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Bioleaching of Black Shale by *Acidithiobacillus ferrooxidans*

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Microbiological leaching has been used as an alternative approach to conventional hydrometallurgical methods of metals extraction. The main concern of the present study was to develop a feasible and economical technique to microbially recover metals such as Fe, U, Mo, Cu and Mn from black shale ore as well as to study the comparative growth of bacteria in a medium containing salts and ferrous sulphate. The microorganism used in this study was potentially important bacteria like *Acidithiobacillus ferrooxidans*, utilizing energy from the oxidation of ferrous iron, sulphur and reduced sulphur compounds. During bioleaching study, bacteria oxidize pyritic phase to ferric iron and sulphuric acid in case of samples supplemented with $FeSO_4$. The pH of the media decreased and redox potential remarkably increased. In bioleached residues, K-jarosite and gypsum were predominant minerals and phologopite grades to biotite. Dolomite transformed to gypsum. Shake flask technique is feasible as bacterial leaching system.

Key Words: Bioleaching, Black shale, Mineralogy, *Acidithiobacillus* sp.

INTRODUCTION

Worldwide reserves of high-grade ores are diminishing at an alarming rate due to rapid increase in the demand for metals. However, there exist large stockpiles of low and lean grade ores yet to be mined. The recovery of metals from low and lean grade ores such as shale and schist etc. using conventional techniques is very expensive due to high energy and capital inputs required. Another major problem is environmental costs due to the high level of pollution from these technologies. Environmental standards continue to stiffen, particularly regarding toxic wastes, so costs for ensuring environmental protection will continue to rise. As a consequence of technological and industrial development, many industrial sites are contaminated with heavy metals and organic compounds, which are recognized to be anthropogenic and toxic to any kind of organisms, particularly human beings. Therefore, industries and public offices are obliged to implement the concepts of structured environmental management system more strictly[1-3].

Microbially metal-extraction processes are usually more ecofriendly than physicochemical processes. They do not use large amount of energy as compared to roasting and smelting and do not produce sulphur dioxide, another harmful gas. This process can be considered with antipollution law. Mostly naturally occurring microbes perform numerous physiologically important reactions that enable them to grow and reproduce. Many *Acidithiobacilli* sp. can oxidize minerals as substrates releasing metals and producing sulfuric acids from these minerals. *Acidithiobacillus ferrooxidans* is the principal bacterium used in mineral biotechnology[4]. This bacterium is metabolically active in pH range of 1.5-5.0 and temperature in the range of 40-42 ºC. It is possible to use the bacteria to leach the desired metals out of deep or low grade deposits without removing them from the ground. In recent years some work has been done to evaluate the specific chemical and microbiological role in weathering of low and lean grade ores, shale and schists[5]. Recently, laboratory bench and pilot scale experiments have shown the amenability of the black shale and schist ore to bioleaching with high metal recoveries, which are of economically important[6]. In natural systems, microbial degradation of organic matter from coal or shale is often accompanied by the release of inorganic compounds like heavy metals. As dissolved organic carbon (DOC) has a high potential to complex heavy metals, it is an important carrier for heavy metals from refractory organic matter (OM) in black shale. Main components of the black shale are loams, carbonate minerals, organic compounds and detritus minerals. The black shale ores differ from others in mineralogical and chemical properties and in a susceptibility of recovery of metals[7-9]. The purpose of the present work is to characterize the leaching of indigenous black shale with *Acidithiobacillus ferrooxidans*.

EXPERIMENTAL

Black shale samples: A representative black shale ore sample was obtained from Pakistan Institute of Engineering and Applied Sciences (PIEAS), Islamabad. The samples were obtained originally from Turbella area. It was marked as M-921. The sample was oven dried ground to ≤ 200 particle mesh size and was used for its chemical and mineralogical analysis and for shake flask bioleaching experiments. Scanning electron microscopic-electron dispersive X-ray spectroscopy (SEM-EDX): spectroscopic analysis was carried out by using Hitachi S-2380 Japan as described by Barzyk *et al.*[10] and Hongtao-Shi[11]. Air dried ore sample was mounted on to the aluminium stub by standard method to observe surface morphology of ore along with electron dispersive X-ray spectroscopy (EDX) to determine the elements present at the surface of the particular area of the ore sample.

Chemical analysis: For the chemical analysis, 1 g sample was added in 40 mL HNO_3 (1:1) in a 250 mL beaker and covered with watch glass. The contents of the beaker was heated at 80-90 ºC for 5 h. On cooling, the contents were filtered out through Whattman filter paper. The filtrate was evaporated to dryness and the residue was again diluted with HNO_3 (5 mL) with the addition of 20 mL distilled water. The volume was made to 200 mL with distilled water. The solution was used for elemental analysis of dissolved metals by atomic absorption spectroscopy.

Mineralogical analysis: X-Ray diffractometer was used to determine the mineralogical composition of black shale ore using Rigaku Rint 2000 series diffractometer and JCDS diffraction software. Finally ground and oven dried sample was analyzed as glass slide mount using the CuK radiation 40 kV and 20 mA and a wide range goniometer equipped with a diffracted beam curved graphite monochromator and 2ºθ compensating slit[12,13].

Culture media for microorganisms and leaching studies: For comparative growth studies, liquid medium was used, three strains of *Acidithiobacillus ferrooxidans* were used for Fe^{2+} oxidation and leaching of black shale. *Acidithiobacillus ferrooxidans* (ATCC 13661), (TF1-35) and (Heap-1) was used in this study. The culture were grown in shake flask (100 rpm) at 30 ºC in mineral salts medium (0.5 g $MgSO_4.7H_2O$, 3 g $(NH_4)_2SO_4$, 0.1g KCl, 0.5 g KH_2PO_4, 0.005 g $Ca(NO_3)_2$ per litre). The media was adjusted to pH 1.9-2.0 with H_2SO_4. Some media were supplemented with ferrous sulphate solution (160 mM) that was used as an energy source for iron oxidizing bacteria. To determine the feasibility of chemical reaction, the samples were withdrawn for pH and redox potential measurements and then centrifuged (12000 rpm for 15 min) filtered to remove the solids before chemical analysis of dissolved metals. The liquid media from each tube was decanted off gently without disturbing the underneath pellets.

