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BIO-PHYSIOCHEMICAL BENEFICIATION OF LOW GRADE COAL FROM LAKHRA MINE, (*Sindh*) PAKISTAN

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ABSTRACT

Low grade coal of Lakhra mines was beneficiated using bioleaching technology. This technique has more advantages over their counterparts such as chemical and physical techniques due to its easy processing, simplicity, having no environmental pollution, economic feasibility and maximum removal of contamination like sulfur and ash present in coal composition. Presently bacterial strain of *Thiobacillus ferrooxidans* was isolated and purified from indigenous sources i.e. Coal mine drainage, hot spring water and soil adjacent to sulphide mineral ore, using simple screening, serial dilution and growth culturing. Isolated microorganisms were applied on low grade reduced size/powdered coal collected from mines site. Chemical evolution of coal revealed that this sample has 44.45% of ash and 7.03% of sulfur along with 2.3% of Iron. Microbial processing was continued for maxim 36 days which successfully reduced up to 57.33% of sulfur, 75.21% ash and 87.7% iron. Optimization of parameters such as pH, grain size, aeration time and pulp density were also been optimized. The results were confirmed by 100, 50 and 20 magnification of scanning electron microscope (SEM) fallowed by Fourier transform infrared spectroscopy (FTIR).

Contribution/ Originality: This study contributes in existing literature about the bio desulphurization of low grade coal having high contaminations. Presently we have selected the sample from Lakhra mines with high percentage of sulfur, ash and associated minerals for bioleaching process. It is found successful for beneficiation of such type of coals.

1. INTRODUCTION

Need of energy is the basic requirement of every living body; various sources are available for fulfillment like solar, wind, electrical, and coal sources. All other sources of energy are comparably neat & clean, however use of coal for energy purpose have adverse effects for our environment, animal health, plants and damaging of solid structures. This behavior is due to presence of contaminations in coal composition such as high sulfur, minerals and burden of ash; on combustion of coal it produces sulfur dioxide, sulfur trioxide and also producing high ash burden

in the kilns of factories and power plants boilers. So the removal of these contents using suitable technique before combustion of coal is the best solution for kilns and as well as for protection of our environment [1].

Various physical and chemical techniques are commonly used for removal of these contaminations such as gigging, froth flotation, chemical leaching and dance media separation. However these techniques found uneconomical due to requirement of high temperature and pressure for removal of contamination and getting desired purity of coal [2].

In present investigation we have used recent growing technology of bioleaching for the removal of these contaminations from the low grade coal collected from Lakhra, Sindh mining area. Bio-desulphurization is the process in which specific microorganisms having the ability to remove the sulfur in presence of air and moisture are utilized for coal processing [3]. Microbial desulphurization of coal have various advantages over their counterparts, as it is less energy consumable, environmental friendly, quick regeneration of microorganisms without damaging the quality of coal and have no harmful or health hazard effects for the manpower working in processing plants [4-6]. The lowering of sulfur in coal body is due to conversion of iron pyrite into ferric state iron and dissolution of sulfur by conversion in sulfuric acid which is water soluble. It is assumed that combination of both *T. ferrooxidans* and *T. thiooxidans* directly or indirectly interaction have more effectively reducing power for sulfur reduction [7].

According to Geological Survey of Pakistan about 187 billion tons of coal reserves are present in the country. Utilization of ingenious coal for energy purpose is still minimum, as only 10% of reserves are being used for electricity production, this less utilization is only due to its low grade and quality [8]. Most of Pakistani coal is of low grade i.e. lignite having high contamination of sulfur and ash in its composition. Our country is still facing energy crisis and unfortunately indigenous coal is useless to reduce this problem. When Pakistani coal burns in kilns of factories and power plants, it produces huge burden of ash along with emission of restricted gases [9].

The Lakhra coal mines are located near Hyderabad Sind area of Pakistan. The estimated coal reserves of these deposits are about 1.33 billion tones. The coal composition of this area is sulfur 5-8%, Ash 18-56%, Volatile matter 27.9% and fixed carbon 30%. Some beneficiation work on the Lakhra coal has been carried out by physical cleaning using dance medium separation technique and they reduced sulfur up to 42.4% [10]. Present investigation involves on up gradation of coal using bioleaching technique on low grade coal of this area having high sulfur and ash contents.

2. EXPERIMENTAL

2.1. Reagents and Equipment's

All the chemicals used for bacterial strains and growth culture were of reagent grade. The bacterial strains were cultured in flasks already sterilized along with cotton plug in autoclave at 120 ºC for 15 minutes. The experimental work was conducted under hygienic condition.

2.2. Coal Sample

Low grade coal sample was collected from Lakhra coal mines Sindh (Pakistan), having estimated deposit about 1.33 billion tons of coking coal. This sample was granular powder having grain size 7 cm to fine powder.

