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Bioleaching of Copper Concentrate and Pyrite by Using Native Bacterium Acidithiobacillus Ferrooxidans IRL.8F and Evaluating the LPS Role in Bioleaching Process

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Abstract - This study was performed to evaluate the ability of native bacterium to extract copper and iron from their ores. This bacterium was isolated from iron mineral springs in Iran's Larzan region and was named *Acidithiobacillus ferrooxidans IRL.8F* based on morphological and physiological characteristics and 16S rRNA molecular analyses. The results from bioleaching of copper concentrate showed that the amount of extracted copper and iron was 71.4% and 29.3%, respectively. Furthermore, in comparison with control samples, these amounts increased by 93.5% and 92%, respectively. In the control samples minor amount of metals were extracted due to spontaneous leaching. To assess the importance of bacterial lipopolysaccharydes(LPS) role, LPS of bacterium was removed. When ethylenediaminetetraacetic acid (EDTA) in concentrations of 5 and 10% was used during the bioleaching process of pyrite, process efficiency decreased to 61 and 70%, respectively. The cells lacking LPS were led to 59.4 % decrease in the amount of bacterial leaching, in contrast to whole cells. Therefore, it can be concluded that: 1. EDTA causes a drastic reduction in the efficiency of leaching process, 2. Bacterial LPS have a key role in attachment to particles of ore and 3.This bacterium is capable of leaching metals through the direct mechanism.

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I. Introduction

Bioleaching is a general term used to refer to the conversion of insoluble to soluble metals (usually in sulfated form) through biological oxidation by using microorganisms (Rawlings., 2002; Makita et al., 2004).

Bacteria of the genus Thiobacillus, like Thiobacillus ferrooxidans retrieve the energy from ores via enzymatic oxidation. Biological oxidation of sulfide ores and the electron transport occur in three forms, including direct (or enzymatic or contact), indirect (mediated by compounds such as Fe^{3+} ions) and cooperative (which includes both direct and indirect) mechanisms. In the indirect mechanism, Fe^{3+} iron plays major role, while in the direct mechanism, the bacterium should have access to the ore, bind it and then the reaction will occur at the ore-water interface (Donati and Sand, 2007; Wolfgang and Edgardo, 2007). In this mechanism, the microbial attachment to the ore surface is necessary for the bioleaching process. As the micro-organism approaches.

The minerall, the cell surface changes and this accompanies with expression of extracellular polymeric substances (EPS) which lead to the attachment (Scobar et al., 1997; Clausen, 2003). EPS forms chemical bonds with the surface and mediates or promotes respiration and nutritional chemical reactions (Scobar et al., 1997). These bonds are made stronger with the attachment of microorganism to the ore and the reactions are followed by oxidation of reduced mineral compounds or reduced Fe^{2+} or sulfate ions. Attaching to the ores may be mediated by forming EPS on the surface of solid particles such as lipopolysaccharide (LPS), phospholipids or other macromolecules like the polypeptides in the outer membrane of the bacterium. These compounds are released by the organism when it is in contact with the ores (Donati and Sand., 2007; Scobar et al., 1997). The Mechanism of the electron transport from pyrite to molecular oxygen has been identified in detail. The primary stages occur in the EPS, in which electrons are extracted by means of the Fe^{3+} ion in complex with glucoronic acid (Rangin and Basu., 2004). Attachment to hydrophobic substrates such as sulfur is mediated by van der Waals forces, while for binding to charged substrates like pyrite, cations or molecules which act as Lewis acids accept the un-charged electron pair of the pyrite sulfur, followed by formation of a complex between different iron species and the exopolysaccharide and finally the attachment of the bacterium to the substrate (Gehrke et al,. 1998)

II. Materials and Methods

a) Media

Types and compositions of the media used for culturing, isolating and screening included: (1)SF or T.F.

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medium containing: Solution A: K_2HPO_4 (0.5g/l), $(NH_4)_2SO_4$ (0. 5g/l), $MgSO_4$ (0.5g/l), H_2SO_4 0.5M (5 ml/l) and D.W (1000ml) ; Solution B: $FeSO_4 . 7H_2O$ (167g/ l), H_2SO_4 . 0.5M (50 ml) and D.W (1000ml).One unit volume of solution B is mixed with four unit volumes of solution A and the pH is adjusted on 2-2.5 by using H_2SO_4 . 0.5M. (2)TSB medium containing: KH_2PO_4 (3g/l), $(NH_4)_2SO_4$. $7H_2O$ (0.4 g/l), $MgSO_4$. $7H_2O$ (0.5 g/l), $CaCl_2$. $2H_2O$ (0.25 g/l), $FeSO_4 . 7H_2O$ (0.01g/l), $Na_2S_2O_3$. $5H_2O$ (5 g/l), Agar powder (16g/l) and D.W (1000 ml).(3)TTB medium which contains (g/l) : ($(NH_4)_2SO_4$ 0.3; K_2HPO_4 , 0.5; $MgSO_4.7H_2O$, 0.5; and 0.5M H_2SO_4 .After autoclaving, sterilizing and cooling the medium, 5% sulfur powder separately sterilized in an aluminum foil, was added(Chen and Lin, 2000; Sasaki et al., 2009). During the preparation of these media, the iron sulfate was sterilized with a (0.22μ) filter and added to the solution. The cells were collected from 10-day media centrifuged in 50 ml falcon tubes at 15000 rpm for 20 min. (Elzeky an

III. The Bacterium

The bacterium used in this study was isolated from mineral springs in Larzan, Qazvin province, Iran. With this purpose, the mixed samples of water and precipitations deposited at the bottom of the spring were collected, transferred to the laboratory and incubated into 250 ml Erlenmeyer flasks containing 50 ml of broth TF and TT media (respectively containing elemental iron and sulfur as the sole sources of electron and energy). The samples were placed in shaker incubator at 30°C and in 200 rpm for 7 days, and then recultured in fresh media. The oxidation power of Fe and S elements were evaluated. During the cell culture period, essential parameters including the daily measure of pH, titration of produced acid, solution rate of elemental sulfur in medium, the amount of oxidized Fe, macroscopic and microscopic study of samples and counting and calculating the cell concentration were also considered or evaluated.

