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Ferrous bio-oxidation by *Acidithiobacillus ferrooxidans* in hydrochloric acid pickling waste liquor

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ABSTRACT

Hydrochloric acid pickling waste liquor (PWL_{HCl}) is generated in the steel surface cleaning treatment. This process is used to chemically remove the iron oxide scale from steel. PWL_{HCl} is classified as toxic and hazardous waste in many countries because it contains high concentrations of corrosive hydrochloric acid, ferrous irons, and other heavy metals. These properties pose difficulties for PWL_{HCl} reclamation and bioavailability of toxic metals in general. The oxidation of ferrous to ferric sediment is a possible route for PWL_{HCl} reclamation. In this study, *Acidithiobacillus ferrooxidans* (*At.f*) was applied to PWL_{HCl} to investigate ferrous oxidation efficiency under low pH and high chloride ion stress. Results indicated that the oxidation ability of *At.f* remained relatively high when PWL_{HCl} and 9K medium mixed ratio to 60% (V/V); the species was able to endure the extreme conditions at pH 1.4, ferrous ions 0.04 mol/L, chloride ion 800 mg/L, and maintain the ferrous oxidation rate at 90%. The pH was a critical factor impacting the ferrous oxidation efficiency. Different types of mineral crystal shapes, determined by SEM, illustrated the possibility of a new mineral formation. XRD analysis revealed main composition of minerals as $(NH_4)Fe_3(SO_4)_2(OH)_6$, and infrared spectroscopy indicated that the existence of C–Cl bond promoted the adaptation of bacteria to PWL_{HCl}.

Keywords: Hydrochloric acid pickling waste liquor (PWL_{HCl}); *Acidithiobacillus ferrooxidans* (*At.f*); Chloride ion stress; $(NH_4)Fe_3(SO_4)_2(OH)_6$

1. Introduction

Pickling solutions are used for cleaning steel surfaces through the removal of iron oxide scale. They are considered spent when their acid concentration decreases by 75–85%, which is accompanied by a metal content increase of up to 150–250 g/dm^3 [1]. Hydrochloric acid is commonly used for steel pickling and it has been proven to be very effective in

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removing rust and mill scale. However, the pickling efficiency is gradually weakened as accumulate of ferrous and ferric chlorides in the bath over time [2]. Hydrochloric acid pickling waste liquor (PWL_{HCl}) is corrosive because it contains high concentrations of acids (pH nearly 0). As an indispensable process in the steel industry, the pickling process discharges vast amounts of waste liquor. Discarding this waste liquor will result in the depletion of a potentially valuable resource and would subsequently enhance the pressures on wastewater treatment [3].

Pickling waste liquors is a highly reclaimable resource containing abundant iron ions and hydrogen ions at concentrations up to 122 g/L and 910 mg/L, respectively [4], which are needed for synthesizing iron oxide. In previous studies, ferrous ion in sulfuric acid pickling solutions was oxidized by using a mesophilic microbe *Thiobacillus ferrooxidans* at room temperature. This was achieved through optimum pH adjustment at 2 with NH_4OH solution and the microbial oxidized pickling solution by microbial heating at 160˚C for 8 h, which resulted in the formation of ammoniojarosite ($NH_4Fe_3(OH)_6(SO_4)_2$) that is a raw material in the production of iron oxides [5]. Fe^{2+} and SO_4^{2-} represented the necessary electron donors needed for the acidophilic autotrophic bacteria such as *Acidithiobacillus ferrooxidans* (*At.f*) [6], *Leptospirillum ferrooxidans* (*L.f*) [7], *Leptospirillum ferriphilum* (*L.fr*) [8], and *Ferroplasma acidiphilum* (*F.a*) [9] to survive in the sulfuric acid pickling liquor. However, for the PWL_{HCl}, oxidation by the microorganisms was difficult because of the high concentration of chloride ions and extreme acid. Shiers et al. reported that the ability of mesophilic iron oxidizers to adapt to chloride was limited [10]. The acidophilic autotrophic bacteria are extremely sensitive to chloride ions and can be inhibited by concentrations well below to that of seawater [11]. *Acidophilus* possess a positive internal membrane potential in the presence of chloride ions, which results in the influx of chloride and hydrogen ions into the cell. This lowers the cytoplasm pH and leads to cell death [12]. Many studies have focused on the effects of initial pH on the bioleaching process in which *At.f* and *Acidithiobacillus thiooxidans* dominated [13]. Ferrous ion speciation and the precipitation of jarosite were greatly influenced by pH. It was also reported that less jarosite precipitation formed in the condition pH < 1.6 [14].