2.3. Microorganisms

Microbial strains source samples were collected from coal mining area of Khost, Shargh, and Harnai deposit. Some samples of hot spring water were collected from Spintangi and soil samples adjacent to sulphide ore were procured from stibnite mines of Qillah Abdullah Baluchistan. These samples were collected in sterilized plastic bottles, priory cleaned & autoclaved. Presence of microorganisms in the samples were determined using handy pH meter (Hanna), having lower pH about 4.6 indicate presence of bacterial strain with ability to reduce sulfur. The

strains were cultured in 100 cm^3 laboratory prepared medium as described below. The pH was adjusted at 2.8 using dilute sulfuric acid.

2.3.1 Medium for Growth Culture

The required medium was prepared in the laboratory as described by Barron and Lueking [11]. The constituents were dissolved in concentration of gram per litter i.e. KCl, 0.116; Ca $(NO_3)_2$, 0.00168; K_2HPO_4, 0.058; $MgSO_4$-$7H_2O$, 0.058; and $(NH_4)_2SO_4$. 4.0. The volume of solution was prepared up to 420 ml and filtered (whatman #42) and autoclaved. Another solution was prepared by dissolving $FeSO_4$-$7H_2O$ (74.67g) in 580 ml of double distilled and this was also autoclaved and cooled. Now both solutions were mixed together, during mixing some precipitation of salts were also observed which removed by centrifugation at 300 rpm for 15 minutes. The solution was used as medium for growth culture of the chemo- autotrophic bacterial strains. All the conditions required was adjusted as per requirement using aerobic fermentation. During investigation all the chemicals that were used are of reagent grade.

2.3.2 Mass Culture and Purification of T. ferrooxidans

About 100 ml of source samples were added in the above mentioned lab prepared medium and incubated at 37 ºC for 12 days in hygienic and favorable conditions. At initial stages no any decrease was detected in the pH of this solution, however after 6^{th} day of incubation solution showed a gradual decrease in pH level. Presence of organisms were also confirmed by slide preparation and examination under optical microscope (Nikon). Gradual decrease in pH of the medium found supportive for purification of microorganisms. After 12^{th} day of incubation, change of color of medium greenish to brown (due to change of ferrous iron in to ferric state) was further confirmed the presence of active microorganisms.

2.3.3 Microscopic Examination

Colonies of bacterial strain were also produced on semisolid agar based medium surface. 25 ml of the nutrient agar (Oxoid) was mixed with above mentioned medium in molten state and poured in sterilized petri dishes and allowed to settle down. The source sample after incubation for 12 days in medium was applied on the surface. At incubation in sterile condition at 37ºC, colonies of microorganisms were observed. A small portion of colony of interest was blended in minute amount of medium and applied on slide for microscopic examination. Studies revealed that it contains various strains along with *T. ferrooxidans*.

2.4. Sample Preparation for the Leaching Studies

The primary and secondary crushing and grinding of ore was carried out using lab scale Jaw crusher fallowed by rod mill. The size of coal was reduced up to 4#.

2.5. Bioleaching activity

All the experimental work was carried out in autoclaved conical flasks, caped with cotton plugs. The coal samples (4#) were added in 150 ml of culture broth media. The conditions were adjusted at aerobic and dynamic using sterilized shaking bath incubator adjusted at 37 ºC. By addition of dilute sulfuric acid pH of liquid medium was adjusted at 2.5. After completion of process, coal was removed through filtration and washed with tap water which after all neutralized by addition of lime. The Head grade, Control and bioleached samples for 15 days and 36 days were analyzed and results are mentioned in relevant portion.

2.6. Estimation Assay

Samples for ferrous iron determination were prepared by filtration of microbiological culture through 0.2-pm membrane (Millipore Corp., Bedford, Mass.). Ferrous iron was determined by titration with potassium dichromate according to ASTM. The pH of solution was also gradually decreases as growth of *T. ferrooxidans* proceed in medium which was continuously measured with a combined pH glass electrode (Metrohm AG, Herisau, Switzerland).

2.7. Scanning Electron Microscopy

The scanning Electron Microscopy for structural images was carried out using SEM (3700N-Hitachi Japan). The powdered samples were introduced and images were obtained before and after bioleaching process.

2.8. Ft-Infrared Spectroscopy

Infrared spectroscopy of coal samples were carried out using FT-infrared spectrophotometer (Bomin- MB 100; Heartmann and Broun, rsqb) and infrared measurements were recorded in the wave number range of 500-4000 cm^{-1}.

3. RESULTS AND DISCUSSION

3.1. Isolation of T. ferrooxidans

The Petri plates of semisolid medium was prepared as discussed in experimental section, the appeared colonies having brownish character was removed and added in broth after serial dilution for further purification. As indicated in Figure 1 after 3rd day of incubation at 37 °C, colonies were observed on agar plates having application of strain while no colony was detected in control.

Figure-1. Different Colonies of bacterial and fungal strains on nutrient agar medium and source sample filtration process.