IV. Oxidation of Fe^{2+} to Fe^{3+}

The oxidation of Fe^{2+} by the bacterium was investigated in a 250 ml Erlenmeyer flask containing 50 ml SFB medium. With this purpose, 5 ml of 14-day bacterium culture (comprising $\sim 9\times10^8$ cells) was inoculated into the medium and incubated at 30°C at 200 rpm. The control was without bacterium inoculation. The initial pH was adjusted to 2.5 using 0.5M sulfuric acid. As Fe^{2+} is oxidized to Fe^{3+} iron, the medium turns from lime green to yellow, brown and brick red. Orth-ophenanthroline method and atomic adsorption spectroscopy analysis systems were used to analyze the iron. The total iron content (Fe^{2+} + Fe^{3+}), the converted Fe^{2+} iron to Fe^{3+} and the Fe^{3+} content of the medium were measured.

V. Sulfur Oxidation and Sulfuric Acid Production

The medium in this study was TTB. The inoculation and growth conditions were similar to those of iron oxidation. The initial pH was adjusted to 4.5. Sulfur oxidation, pH reduction and sulfuric acid production were measured. The control was prepared in a similar way without bacterium inoculation. The sulfuric acid content of the medium was measured after the drastic decrease of pH by titration using 0.1 M NaOH.

VI. Bacteria Identification By 16s Rrna

To identify the bacteria, the sequencing of 16S rRNA gene fragments was applied. Considering that these bacteria have a slow growth rate and are extremophilic species, the alkaline lysis and lysosyme methods were used in hybrid to extract their genomes. At first, the bacterial genomes were purified. With this purpose, 500 ml of 7-day culture of bacteria was prepared in TTB medium. The sample was centrifuged at 15000 rpm and the bacterial biomass was obtained. One hundred microliter of the SET cold buffer was added to the bacteria and 100μl of lysosyme was added to the above mixture and it was vortexed thoroughly. The mixture was incubated at 37°C for 30 min, and then, 200μl of lysis buffer (NaOH (5M), SDS (10%), H_2O) was added to the mixture and placed in ice for 10 min. In the next stage, as much phenol as the volume of the solution in the tube was added. The mixture was blended thoroughly and centrifuged at 10000 rpm and 4°C for 3 min. Furthermore, the supernatant was transferred to another tube and as much as its volume, phenol-chloroform (1:1) was added. The mixture was centrifuged again in a similar way as mentioned above. The supernatant was transferred to another tube, chloroform was added as much as its volume and the sample was again centrifuged as above. The supernatant was removed and isopropanol stored at -20°C was added as much as 0.6 of volume of the supernatant. This solution was stored at -20°C for 1 h. In the next stage, the sample was centrifuged at 14000 rpm in 4°C for 15 min. Isopropanol was immediately removed and 1 ml of 70% alcohol was added and then the sample was centrifuged at 14000 rpm at 4°C for 10 min. The sample was drought at room temperature and 20 to 30μl of TE buffer or distilled water and 3 to 5μl of RNase A was added. The tube containing the sample was stored at 37°C for 1 h and the sample was then stored at 4°C(Ohba and Owa., 2005). After electrophoresis, the PCR was performed. The primers required to identify the bacteria were universal primers with the following sequences (Hong et al.,2006 ; Yeats et al 1998): Forward: FORB: 5ó AGAGTTTGATCCTGGCTCAG3ó

reverse : REVB: 5ó GGTTACCTTGTTACGACT3ó. Using the purified genome as the template and the *Taq* polymerase, the 16S rRNA gene fragment was amplified as the defined program and the final product was investigated on agarose 1% gel. After confirming the quality of the PCR product, the samples were sequenced using Genetic analyzer 31030 - Accessories Applied Biosystem.

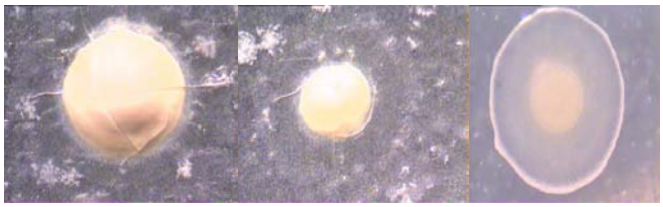

Figure 1 : A. ferrooxidans IRL.8F micro-colony and production of exopolysaccharide (white corona) on TSA medium (magnified ×100 – by A.M. Latifi). From right to left: fresh colony, semi- dried colony, dried colony.

The ore

The pyrite and copper ore were used in this study. The ore was powdered using the crusher or mortar-and-pestle, and the samples were prepared using special sieves with appropriate gridding. The elemental and compositional analysis of the ore powder

VII. Bioleaching Media and Conditions

To measure the metal produced from ore, 10 g of ore powder in flasks containing 250ml medium without any of energy resources (iron, sulfur, etc) was used. The base medium for all samples was 100ml water. The gridding of the particles was 200 and their

VIII. Study of The Effect of EDTA on Bioleaching Process

To study the effect of EDTA on bioleaching process, four samples were prepared as followed: Sample 1: leaching medium without bacterium inoculation (as control), Sample 2: leaching medium inoculated with bacterium without EDTA, Sample 3: leaching medium inoculated with bacterium and EDTA (5%) and Sample 4: leaching medium inoculated with bacterium and EDTA (10%) test samples, the 0.2M EDTA solution was used.

All samples were placed in a shaker-incubator at 28°C and 200 rpm. Bacterium compatibility with new media was investigated by measuring pH of media during the bioleaching process. The initial pH at the start time of the process was also recorded. During its growth period, the bacterium reduces the pH and produces sulfuric acid through oxidation of the sulfur ore.

