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Full Length Research Paper

Removal of phosphorus from Nigeria's Agbaja iron ore through the degradation ability of *Micrococcus* species

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Study on the potential of *Micrococcus* species to remove phosphorus (P) from Nigeria's Agbaja iron ore was carried out by submerged culture technique. Findings reveal that *Micrococcus* species which was originally isolated from the ore samples solubilized phosphorus with 69.66% phosphorus removal rate. The microbe also completely accumulated iron (Fe) and cadmium (Cd) ions found in the medium while the uptake of copper (Cu), zinc (Zn) and manganese (Mn) were equally remarkable. However, microbial mortification occurred over time as a consequence of over-accumulation of trace metals and other antimicrobials which reduced further solubilization. The study shows that phosphorus can reasonably be removed by the agent *Micrococcus* species but its capacity was acutely hampered by a rapid decline in microbial population after the 4th week of experimentation. Further work is suggested with respect to possibilities of microbial metabolic wastes timely removal and disposal which may prolong the phosphorus removal capability of the microbe.

Key words: Ore, culture, microbes, biodegradation, phosphorus, serial, dilution.

INTRODUCTION

The Nigeria's Agbaja iron ore reserve, which according to Uwadiale (1991) is over 1.2 billion tons, is part of a much larger formation called the 'Lokoja Ironstone', covering a surface area of 400 km^2 and contains at minimum 2,300 million tons (Astier et al., 1989). The Agbaja iron ore reserve with an estimated 47% Fe content is, however, also associated with high phosphorus (P) content and has been categorized as nonbeneficial (Amadi et al., 1982; Uwadiale and Nwoke, 1983). Phosphorus is a deleterious inclusion in steel as it causes brittleness and fracture at low stress values. Allowable phosphorus concentration in high quality steel is in the range of 0.03 to 0.02 wt.% or less (Kudrin, 1985). The twin problems of the high-phosphorus content and beneficiation difficulties, which were subjects of sustained investigation by many researchers in the early 1980s were not addressed and it led to the abandonment of the reserve.

The purpose of this study is to remove phosphorus from Nigeria's Agbaja iron ore through degradation ability of *Micrococcus* species. The works of investigators on mineral processing through the use of microorganisms abound and are well documented (Rawlings, 2002; Delvasto et al., 2005; Anyakwo and Obot, 2008, 2010). The choice of *Micrococcus* species of bacteria as the removing agent was prompted by the fact of their common environmental association with the ore, and it is expected that for the same reason, it should be able to remove it. The future prospects of this approach are enormous both to the environment and metallurgy for as long as wastes from this approach pose no threats and phosphorus can be removed in a comparatively less expensive way.

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MATERIALS AND METHODS

Raw iron ore was obtained from Agbaja in Kogi State of Nigeria. It was crushed with hammer and anvil and sieved with Shital test kits to generate 0.50/0.25 mm particle size distribution. Precisely, 20 g of this sample was subjected to compositional analysis. The concentrations of Fe, MgO, Cu_2O, ZnO and MnO_2 were determined

Table 1. Composition (%) of Nigeria's Agbaja iron ore

Component	FeT	SiO_2	P_2O_5	MgO	Cu_2O	ZnO	S	MnO_2	Al_2O_3
Level	51.50	0.57	1.25	0.08	0.005	0.091	3.25	0.001	34.77

using AAS (UniCam 939). The level of sulfur (S) was determined by Eschaka method, while Al_2O_3 by titrimetry. Also measured were SiO_2 and P_2O_5 by colorimetry method using ammonium molybdate and ammonium vanadate, respectively.

To isolate microbes with strong solubilizing potential, 10 g of sample was placed in 90 ml distilled water in 250 ml conical flask and then serially diluted to 10^{-6}, in order to decongest possible available microbes and allow for a moderate growth to occur. At the end of 14 days bacteria found on the ore surface were cultured by spread plate technique on Bacto - Nutrient Agar (NA). The NA plates were incubated appropriately at room temperature (28±2°C). Specific colonies were then sub-cultured on freshly prepared basal media and the pure cultures were characterized according to recommended procedures (Sneath and Holt, 1986; Alsina and Blanch, 1994).

