Biodephosphorization of Iron Ore using *Acidothiobacillus ferrooxidans.*

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ABSTRACT: An attempt has been made to investigate the removal of phosphorus from Agbaja iron ore using *Acidothiobacillus ferroxidans* bacterium. The bacterium strain was isolated from Agbaja high phosphorus iron ore. Various bacterial loads of the bacterium were cultivated in a crushed iron ore of 60μm particle size. The effects of time and microbial population on the biodephosphorisation of the ore were studied at a temperature of 25^{o}C. The maximum degrees of biodephosphorization and beneficiation were 79.34% and 71.84% respectively, in 40 days using 10^8 microbial populations. The results obtained show that as the leaching time increases the rate of biodephosphorization and the degree of beneficiation increases. Furthermore, as the microbial population increase, the rate of biodephosphorization and degree of beneficiation increase marginally.
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INTRODUCTION

Iron ore resources are fast depleting as a result of pressure originating from population and industrial development requirements. However there exist large stockpiles of low and lean grade ores to be mined of which Agbaja iron ore is one of such ores. The ore is low grade because of the high phosphorus content.

Phosphorus may be incorporated either into the crystal lattice of iron oxides or into the gangue minerals (Dukino *et al*, 2000). This element has a deleterious effect on the workability of steel (Muhammed and Zhang, 1989). For that reason, in most places only premium low phosphorus ores (less than 0.08 wt%) are extracted leaving many iron mines around the world enriched in un-tradable, high phosphorus iron ore (Cheng *et al*, 1999, Dukino *et al*, 2000).

If steel is produced at high level of phosphorus that steel will be brittle and can easily crack hence the need for dephosphorization. In the last eight years, the situation of iron ore markets has changed dramatically due to an increase in the world steel consumption, pushed up mainly by the economic growth of China and other Asian emerging markets.

Conventional technologies involved in metal extraction from metallic ores are economically expensive in monetary terms and human labour and are also environmentally unfriendly. Many of the by-products of extraction by thermal and chemical means are toxic to man and his environment and constitute sizeable environmental pollutants in cities and industrial layouts.

On the search for more environmentally sound technologies for the mining industry, biological processes to extract metals from ores, pre-treating metallic ores or removing contaminants from metallic ores or industrial wastes have been developed for different metallic mineral resources (Jain and Sharma, 2004).

Bacterial leaching is regarded as one of the most promising and certainly the most revolutionary solution to these problems compared to pyrometallurgy or chemical metallurgy. This leaching process is carried out under mild conditions, usually without addition of toxic chemicals. The biological treatment of ores to remove contaminants often referred to as bioleaching (Jain and Sharma, 2004) is another variant of chemical processing. In such a process the microorganisms produce, as a consequence of their metabolism, a chemical by-product mineral acid, organic acids, polymers and enzymes. The chemical by-products attack the gangue minerals contained in the ore, dissolving them and thus producing their selective removal (Jain and Sharma, 2004).

In phosphorus limited environment, microorganisms will be obligated to extract phosphorus from mineral sources to supply their growth needs (Banfield *et al*, 1999) and this is the theoretical base for the biodephosphorization of high phosphorus iron ores. Organic acids producing filamentous fungi have been used to remove phosphorus from ores in series of reports (Parks *et al*, 1990; Buis, 1995; Delvasto *et al*, 2005).

The use of *Acidothiobacillus ferroxidans* in the isolation/metal extractions including iron in different media have been extensively reported

(Bartels *et al*, 1989; Boon *et al*, 1988). Some researchers previously investigated the simultaneous leaching of metal oxides and sulphides. Ghosh and Imai (1985) have reported that iron-oxidizing bacterium, *Thiobacillus ferrooxidans*, leached manganese from manganese dioxide in the presence of the sulphide ores of copper.