The environmental factors such as initial pH and anion influencing iron transfer from solid to be dissolved phase through *At.f* in the bioleaching process have been previously investigated. The role of these factors in affecting the ferrous bio-oxidation process has been of lesser concern. The purpose of this study is to examine experimentally the possibility of ferrous ion bio-oxidation in the PWL_{HCl} by *At.f*. The effect of chloride and hydrogen ions on the ferrous ion oxidation efficiency was evaluated; the morphology and groups of ferric precipitants formed under high chloride concentrations were also examined.

2. Materials and methods

2.1. Strain isolation and identification

The strain was isolated from a discharge outlet of polymerization ferric chloride manufactory plant in Tianjin, China; one gram of the sediment near the outlet was collected and cultivated in the sterile 9K medium at pH 2.0; $(NH_4)_2SO_4$ 3 g/L (Tianjin Tianda Chemical Experimental Factory, China), $MgSO_4{\cdot}7H_2O$ 0.5 g/L (Tianjin Bei Fang Tian Yi Chemical Reagent, China), K_2HPO_4 0.5 g/L (Tianjin Guangfu Science and Technology Development Co., Ltd., China), KCl 0.1 g/L (Tianjin Bodi Chemical Co., Ltd., China), $Ca(NO_3)_2$ 0.01 g/L (Tianjin Red Rock Chemical Reagent, China), and 0.5 g/L $FeSO_4{\cdot}7H_2O$ (Tianjin Bodi Chemical Co., Ltd., China) were added for energy. The cultures were incubated in 250 ml Erlenmeyer flasks containing 50 ml of sterile 9K medium on a rotary shaker (HWY-100A, China) at 100 rpm at 30˚C until turned rusty, then the cultures were spread in the dried 9K agar (Gelrite® gellan gum) plates. The rusty colonies appeared in the agar were inoculated in the liquid 9K medium and cultivated to rusty, then the inoculants were spread in the 9K agar. This procedure was repeated until single pure colonies presented and the strain was sequenced and identified to be *At.f* (NCBI No: KF378735).

2.2. The chemical composition of PWL_{HCl}

PWL_{HCl} was collected from pickling bath of a steel processing plant; main compositions were (1L): Cl^- (310 mg); Fe^{2+} (250 mg); Fe^{3+} (67 mg); Cd^{2+} (7 mg); Ca^{2+} 34 mg; pH (0).

2.3. Ferrous bio-oxidation of PWL_{HCl}

PWL_{HCl} was filtered by Millipore filters of 0.22 μm and mixed with 9K medium at a volume ratio of 20, 40, and 60 (v/v) until 100%. The ferrous content of the 9K medium was maintained at the same level as the PWL_{HCl} mixed medium. The isolated bacteria in late exponential phase were inoculated into 9K medium and PWL_{HCl}− 9K mixed medium, and ferrous ion concentrations were monitored every 5 h.

2.4. The effects of growth-limited factors on the PWL_{HCl} bio-oxidation

pH, ferrous ion, and chloride ions concentration are the main growth-limiting factor that can affect ferrous oxidation efficiency in PWL_{HCl}. According to the results of Section 3.1, single-growth factor experiments were designed as Table 1, to determine the extreme conditions in which bacterial growth and ferrous bio-oxidation were hindered. Single-factor experiment of every value was repeated three times.