The ability of the bacterial isolates to utilize the ore as their sole source of energy for growth was determined by the method of Okpokwasili and Okorie (1988) and Itah and Essien (2005) using the mineral salt medium (MSM) of Zajic and Supplison (1972). Briefly, 10 ml of MSM were dispensed into each test tube and then 2% (0.2 g) of the milled ore sample was added. The ore-supplemented medium was then sterilized by autoclaving at 121°C for 15 min under 15 psi atmospheres. Thereafter, 1 ml of 18 h old tryptone soya broth culture of the bacterial isolates were aseptically seeded into the ore-supplemented MSM and then incubated undisturbed at 28±2°C for 3 weeks. Un-inoculated tubes were included for each test isolate to serve as controls.

The population dynamics of the bacteria test isolates was used as the index of ability to utilize the ore medium for growth. The growth rate of the isolates was graded as high (+++), moderate (++), minimal (+) and no growth (-). Among the isolates with strong capability to utilize ore based substrate for growth, *Micrococcus* species was one of the most prevalent and was subsequently selected for the phosphorus removal studies.

In order to determine the phosphorus solubilizing capability of *Micrococcus* species, the submerged culture technique was adopted. In this procedure, 100 ml of nutrient broth (NB) was dispensed into 250 ml conical flasks and thereafter supplemented with milled ore. The ore supplemented NB was sterilized as before and allowed to cool after which 1 ml of the solubilizing agent was seeded into each flask. Un-inoculated flasks and flasks inoculated with the test organisms but without ore samples served as the control. They were left to stand for 10 weeks, during which representative samples were removed at weekly basis for the determination of phosphorus content, pH and growth of the solubilizing agent.

The amount of phosphorus in ore-NB was determined by volumetric analytical technique (Jain, 1982) in which, ammonium phospho-molybdate precipitate was obtained and phosphorus concentration in it analyzed by titrating the precipitate with 0.1 N HCl using 4 to 5 drops of phenolphthalein as indicator. The pH of the fermentation broth was determined using a pH meter (EIL 7020, Kent Industrial measurement Ltd). The growth of the solubilizing agent, *Micrococcus* species, in the ore supplemented broth culture was determined by pour plate using freshly prepared NA. The NA plates were incubated for 24 h at room temperature after which the number of cells in colony forming unit per mili-litre was determined with the aid of colony counter.

Also determined before and at the end of phosphorus removal experiment, was the trace metals levels in the ore-supplemented medium (ore+NB) and in ore+NB inoculated with *Micrococcus* species. The concentrations of iron (Fe), copper (Cu), cadmium (Cd), zinc (Zn), nickel (Ni), manganese (Mn) and lead (Pb) in the substrates were determined with AAS after digestion with a solution of concentrated HNO_3 (0.3 ml) and HCl (6.0 ml) (Binning and Baird, 2001).

RESULTS AND DISCUSSION

The results of ore compositional analysis data are shown in Table 1. Loss on ignition was taken at 939°C. The results revealed that Agbaja ore was rich in Fe content and as well confirmed the high-phosphorus and high-alumina status of the ore which had earlier been reported by Uwadiale, (1989).

The curve of the weight percent phosphorus content during phosphorus removal from the ore by *Micrococcus* species for 10 weeks is shown in Figure 1. It shows that the phosphorus removal started rapidly and slowed down between 1 and 2 weeks.

Thereafter, a sinusoidal removal tendency was observed possibly due to the effect of the life cycle of the bacterium on the removal process, with the upper and lower curves coinciding for the periods of maximum and minimum cells population, respectively. Not much removal was observed from 8th week till the end of the experiment.

The growth curve of *Micrococcus* species during phosphorus removal for 10 weeks is shown in Figure 2. The cells population grew exponentially to a maximum at about 4th week and soon after declined till the 6th week. A period of poor growth was observed from the 6th to 8th week when the population suddenly declined and finally remained stagnant to the end.

