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Microbial Extraction of Antimony from Stibnite of Qillah Abdullah

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Abstract

The study involve bioleaching of antimony ore collected from Qillah Abdullah Balochistan (Pakistan) by using *Thiobacillus ferrooxidans* isolated from coal mine drainage of district Harnai. Analysis of ore revealed that it has 19.54% antimony along with 42.24% SiO_2 as gangue material. Trails for bioleaching were conducted in shake flasks having 10% (w/v) ore and growth medium. Isolated microorganisms were found more efficient for liquification of antimony contents at grain size -150#. Liquefied antimony was recovered as antimony sulphide by precipitation with H_2S. Parameters affecting the process of bioleaching such as aeration, time period, temperature, grain size of ore and pH were also optimized. Overall recovery of product was estimated as 73% in 120 days.

Keywords: Bioleaching; Antimony; *Thiobacillus ferrooxidans*; Harnai; Qillah Abdullah.

1. Introduction

Bioleaching is simple and economically feasible process for extraction of metals from mineral ores by using bacterial and fungal strains. Bacterial strains such as *T. ferrooxidans* and *T. thiooxidans* have been used for leaching of valuable mineral contents from ores [1]. These microorganisms are gram-negative, rod shaped, having flagellum autotrophic bacterium which grows speedily in inorganic mining environment [2].

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The prior investigation shows that these microorganisms attack directly as well as indirectly on metallic contents of ore with producing iron oxidase enzyme along with sulphuric acid at low pH, which permits to metabolize metal ions, such as ferrous iron and sulfur [3]. Therefore, they achieve energy for its growth from oxidation of ferrous iron or reduction of inorganic sulfur compounds present in medium and sulphide ores [4,5].

These microorganisms have ability to convert insoluble sulfides of metals in to their soluble metal sulfates in presence of moisture, air and nutrients such as copper, lead, zinc, antimony and nickel. It is previously reported that copper, uranium and gold have been successfully leached from their low grade ores [6]. As per literature review a limited work was found on bioleaching of stibnite. Initially efforts were being made by Troma and Gebra for leaching of antimony from stibnite [7].

In present investigation antimony ore of Qillah Abdullah has been successfully leached by laboratory purified bacterial strain of *T. ferrooxidans*, isolated from coal mine drainage of Harnai Balochistan in 120 days of incubation period under optimized conditions. The overall recovery was estimated as 73%. In Pakistan ore of antimony occurs in Kharan district, salt range of Krinj in Chitral division, Kurram agency, Khuzdar, and Qilla Abdullah, District Pashin [8]. Occurrence of antimony ore in Qilla Abdulla and Qillah Saif-ul-Allah is reported in combination of quartz as oxidized slate [9].

2. Method & Material

2.1 Antimony Ore

The studies have been performed on representative sample collected from depth of 10 to 20 meters stibnite of Qillah Abdullah Balochistan. Primary and secondary crushing up to -150# grain size was accomplished on lab scale jaw crusher, roll mill followed by its sieving through -150 mesh. The chemical analyses were carried out on AAS, Hitachi Z-8000.

2.2 Microorganisms

The mine water samples were collected taking necessary precautionary measures in sterilized duly packed/sealed bottles for shifting to laboratory from various coal mines area of district Harnai- Balochistan. Agar spread plates method was used for growth culture of bacterial strains, plates were prepared by addition of nutrient ager (1.25%), $FeSO_4$ (0.5%), $CaCl_2$ (0.025%), $MgSO_4.7H_2O$ (0.05%), $Na_2S_2O_3$ (0.5%) in distilled water and autoclaved at 120ºC for 15 minutes. The collected source sample was spread for producing colonies.

2.3 Culture Medium Preparation

The modified (9K) medium was prepared as described in previously reported literature [10]. Basal solution was prepared separately by desolation of 74.67g $FeSO_{4.}\ 7H_2O$ in 580 mL of double distilled autoclaved water. Another medium solution was prepared by dissolving salts KCl, 0.116g; $Ca(NO_3)_2$, 0.00168 g; K_2HPO_4, 0.058 g; $MgSO_{4.}7H_2O$, 0.058 g; and $(NH_4)_2SO_4$ 4.0; g/litter in 420 mL distilled and autoclaved water. Both solutions were mixed together and used as medium for the growth culture of the bacterial strain. pH of medium was adjusted at 2.8 using 0.1M H_2SO_4. The chemicals and reagents were used are of analytical grade and used as such without further purification.