The pellets containing cells were re-suspended in presterilized distilled water at pH 2 and allow to stand overnight at 4 ºC. The upper milky layers, were transferred into separate tubes and were used for comparative growth studies of microbes[14] and bioleaching studies of black shale.

For the determination of total iron, Fe^{2+} and Fe^{3+}, a standard UV/Vis spectrophotometer method was used by using 1,10-ortho-phenanthroline as a complexing reagent. For the determination of Fe^{2+}, 1 mL of the sample (collected at regular intervals) was added in 25 mL volumetric flask. Then supplemented successively 0.5 cm^3 of 1,10-ortho-phenanthroline solution (1 %) and the volume was made up to mark with distilled water (pH adjusted to 2.0 by H_2SO_4). Samples were allowed to stand for 0.5 h and then the absorbance was measured on spectrophotometer at 510 nm wavelength against reagent blank. For the determination of total iron, the procedure was same as that in case of ferrous iron estimation but in case of total iron determination, 1 mL of hydroxylamine hydrogen chloride was added before the addition of 1,10-ortho-phenanthroline. It reduced ferric (Fe^{3+}), if present to ferrous (Fe^{2+}). The amount of ferric (Fe^{3+}) present in the medium was measured by subtracting ferrous iron (Fe^{2+}) concentration from total iron. In each case, oxidation rate was determined using the average of two readings[15]. Leached metals were analyzed using atomic absorption spectrophotometer. Centrifuged sample (1 mL) were taken and diluted with 1 M HCl and stored at room temperature. Soluble metals were analyzed using atomic absorption spectrophotometer[16].

RESULTS AND DISCUSSION

Black shale ore is black in colour and alkaline in nature. It is insoluble in water. The colouring material in individual shale is carbon, hydrocarbon or finely divided iron sulfide.

Scanning electron microscopic-electron dispersive X-ray spectroscopy (SEM-EDX): Scanning electron microscopic study (SEM) was carried out to observe the surface morphology of sample along with electron dispersive spectroscopy (EDX) to detect the element present at the surface of particular area of ore concentrate. The major elements identified were Al, C, Si, Ti, S, O, Fe and K. SEM micrograph of the representative black shale sample are shown in Fig. 1.

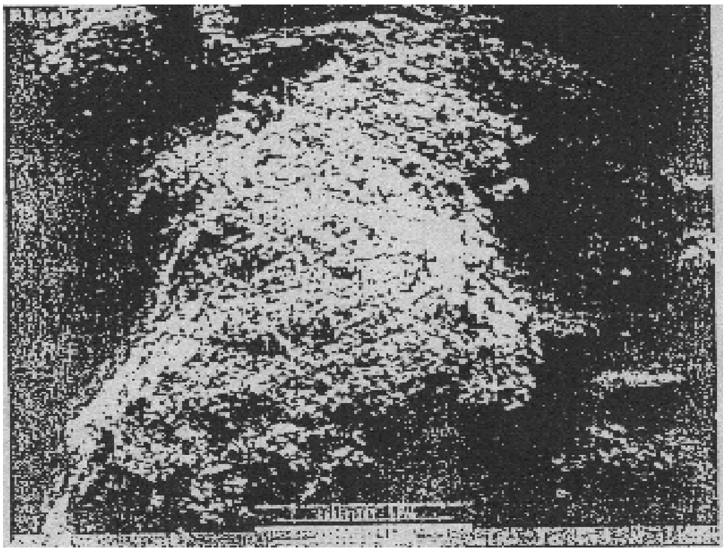

Fig. 1. SEM micrograph of black shale ore sample

Analysis of black shale samples: Samples were analyzed by standard atomic absorption spectrophotometric technique. The elemental analyses of the samples are reported in Table-1. The readings obtained were translated into respective element concentration. It was noted that the concentration of Ni in black shale sample was below the level of detection. The concentrations of Cu, Mn and Fe were 46, 34 and 2510 mg/L, respectively.

TABLE-1
ELEMENTAL ANALYSIS OF BLACK SHALE ORE SAMPLE

Results (mg/L)	Elements
Cu	46
Mn	34
Fe	2510
Ni	ND

Mineralogical analysis: Mineralogical composition of black shale ore sample was examined by powder X-ray diffraction analysis as reported earlier[12]. XRD data for finely ground black shale ore is given in Table-2.

TABLE-2
XRD DATA OF BLACK SHALE ORE

Mineral name	Chemical formula
Orthoclase	$KAlSi_3O_8$
Muscovite	$(K, Na) Al_2(Si, Al)_4O_{10}(OH)_2$
Illite	$KAl_2(Si_3Al)O_{10}(OH_2)$
Biotite	$(KMg_3(Si_3,Al)O_{10}(OH)$
Albite disordered	$Na,(Si_3,Al)O_8$
Phlogopite	$KMg_3(SiAl)O_{10}(OH)$
Muscovite	$(KAl_2,Si_3Al,O_{10}(OH,F)_2$
Albite ordered	$(NaAl,Si_3O_8$
Pyrrhotite-4M	$Fe_{1-x}S$
Chalcopyrite	$CuFeS_2$
Wollastonite	$CaSiO_3$
Dolomite	$CaMg(CO_3)_2$

The black shale ore sample contained aluminosilicate minerals such as orthoclase, muscovite, illite, biotite, albite disordered, phlogopite, microcline and wollastonite as reported earlier[17,18].