The substrates pH obtained during phosphorus removal from the ore for 10 weeks is shown in Figure 3. As shown in Figure 3, the pH which began in a relatively weak acid region gradually progressed into a completely basic region approaching pH 10 by the end of the experiment in 10th week.

The variation of phosphorus content, substrate pH and the log of density of *Micrococcus* species during phosphorus removal from the ore for 10 weeks is shown in Figure 4. The Figure 4 shows that the initial phosphorus removal was less rapid at the end of 1st week. Phosphorus seemed to have reverted to the ore in 2nd week thereafter, the phosphorus removal continued smoothly becoming insignificant from 9th week and terminating at 0.267 wt.% by the end of the experiment. Figure 4 also shows that the cells population grew rapidly and climaxed in 4th week. The maximum phosphorus

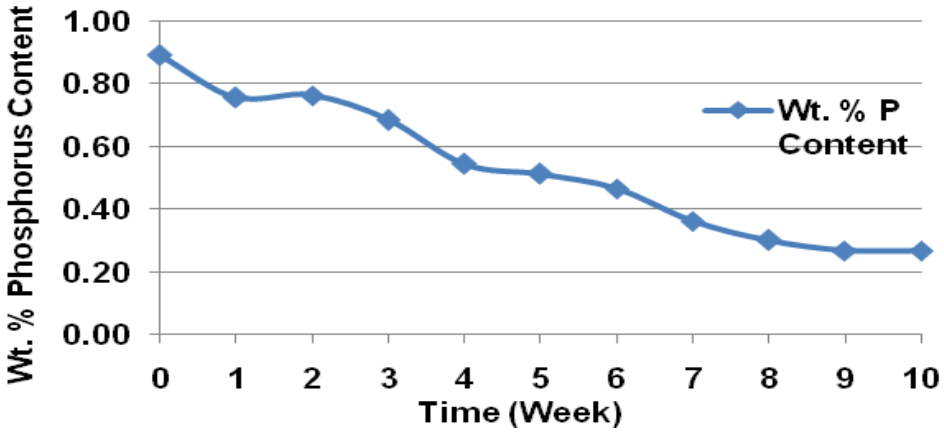

Figure 1. Curve of weight percent phosphorus content versus time during phosphorus removal from Nigeria's Agbaja iron ore 0.50/0.25 mm by *Micrococcus* species for 10 weeks.

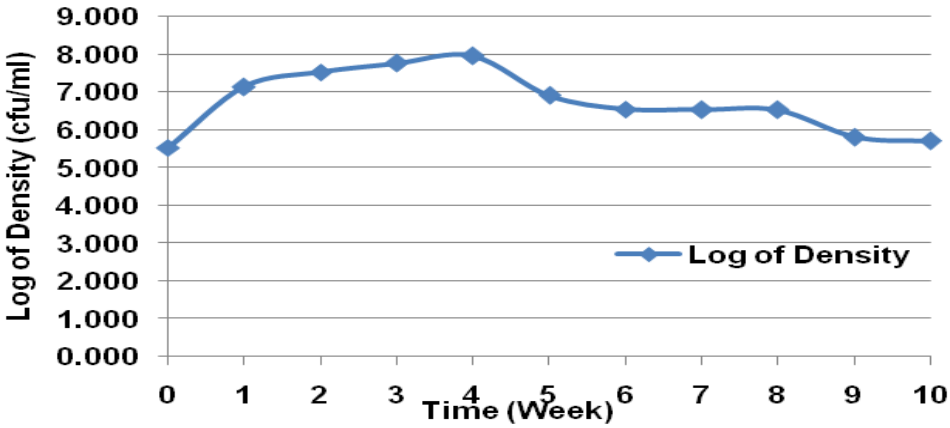

Figure 2. Growth of *Micrococcus* species during phosphorus removal from Nigeria's Agbaja iron ore 0.50/0.25 mm for 10 weeks.