3.2. Conformation and Isolation of T. ferrooxidans on Semisolid Medium

The colonies shown positive for the presence of *T. ferrooxidans* were collected and dissolved in 9k medium and further applied on iron containing agar based semisolid medium. After 5-7 days incubation at 37 °C colonies of *T. ferrooxidans* were appeared on surface of agar as shown in Figure 2. The conformation of strain and purification was indicated by producing similar colonies of reddish brown color on solid medium surface.

Figure-2. Colonies of *T .ferrooxidans* cultured on nutrient agar showing conversion of Fe++ in Fe+++ ferric.

3.3. Growth of Isolated Strain in Broth Culture

As these microorganisms are mostly using in the bioleaching of minerals and coal desulphurization, so these are usually required its presence in broth culture. The liquid medium was prepared as described in experimental section. The colonies produced on the semisolid were added to liquid medium. The solution was remained on continuous circulation by passing air from bottom of container, as it is vital for oxygen and carbon requirement of microorganisms. The pH of solution was maintained by addition of dilute sulfuric acid throughout these studies. The readings were noted after every 24 hours and temperature was kept at 37 °C. After 216 hours it was observed that Fe^{+2} begin to start changing in Fe^{+3} as shown in Figure 3. The pH of the solution medium gradually decreased up to 1.47. The total ferrous Iron was completely changed in ferric state on 12^{th} day. After 15^{th} days of incubation strain solution was removed and preserved for bioleaching and coal desulphurization trials.

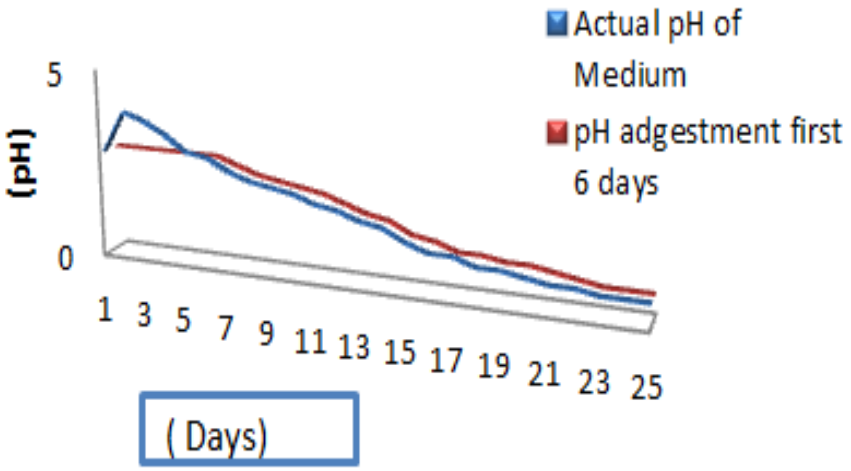


Figure-3. Growth of T. ferrooxidans showing lowering pH of medium.

3.4. Microscopic Structure of Isolated T. Ferrooxidans

After identification based on colony morphology and pH lowering characteristic, isolates were examined under optical microscope which revealed that these microorganisms are somewhat rod shaped, gram negative and chemoautotrophic in nature as illustrated in Figure 4.

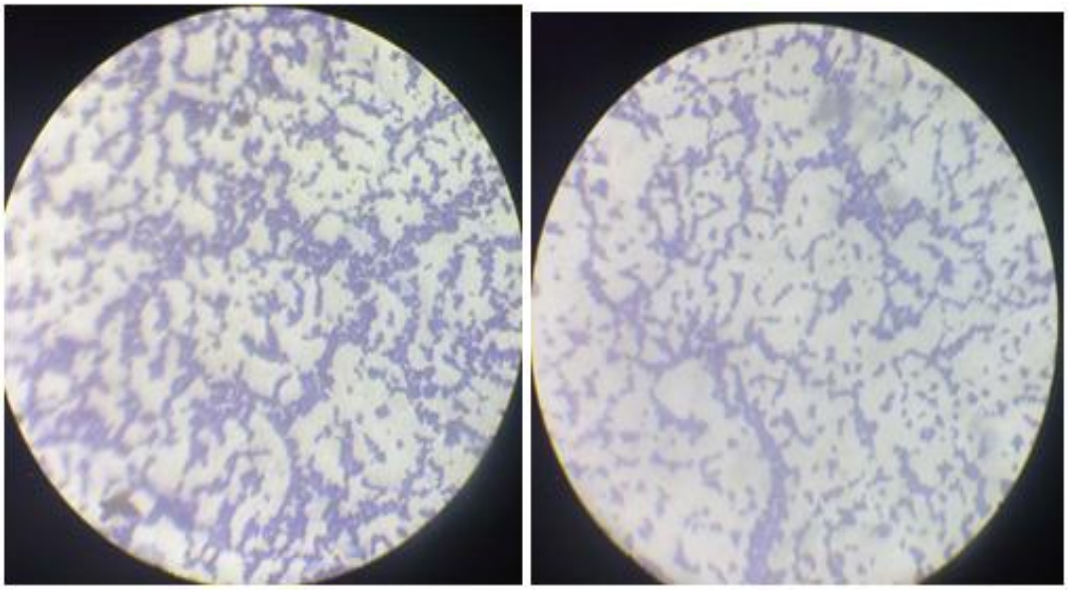


Figure-4. Microscopic view of isolated bacterial strain.