IX. LPS Removal In *Acidithiobacillus Ferrooxidans* IRL.8F

To remove the bacterial LPS, 0.2M EDTA and Tris-HCl at pH4.5 were used. (Ramadas et al., 1991; Scobar et al.,1997). Bacterial biomass was collected from 50 ml of bacterial suspension comprising a 10-day culture. The biomass was converted to a homogenous suspension in the EDTA and Tris-HCl solution and incubated at 37°C for 1 h. The tube containing the sample was then centrifuged at 11000 rpm and the supernatant containing EDTA, Tris-HCl and lipopolysaccharide (LPS) was removed. Bacterial cells lacking LPS were extracted from the solution containing EDTA and LPS by centrifugation at 11000 rpm for 10 min, and were inoculated with the bioleaching medium previously prepared .

X. Assessing The Activity of Lps-Lacking Bacteria In Oxidation of Fe^{2+}

Two samples containing TF medium with Fe^{2+} as the source of energy, were inoculated as follows: (1) The control in which the normal *A. ferrooxidans* IRL.8F was inoculated into the medium without any treatment, and (2) The test sample with LPS-lacking *A. ferrooxidans* IRL.8F inoculated into the medium.

To investigate the restoring of LPS production ability of the bacteria, they were collected from 10-day culture of the second sample and inoculated into the fresh TF medium.

XI. Assessing The Activity of Lps-Lacking Bacteria In Bioleaching Of The Iron From Pyrite Soil

To assess such an activity, the pyrite ore with mesh of 200 and mesh size of 0.074mm was used and 10g/l of the ore was added to each of Erlenmeyer flasks. The base medium of all samples was water. pH of all samples was adjusted on 4.5. Prepared samples included: leaching medium inoculated with normal bacteria (having LPS), leaching medium inoculated with LPS-lacking bacteria and leaching medium without any bacterium.

The samples were placed in a shaker-incubator at 200 rpm, and 25°C for 14 days, and after precipitation the supernatant was used to analyze the amount of

XII. Results

During screening stage, we could isolate a bacterial strain with remarkable enzymatic ability to oxidize the iron and sulfur as its sole energy and electron source. It is noteworthy that the mineral spring

from where the bacterium was isolated have a brick-red solution and fawn deposits. This results from the natural activity of the bacterium in oxidation of Fe^{2+} iron in the nearby soils to Fe^{3+}, leading to color change and generation of jarosite (iron hydroxide) deposits. The isolated bacterium produces small colonies similar to fried egg in TSA agar medium which are hardly visible with naked eyes. Applying an innovative method using optical microscope and simultaneous lighting from up and down in this study, we could produce high-quality pictures of bacterial colonies (Fig. 1).

Results obtained from morphological, physiologic and molecular identification based on 16S rRNA revealed that this bacterium is mostly similar to A. ferrooxidans strain. Therefore, the bacterial strain was named A. ferrooxidans IRL.8F. It is chemautotroph and uses CO_2 in the air as its carbon source. Fig 2 shows what was obtained from extraction of bacterial genome.

XIII. Fe Oxidation

The medium was observed to turn from lime green to brick red (confirming the conversion of Fe^{2+} to Fe^{3+}) (Fig. 3). After 18 h, the medium turns to yellow as a result of bacterial activity and the brick red color observed within 48 to 72 h represents complete oxidation of Fe^{2+} to Fe^{3+}. Results of cell counting showed that bacterial cell concentration has begun to increase when the color changes started and it increased from 9×10^8 cells per ml in the first 24-h period to 18×10^8 in the second day. Within this period, pH of the medium decreased from 2.5 to 2. Deposits in fawn color were observed on the wall of flask, which increased in amount daily. . They can trap the leached metals in the solution in their lattices and thereby disturb the bioleaching process. Meanwhile, this problem can be overcome by retaining the low pH.

This experiment demonstrates the bacterial capability to leaching the minerals containing iron compounds.

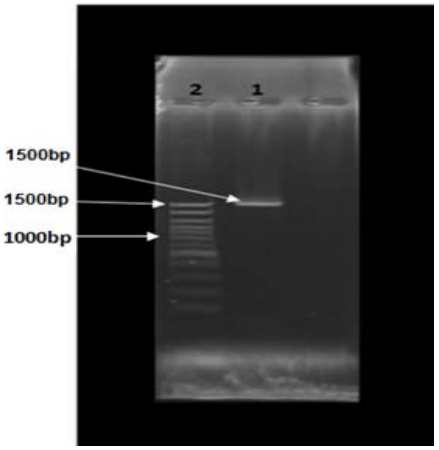

Figure 2 : Electrophoresis of the PCR product of IRL.F8 bacterium on 1% agarose gel. 1. PCR product, 2. DNA Ladder.

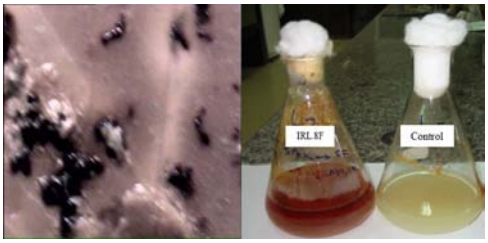

Figure 3 : Oxidation of Fe^{2+} to Fe^{3+} in SF broth medium and iron oxide particles formed on the colonies surface in the solid TSA medium (magnified $\times$100 – by A.M. Latifi).