2.4 Isolation of strain

The growth and isolation of the bacterial strain was carried out by screening of colonies having radish brown color on plates due to change in ferrous to ferric state of iron. The colonies were collected and introduced in 100 mL of broth media. The flasks were incubated at 25°C with continuous shaking at 100rpm. Maximum growth was attained after 12 days of incubation.

2.5 Bioleaching activity

Bioleaching trails were performed in 500mL Erlenmeyer flasks containing 150mL of culture medium (9K) caped with cotton plugs along with 10 g of (-150#) stibnite. 10 mL inoculums having lab isolated bacterial strain *T. ferrooxidans* were added in flasks, conditions were adjusted at aerobic and dynamic using rotary shaking bath at (37±1)°C and pH of liquid medium was adjusted at 2.8 by addition of 1N sulphuric acid. The leaching process was allowed for 150 days, aliquots were taken after time intervals and gangue was removed through centrifugation, in supernatant solution antimony contents were estimated by AAS.

2.6 Trail batch for antimony bioleaching.

Roll milled crushed sample of ore (10g) was introduced in Erlenmeyer flask having 100mL of *T. ferrooxidans* containing broth medium. The pH was adjusted at 2.8 and flasks were capped with cotton plug. The blank was also prepared in same manner without bacterial strain. The flasks were incubated at 25°C, with continuously shaking at 80 rpm. The leachate was estimated for antimony and 53% of liquefied metal found in solution.

3. Results and Discussions

3.1 Analysis of ore material

Chemical analysis of ore revealed that it has antimony (19.54%), SiO_2 (42.24%) Fe (13.9%), S (15.1%), Pb (2.23%), and Zn (0.25%). Fig. 1 shows disc graph of ore composition.

3.2 Growth of microorganisms

As described in methodology by addition of ferrous iron in semisolid medium is the cause of *T. ferrooxidans* presence which was indicated by its radish brown color of colony as shown in Fig 2. Slides were prepared and examined under optical microscope (Fig 3); these microorganisms were found similar in shape and structure as reported previously in literature [11].

These colonies were removed and added in broth culture medium. Isolated strain showed rapid growth and color of medium was changed from aqueous light green to radish brown in twelve days of incubation at 37± 1°C, (Fig.4) illustrated the presence and activity of *T. ferrooxidans*.