However the main draw back of these investigations was that the used strains were not associated with the ore being treated. When artificially inoculated in a particular environment, indigenous microorganisms, as a general rule compete better in terms of adaptation and cause fewer ecological distortions than exogenous micro organisms. Consequently, if an efficient bio dephosphorization process has to be implemented for treating a determined raw material, studies on the micro biota naturally living in such a substratum and evaluation of its desired properties should be the starting step.

This investigation attempts to use bacterium harvested from the raw iron ore of Agbaja to dephosphorize the ore.

MATERIALS AND METHODS

The iron ore sample was obtained from Agbaja, Kogi State, Nigeria. The sample was pulverized with hammer mill into finer size and sieved to obtain a particle size of 60 microns. 20kg of the sample was weighed out. It was divided into two parts of 14kg and 6kg respectively. The 6kg was subjected to immediate microbial and chemical analysis. The remaining 14kg portion was subjected to scrubbing, and subsequent post scrubbing microbial and chemical analyses.

Microbial reagents (Nutrient agar)

A 25g of dehydrated nutrient Agar was dissolved in 1 liter of distilled water and the solution heated in a water bath until completely dissolved and then dispersed in 25ml bottles, corked and autoclaved at 120^{o}C for 15 minutes. The sterile molten nutrient Agar was poured into sterile Petri dishes and allowed to solidify. They were then oven dried in the incubator at 50^{o}C for 15 minutes and thereafter incubated for 24 hours to ensure complete sterility before use.

Microbial culture.

The raw iron ore was mixed with sterilized water and stirred vigorously with glass stirring rod. A loop full of the ore suspension was streaked on the oven dried sterile nutrient agar in a Petri dish with sterile inoculating loop pre heated to redness. The streaked culture Petri dish was incubated at 37^{o}C for 24 hours. 24 hours incubation yielded moderate growth in the culture plate. On examination 5 colonies of distinctive features were revealed.

Isolation of bacteria

From each colony forming unit, a streak was taken and transferred into 20ml agar slant tube for pure culture. Pure cultures obtained from the first colonies were properly stored in an incubator at 37^{o}C for onward identification and confirmatory tests.

Microscopic examination of isolation.

Each isolate was subjected to stain gram test. The grain stain revealed single rods shaped organisms arranged in chains with various colours, some flagellated and discoid. Some of the isolates were also gram negative, but with sporation and rod shaped. These features pre supposed the presence of bacilli specie. The first colony was identified and confirmed as *Acidothiobacillus ferroxidans* and was used in this work.

Preparation of innoculum and leaching of scrubbed sample

A standard culture of 10^9 was made from pure culture in the agar slant containing *Acidothiobacillus ferroxidans*. This 10^9 standard culture solution was made by pouring 5ml of sterile water on the surface of the agar slant and stirred. It is then poured into a sterilized test-tube. Another 5ml of sterile water was again poured on the surface of agar slant and stirred again and this is poured into the same test tube. This result to 10ml concentration of the micro-organism in the sterilized test tube and this forms the standard 10^9 colony forming unit of *Acidothiobacillus ferroxidans*. Subsequent serial dilutions were made from the standard culture to obtain, 10^8, 10^7, 10^6, 10^5, 10^4, 10^3, 10^2, 10^1, microbial population. Each of the microbial population was inoculated into 250g of scrubbed iron ore and put into incubating bottles. These samples were incubated for 88 days at 25^{o}c. Samples were collected from the bottles after 8 days interval and were analyzed for phosphorus and iron contents.

Degree of dephosphorization % =

$$\frac{As\ \text{received value P (wt\%) - Final value P(wt\%)}}{As\ \text{received value P(ppm)}} x \frac{100}{1}$$

Degree of beneficiation % =

$$\frac{Final\ value\ Fe(wt\%) - As\ received\ value\ Fe\ (wt\%)}{Final\ value\ Fe(\ ppm)} x \frac{100}{1}$$

RESULTS AND DISCUSSION

Table 1 shows the chemical analysis of as received Abgaja iron ore. The iron content shows that

the iron ore if properly beneficiated and dephosphorized can be used in steel making.