XIV. Oxidation of Sulfur and Production of Sulfuric Acid

Results obtained from this experiment (Figure 4) revealed the high capability of the bacterium to produce acid, reduce the pH and make strong acidic conditions in TTB medium, such that in the third day the pH reached 1.5, in tenth day it decreased below 1 and in the 18^{th} day it was 0.75. As the pH decreases, the number of bacterial cells progressively increased, such that it doubled (to 6.13×10^8) with pH decrease from 4 to 1.6 and it triples with pH decrease from 1.6 to 0.9. For the fact that the most populated cell colony is observed in 14^{th} day, we used the 14-day suspension to produce the bacterial seed. The maximum acid production rate in 25^{th} day is 20 g/l. The pH changes and the sulfuric acid production are shown in the plot(Figure 4) .

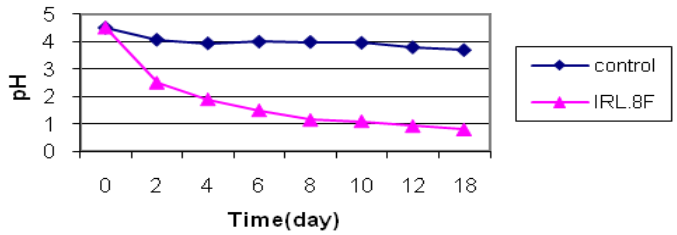

Figure 4 : Decrease of pH by the bacterium in TT broth medium.

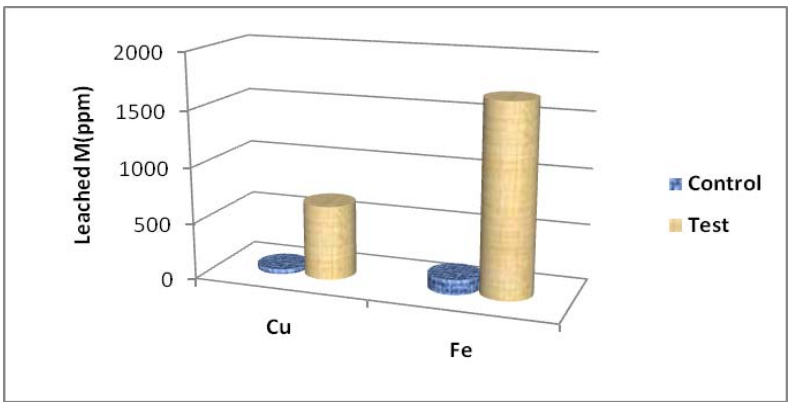

Figure 5 : Bioleaching of Cu and Fe from Copper concentrate.

XV. Bioleaching of Iron And Copper From Its Concentrate

X-ray fluorescence (XRF) and X-ray diffraction (XRD) analyses of pyrite ore showed that it contains 23.91% iron, 23.11% copper, 0.052% manganese, 0.001% nickel, 5.55% gold, 0.014% molybdenum, 22.79% sulfur.

Bioleaching of this ore for copper and iron elements showed that this mine is a highly appropriate medium for growth and activity of this bacterium. The bacterial cells consume and oxidize the sulfur element available in the mineral soil to sulfuric acid and drastically decrease the pH to as low as 1.98; thereby they provide.

Appropriate conditions for extraction of insoluble metals in the mineral soil. Analysis of the leaching solution showed 1690 and 663ppm rates of iron and copper extraction, which equal 29.3 and 71.4%, respectively. The values showed 92 and 93.5% increase in comparison with control samples, respectively (Fig.5).

The small amounts of extracted metals in control samples have resulted from spontaneous leaching. Note that in comparison with control samples (without inoculated bacteria), bioleaching medium of copper concentrate came in green and with development process the intensity of color was increased (Fig.6) .

Figure 6 : Color change due to bacterial activity and extraction of Cu in medium.

Bioleaching of pyrite ore

XRF and XRD analyses of pyrite ore showed that it contains ti iron and 25% sulfur. The mineralogical analysis showed its composition as $CaCO_3$ (Calcite), FeS_2 (Pyrite), ZnS (Sphalerite) and $CaMg(CO_3)_2$ (dolomite). Bioleaching of this type of ore demonstrated the decrease of pH as a consequence of sulfur consumption, resulting in the efficient metal extraction. The amount of metal extracted from the test sample showed a 60 to 70% increase in comparison with control sample (without bacterium).

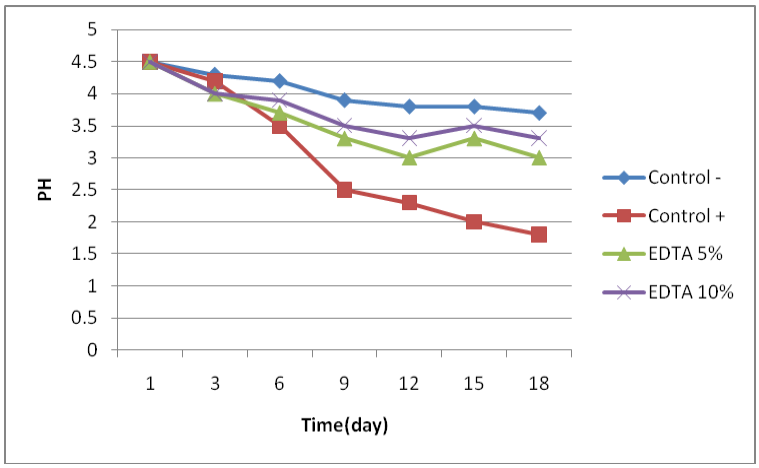

Figure 7 : pH changes in the investigation of EDTA effect on bacterial activity of *A. ferrooxidans IRL.8F* and rate of Fe^{3+} extraction from pyrite ore.

xvi . Study of Edta Effect on Lps In The Outer Membrane of *A. Ferrooxidans* Irl.8f And on Rate Of Fe^{3+} Extraction From The Pyrite Ore

To investigate the effect of EDTA on bioleaching through damaging the bacterial cell membrane, 5 and 10% concentrations of EDTA were simultaneously added to the leaching medium (the base medium of water). Decrease of pH in the sample without EDTA was observed to follow a slower slope and it directly depends on rate of bioleaching process (Fig.7). The results also confirm the severe reductive effect of EDTA on efficiency of the bioleaching process, such that the bioleaching rate in 5 and 10% concentrations of EDTA decreased by 61and 70%, respectively in comparison with the sample without EDTA (Fig.8).