Sulfide minerals found in black shale ore sample were pyrrhotite ($Fe_{1-x}S$) and chalcopyrite ($CuFeS_2$). Dolomite ($CaMg (CO_3)_2$ was the only carbonate mineral detected in the ore-matrix. Sulfide minerals in black shale generates a chemically reducing environment with in these rocks as described previously[19].

Oxidation of Fe^{2+} by *Acidithiobacillus ferrooxidans*: *Acidithiobacillus ferrooxidans* derives its energy for growth by the oxidation of reduced Fe- and S-compounds. For the comparative growth studies of three bacterial strains, the ratio of Fe^{2+}/Fe^{3+} was estimated in all the samples collected at regular intervals by a spectrophotometric method using 1,10-ortho-phenanthroline as colouring agent. This dye forms a brick-red complex with Fe^{2+}. It was investigated that during the lag phase of bacteria, the ratio of Fe^{2+}/Fe^{3+} remaining almost constant, but later a rapid decrease in the Fe^{2+}/Fe^{3+} ratio was observed which indicated the log phase of bacterial growth (Figs. 2 and 3). It was noted that the ratio of Fe^{2+}/Fe^{3+} was almost comparable in all the cases of bacterial strains. However, decrease in Fe^{2+}/Fe^{3+} ratio was very small in case of chemically controlled flasks. The small decrease observed in Fe^{2+}/Fe^{3+} ratio was only due to the air oxidation. The biomass specific oxygen consumption rate was dependent on the Fe^{2+}/Fe^{3+} ratio in the culture[20]. In contrast to present results, an increased Fe^{2+}/Fe^{3+} ratio has been reported due to pyrite dissolution and the presence of S^o as reductant[16] for Fe^{3+}.

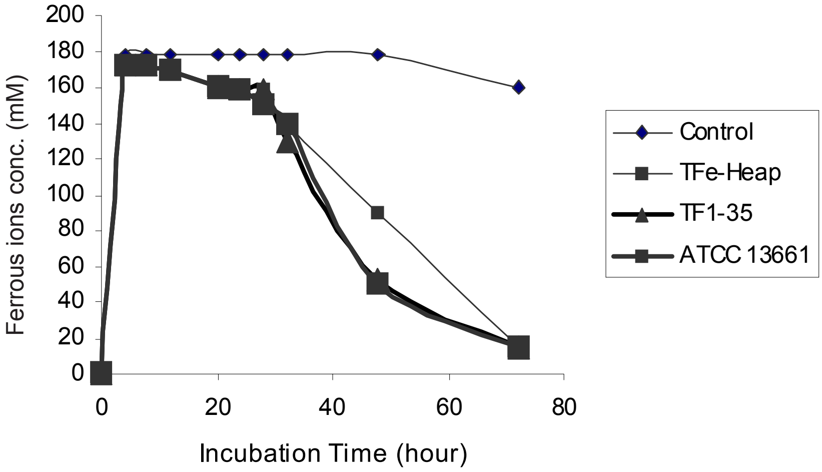

Fig. 2. Ferrous ions oxidation by various strains of *Acidithiobacillus ferrooxidans*

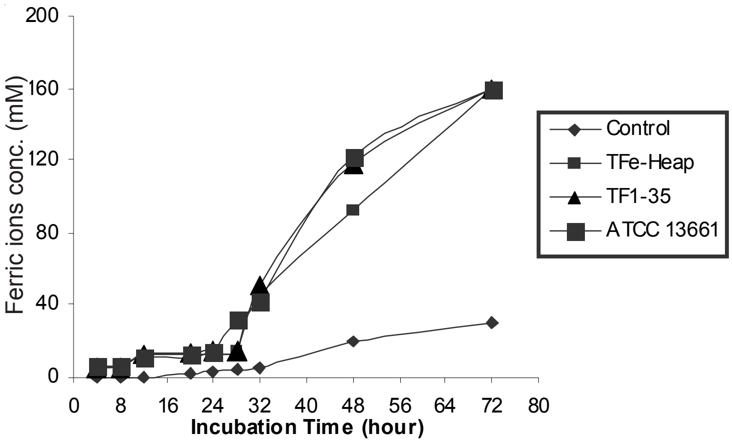

Fig. 3. Ferric ions formation during bacterial oxidation of ferrous ions by *Acidithiobacillus ferrooxidans*

For the comparative growth studies of three strains, the number of cells per mL present in the cell suspension were determined by taking absorbance at 400 nm by using UV-visible spectrophotometer. *A. ferrooxidans* ATCC-13661 was found to be more adopted in the supplied media and show maximum growth as compared to other strains used.

Redox potential: Redox potential of all the samples collected at regular intervals was measured to follow the growth studies of three strains of *Acidithiobacillus ferrooxidans*. Initially, it was noted that redox potential remained almost constant in all the flasks indicating the lag phase of bacteria but later on sharp increase in redox potential was noted indicating the log phase of bacterial growth (Fig. 4). As bacteria derive energy for growth by the oxidation of Fe^{2+} making the environment of media highly oxidative.

$$4Fe^{2+} + O_2 + 4H^+ \longrightarrow Fe^{3+} + H_2O$$

It was noted that increase in redox potential was comparable in case of three bacterial strains of *Acidithiobacillus ferrooxidans*. However, increase in redox potential was almost constant in case of chemical control flasks as shown in Fig. 4.