3.5. Proximate & Ultimate analysis of Coal

Table-1. Proximate analysis.

Sr. No.	Test	Head grade	Control	After bioleaching for 15 days	After bioleaching for 36 days
1.	Moisture %	4.86	4.4	8.60	8.87
2.	Ash%	44.48	24.96	17.19	9.49
3.	Volatile Matter%	27.08	39.50	39.24	39.96
4.	Fixed carbon%	23.58	31.13	34.70	41.75

Table-2. Ultimate analysis using CHNS.

Sr. No.	Test	Head grade	Control	After bioleaching for 15 days	After bioleaching for 36 days
1.	Carbon %	31.75	50.48	53.82	61.71
2.	Hydrogen%	5.02	5.35	5.35	5.36
3.	Nitrogen%	1.77	1.63	1.62	1.63
4.	Sulfur%	7.03	6.09	5.12	4.25
5.	Oxygen%	54.43	38.29	34.08	25.22

Table-3. Distribution of sulfur.

Sr. No.	Test	Head grade	Control	After bioleaching for 15 days	After bioleaching for 36 days
1.	Sulphate Sulfur	1.26	1.50	0.82	0.26
2.	Pyritic Sulfur	3.16	2.05	2.284	2.123
3.	Organic	2.61	2.54	2.016	1.867

