(SO_4)_2(OH)_6 \downarrow +8H^+. \quad (4)$$

The toxicity of flotation reagents is well documented and the presence of their substrates in the flotation products indicated many environmental issues which may inactivate bacteria metabolism [71–73]. Thus, frothers can change the surface properties of energy resources in the culture, limit the surface tension of the media, and inhibit microorganism activities [74,75]. The toxicity of flotation reagents depends on their chemical composition, and their dosages [25–27,32,76]. When flotation concentrate of sulfides is subjected for metal extraction via bioleaching, presence of frothers in the solution can increase the pH at the initial stage of the process [28,29,43,77]. Loon and Madgwick reported that flotation reagents reduced the bacteria growth and limited the formation of soluble iron in the bioleaching process. Since MIBC and PO are unstable at pH below 3, therefore, they may consume H^+ from the solution, decompose, and increase the pH. Thus, presented results in Figures 4 and 5 are in good agreement with the literature where by increasing the dosages of these frothers, the rate of decreasing in pH and Fe^T value into the solutions are slowing down [29].

It was reported that the growth rate of L.f is lower than T.f (around half of T.f) [58]. This can translate as the rate of its activities also lower than two other examined cultures in a certain period (21 days) of the process (Figure 1 and Table 5). These mean that the rate of the negative effect of reagents can be related to the bacteria sensitivity. Okibe and Johnson reported that L.f is more sensitive than T.f in the presence of flotation reagents which comprises the presented results in Figures 4 and 5 [45]. In general, in the mixed culture, bacteria show a better activity and lower sensitivity than other cultures to the toxicity of frothers while the sensitivity of T.f to the frothers in their highest dosages (1 g/L) is lower than two other bacteria (Figures 4 and 5). This can be as a result of the simultaneous presence of iron and sulfur-oxidizing bacteria in the mixed culture that positively improves microorganism activities. Zhang et al. reported that the oxidation activity of the mixed culture (*Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans*, and *Leptospirillum*) is higher than that of the pure culture, and the mixed culture has the highest adaptability to the bioleaching conditions [78]. Furthermore, meanwhile, the solubility of MIBC is six times higher than PO (at the same condition) [47], its inhibitory effect on bacteria activity can be higher than PO. This also is in a good agreement with the outcome of analyses (Figures 4 and 5). Thus, although PO produces larger and less stable bubbles than MIBC within flotation separation, its toxicity in terms of bioleaching and environmental issues is lower than MIBC.

5. Conclusions

A comparison between the mixed and pure cultures during 21 days of monitoring indicated that bacteria concentration of the mixed culture is higher than pure ones. These results indicated that the insensitivity of the mixed culture to the toxicity of MIBC and PO as conventional flotation frothers in low dosages (0.001 and 0.01 g/L) is more than pure cultures. MC showed the highest population in the presence of frothers (0.001 and 0.01 g/L). Mutual information and Pearson correlation assessments released that pH value and total iron in the solution are the main parameters during bacteria activities.

There is a significant negative correlation between bacteria population and pH (as the most important factor of bioleaching). Presence of frothers disrupted bacteria activities; thus, the rate of pH reduction and oxidation-reduction of iron were decreasing by increasing the dosage of frothers. In the absence and presence (0.001 g/L) of the flotation frothers the rate of pH reduction during the process has the following order for the examined cultures: mixed > T.f > L.f. In general, the mixed culture has the highest Fe oxidation-reduction ratio in both the absence and presence of frothers. Results demonstrated that although during flotation MIBC can produce smaller and more stable bubbles than PO, its toxicity is higher than PO for various microorganisms.

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