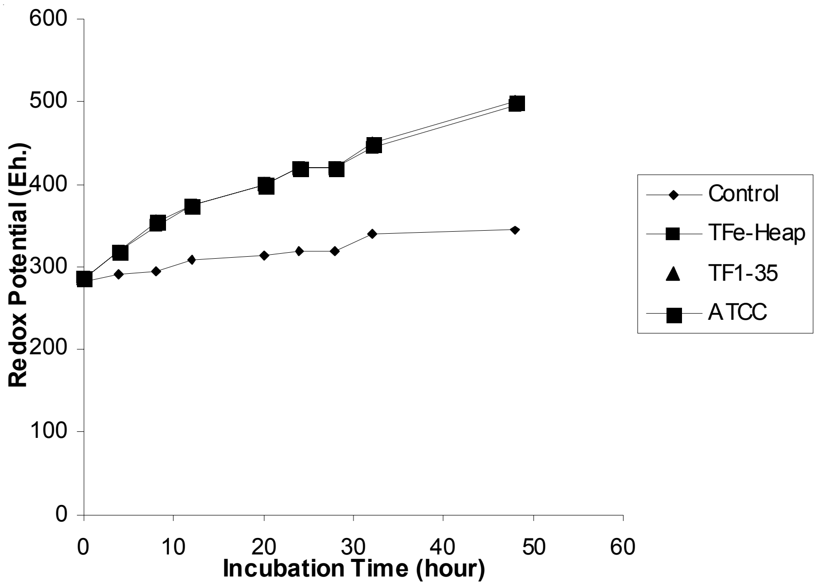

Fig. 4. Redox potential (Eh) profile of growth media during bacterial oxidation of ferrous iron by *Acidithiobacillus ferrooxidans*

Bioleaching studies of black shale: Leaching studies of black shale sample (1 %, w/v) were carried out in 250 mL shake flasks with or without supplemented Fe^{2+} in medium using locally isolated strains of *Acidithiobacillus ferrooxidans* Heap-1. The chemically controlled samples were also included in the experiments for comparison. All the flasks were incubated at 30 ºC and 100 rpm. Leach slurry samples were collected at regular intervals to monitor pH, redox potential and the soluble leached metal ions.

Scanning electron microscopic studies of treated sample of black shale was carried out with electron dispersive X-ray spectroscopy (EDX). SEM micrograph of the representative black shale treated sample are shown in Fig. 5.

Chemical analysis of leach liquor: During the bioleaching of black shale, 1 % pulp density was used by *Acidithiobacillus ferrooxidans* Heap-1. Leach samples of black shale were collected at regular intervals and used the leachate for their chemical analysis. The pH values of the leach suspension are given in Fig. 6. It was observed that there was a decrease in the pH values of the inoculated flasks containing growth media of the initial pH values. Results are shown in Fig. 6 and 7. The effect of ferrous iron on the pH of the growth media was evident in Fig. 8.

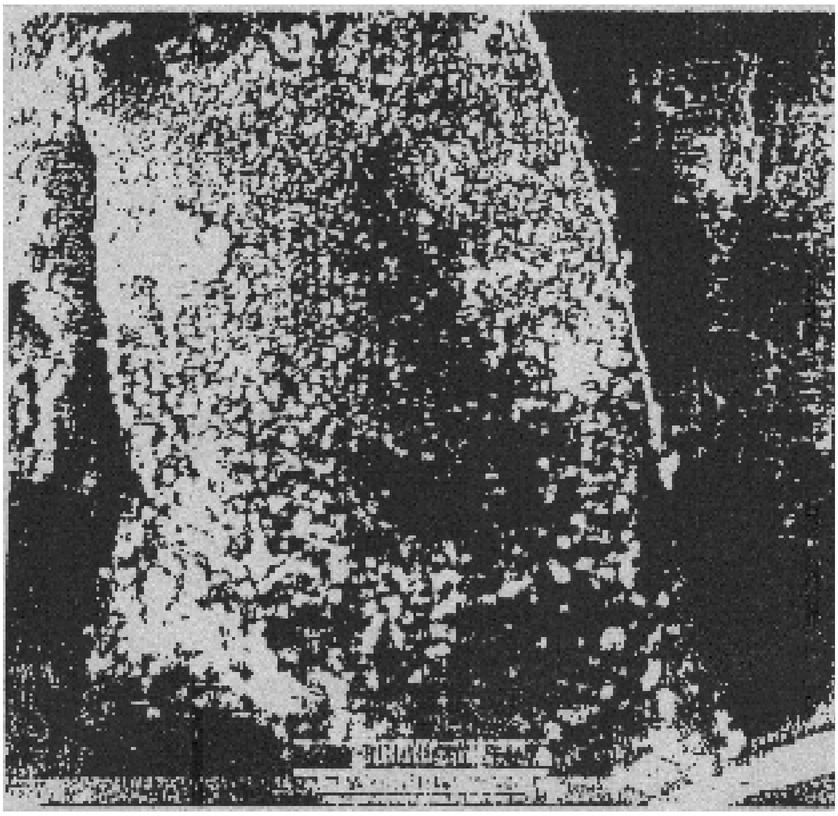

Fig. 5. SEM micrograph of bacterially leached residue of black shale ore sample

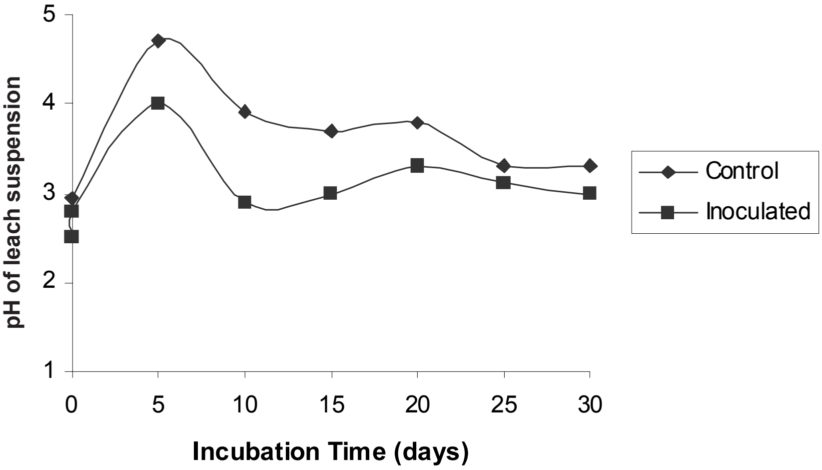

Fig. 6. pH profile of leach suspensions during bioleaching of black shale with *Acidithiobacillus ferrooxidans* Heap-1