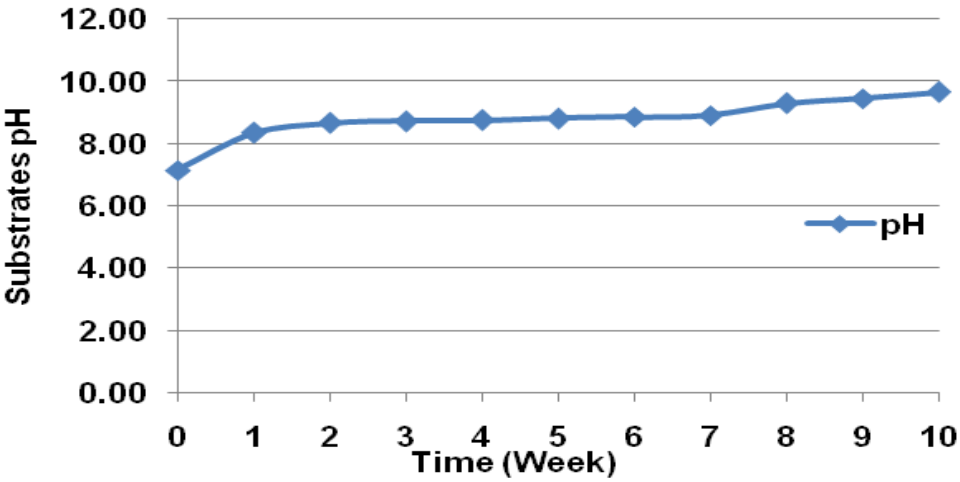

Figure 3. Curve of substrates pH versus time during phosphorus removal from Nigeria's Agbaja iron ore 0.50/0.25 mm by *Micrococcus* species for 10 weeks.

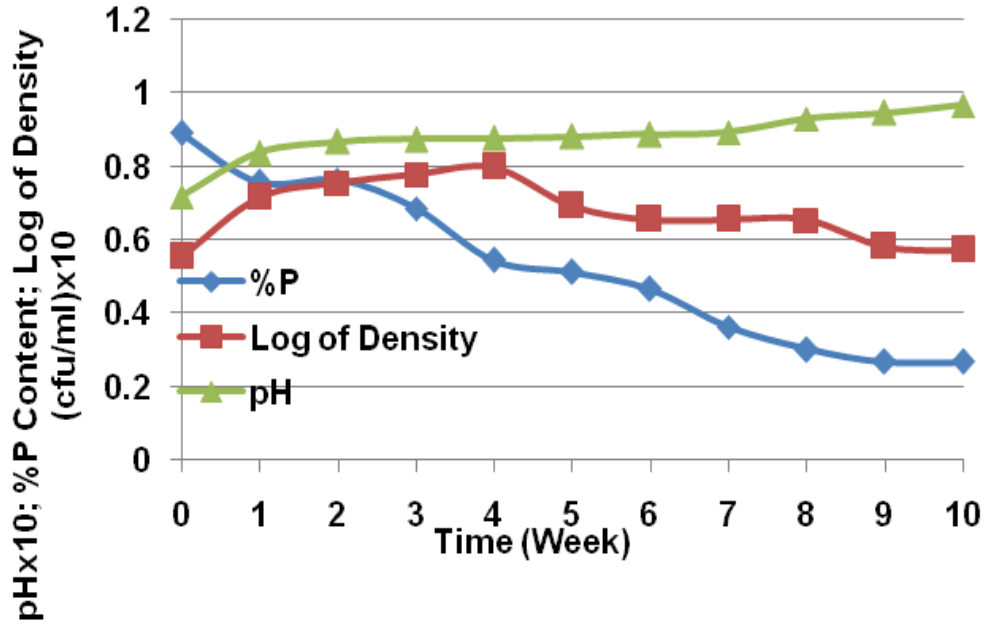

Figure 4. Variation of phosphorus content, substrates pH and log of density of *Micrococcus* species during phosphorus removal from Nigeria's Agbaja iron ore 0.50/0.25 mm for 10 weeks.