The high phosphorus content adduces the reason why the iron ore has not been exploited. The sulphur content, though small, can constitute cog in the wheel of making steel using the iron ore. The other components of the iron ore are quite insignificant and they constitute little or no problem in steel making.

In Table 2, the chemical analysis of scrubbed iron ore is depicted. The effect of scrubbing or desliming is not significant though it increased the degree of beneficiation by 0.01%. It can also be observed that the phosphorus content reduced from 0.79% to 0.69% showing 0.13% degree of dephosphorizarion. It can be inferred that scrubbing helps in removing extraneous materials like sand and dirts but contributes quite insignificantly to beneficiation and the dephosphorization. Sulphur content was reduced from 0.12% to 0.05% showing 58% reduction which is quite significant.

In Table 3 the preliminary and confirmatory tests are shown. The gram test showed rod -like shape with flagellum as viewed under the microscope.

The regular and undistorted rod-like shape with flagellum suggest that extra cellular networks observed could actually be due to the interconnection of proteinaceous cell appendages such a fibrils and anchors (Ishii *et al*, (2004) or pili (Chung *et al*, 2003; Tomish and Mohr, 2003). These structures are used by bacteria to strongly attach to surfaces during initial and intermediate stages of surface colonization (Davey and O'toole, 2000; Dunne, (2002). As reported elsewhere Miron *et al*, (2001); Levy *et al* (2003); Ishii *et al* (2004); Mora Bejarano and Schneider, (2004), cell appendages involved in surface colonization can give rise to clear extracellular networks.

The bacterium is arranged in chains and the colour is red. The strain character is negative showing gram negative specie. The confirmatory test was carried out to authenticate the particular specie. The coagulase and manitol tests showed positivity in the result. The litmus milk test was acidic with appearance being curdy. Sulphide oxidase was positive and oxidation of ferrous into ferric ion was also positive.

The organism in table 3 was a gram negative flagellated bacterium which reacted positive (+) to acid test, oxidizing sulphide and ferrous to ferric ion, suggestive of acid – sulphur – iron susceptive bacillus. The above description confirms the bacterium as *Acidothiobacillus ferroxidans*.

Table 1: Xray fluorescence chemical analysis of the Agbaja Iron Ore before Desliming

Component	Average Composition %
Fe	56.34
SiO_2	5.16
S	0.12
Al_2O_3	6.60
CaO	0.23
MgO	0.07
MnO	0.18
TiO_2	0.15
K_2O	0.04
P	0.79
H_2O	2.06

Table 2. Xray fluorescence chemical analysis of the Agbaja Iron Ore after Desliming

Component	Average Composition %
Fe	56.90
SiO_2	5.02
S	0.05
Al_2O_3	5.2
CaO	0.21
MgO	0.3
MnO	0.17
TiO_2	0.25
K_2O	0.007
P	0.69
H_2O	2.81

Table 3: Result of microbial characterization of acidothiobacillus ferrooxidans.

Stain	Gram Stain
Shape	Rod-like with flagellum
Arrangement	In chains
Colour	Red
Stain character	Negative
Presumptive test	Bacilli specie suspective
Confirmatory test	
Coagulase	+
Manitol	+
Litmus milk	Acid curdy in appearance
Sulphide oxidase	+
$Fe^{2+} \rightarrow Fe^{3+}$	+

In figure 1, the degree of dephosphorization follows Michaelis – Menten equation. Between the 8^{th} day and 16^{th} day the quantity of phosphorus removed was about 7.06% and 8.37% respectively. The percentage degree of dephosphorization was small due to the fact that lag phase predominates showing that the cell division has not fully commenced. The small rate of dephosphorization may also be attributed to the fact that the microorganism was not fully adapted to the new

environment because of limiting nutrient which is in line with unreacting core model.