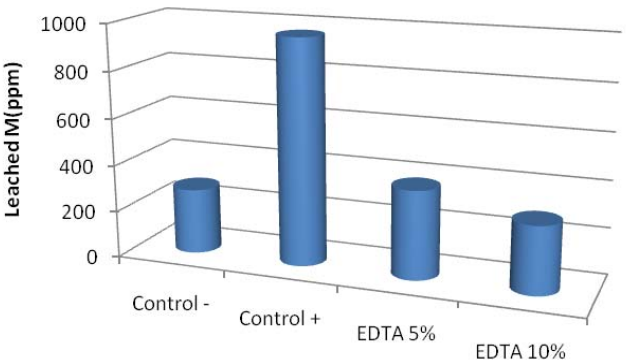

Figure 8 : EDTA effect on extraction of iron from pyrite ore
Control - = leaching medium without bacterium and EDTA
Control + = leaching medium + bacterium without EDTA.

XVII. Study of The Activity of Lps-Lacking Bacteria And Oxidation of Fe^{2+} Iron In Tf Medium

For this purpose, the bacterial LPS was first removed and the LPS-lacking bacteria were inoculated into the TFB medium. No color change was observed after 10 days, which represents the inability of the bacteria in the oxidation of Fe^{2+} to Fe^{3+}, whilst in the control sample with normal bacterium, the color began to change in the 5 day and it remarkably turned from green to red after the 10^{th} day (Fig.9, right). To ensure that the EDTA + Tris-HCl treatment has not killed the bacteria and the cells just have lost their LPS, in the second stage, the LPS-lacking bacteria used in this experiment were transferred to a fresh TF medium. After 10 days, the bacterium turned the medium from lime green to red, indicating that the bacteria have restored the ability of LPS synthesis and have oxidized Fe^{2+} to Fe^{3+} (Fig.9, left).

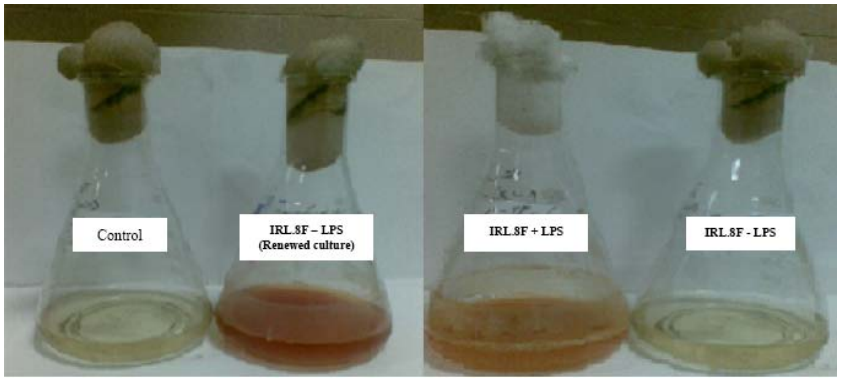

Figure 9 : Two stages of bacterial cell culture in TF medium. Right: the initial culture of LPS-lacking bacteria in TF medium, Left: re-culturing the LPS-lacking bacteria in the fresh TF medium.

XVIII. Study of The Capability of Lps-Lacking Bacteria in Leaching of Fe^{3+} From Pyrite or

The samples were analyzed after 14 day from beginning of the process and the amount of leached iron was obtained as shown in Figure 10. As it can be observed from the figure, LPS-lacking bacteria have remarkably lost their ability to leaching the iron.

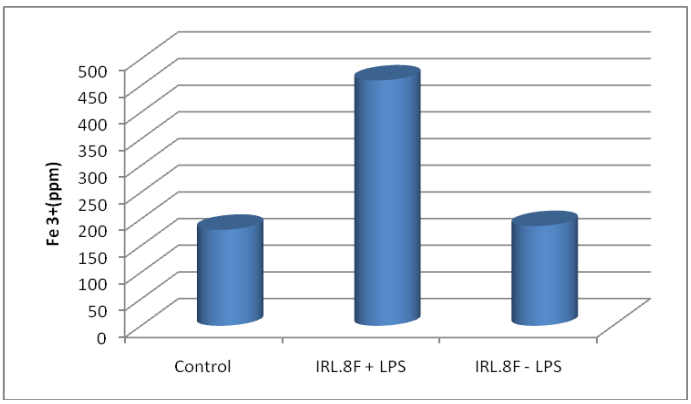

Figure 10 : Concentration of extracted Fe^{3+} from pyrite ore, in presence of LPS-lacking *A. ferrooxidans IRL.8F.*

XIX. Discussion

In the present study with the aim of evaluating the ability of native bacterium to extract copper and iron from their ores, an acidophilic strain was isolated from an iron mineral spring in Larzan, Iran. The isolated strain shows a remarkable enzymatic activity in Fe and S oxidation and is highly capable with bioleaching the copper and pyrite.

The results from bioleaching of copper concentrate showed that the amount of extracted copper and iron was 71.4 and 29.3%, respectively. Furthermore, in comparison with control samples, without bacteria, these amounts increased by 93.5 and 92%, respectively. In the control samples, however, a minor amount of metals were extracted due to spontaneous leaching.

One of the factors influencing the quality and quantity of the bioleaching process is the bacterial ability to attach to the mineral surface. The microbial contact with the ore surface stimulates the expression and production of extracellular polymers which entrap the bacterium at the side of the ore and attach it to the mineral surface (Dispirito et al., 1983; Bagdigian and Meyerson, 1986).