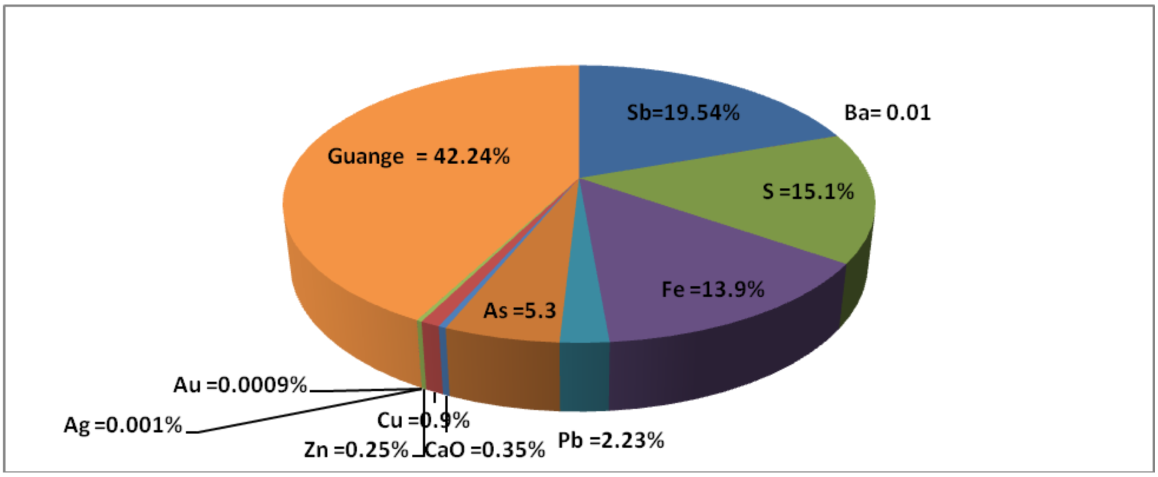

Figure 1: Chemical **a**nalysis of representative sample of stibnite

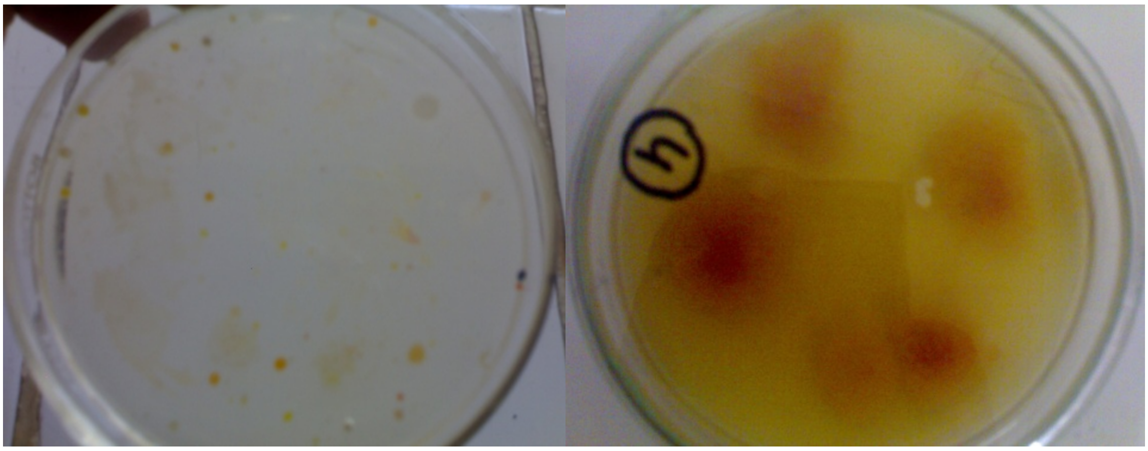

Figure 2: Colonies of bacterial strain on semisolid medium

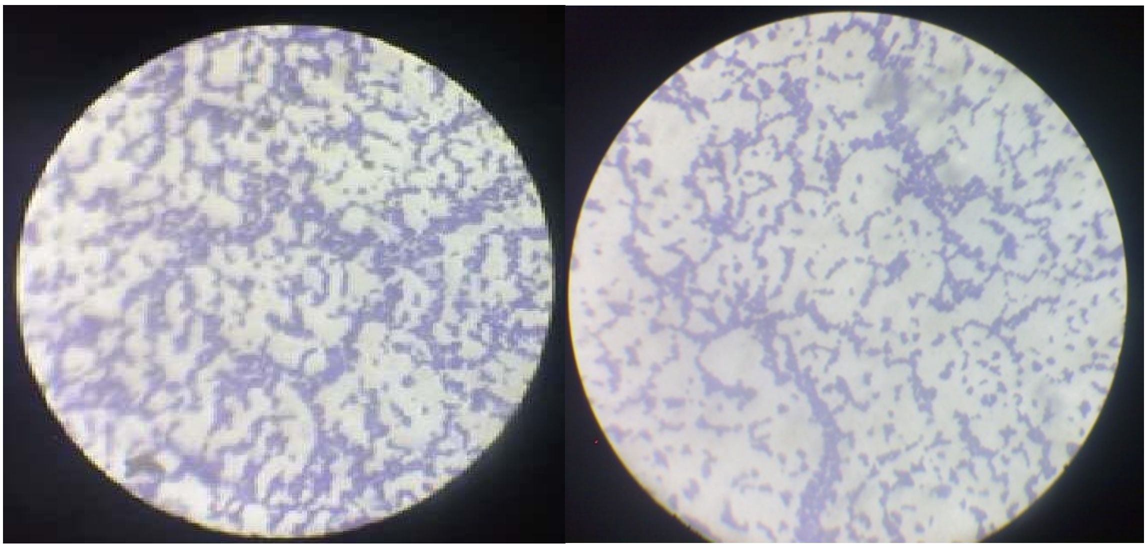

Figure 3: Microscopic view of isolated *Thiobacillus ferrooxidans*

Figure 4: Initial to final stage of broth culture of bacterial strain

3.3 Application of strain on stibnite for adaptation

Antimony ore was crushed and ground in roll crusher, one liter of medium containing bacterial strain was directly applied on 100 grams of crushed sample of stibnite, pH was adjusted at 2.8. In initial 7 days pH was began to increased from 2.8 to 3.4 and no metal was detected to liberate in liquid form in liquid medium. After 12th days a slight decrease has been seen in pH of reaction mixture, this is same as described by Hector [12]. The extraction of metals from stibnite also confirmed by increase in libration of radish brown color with passage of time as illustrated in Figure 5.

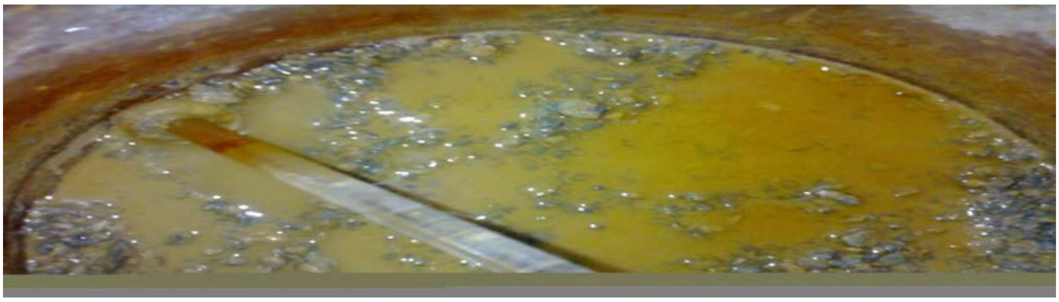

Figure 5: Direct application of microbial strain on roll crushed ore of antimony

After a time period of 120 days leached antimony contents were separated by filtration and precipitated as antimony sulphide. The recovery of antimony was estimated as 53%, after this period no further removal of antimony was detected. The filtrate after removing antimony was again added in (9K) medium and used for further experiments.

4. Optimization of parameters