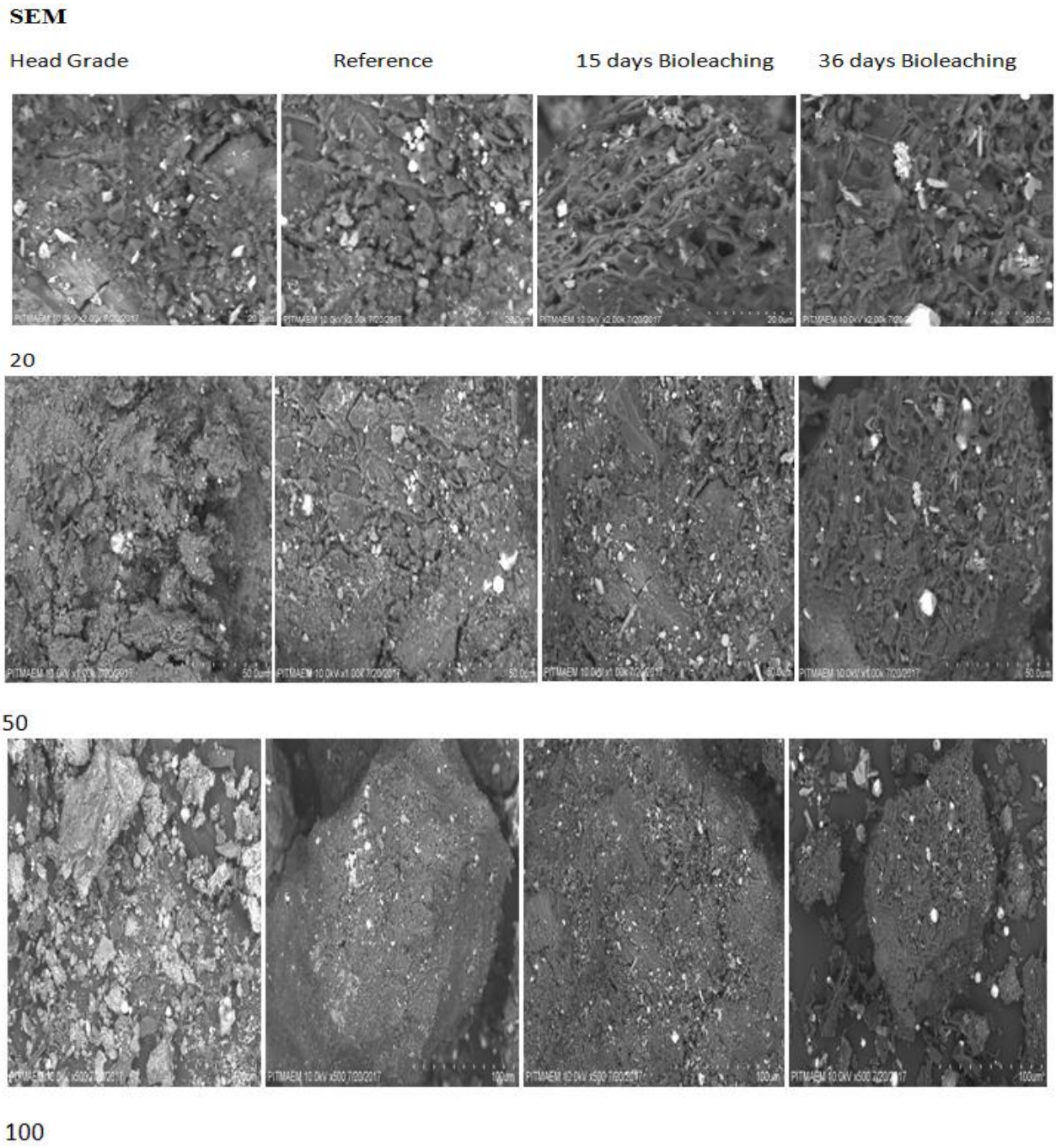


Figure-5. Image of coal on (20,50, 100, magnification) indicating differences due to bacterial action using SE.M.

3.6. Conformation of Results Using SEM

SEM images were obtained using, scanning electron microscopy (3700 N, Hitachi, Japan) for head grade and leached samples before and after bioleaching experiments. These images were digitalized under 2000, 1000 and 500 pixel; voltage 10.0 kV, Scale 20 um, 50 um and 100 um with magnification, 500 X, 1000 X and 2000 X.

As per investigation pyrite is the dominant sulphide in this low rank coal. It typically occurs as an antigenic component in calcite within fascinates as lumen fill in inordinate; as pyritic frame holds; as a cell –infilling or as replacement of the mineral components; and as cubic crystals. The SEM image of the virgin coal shows a bulk microstructure composed of homogenously distributed network of small crystallites showed the presence of minerals. These features indicate about the presence of minerals which distributed in the organic matrix.

Scanning electron Microscopy (SEM) has been extensively used to illustrate the attachment of bacterial species to mineral surfaces [12-14]. SEM is valuable technique to understand the oxidation mechanism during bioleaching

process. The SEM images indicate the pyrite bands in coal sample before and after treatment. The pyrite surface is smooth prior to leaching in head grade (origin) sample as shown in Figure 5. The pyrite surface become rough with holes of 1- 2 mm in length after fifteen days bioleaching as the process proceeds and bacterial cells were added during oxidation while such holes were not found in head grade sample, generally the holes became larger and larger with increasing leaching time however during this investigation the accurate surface area shows slight difference after 36 days bioleaching process probably due to coal ranking and pyrite content in virgin coal. The SEM images clearly showed that the species used for bioleaching actively reduce the pyrite sulfur as the data shown in Table 1 & 2.

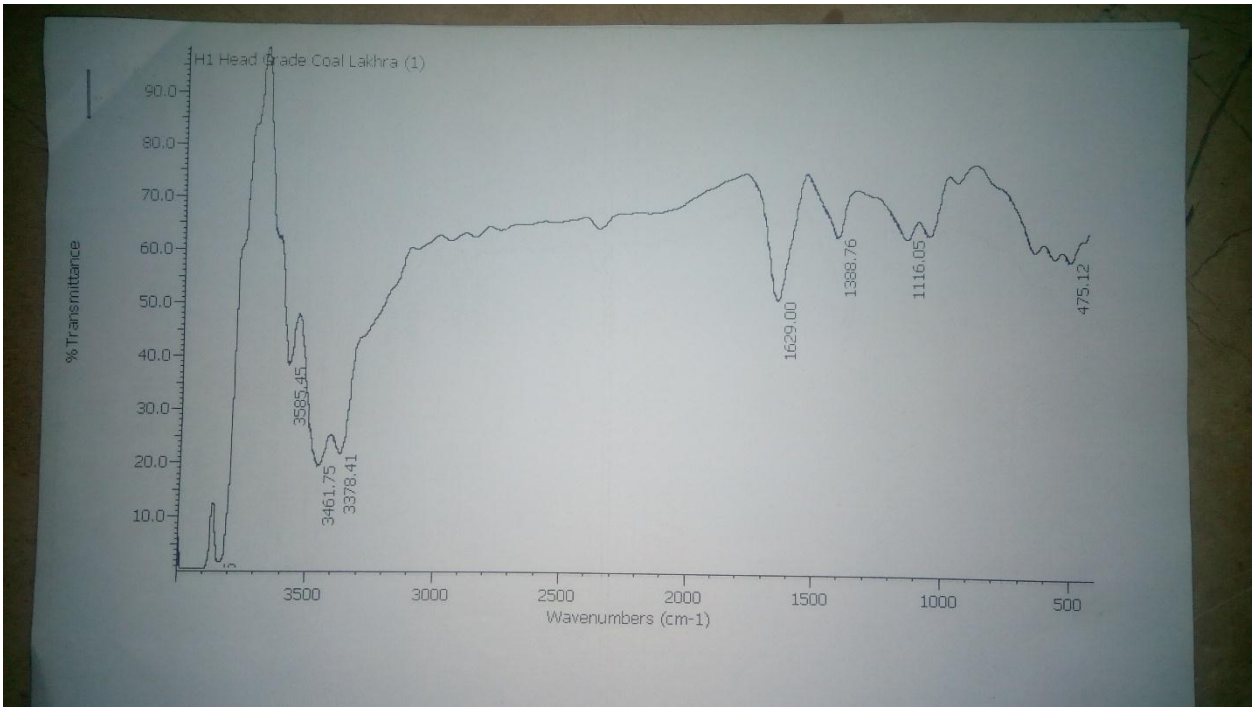
The major form of pyrite in coal samples were massive and vein lets as shown in Figure 5 a. After 15 days of bio processing, the massive pyrite in the coal samples decreases intently as shown in Figure 5c-d. However, the coal of sterile (reference) control almost remains unchanged as shown in Figure 5 b. The bacterium species have strong ability to remove massive pyritic in comparison with microscopic form of sulfur (Organic) as reported in literature [15] which seems true in this study as similar phenomena observed. The visible corrosion of pyrite observed in form of 1-2 mm length holes shows the adsorption of bacterial cells in mineral surfaces.