In addition, EPS can form chemical bonds with the mineral surface and mediate or promote respiration and nutritional reactions (Scobar., 1997; Ehrlich and Brierley., 1990). The molecules constituting the EPS can be made of LPS, phospholipids or other macromolecules such as poly-peptides. These compounds are released by the organism when it is attached to the mineral (Scobar, 1997).

There are varioustechniques to isolation of LPS from bacteria, Such as phenol-water method or by the phenol-chloroform-petroleum ether extraction methods, but these methods usually cause cellular damag or death. (Ramadas et al., 1991).The purpose of this study was to isolate and remove bacterium LPS without causing bacterial cell damage or death. Studies show that EDTA treatment negatively affects the adherence of the cell to mineral by the loss of part of LPS, without cell lysis. (Arredondo et al., 1994; Scobar et al 1997).

Scobar et al (1997) investigated the effect of EDTA on iron extraction from the pyrite ore. They believed that this substance removes the LPS from bacterial outer membrane and this leads to a remarkable decrease in the attachment of bacterium to its substrate. In the investigation of chalcopyrite and pyrite ores, they observed 85 and 77% decrease in attachment, respectively for bacterial cells treated with EDTA (Scobar, 1997). Such substances as EDTA absorb bivalent cations attached to phosphate groups in LPS and transform it from natural form to aggregated form, which obstruct the subsequent reactions (Rangin and Basu, 2004). Results of the present study revealed that samples with EDTA treatments show a remarkable decrease in bioleaching rate of the metal of interest. In direct mechanism where contact and attachment of the bacterium to the mineral surface is mediated by releasing exopolymers (Vandevivere and Kirchman., 1993) , EDTA removes part of this exopolymer and thereby, to a great extent decreases the efficiency of iron extraction from pyrite ore (Arredondo et al., 1994; Scobar et al 1997).

Since the bioleaching drastically decreased with LPS removal, in the present study, it can be concluded that the most amount of metal has been extracted through direct mechanism. The bacterium secretes such substances as LPS when approaching the mineral surface in order to be able to attach to the mineral surface; however, when LPS is removed the attachment cannot occur and the leaching by the bacterium will decrease (Pogliani and Donati., 1999; Arredondo et al., 1994; Scobar et al 1997). In the next experiments, to ensure that the decrease in bioleaching has resulted from LPS removal by EDTA, the bacterial LPS was removed by use of EDTA treatment and LPS-lacking bacteria were transferred to the leaching medium. Cultures of these bacteria in leaching media containing pyrite soil also significantly showed the decrease in extration of Fe^{3+}. These bacterial cells were also cultured in iron-containing TF medium. As it was expected, the treated bacteria with EDTA could not oxidize the iron, whereas the iron oxidation was observed in the Erlenmeyer flask containing non-treated bacteria.

Furthermore, EDTA in the leaching medium may act as a chelator absorbing the iron cations and decrease the oxidation of iron from Fe^{2+} to Fe^{3+}; therefore, the bacterial LPS may be of no role in decrease of leaching. LPS removal and inoculation of LPS-lacking bacteria into the leaching medium led to a 60.1% decrease in extracted metal. The less decrease in metal in comparison with when EDTA was used can be attributed to three possible reasons: 1) Some bacterial cells have restored their ability to produce LPS, 2) EDTA has acted as an iron chelator, or 3) EDTA has decreased the enzymatic oxidation of the iron.

In bacterial bioleaching, Thiobacillus Thiooxidans is used together with T. ferrooxidans, for the following reasons: It can release metallic elements by oxidation of reduced and semi-reduced sulfur compounds of the minerals and can promote the leaching of metals by producing the sulfuric acid as an oxidant. In addition, it provides the optimal acidic conditions for growth and activity of T. ferrooxidans. In bioleaching processes, that bacterial strain is of the greater importance which produces more amount of acid (Qiu et al., 2005).

In conclusion, results obtained from the present study indicate that: 1) EDTA drastically

decreases the efficiency of the bioleaching process, 2) LPS in the isolated bacterial strain in this study has a key role in bacterial attachment to mineral particles, and 3) the bioleaching process in this case promotes through the direct mechanism.

In Fe oxidation in TFB , pH of the medium decreased from 2.5 to 2, Probably because of consumption of sulfur compounds in the medium. Deposits in fawn color were observed on the wall of flask, which increased in amount daily; probably the iron hydroxide(jarosite) appeared in pH<2 (Qiu et al., 2005).

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Prevalence of Parasitic Eggs and Parasites Cysts on Computer Mouse and Keyboard in School of Science and Computer Studies of Federal Polytechnic, Ado-Ekiti, Nigeria

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Abstract - The prevalence of parasitic eggs and parasite cysts on computer mouse and keyboard in School of Science and Computer Studies, Federal Polytechnic, Ado-Ekiti, Nigeria was investigated. The total number of samples examined was one hundred and eighty (180) of which twenty nine (29) were positive. The result showed that the samples collected during the first, second, and third weeks had 13, 10, and 6 numbers of cysts and ova respectively. The highest incidence was observed during the first week. Some bacteria such as *Staphylococcus aureus, Streptococcus pyogenes, Pseudomonas aureginosa* and *Enterococcus faecalis* were also isolated from the sample with the highest incidence found in *Staphylococcus aureus*. It was concluded that the mouse and keyboards could be a source of disease transmission and should be disinfected appropriately and often.

Keywords : Parasitic eggs, Parasite cysts, Staphylococcus aureus, Computer keyboard and mouse.