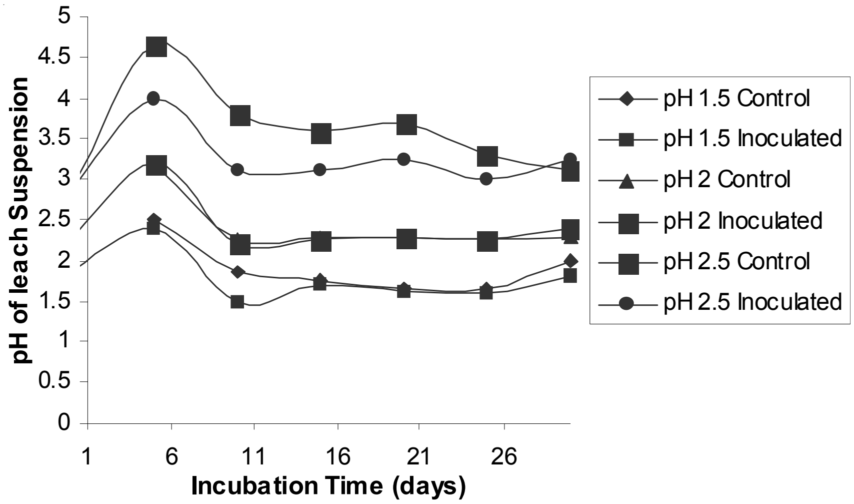

Fig. 7. pH profiles of leach suspensions during bioleaching of black shale with *Acidithiobacillus ferrooxidans* Heap-1 at different initial pH values of the mineral salts (with out $FeSO_4$ supplement) media

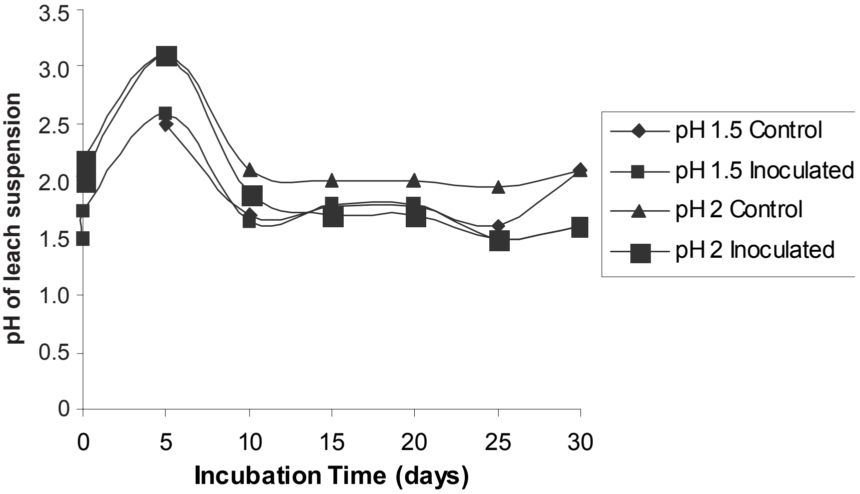

Fig. 8. pH profile of leach suspensions during bioleaching of black shale with *Acidithiobacillus ferrooxidans* Heap-1 at different pH values of the (with $FeSO_4$ supplement) mineral salts medium

It was noted that increase in concentration of total iron was much more in case of samples supplemented with $FeSO_4$ and inoculum. Initially the concentration of ferric iron was small but later on it was noted that there was sharp increase in concentration of ferric. As *Acidithiobacillus ferrooxidans* is capable to oxidized Fe^{2+} to Fe^{3+}. These observations were in good agreement with that of Andres *et al.*[16].

Change in pH during bioleaching studies: Biological oxidation of inorganic sulphur, sulfides and reduced iron form (Fe^{2+}) requires the presence of hydrogen ions as well as electrons. When chemolithoautotrophic bacteria (*Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans* and *Leptospirillum ferrooxidans*) are present, derive their energy by oxidation-reduction reaction using insoluble sulfidic minerals as growth substrate, including pyrite, chalcopyrite, chalcite and sphalerite[21,22]. Acid is generated *in situ* by biological oxidation of sulphur and metal sulfide. This acid lowers the pH of media in the flask while releasing the metals of values from minerals into solution. Initially, at the time of inoculation, pH values were raised due to the fact that initially bacteria were unable to produce acid at the rate required for the neutralization of chalcite ($CaCO_3$) and other acid consuming minerals like dolomite [$CaMg(CO_3)_2$], clay minerals and silicate minerals like wollastonite ($CaSiO_3$) present in shale matrix[23]. Lowering of pH value was observed after 7th days of inoculation as bacteria now was able to produce enough acid required to decrease the pH values. However, in control flasks, acid consumption rate was greater and appreciable increase in pH was observed.

In case of inoculated flasks, it was noted that there was remarkable decrease in pH of media supplemented with $FeSO_4$ but decrease in pH was very less in the inoculated media containing medium without $FeSO_4$. Initial pH observed that was 2.0 (Figs. 8 and 9). This pattern suggested the possibility of quantitative relationship between pH and amount of ferrous sulphate ($FeSO_4$) to be oxidized.