2.5. FTIR measurements

Samples were analyzed through infrared spectrum using a Fourier Transform Spectrometer (Bruker Tenson37, Germany) with a diffuse reflectance attachment. Radiation was measured with a TGS detector against a non-absorbing potassium bromide matrix, used as a reference. The samples for diffused reflectance were prepared by dispersing 10 mg of the sample in 100 mg of potassium bromide. Typical measurement time while recording the spectra was about 32 scans at a resolution of 4 cm^{-1}.

2.6. Analytical determinations

The ferrous ion concentration was determined using the potassium dichromate titration method. X-ray diffraction patterns on powdered samples were obtained in the 9K and PWL_{HCl} mixed medium. The morphology of the bio-oxidation products in the 9K and PWL_{HCl} mixed medium were observed by SEM (JSM-7500F, Japan). Chloride ion concentrations were determined by Ion Chromatography (DIONEX, ICS-2000, USA).

3. Results and discussion

3.1. Bio-oxidation possibility of ferrous in the PWL_{HCl}

Results in Fig. 1(a) for the 20% PWL_{HCl}− 9K mixed medium show that during the first oxidation stage (0–45 h), the ferrous oxidation rate in mediums increased to reach 96.7 and 97.5%. Starting at 45 h, both mediums (9K and 20% PWL_{HCl}− 9K mixed) showed a steady increase to 99.9%. It was found that under these initial conditions: C_{Fe}^{2+} 7.13 g/L, pH 1.83 and C_{Cl}^{-} 99.74 mg/L, ferrous oxidation was not inhibited.

Fig. 1(b) presents the ferrous oxidation rate in the medium. Starting at 1 h, slow ferrous oxidation was observed and bacterial growth was temporarily stagnant. From 84th h onward, *At.f* entered the exponential growth phase and the ferrous oxidation rate of the mixed medium increased to reach 99.97% at the 80 h. However, the rate was slower than that of the 9K medium, which reached the same value at 54 h. The trends in the iron oxidation rate in the 40%. PWL_{HCl} indicated that under these conditions: C_{Fe}^{2+} 5.35 g/L, pH 1.75, and C_{Cl}^{-} 149.62 mg/L, bio-oxidation was inhibited.

Results of the ferrous oxidation rate of the 60% PWL_{HCl}− 9K mixed medium are shown in Fig. 1(c). On the 4th day, the oxidation rate of the 9K had reached to 99.9%, but that of 60% PWL_{HCl} reached only to 10%. This slow and steady growth in the 60% PWL_{HCl} continued until day 10, and the culture was rusty colored with little sediment formation. The ferrous oxidation rate in the 60% PWL_{HCl} reached to 99.9% on day 17. It was shown that the iron bio-oxidation in the 60% PWL_{HCl} was significantly inhibited under these conditions of C_{Fe}^{2+} 3.57 g/L, pH 1.42, and C_{Cl}^{-} 200.78 mg/L.

The *At.f* was also inoculated into the 70 and 80% PWL_{HCl}− 9K mixed medium, but no ferrous oxidation was observed. These results showed that ferrous bio-oxidation by *At.f* in the PWL_{HCl} gradually stagnated as the ratio of PWL_{HCl}− 9K increased; the possibility of ferrous bio-oxidation by *At.f* in the PWL_{HCl} was prevented when the ratio of PWL_{HCl}− 9K was more than 60%.

3.2. Effect of pH and C_{Cl}^{-} on the ferrous bio-oxidation

Fig. 2 shows that rate of iron (II) oxidation by *At.f* shifted with different growth stages from pH 2 to 0.5.