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Prevalence of Parasitic Eggs and Parasites Cysts on Computer Mouse and Keyboard in School of Science and Computer Studies of Federal Polytechnic, Ado-Ekiti, Nigeria

Ajenifuja, Oluwafemi A[α] & Ajibade, V.A[σ]

Abstract – The prevalence of parasitic eggs and parasite cysts on computer mouse and keyboard in School of Science and Computer Studies, Federal Polytechnic, Ado-Ekiti, Nigeria was investigated. The total number of samples examined was one hundred and eighty (180) of which twenty nine (29) were positive. The result showed that the samples collected during the first, second, and third weeks had 13, 10, and 6 numbers of cysts and ova respectively. The highest incidence was observed during the first week. Some bacteria such as *Staphylococcus aureus, Streptococcus pyogenes, Pseudomonas aureginosa* and *Enterococcus faecalis* were also isolated from the sample with the highest incidence found in *Staphylococcus aureus*. It was concluded that the mouse and keyboards could be a source of disease transmission and should be disinfected appropriately and often.

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I. Introduction

Parasite is an organism that lives in or on a second organism, called a host, usually causing it some harms. It is generally smaller than the host and of different species (Yusuf, 1990). Parasites are dependent on the host for some or all of their nourishment (Martins *et al*, 1980). Parasite can also be seen as an organism that has a deleterious symbiotics relationship with another organism or host species. A flea or tick is a parasite, bacteria can be parasitic, mistletoe is a parasite (Tanko *et al*, 1999). Parasite sometimes cause the eventual death of the host although not always and this can lead to the parasites demise if it cannot leave or find a new host (Kramer, 2006). Parasites are just about everywhere in our environment, so it's easy to become infected (World Health Organization).

In the 1993, world development report intestinal helminthes rank first as the main cause of disease burden in children aged 5 – 4 years and also rank highly as the disease that can be efficiently control by cost effective intervention (Lawande, 1983). Multiple infectious with several different parasites e.g. hookworms, roundworms and amoebae are common, and their harmful effects are often aggravated by co-existence malnutrition or micronutrient deficiencies (Akogun, 1989).

In America, parasitic infections are not as widespread but these infections are on the rise for various reasons. For example people bring parasites with them when they migrate to the U.S and soldiers often return to the U.S bringing parasites with them from overseas (Kucik *et al*, 2006). Parasitic infections are common in rural or developing areas of Africa, Asia, and Latin America and less common in developed areas. A person who visits such an area can unknowingly acquire a parasitic infection when the person returns home. In developed areas, parasite infections may also affect immigrants and people with a weakened immune system (such as those who have AIDS or who take drugs that suppress the immune system).

The infections may occur in places with poor sanitation and unhygienic practices. Parasites increase their fitness by exploiting host for resources necessary for the parasites survival i.e. food, water, heat, habitat, soil and dispersal. Parasites reduce host fitness in many ways, ranging from general or specialization pathology such as parasitic castration, impairment of secondary sex characteristic, to the modification of host behaviour (Rufala, 2006).

The Nigeria environment has been described as poor, based on personnal, community and environmental hygiene (Akogun *et al*, 1989). This poor state of hygiene is accounted for by the presence of immature stages of parasite (egg and cysts) in the soil (Ali, 1993), in the air (Lawande, 1983) on toilet door handles, on water closet handles (Nock and Geneve, 2003), on becks and legs of domestic chicken (Abuja, 1997) and on the sole of shoes (Tanko, 1999) demonstrating the indiscriminate nature of faecal disposal system. As these show the dynamic transmission network that exist in the Nigeria

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environment, through which parasites infect human and animal hosts; because once they are introduce into the soil, parasites eggs and cysts can be transported on contact with any subject. This accounted for the high prevalence and incidence of parasitic infection in both humans and animals (Hopkins, 1992).

The internet is progressively becoming an effective means of communication in Nigeria, thus there is an upsurge of people visiting the internet cafes, some reason to browse. During the use of the computer, the keyboard and mouse are used for input of commands with the fingers and palms of the hands, thus acting as points of contact between the internet and its users. The internet café is proposed, as a suitable model to test the role it plays in the transmission of parasite cysts and eggs in Federal Polytechnic, Ado-Ekiti, Nigeria.

II. Materials And Methods

a) Collection of Samples

A total number of 180 samples were collected from keyboard and mouse in School of Science and Computer Studies of Federal Polytechnic, Ado-Ekiti, Nigeria, over a period of three weeks. Sixty (60) samples were collected in each of the three weeks, 10 samples in the morning and 10 samples in the afternoon, which made up of 20 samples for each week.

b) Preparation of Culture Medium

2.8g of Nutrient Agar was dissolved in 100ml of distilled water and heat to melt. The conical flask was plugged with cotton wool and it was wrapped with foil paper and autoclave for 15mins. It was allowed to cool to between 45°C – 50°C after autoclaving it was poured into sterile Petri dishes and allowed to solidify. It was also poured into McCartney bottles which was half filled and the bottles were placed slantingly on the bench tops to allow the agar to set inform of slopes. The plates were labeled with Date and Name of the organisms to be inoculated. The swab samples collected from computer accessories were inoculated into the various grow media by streaking each nutrient agar plate and the plate were incubated at 30°C for 2 days. The plates were observed after incubation.

c) Preparation of Unstained Wet Mount

A sterile swab stick moistened with normal saline solution was moved over the keyboard and the buttoms of computer mouse. Special attention was given to the swabbing of the most commonly used keys for examples 'Enter', 'Spacebar', 'Delete', 'Shift key', etc. These swabs were taken to laboratory in sterile test tube containing 10ml of normal saline and each samples were labeled Day 1, 2, 3, etc. Each sample was further centrifuged at 2000rpm for 3 minutes. The supernatant was discarded and the sediment re-suspended. Little quality was taken with a Pasteur pipette and placed on a clean microscope glass slide. A drop of lugol's iodine solution was added and a clean cover slip was placed on the surface and examined under florescent microscope x400 magnification.

d) The Gram Staining

The bacterial smear was taken from the prepared Nutrient agar plates into the slide. The slide was placed on the staining rack and a drop of distilled water was added and mixed with the bacterial smear. The smear was flooded with crystal violet stain and leaf for 60secs. The smear was flooded again with Gram iodine and leaf for 60secs, after which the iodine was washed off with distilled water. Acetone-alcohol was added until no more colouration is seen to come up; it was washed immediately with distilled water and left for 10 – 15secs. The slides were flooded with carbol fuchsin and left for 1 minute, it was then washed off. It was gently dried between sheets of clean blotting paper and allowed to air-dry. It was examined under the florescent microscope x100 oil immersion.