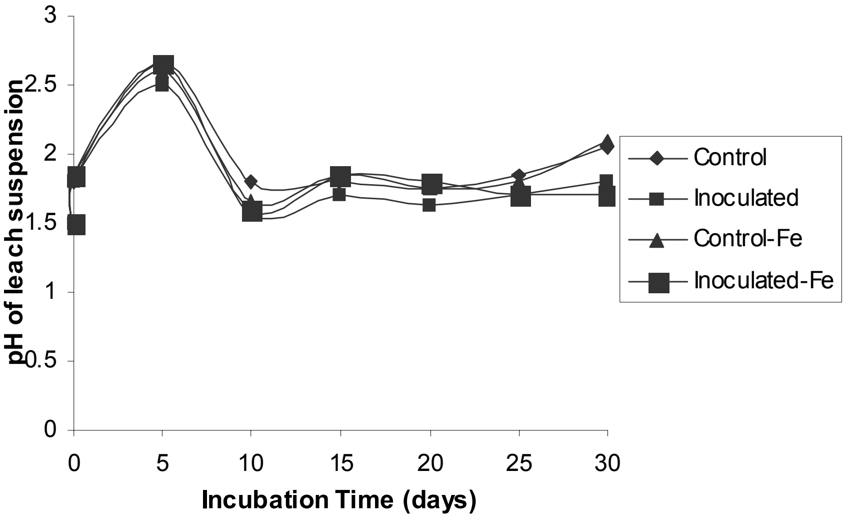

Fig. 9. pH profile of leach suspensions during bioleaching of black shale with *Acidithiobacillus ferrooxidans* Heap-1 in medium with and without $FeSO_4$

Change in redox potential: To determine the feasibility of chemical reaction, redox potential of samples collected at regular intervals were noted. Initially at the time of inoculation redox potential of media in all the flasks was comparable. On both days, it was noted that redox potential of samples supplemented with $FeSO_4$ was remarkably increased (Figs. 10-12). It was the indication that the conditions of the media in flasks were highly oxidative. Change in redox potential of these flasks was not noticeable during next 15th days of leaching experiment. However, the increase in redox potential of media without $FeSO_4$ and inoculum was very small. Ferric iron produced during oxidation is a chemical oxidizing agent of pyrite and thereby enhances the bacterially mediated oxidation. Oxidative dissolution of pyrite from shale samples has been evaluated with *Acidithiobacillus ferrooxidans* earlier[24,25]. The abiotic redox reactions reduce Fe^{3+} to Fe^{2+}, which is then reoxidized by *Acidithiobacillus ferrooxidans.*

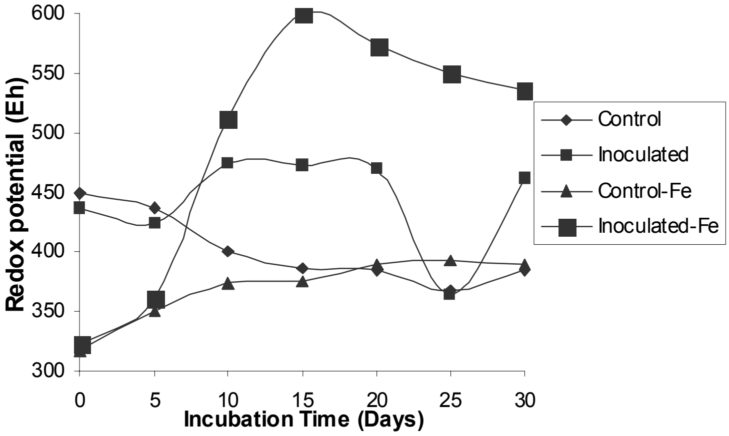

Fig. 10. Redox potential profiles of leach suspensions during bioleaching of black shale with *Acidithiobacillus ferrooxidans* Heap-1 in mineral medium with and without $FeSO_4$

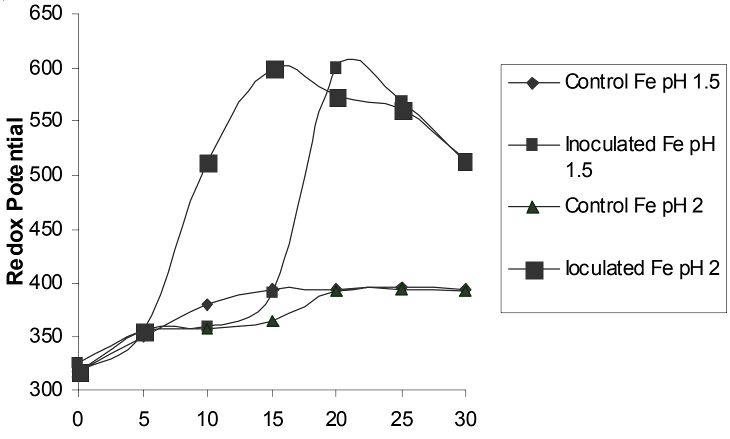

Fig. 11. Redox potential profiles of leach suspensions during bioleaching of black shale with *Acidithiobacillus ferrooxidans* Heap-1 in mineral medium supplemented with $FeSO_4$ at different pH values of the media

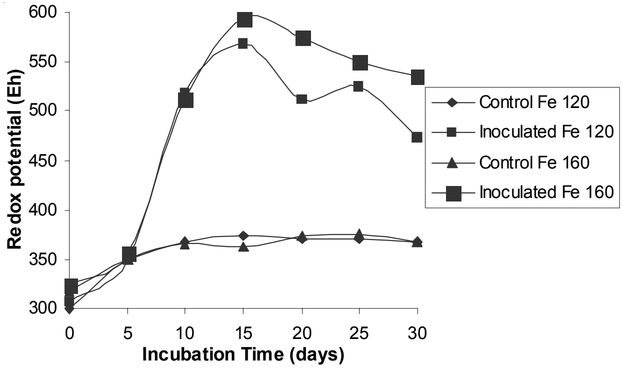

Fig. 12. Redox potential profiles of leach suspensions during bioleaching of black shale with *Acidithiobacillus ferrooxidans* Heap-1 in mineral medium supplemented with different concentrations of $FeSO_4$