3.7. FTIR Conformation

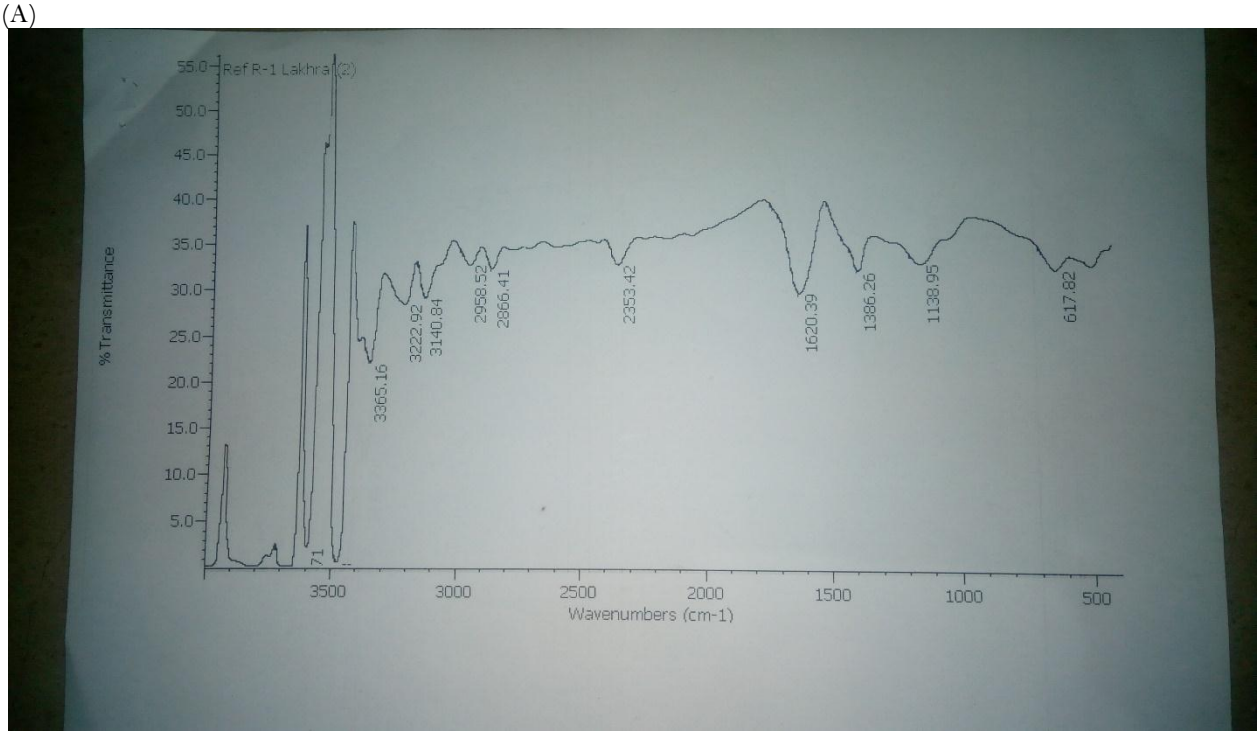
The (Spectra 1) sharp bands observed in region at 475-490 Cm-, resulting from clay and silicate minerals, at 3618 Cm^{-1} attributed to Kaelinte and illite minerals [16] Figure 6. As expected the above bands will be prominent in FTIR Spectra due to high ash content (44. 48) as shown in Table 1. In the aliphatic starching region (30.00-2800), there are distinct peaks observed at 2910 Cm^{-1} and 2851 cm^{-1} symmetric and asymmetric $-CH_2$ stretching respectively. The absorption intensities O=S=O and S=O bands in sulfur containing compounds are prominent as observed at 1040 cm^{-1}, which is gradually decreased in intensity in treated coal samples (T 01 and T 02) due to bioleaching proceeds as coincide with results observed in elemental analysis shown in Table 2. Furthermore, the distribution of sulfur as shown in Table 3, consistent with FTIR data as the total sulfur peak 05 decreased with passage of time due to bio-demineralization. The C-O group in region of 1116-1118 Cm^{-1} is distinct in spectra 1. While it is disappeared in treated samples due to cleavage of oxygen containing bands as observed in reported in elemental analysis e.g. 54. 43- 34.08 – 25.22. The absorption peak at 1130 – 1133 Cm^{-1} in the FTIR Spectroscopy of treated coal samples were too weak to identify likely due of their negligible level in low rank coal (lignite) and swamping by various oxygen containing group and the peaks attributed to the in plane bending vibration of C-H in treated coal sample (T 01, T 02) and coincide with reference sample (R 01).