Organisms isolated are;

- Staphylococcus aureus
- Streptococcus pyogenes
- Pseudomonas aeruginosa
- Enterococcus feacalis

e) Catalase test

A loopful of the isolate was placed on a clean sterile slide and a drop of hydrogen peroxides was added. The effervescence of gas is shown by bubbling.

f) Oxidase test

An oxidase strip (i.e. a strip that has been impregnated in the reagent) was smeared with the test organism and left for 10 seconds. Purple colouration is a sign of oxidase.

III. Results And Discussion

Table 1 : Prevalence of parasite eggs and cysts on keyboard, and mouse for three days per week in the first week

Days	Number of samples	Positive No.
1	20	6
2	20	3
3	20	4
Total	60	13

Table 2 : Parasite eggs and cysts on keyboard, and mouse for three days per week in the second week.

Days	Number of samples	Positive No.
1	20	4
2	20	3
3	20	3
Total	60	10

Table 3 : Parasite eggs and cysts on keyboard, and mouse for three days per week in the third week.

Days	Number of samples	Positive No.
1	20	3
2	20	3
3	20	0
Total	60	6

Table 4 : Bacterial encountered during the study.

Bacterial isolated	No (%)
Staphylococcus aureus	8 (57)
Streptococcus pyogenes	2 (14)
Pseudomonas aeruginosa	3 (21)
Enterococcus feacalis	1 (7)

Table 5 : Characteristic of the test bacteria.

Test bacteria	Shape	Size (μm)	Motility	Gram reaction	Appearance	Temperature (^{o}C)	Characteristic
S. aureus	Cocci	0.7-1.0	+ve	+ve	White, yellow	37	Anaerobic
Str. pryogenes	Cocci	0.6-1.0	–ve	+ve	Greenish	37	Anaerobic
P. aeruginosa	Rod shape	0.6-1.0	+ve	–ve	Pink-red	37	Aerobic
Ent. feacalis	cocci	1 – 2	–ve	+ve	Yellow pigment	37	Aerobic

IV. Discussion

Overall samples examined were one hundred and eighty (180) and twenty nine (29) of the samples were positive. The results show that first week samples had (13) highest occurrence number of positive samples having eggs and cysts, then followed by second week samples has (10), followed by third week samples which had (6) with the lowest prevalence. Bacteria encountered during the study are; Staphylococcus aureus (08) with the highest occurrence number, then followed by Pseudomonas aeruginosa (3), followed by Streptococcus pryogenes (2) and Enterococcus feacalis (1) with the lowest occurrence number.

Computer technology for the management of individual has become an essential part in all aspect of modern medicine (Fukatat *et al*, 2008). Consequently, the computer keyboard and mouse in the Departments of School of Science and Computer Studies' laboratory in Federal Polytechnic may act as a reservoir for microorganisms. And contribute to the transfer of pathogens from one individual to the other unknowingly. (Hartman *et al*, 2004).

Most of the keyboards examined in the study were contaminated with non pathogenic microorganisms such resident skin flora or environmental bacteria. Long survival time of potentially pathogenic microorganism, particularly on desks, contribute to the hypothesis of computers acting as reservoir of pathogenic (Kassem, 2007). Hence, the process of correct hand disinfection is still the main stay of any preventive measure for the reduction of infections. Hand disinfection policy should not be reserved to student or internet users (Nock and Geneve, 2002). Beside to improve hand hygiene compliance, improvement of cleaning service could admonished as an infective infection control measure (Nock and Brown, 1994). Disinfectant including chlorine, alcohol, phenol and quarternary ammonium are all effective against Staph. aureus and Enterococcus spp. Species on keyboards of computers and even sterile water is effective to remove more than 95% bacteria (Rutala, 2006). Although keyboard can be safety and successfully disinfected, the need to clean computer interface surface as routine practice is generally accepted, no specific cleaning and disinfection frequency and procedure for computer accessories has been defined. Daily cleaning and hygiene regularly for using computer is of great significance and could help in the reduction of parasite eggs/cysts and pathogenic bacteria and also reduce keyboard contamination (Williams, 2006).

Computer should be disinfected daily and well visibly soiled, Health care workers should not touch computer keyboard and mouse with contaminated hands. Preventive measure should be adopted particularly when the number of people visiting the operating room daily are considered.

The isolation cysts from samples collected on keyboard is an indication that it could be source of transmission of pathogens Krammer *et al*, 2006). These findings correlate with that of (Hartman *et al*, 2004) where it was observed that keyboard houses a lot of parasites.

Staphylococcus aureus which are antibiotic-resistant are found to be predominance bacteria found on keyboard and mouse because they are normal floral of humans found on nasal passage, skin and mucurs membrane, pathogen of humans, causes a wider range of superlative infections, as well as food poisoning and toxic shock syndrome.

The isolation of some bacteria from the keyboard and mouse is an indication that they could be a source of the transmission of diseases. The predominance of Staphylococcus aureus explains the

long standing believe that the skin houses Staphylococcus aureus. The isolation of Streptococcus pyogens which is found in nasal passages is an indication that the bacteria could have been dispersed through droplets from the mouth.

V. Conclusion

This study showed that a fairly large number (i.e. 95%) of the computer keyboard and mouse devices which are in use in various areas of the school is contaminated and the discovery of Staphylococci on computer keyboards draw much needed attention to good sanitary habit after utilizing the keyboard and mouse. Additionally, touch of the mouth or the nose while operating the keyboard could have contributed to the contamination because humans can transport staphylococci from the nasal passage.

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