Table 1
Experiment arrangement of single-growth factor

Factor		Different value					Initial conditions
pH	2	1.7	1.4	1.1	0.8	0.5	C_{Fe}^{2+} = 0.159 mol/L C_{Cl}^{-} = 47.6 mg/L
Fe^{2+} (mol/L)		0.12	0.1	0.08	0.06	0.04	C_{Cl}^{-} = 47.6 mg/L pH 2
Cl^{-} (mg/L)		NaCl	200	400	600	800	C_{Fe}^{2+} = 0.159 mol/L
		KCl	200	400	600	800	pH 2

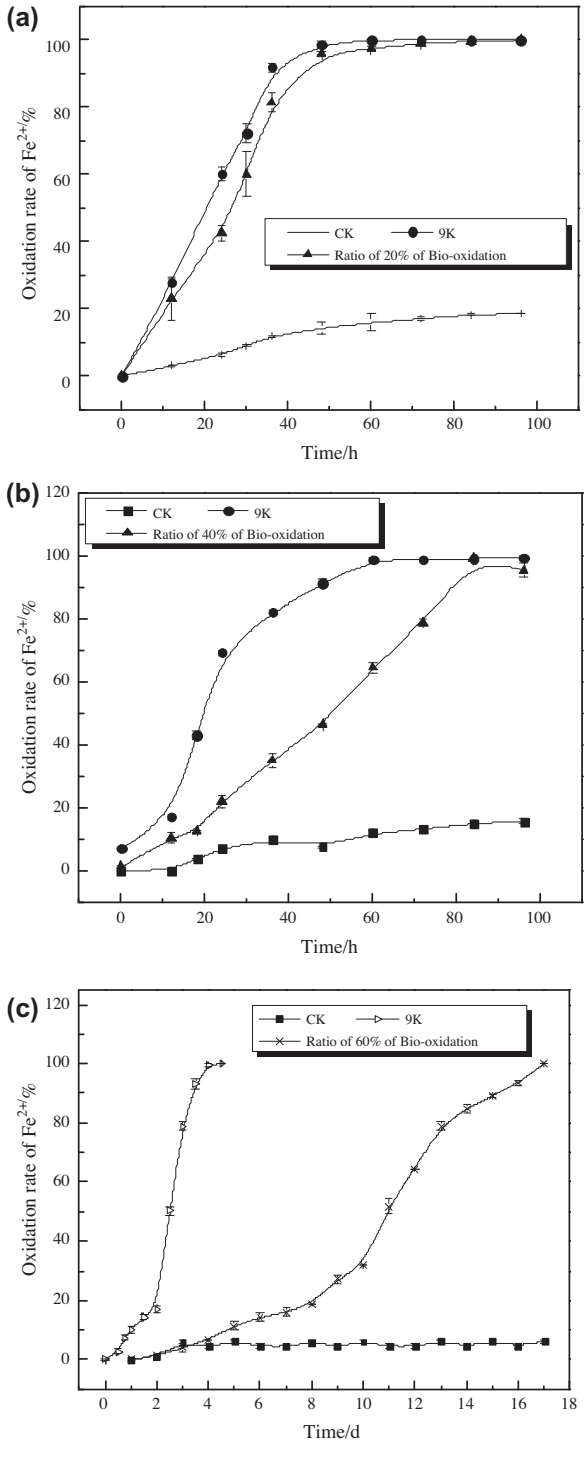

Fig. 1. Fe(II) oxidation rate during the bio-oxidation process in different percent medium (a) in 20% PWL_{HCl}− PWL_{HCl}− 9K (b) in 40% PWL_{HCl}− 9K (c) in 60% PWL_{HCl}− 9K.