The factors affecting the rate of leaching, product recovery and purity of product such as grain size/particle size, temperature, supply of air, shaking speed and pH of the system were optimized to get maximum recovery.

4.1 Effect of pH

The effect of pH on the antimony bioleaching was investigated in acidic medium by gradually increasing it from 1-7. 10 grams of ore sample having -150# size was added in each flask, having 100 ml of growth medium along with 10 % (v/v) bacterial strain. The adjustment of pH was carried out by (0.1M) sulphuric acid from 1.0 to 7.0 at temperature of 37°C. After 70 days the reaction mixtures were removed, filtered and antimony contents were estimated by described method. The investigation showed that pH 3-1 is the best for maximum liquification of antimony as mentioned in Figure 6.

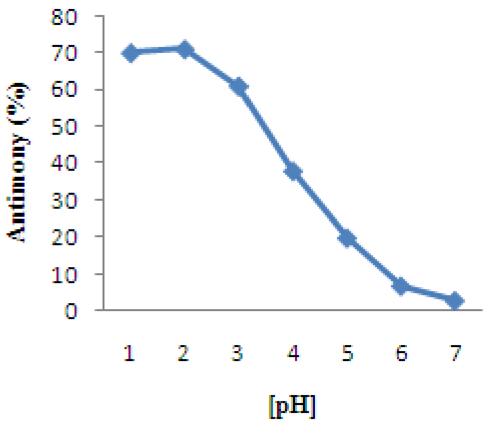

Figure 6: Effect of pH on bioleaching of stibnite

4.2 Effect of time period

Optimum time period for maximum bioleaching process was estimated by incubation shake flasks at varying time period intervals. All other parameters such as temperature, pH, and shaking were kept constant. The reaction was stopped by addition of 1ml of chloroform in the flask when it was removed from system. The optimum leaching time was estimated after 120 days for the maximum recovery of metal in the form of soluble and insoluble sulfates, results are shown in Figure 7. The decrease in leaching after 70 days shows conformation of production of insoluble antimony sulpahte as reported earlier [13].