Amount of the Fe^{2+} present in leach liquor was analyzed in control as well as experimental flasks using 1,10-phenanthroline. Results are shown in Figs. 13-15. It was observed that ferric iron concentration in the leach suspension was higher in the inoculated flasks as compared with abiotic control (sample without microbes). In the case of control flasks (without supplement), the ferric iron concentration was higher. Leaching data of total iron solubilization are shown in Fig. 16. Leach liquor of both medium supplemented with ferrous iron and without ferrous iron on 20th day was also analyzed by atomic absorption spectrophotometer. Elements to be analyzed were Fe, Cu and Mn in medium supplemented with Fe^{2+} whereas in case of medium without Fe^{2+}, only iron was analyzed which indicate that $FeSO_4$ increase the recovery of metals during bioleaching. Most of the copper contained in shale was found to be probably pyrite and chalcopyrite. According to Teresa *et al.*[26] the metals such as Cu in the form of chalcopyrite and Ni could be extracted from black shale ore by bioleaching in an acid medium. Elzbieta *et al.*[27] recovered the trace metals like U, V, Mo, Zn and Pb from black shale with *Acidithiobacillus ferrooxidans* in acidic medium supplemented with $Fe_2(SO_4)_3$ and H_2SO_4. The enhancement of metal leaching from shale due to the treatment with Fe- and S-oxidizing bacteria has been reported for Polish and Russian black shale[28]. In contrast to these, Kolahdoozan *et al.*[29] reported that pyrite was depressed whereas chalcopyrite and other sulfide minerals were unaffected and also the pulp pH remained unchanged during bioleaching. The elemental analysis of leached solution obtained on 20th day leaching experiment is summarized in Table-3.

Mineral transformation in bioleaching studies of black shale: To study the mineralogical transformation, small amount of leached residue was separated by centrifugation on the 20th day of leaching experiment. It was observed from XRD data that the formation of K-jarosite and gypsum was the predominent minerals found in the leached residues of both inoculated and abiotic control. The formation of K-jarosite was indicative of Fe^{2+} oxidation[12].

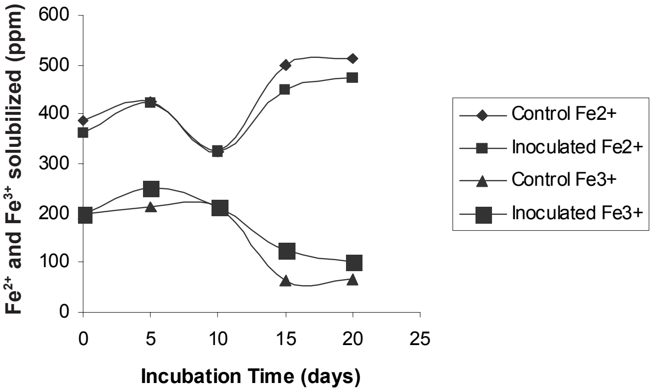

Fig. 13. Dissolution of ferrous and ferric ions during bioleaching of black shale with *Acidithiobacillus ferrooxidans*

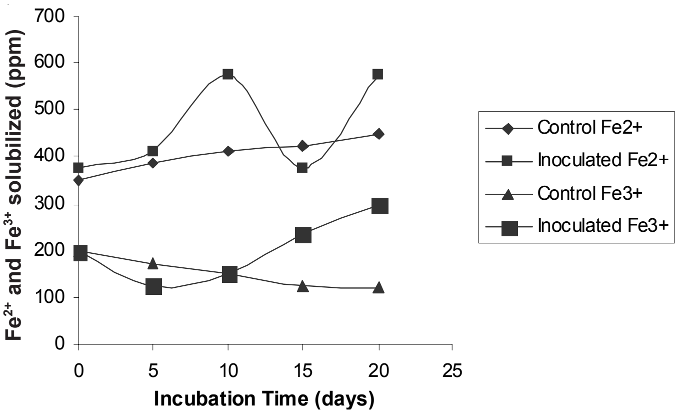

Fig. 14. Dissolution of ferrous and ferric ions during bioleaching of black shale with *Acidithiobacillus ferrooxidans* in medium supplemented with $FeSO_4$

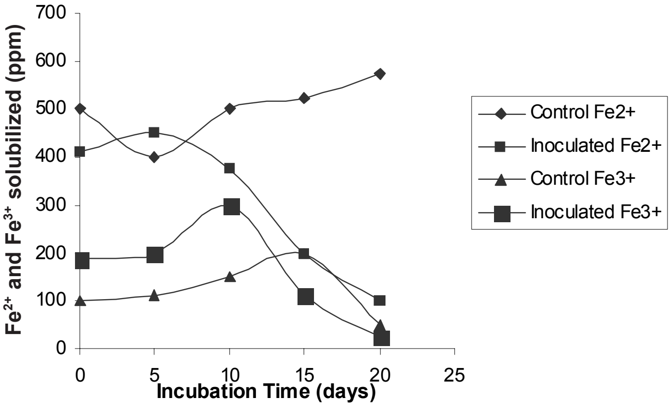

Fig. 15. Dissolution of ferrous and ferric ions during bioleaching of black shale with *Acidithiobacillus ferrooxidans* in medium supplemented with $FeSO_4$ at different pH values