The lower the original pH of the culture, the longer the lag phase was. The greatest lag phase (approximately 60 h) was observed for iron (II) oxidation at

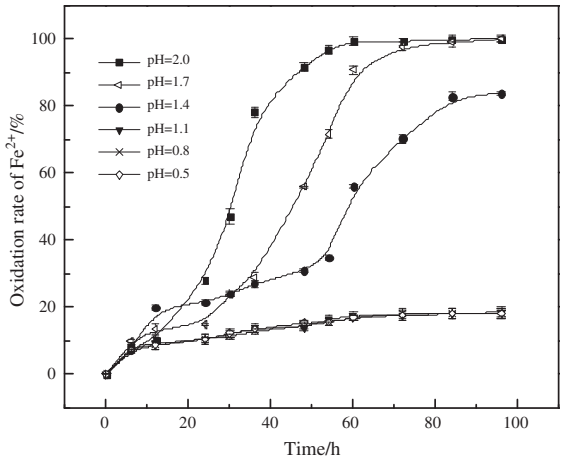

Fig. 2. Fe(II) oxidation rate at different initial pH.

pH 1.4. However, starting at the 60th h, the bacteria began to oxidize iron (II) efficiently, reaching a rate of 80.5%. Cells grown at different pH values showed significant differences in iron (II) oxidation rates. Cells grown at pH 2.0 achieved a max oxidation rate (99%) at the 60th h, while it took cells grown at pH 1.7 a total of 80 h to reach the same rate. Bacteria from cultures grown at pH 1.1 could not oxidize iron (II), as expected, due to the fact that iron (II) oxidation is inhibited below pH 1.2 [15,16].

The effect of C_{Cl}^{-} on the ferrous bio-oxidation is shown in Figs. 3 and 4. Chloride ion inhibition was observed when sodium chloride was added. Zammit et al. also found that elevated chloride levels in leach solutions were inhibitory to iron- and sulfur-oxidizing microorganisms [17]. Iron (II) oxidation rate slowed down obviously while the time needed to reach the

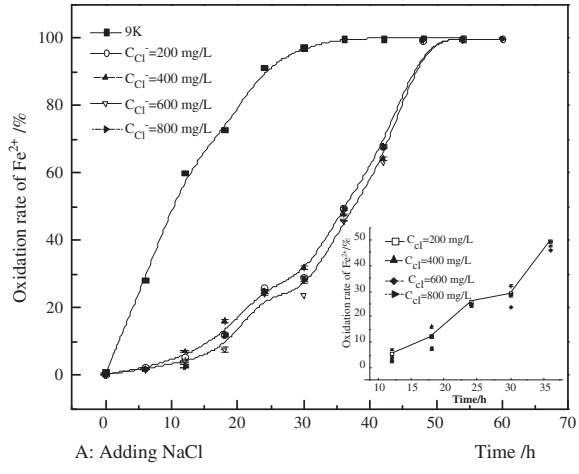

Fig. 3. Fe(II) oxidation rate at different initial NaCl.

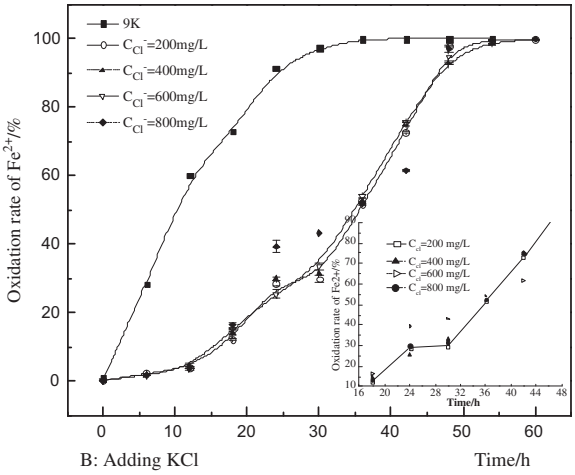

Fig. 4. Fe(II) oxidation rate at different initial KCl.

max rate (99%) retarded from 36 to 50 h (KCl) and 54 h (NaCl). The iron (II) oxidation rate in KCl was faster than that in NaCl because K^+ could be utilized to form jarosite ($KFe_3(SO_4)_2(OH)_6$). No significant difference in oxidation rate was observed when the concentration of KCl and NaCl increased from 200 to 800 mg/L. Cell growth was slower but nearly all ferrous irons were still oxidized. The same phenomenon was reported for the chalcopyrite bioleaching process, a lag period preceding iron oxidation was also observed in the combination of 120 mM Fe(II), 2.5% chalcopyrite, and 100 mM Na–chloride in the inoculated medium. The lag period was attributed to the increased inhibition of *At.f* caused by the increase in salinity (addition of sodium chloride) [18].