The peak at 1384-1389 Cm^{-1} attributed to starching mode of methyl group in all spectra as methyl linkages are common in low-rank coal framework which is slightly decreased in treated samples due to up gradation (biological Cleaning) of coal samples (T 01, T 02) as compared to raw coal (H 01) consistent with elemental analysis as shown in Table 2 and proximate analysis reported in Table 1. The weak absorption band at 475 Cm^{-1} as observed in spectra corresponds with the presence of FeS_2 in coal sample, consistent with the result reported in Table 3 for distribution and sulfur types (PS, OS, SO_4^{-2}) during bioleaching. The intensity of peak decreased in treated coal samples following biological desulphurization, although the bond strength of organic part of sulfur as C-S, S-S and S-H bonds were too weak to accurately identify. The dance peak at 1629 Cm^{-1} raw coal correspond to oxygen containing functionalities derive from the stretching vibration of the carboxyl group is prominent in composition with treated coal sample due to decrease in oxygen content during treatment. The peaks in the range 3600-3300 Cm^{-1} assigned to the hydroxyl group (-OH) hydrogen bonds and mineral matter in coal which is very prominent in raw coal and reference sample (R 01) in comparison with decrease in mineral matter as reported in Table 1.

The FTIR results were confirmed by elemental analysis and sulfur distribution in raw and treated coal samples [17, 18].



(A)



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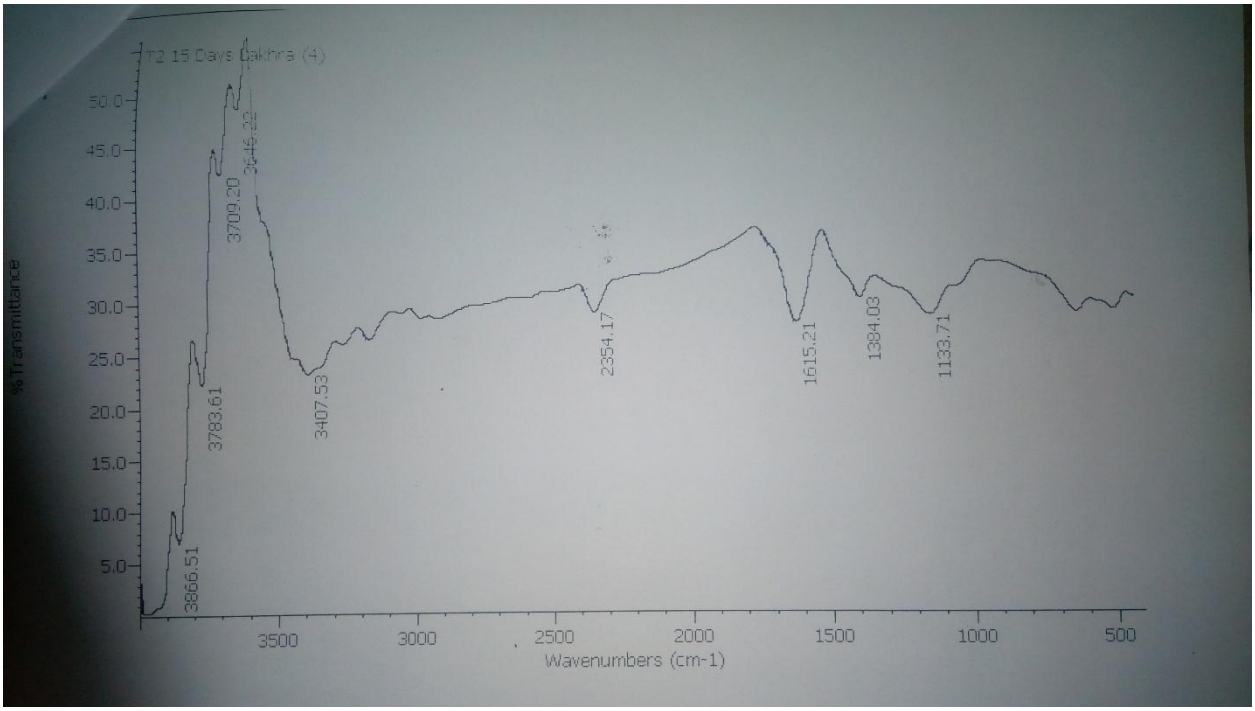