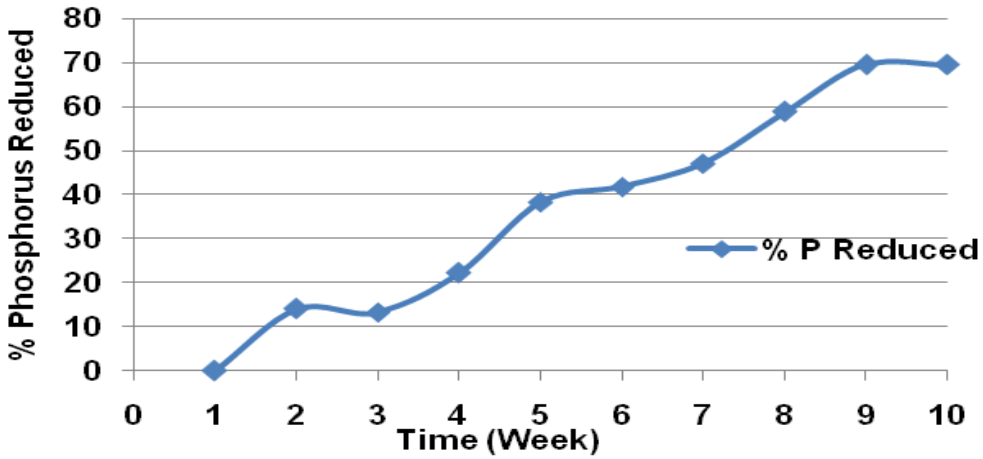

Figure 5. Curve of percent phosphorus removed versus time for Nigeria's Agbaja iron ore 0.50/0.25 mm by *Micrococcus* species for 10 weeks.

removal was sustained during the period of cumulative cells growth which lasted till 9th week. The pH of the NB medium after the initial week shows that the removal proceeded in a basic medium permanently till the end of the experiment in 10th week.

Figure 5 shows the percentage phosphorus removed by *Micrococcus* species in the course of 10th weeks and 69.66% was the maximum. It is observed that apart from the smooth removal gaps encountered between 2nd and 3rd weeks, and also between 9th and 10th weeks when the cells population might have suffered some set back, the bacterium progressively removed phosphorus from the ore sample during the period of experimentation.

The fluctuation in trace metals concentration in the ore-supplemented NB for 10 weeks during phosphorus removal by *Micrococcus* species is shown in Figures 6 and 7. Comparing the analytical results of control (NB-pure) and the NB+*Micrococcus* species without ore (control), it is observed that *Micrococcus* species merely accumulated 0.80% Zn and released more ions of Fe, Cu, Cd which led to a respective increase in their concentrations from 3.0782 to 10.1399 ppm, 0.0907 to

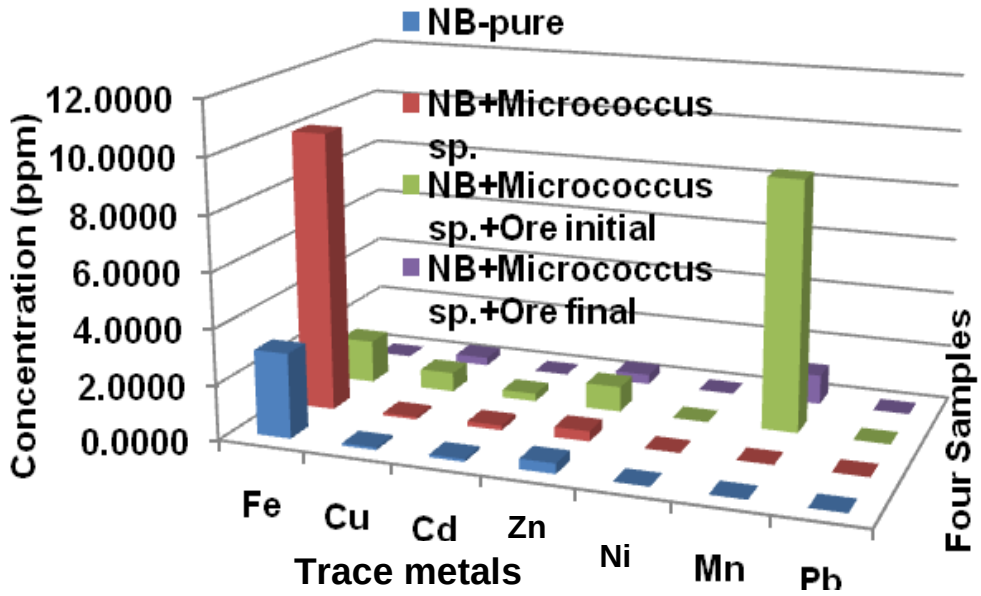

Figure 6. Analytical results of NB medium cultures during phosphorus removal from Nigeria's Agbaja iron ore 0.50/0.25 mm by *Micrococcus* species for 10 weeks