Iron (II) bio-oxidization of the PWL_{HCl} depends on the pH and the chloride ions (Figs. 2–4). pH was found to be the key factor influencing iron (II) bio-oxidization with no growth observed at pH 1.3. No inhibition effect on iron (II) bio-oxidization was observed when C_{NaCl} or C_{KCl} < 800 mg/L, correspondingly to the concentration of chloride ions in the 60% PWL_{HCl}− 9K mixed medium. Lag phases observed in the experiments could represent the response of the cells to external pH changes rather than to differences in the culture substrates. Cells grown at low pH have a large amount of protons attached to the cell surface. They exhibited longer lag phases in their ferrous oxidation

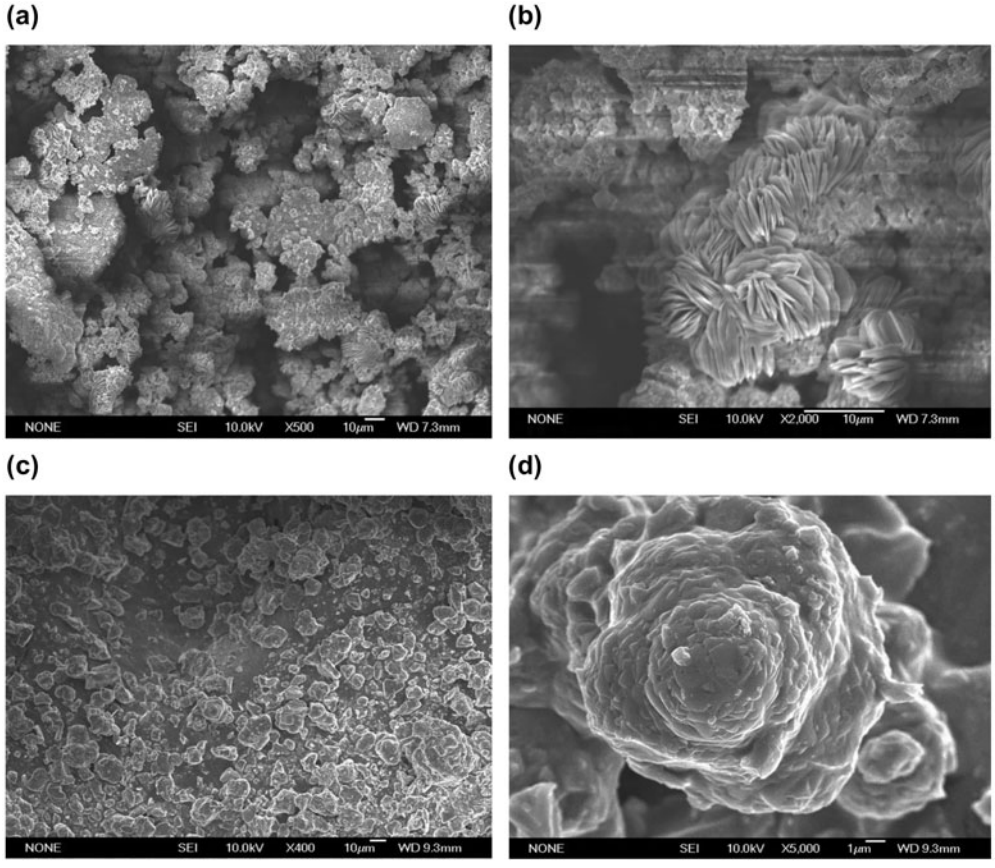

Fig. 5. SEM images of bio-oxidants from 60% PWL_{HCl}− 9K mixed medium and 9K medium.