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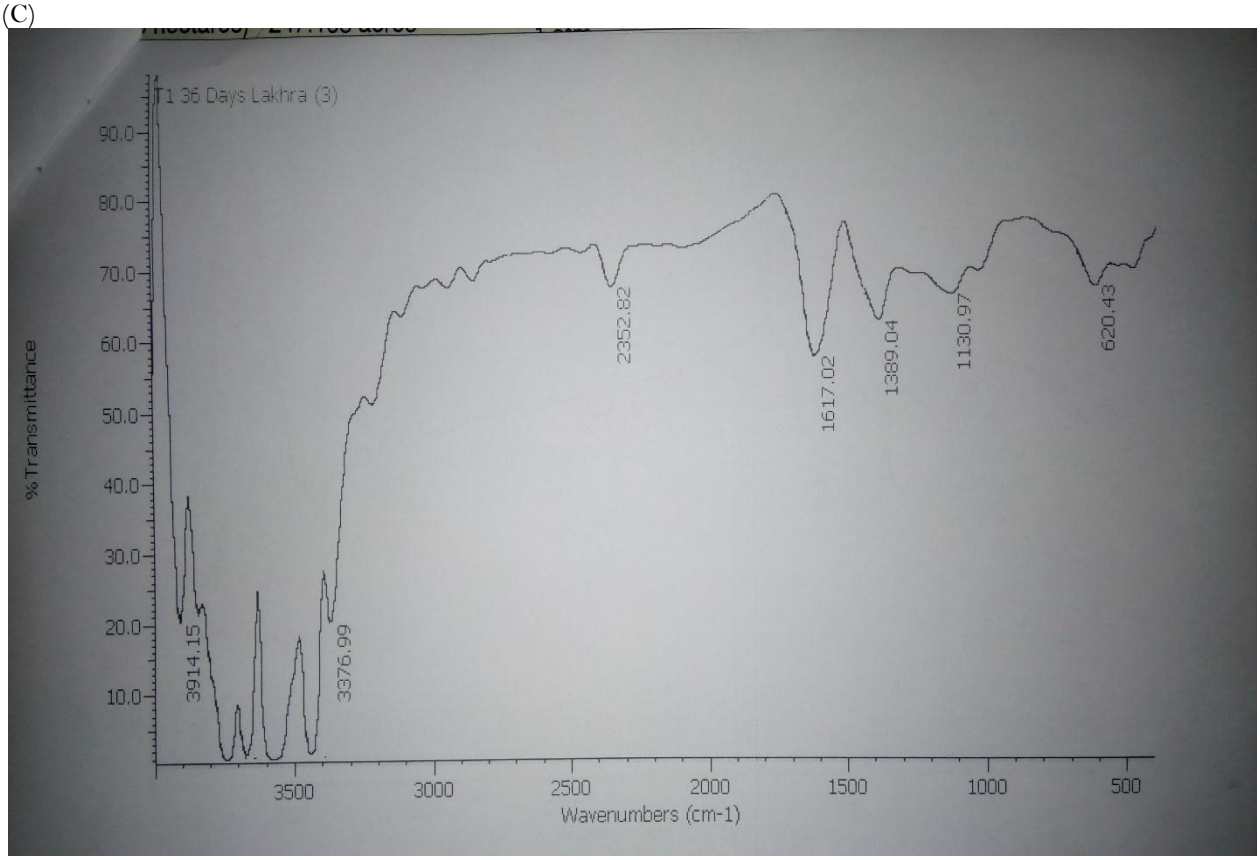

(d)

Figure-6. FTIR Image of (a) Head Grade (T01), (b) Reference (R01), (c) 15 days bioleached sample (H02) and (d) 36 days bioleached sample.

4. CONCLUSIONS

The author took the advantage of bioleaching technology to reduce sulfur, ash and minerals contaminations present in the coal body of Low grade coal of Lakhra and successfully upgraded up to usable quality. Bioleached coal samples showed environmental suitability, low ash production during combustion and no coagulation of particles in kiln. The indigenously isolated bacterial strain was showed good activity for desulphurization process. The results

were also conformed using SEM and FTIR. The optimization of bioleaching parameters were also studied, reduction in processing time period is also possible by further adoptability of microorganisms along with increase in particle surface area. Application of process on heap and pilot plant scale is still required for low grade indigenous coals of Pakistan.

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Competing Interests: The authors declare that they have no competing interests.
Acknowledgement: All authors contributed equally to the conception and design of the study.

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