Effect of leaching time on degree of biodephosphorization at 25°C.

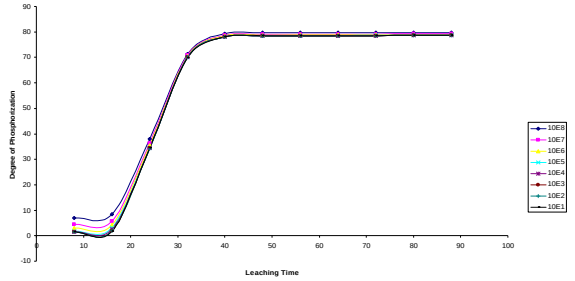

Fig.1 Effects of Leaching time on degree of Biodephosphorization

In nutrient limited environments, such as the one in our experiments, bacteria must colonize mineral surfaces where phosphate is located in order to scavenge it (Bemfiel et al, 1999). They accomplish this through biofilm formation. Biofilms are complex aggregates of bacterial cells, bacterial exopolymers, mineral debris and other metabolites attached to a surface.

The degree of biodephosphorization at 32nd day of experiment was 71.40% showing full mobilization of acidothiobacillus ferrooxidans.The rate of dephosphorization increases from day 16, showing exponential growth rate of the microorganisms, as shown in Michaelis-Menten formular. This exponential growth rate continued until the 40th day where the maximum rate of biodephosphorization of 79.34% was attained. Jain and Sharma (2004) discovered that at a higher pulp density, bacterial biomass encapsulates solids making it difficult for solids to be accessed thereby decreasing biodephosphorization yield. This was carefully avoided in this work by ensuring low pulp density.

A fairly constant degree of biodephosphorization was obtained between 40th day and 88th day. This is as a result of the dynamic balance between growth and death of microorganisms during metabolism. In other words the rate of growth corresponds to the rate of death of these organisms. It can then be inferred that almost the same quantity of acidothiobacillus ferrooxidans were present between 40th and 88th producing fairly constant values. The work of Delvasto et al (2007) using aspergillus niger posted a maximum of 33.2% degree of biodephosphorizaton in 21 days treatment. This present work posted 25% degree of biodephosphorization in 21 days, with a maximum degree of dephosphorization of 79.34% in 40 days. It can be observed that the values of phosphorous in the ore decrease as leaching time increase, that is, as the leaching time increases the rate of dephosphorization increases.

Effect of leaching time on degree of beneficiation at 25°C.

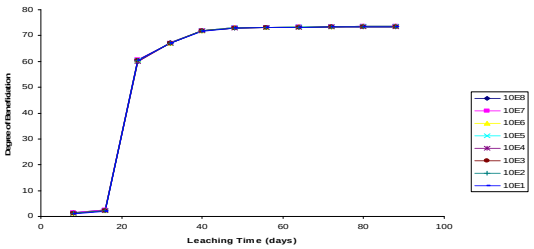

Fig. 2 Effect of Leaching time on degree of Beneficiation

Figure 2, shows the effect of leaching time on degree of beneficiation. There is an overlapping of the degree of beneficiation profiles showing closeness at different microbial populations. It is noted that between 8th day and 16th day a fairly constant degree of beneficiation is obtained. A sudden rise of degree of beneficiation existed from day 16 to day 24 that is from 2.55% to 60.06% as shown as figure 2. This is as a result of intensive degrading activities of Acidothiobacillus ferrooxidans on the deleterious substance in the iron ore, thus increasing iron content of the ore. Between 14th and 40th days, the activity of the bacterium has been reduced resulting in gradually increase of rate of beneficiation from 60.06% to 71.87%. The maximum degree of beneficiation is 71.87% in 40 days. The activity of the bacterium maintained a fairly constant degree of beneficiation from 40th day to 88th day.