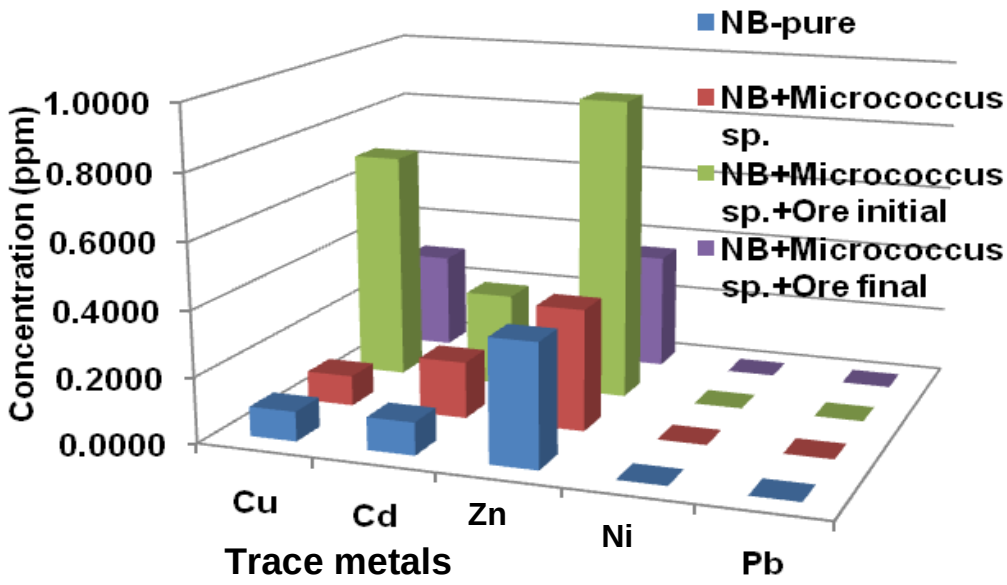

Figure 7. Analytical results of NB medium cultures during phosphorus removal from Nigeria's Agbaja iron ore 0.50/0.25 mm by *Micrococcus* species for 10 weeks.

0.0929 ppm and 0.0995 to 0.1736 ppm. Ni, Mn and Pb ions were absent in the controls. In comparing the NB + *Micrococcus* species + ore's initial and final results, it was also apparent that the same microorganism which demonstrated very poor sensitivity earlier on to Fe, Cu and Cd ions after adjusting to the medium's environment, became highly sensitive to and accumulated 100% Fe from its initial 1.5614 ppm, 58% Cu from 0.7014 ppm, 100% Cd from 0.2869 ppm, 61.49% Zn from 0.9247 ppm and 88.15% Mn from 9.0823 ppm. Ni and Pb ions were absent in the medium. A fact which the above comparisons has established is that *Micrococcus* species actually accumulated most of the trace metals in the fermented broth medium and in the test medium and in some cases accumulated all available ions of a metal. This development therefore, may be the reason the cells population was declining which consequently may have affected the removal process due to cells lysis (Mohapatra, 2008). Figure 7 is a scale-up modification of Figure 6 without the values for Fe and Mn.

CONCLUSION AND RECOMMENDATION

The present study on the phosphorus removal from Nigeria's Agbaja iron ore samples using a biological agent, *Micrococcus* species has revealed positive effects. 69.66% of phosphorus was solubilized by the microorganism. Phosphorus utilization by the bacterium resulted in growth and concomitant production of basic metabolic products and adsorption of detectable concentrations of Fe, Cu, Pb, Zn, Cd and Mn from the samples. The metabolic activity of the bacterium in the fermentation broth was remarkable but later was reduced plausibly as a result of over-accumulation of toxic metabolites and or exhaustion of available nutrients. These resulted in cells lysis and death. What this means is that the microbial route for the removal of phosphorus is successful and encouraging provided the metabolic wastes associated with the process can be well managed. It is possible that removal can be continuous in view of microbial exponential growth rate.