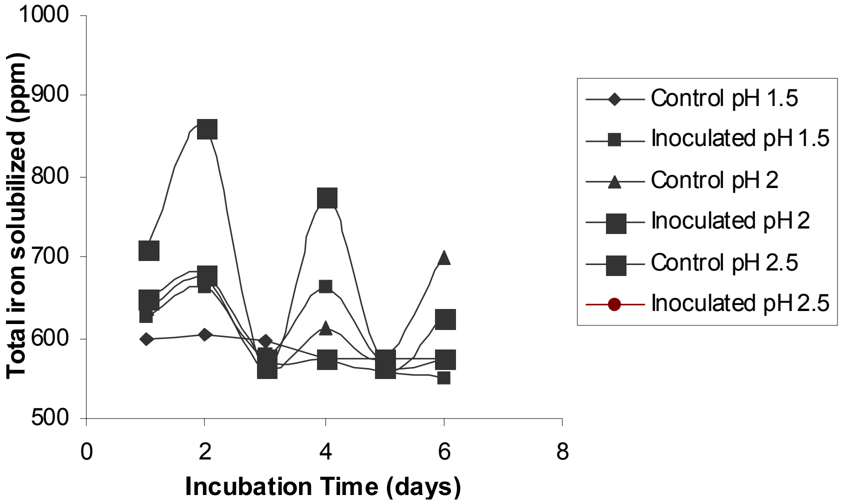

Fig. 16. Dissolution of total iron during bioleaching of black shale with *Acidithiobacillus ferrooxidans* in medium without $FeSO_4$ at different pH values of the media

TABLE-3
ELEMENTAL DATA OF 20th DAY OF LEACH LIQUOR OF BLACK SHALE

Sample	pH	Eh	Results (mg/dm^3)			
			Fe	Mn	Cu	Ni
Medium without Fe^{2+} ion						
Control, pH 1.5	1.78	405	74.25	ND	ND	ND
Inoculated, pH 1.5	1.63	413	103.00	ND	ND	ND
Control, pH 2.0	2.30	390	34.25	ND	ND	ND
Inoculated, pH 2.0	2.32	465	37.75	ND	ND	ND
Control, pH 2.5	3.80	400	15.25	ND	ND	ND
Inoculated, pH 2.5	3.30	400	20.25	ND	ND	ND
Medium supplemented with Fe^{2+} ion						
Control, pH 2.0	2.00	382	158.25	19.5	1.75	ND
Inoculated, pH 2.0	1.65	570	298.00	25.3	1.75	ND
Control, pH 1.5	1.77	390	76.75	24.0	1.75	ND
Inoculated, pH 1.5	1.80	596	171.25	26.8	1.75	ND
Control, pH 2.5	1.96	380	175.00	17.85	1.75	ND
Inoculated, pH 2.5	2.70	513	370.00	15.8	1.75	ND

Eh = Redox potential.

In addition to the octahedral mica minerals (phlogopite and biotite) present in the ore matrix underwent mineral transformation during the bioleaching studies[12]. Phologopite is monoclinic and it has high power cofactor than biotite. With the increase of Fe^{2+} it grades to biotite. Biotite and phologopite are interconvertible and it is difficult to distinguish these. However, these results are not included for the present studies. Muscovite is mica mineral and does not undergo chemical dissolution. It is dioctahedral. Kaolinite is 7 Å clay mineral. Its electron micrograph shows a well formed 6 sided flakes. It is formed from the constituents containing alkali and alkaline earth metals at low temperature and acidic conditions during acidic conditions alkali and alkaline earth metals are removed. Pyrrhotite (mono-sulfide mineral) was disappeared due to bacterial oxidation during the bioleaching studies. Dolomite also disappeared during the leaching process and it was transformed to gypsum ($CaSO_4$). Dolomite [$CaMg(CO_3)_2$] are mostly replaced limestone. The replacement is the product of reaction between Mg^{2+} ions in the interstitial water and calcite to form the double salt dolomite.

$$CaCO_3 + Mg(OH)_2 \longrightarrow CaMg(CO_3)_2 + CO_2$$

Wollastonite is triclinic and occurred characteristically as a clay metamorphic mineral in limestones where it is associated with diopside.

$$CaMg(SiO_3)_2 \longrightarrow CaSiO_3 + MgSiO_3$$

Fe, Mn commonly while Co, Pb and Zn rarely replace Mg in dolomite, calcite ($CaCO_3$), dolomite [$CaMg(CO_3)_2$], diopside [$CaMg(SiO_3)_2$] and silicate minerals like wollastonite ($CaSiO_3$) are acid consuming minerals.

$$CaCO_3 + H_2SO_4 \longrightarrow CaSO_4 + H_2O + CO_2$$

Pyrrhotite is an iron sulfide mineral crystallizing in the hexagonal system. The chemical composition of pyrrhotite is written as $Fe_{1-x}S$ with x between 0-0.5. In some basic igneous rocks it is associated with chalcopyrite ($CuFeS_2$) and pentalantdite (Fe, $Ni)_9S_8$. From XRD data of leached residues, it was investigated that remarkable decrease in pH of media in some flasks was due to the formation of iron mineral, jarosite [$KFe(SO_4)_2(OH)_6$]. It was found that black shale sample also contain calcite as acid consuming gangue mineral. So acid consumption due to carbonate dissolution leads to increase pH values and precipitation of $CaSO_4$ and this is counteractive to the bacterial leaching.

$$CaCO_3 + 2H^+ + SO_4^{2-} + H_2O \longrightarrow CaSO_4.2H_2O + CO_2$$

Conclusion

Bioleaching is an economical alternative to conventional hydrometallurgical methods. This study suggest the factors which influence the amenability of metals of low grade ores to bacterial leaching include electrosorption and chemical structural changes in the mineral phase induced by the leaching reagent sulphuric acid supplemented with $FeSO_4$. It appears that it is the latter factor which controls the difference in leaching recovery observed from black shale. Leaching recoveries of metals such as Fe, Mo, Cu and Mn are found to improve when bacteria supplemented with salts and ferrous sulphate.

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