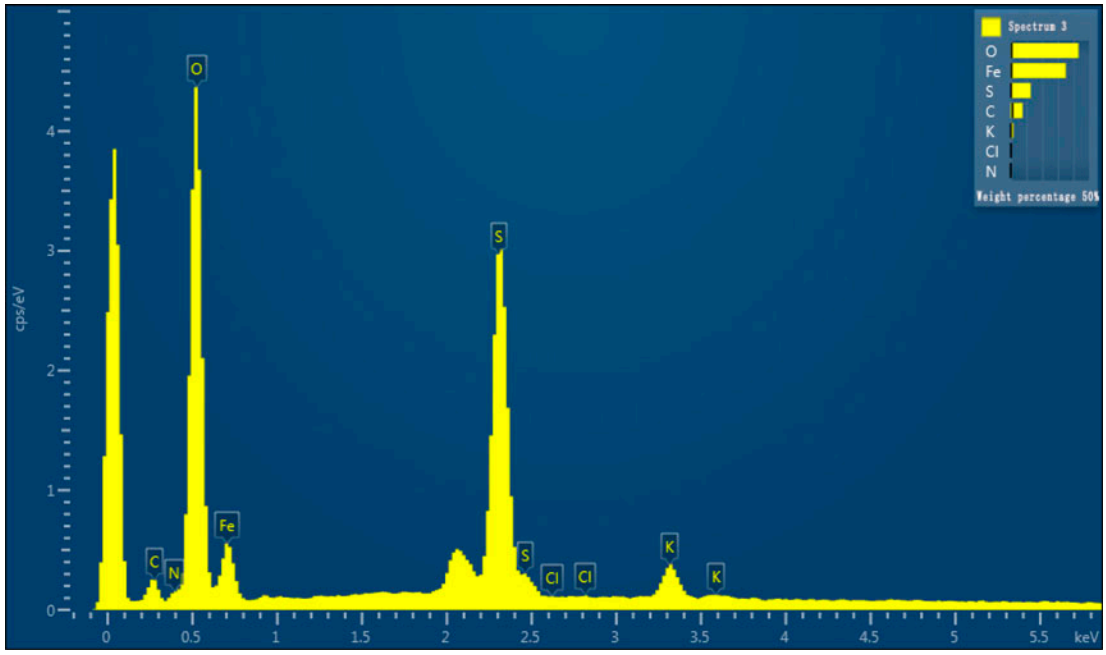

Fig. 6. EDS analysis of sediments from 60% PWL_{HCl}− 9K mixed medium.

indicating a pH stress response. Cell surface proteins changed to allow for the adaptation of the cells to pH changes more than to the substrate changes [19].

3.3. Characterization of PWL_{HCl} bio-oxidants

3.3.1. SEM-EDS and XRD analysis

Magnified SEM images of the bio-oxidants from the 60% PWL_{HCl}− 9K mixed medium are shown in Fig. 5(a) and (b). Compared to the amorphous aggregates (Fig. 5(c)) of the bio-oxidants from the 9K medium, which were piled up rhombus piece (Fig. 5(d)), new crystal structures like "chrysanthemum" were observed here (Fig. 5(b)) and the main elements revealed by EDS (Fig. 6) were O (43.19%), Fe (35.11%), S (12.65%), and C (7.49%), and the composition percent of Cl was only 0.04% which illustrated the new crystals were possibly not chlorides. It has been reported that the presence of chloride ions increases the crystallinity and porosity of sulfur formed during chalcopyrite leaching, giving better oxidant access to the mineral surface [20]. XRD (Fig. 7) proved that the main component of the bio-oxidants from the 60% PWL_{HCl}− 9K mixed medium was potassium jarosite ($(NH_4)Fe_3(SO_4)_2(OH)_6$).