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REFERENCES

Alsina M, Blanch AR (1994). A set of keys for biochemical identification of environmental Vibrios species. J. Appl. Bacteriol., 74: 79-85.

Amadi NJ, Odunaike AA, Mathur JP (1982). Preliminary Bench Scale Beneficiation Studies with Three Lumps of Iron Ore Sample from Agbaja. (Tech. Rep.). Central Metallurgical Research and Development Centre, Jos, Nigeria.

Anyakwo CN, Obot OW (2008). Phosphorus Removal from Nigeria's Agbaja Iron Ore by Aspergillus Niger. IREJEST. 5(1): 54-58.

Anyakwo CN, Obot OW (2010). Phosphorus Removal Capability of *Aspergillus terreus* and *Bacillus subtilis* from Nigeria's Agbaja Iron Ore. JMMCE. 9(12): 1131-1138.

Astier JE, Donzeau M, Uwadiale GGOO (1989). The Loklja Oolitic Ironstone Deposit: Possible Use in the Ajaokuta Iron and Steel Plant. J. Min. Geol., 25: 1-2.

Binning K, Baird D (2001). Survey of heavy metals in the sediments of the Swatkop River Estuary, Port Elizabeth South Africa. Water SA 24(4): 461-466.

Delvasto P, Ballester A, Muñoz JA, González F, Blázquez ML, García-Balboa C (2005). Exploring the Possibilities of Biological Beneficiation of Iron-ores: The Phosphorus Problem. Proceedings of the 15th Steelmaking Conference, 5th Ironmaking Conference & 1st Environment and Recycling Symposium IAS (CD-ROM). Argentinean Steelmaking Institute (IAS). San Nicolás, Buenos Aires, Argentina, pp.71-82.

Itah AY, Essien JP (2005). Growth profile and hydrocarbonoclastic potential of microorganisms isolated from tarballs in the Bight of Bonny, Nigeria, World J. Microbiol. Biotechnol., 21: 1317-1322.

Jain SK (1982). An Introduction to Metallurgical Analysis: Chemical and Instrumental, India, New Delhi, Vikas Publishing House.

Kudrin V (1985). Steel Making. Moscow: MIR Publishers.

Mohapatra PK (2008). Textbook of Environmental Microbiology. I.K. International Publishing House Pvt Ltd, New Delhi.

Okpokwasili GC, Okorie BB (1988). Biodeterioration potentials of microorganisms isolated from car engine lubricating oil. Tribol. Int. 21: 215-220.

Rawlings DE (2002). Heavy Metals Mining using Microbes. Annu. Rev. Microbiol., 56: 65-91. Downloaded 2 December, 2010 from www.ncbi.nlm.nih.gov/pubmed/12142493.

Sneath HAP, Holt GJ (1986). Bergey's Manual of Systematic Bacteriology, Vol. 2, Williams and Wilkins, Baltimore, London, 1599pp.

Uwadiale GGOO (1989). Upgrading Nigerian Iron Ores. J. Miner. Metallurgical Process., AIME, 117-123.

Uwadiale GGOO (1991). Electrolytic Coagulation and Selective Flocculation of Agbaja Iron Ore, J. Min. Geol., 27(1): 77-85.

Uwadiale GGOO, Nwoke MAU (1983). Beneficiation of Agbaja Iron Ore by Reduction Roasting- Magnetic Separation: Semi Pilot Plant Scale-up and Establishment of Residence Point of Phosphorus, National Steel Council, Metallurgical Research and Tests Division, Jos, Nigeria.

Zajic JE, Supplison B (1972). Emulsification and Degradation of Bunker C. fuel oil by microorganisms. Biotechnol. Bioeng., 14: 331-343.