The quantities of phosphorus removed as a result of microbial population are shown in figure 3. The degree of dephosphorization for 8 days and 16 days remained fairly the same for microbial population of 10^1 to 10^3. From 10^3 microbial population, the degree of biodephosphorization increases slightly as the microbial population increases. For 24 days, the quantity of phosphorus removed was almost the same between 10^1 to 10^5 microbial populations, while from 10^5 there was a slight increase in degree of dephosphorization as shown in figure 3. The profile for 32 days showed similar trend as the profile of 24 days. The degree of dephosporization is fairly constant between 40 days and 88 days as microbial population increases. It can be inferred that increase in microbial population after 40 days will show little or no appreciable increase in degree of dephosphorization.

Effect of microbial population on degree of biodephosphorization at 25°C.

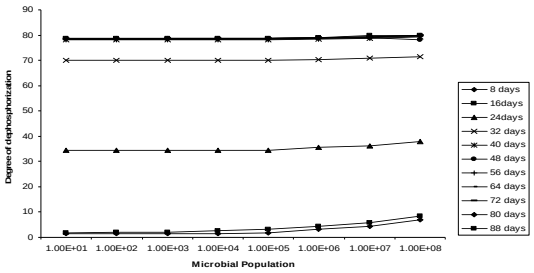

Fig. 3 Effect of Microbial Population on Degree of Biodephosphorization

Delvasto et al, (2009) obtained a maximum of 20.3% dephosphorization in 3 weeks using Burkholderia caribensis $FeGLO_3$, which is slightly below the result 25% at 10^8 populations obtained in this work in 3 weeks.

Effect of microbial population on degree of beneficiation at 25°C

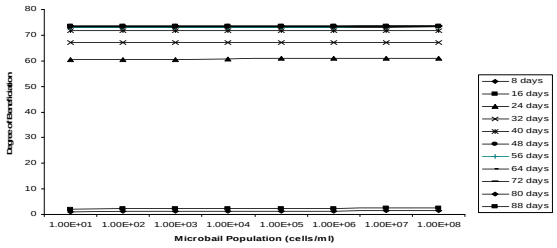

Fig. 4 Effect of Microbial Population on Degree of Beneficiation

Figure 4 shows the effect of microbial population on degree of beneficiation. Released metals can be accumulated in biofilms by complexation with active moieties present in exopolymeric substances (Corzo et al, 1994; Comte et al, 2006). For example, extensive accumulation of ferric ion in biofilm exopolymeric substance through a complexation mechanism has been reported in the pyrite – Acidothiobacillus ferrooxidans system and this feature modulated the bacterial colonization of the pyrite surface (Kinzler et al, 2003).

This accounts for the initial drop in the degree of beneficiation between 8th day and 16th day of treatment as depicted in the graph. There was no change in degree of beneficiation as the microbial population increases. The increase in microbial population did not show appreciable increase in degree of beneficiation between 16th and 24th days is due to increased activities of bacterium as earlier reported.

Effect of fungal or bacterial strains on dephosphorization.

From a biogeochemical standpoint, these fungal filaments or bacteria accomplish three main functions during the bio dephosphorization process Gadd, (2006) namely: i. Sensing the environment, in order to find nutrient sources (i.e phosphorus bearing phases in the ore); ii. Exempting metabolites that may help to exploit the nutrients sources and release nutritive elements. (For example, organic acids that attack the phosphorus bearing phases in the ore and liberate phosphorus in a soluble form) and iii. Taking in solubilized nutrients for bacterial or fungal growth.

Conclusion

The phosphorus removal was found to be feasible using bacterium, identified as Acidothiobacillus Ferroodxidans. Interpretation of the information supplied by the examination of the bacterium mineral interactions indicated, however, that the dephosphorization process was affected. Dephosphorization yields were high and long term treatments of 40 days were needed to achieve 79.34 % degree of dephosphorization and 71.84% of degree of beneficiation using 10^8 microbial populations at 25°C.

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