3.3.2. FTIR analysis

The FTIR spectra of *At.f* in the 9K and PWL_{HCl} mixed medium were used to investigate the possible cell–Cl ion interactions (Fig. 8(a) and (b)). In Fig. 8(a), the FTIR spectrum at 3,399 cm^{-1} was indicative of the existence of –OH and –NH groups in the biomass [21]. The peaks observed at 1,633 cm^{-1} (mainly CO stretching) can be attributed to amide I and II bands of protein [22]. The group of bands at 1,430 cm^{-1} indicates the presence of –CH groups [23]. The bands at 998 and 1,193 cm^{-1}centered on 1,083 cm^{-1} could be assigned to the SO_4^{2-} v3 stretching and the band at 1,083 cm^{-1} can be attributed to the –CN stretching vibrations of the protein fractions [24]. The existence of C–Cl groups was revealed by the peak at 626 cm^{-1}.

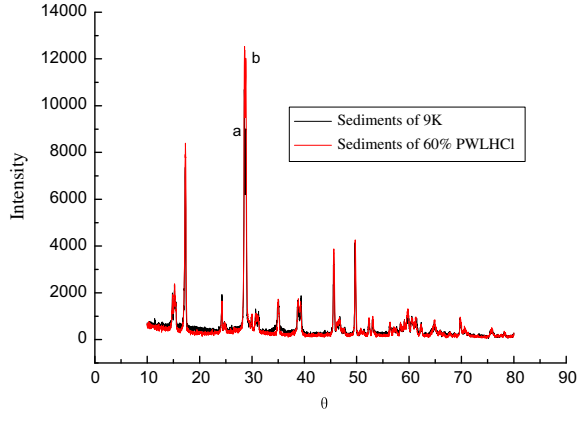

Fig. 7. XRD analysis of sediments from 9K and domesticated solution.

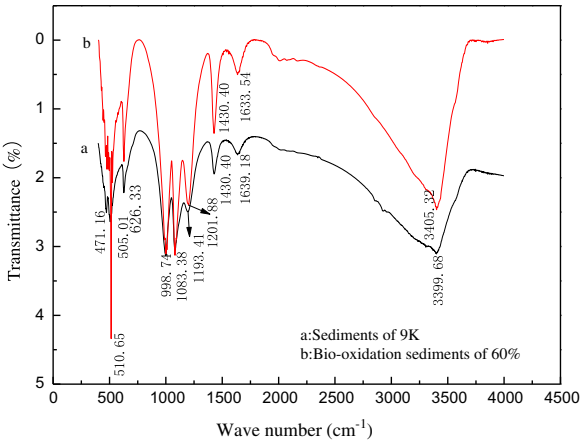

Fig. 8. FTIR analysis of sediments from 9K and 60% PWL_{HCl}− 9K mixed medium.

The peak at 505 cm^{-1} emerged because of the FeO_6 octahedral vibrational absorption. Fig. 8 (spectra b) shows the changes in the FTIR spectrum of the biomass cultivated in the PWL_{HCl} mixed medium. The peaks at 3,399, 1,639, 1,193, and 505 cm^{-1} shifted to 3,405, 1,633, 1,201, and 510 cm^{-1}, respectively. The intensified C–Cl peak indicated that the functional groups on the surface of the biomass were able to interact with chloride ions.

4. Conclusions

At.f was applied to examine the possibility of iron oxidation in the PWL_{HCl}. It was determined that ferrous bio-oxidation by *At.f* in the PWL_{HCl} was prevented when the ratio of PWL_{HCl}− 9K was more than 60%. The chloride and hydrogen ions affected ferrous ions oxidation; pH was a key factor in iron (II) bio-oxidization with no growth observed at pH < 1.3. No inhibition effects on iron (II) bio-oxidization were observed when C_{NaCl} or C_{KCl} < 800 mg/L. The different types of mineral crystal shapes identified by SEM illustrated the possibility of new mineral formation and XRD analysis revealed that the main composition of the minerals was $(NH_4)\ Fe_3\ (SO_4)_2\ (OH)_6$. Infrared spectroscopy indicated the existence of the C–Cl bond supporting the possible adaptation of the bacteria to PWL_{HCl}.

Acknowledgments

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