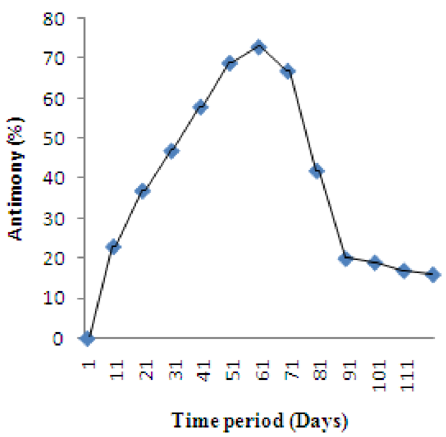

Figure 7: Effect of time period on bioleaching of stibnite

4.3 Effect of temperature

The effect of temperature on bioleaching process was estimated by incubation of shake asks on 30 to 70°C. The other parameters such as grain size, pH, shaking and incubation time period were kept constant. During optimization temperature is detected on pre heated incubator, i.e. when temperatures reached on its optional temperature then flasks were introduced for 70 days as mentioned in Figure 8.

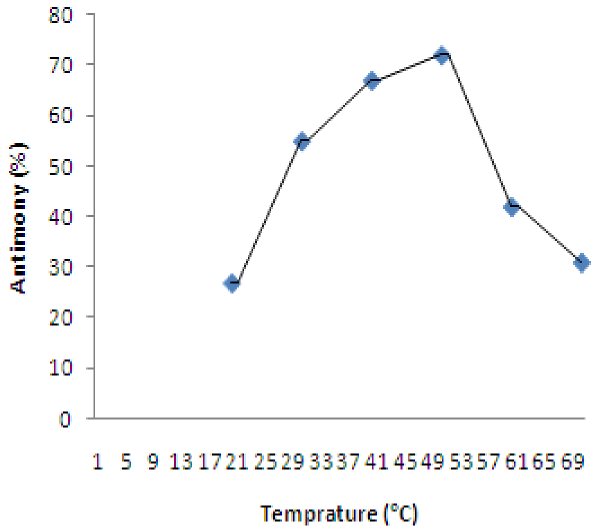

Figure 8: Effect of temperature on bioleaching of stibnite

4.4 Effect of grain size

Grain size has great importance due to increase in surface area of particles, suitable grain size were estimated at variable particle size, #4 to #250. 10 grams of fractions were introduced in different Erlenmeyer shake flasks having bacterial medium 100 ml. All other parameters that affect the recovery and grad of metal were kept constant. The maximum recovery was obtained when ore was crushed 100% passing from #150. The graphical representations of results are shown in Figure 9.

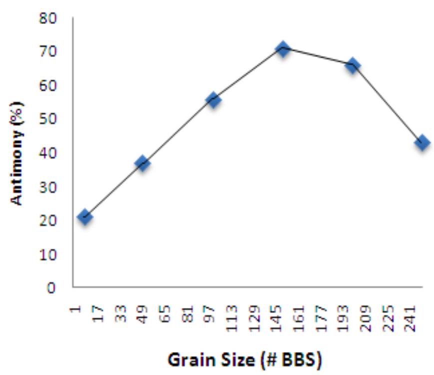

Figure 9: Effect of particle grain size for bioleaching of stibnite

4.5 Shaking speed effect

The shake flasks having the 10 grams of antimony ore and 100 ml of medium containing bacterial strains were shaken with different speed and effects were noted. Each flask was separately shacked in the range of 20 rpm to 200 rpm. It was observed that the shaking speed may also affect the rate of bioleaching. The results are mentioned in figure 10. The other parameters of affecting the rate of reaction were kept constant.

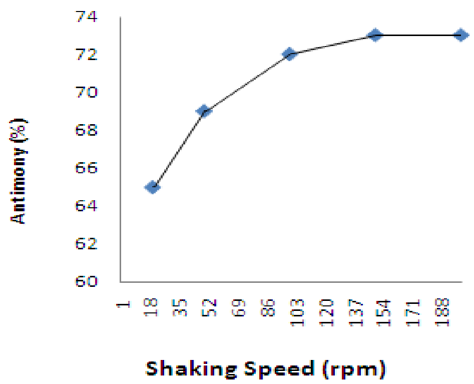

Figure 10: Effect of shaking speed for bioleaching of stibnite

5. Bioleaching of stibnite, at optimal conditions

A weighed quantity of ground antimony ore (100g), having grain size (-150#) was taken in 1liter of *T. ferrooxidans* containing medium shake flask. The pH was adjusted by sulphuric acid at 2.0 at temperature of 37°C with shaking speed of 100 rpm. The process was continued for 120 days. Reference was used having no bacterial strain. The leaching process of antimony was observed after 48 hours of intervals. About half of the solution was removed after each 30 days and again fresh solution of bacterial strain was introduced up to the mark. The results are mentioned in table 1.

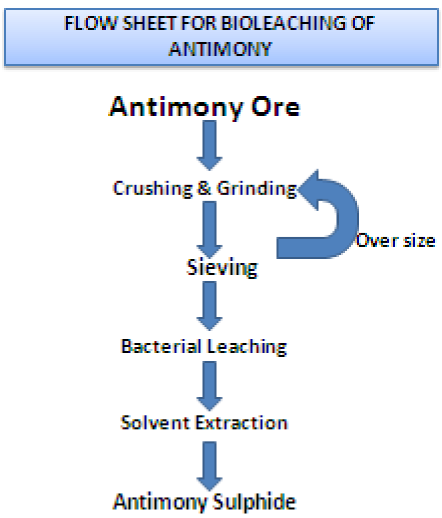

Figure 11: Flow diagram for processing for bioleaching of antimony

Table 1: Recovery of antimony on optimal conditions

Weight of the ore	**Volume of Bacterial Strain**	**Grain size**	**Shaking speed**	**Temperature (°C)**	**Time period (Days)**	**Recovery of metal**
100 grams	1000ml	-150#	100 rpm	37°C	120 days	73%

Figure 12: Final product antimony sulphide

The studies conformed that leaching of antimony is possible by using bacterial strain *T. ferrooxidans* as reported earlier [14]. The strains were obtained from local coal mining area of Balochistan. These microorganisms were simply isolated and cultured in laboratory by applying on semisolid nutrient medium. During bioleaching process it is revealed that leaching of stibnite began to be starts after 48 hours of incubation at suitable temperature, aeration and medium. The process remained continue until production of insoluble sulpahtes began to be produced in liquid medium, as the concentration of insoluble sulpahtes goes up, the percentage of antimony in liquid medium goes down in same manner as reported previously in literature [13]. The regular removal of liquefied antimony along with associated minerals and addition of (medium having strain) results in continuation of leaching of metal till its maximum recovery. The investigation also showed that bioleaching of antimony is dependent of composition and nature of ore. In the present studies ore sample was successfully leached using isolated microorganisms and antimony was separated as antimony sulphide. The other techniques available for the extraction of antimony is pyrometallurgical and alkaline leaching are responsible to create environmental pollution, however the present technique have advantage of no environmental pollution and economically feasible. This root of ore processing is very simple and helpful for extraction of continues and streamline supply of liquefy antimony which may be used for preparation of antimonial compounds for therapeutic medicines.

6. Conclusion

During investigation it is confirmed that bacillus family of microbes have ability to leach minerals present in sulphide type of ores. These strains have great effect for leaching of Iron and Sulphur but also effective for liquification of valuable associated minerals such as antimony. These long period trails were conducted on the deposit of ore samples of Qillah Abdullah Balochistan. The technique used here, has some advantages due to its low consumption of fuel and no environmental pollutions and makes it viable for extraction of antimony from stibnite. The investigation covers optimal conditions for best recovery and grade of product (Sb_2S_3), with weight recovery of 73%. Experimental studies further conformed that continues removal of liquefied antimony is required, during process otherwise antimony produces insoluble sulphate that decrease the concentration of liquefied antimony as well as bioleaching process. It is further concluded that low grade stibnite may directly be leached if there is no or little amount of lead present because it presents difficulties in separation process. Further work is still required for reducing time period of bioleaching process and purity of product.

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