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Comparative Phosphorus Removal Capabilities of *Eurotium herbarorium* and *Clostridium* Species on Nigeria's Agbaja Iron Ore

Obotowo William Obot[1], Charles Nwachukwu Anyakwo[2]
[1]Department of Mechanical Engineering, Faculty of Engineering, University of Uyo, Uyo, Nigeria
[2]Department of Metallurgical and Materials Engineering, Federal University of Technology, Owerri, Nigeria
Email: obotowo2004@yahoo.com, charlesanyakwo@yahoo.com

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ABSTRACT

A study of phosphorus removal capabilities of *Eurotium herbarorium* and *Clostridium* species from Nigeria's Agbaja iron ore was carried out. Iron ore sample was crushed, sieved to obtain 0.50 mm/0.25 mm particle size distribution and cultured with mineral oil medium to facilitate microbial growth. Fungi and bacteria that concurrently grew were sub-cultured in Sabouard dextrose agar and nutrient agar solutions that support fungal and bacterial growth, respectively, and characterized using standard procedures. Ore was exposed to these microbes to effect phosphorus removal in standard media and later analyzed at weekly interval using the standard volumetric ammonium phospho-molybdate method. The fermentation broth media were analyzed for iron, copper, cadmium, zinc, nickel, manganese and lead using the atomic absorption spectrophotometer. The microorganisms markedly removed phosphorus from the ore with 61.48% and 69.20%, respectively. For the fungus pH remained in the acidic region and basic for the bacterium. Trace elements analyses of the initial and final ore-containing media recorded marked reduction in the concentration of these elements. A plausible explanation that is supported by literature is that the microorganisms accumulated them. This probably accounts for the drastic decrease in fungal biomass and bacterial density with the concomitant decrease in phosphorus removal observed towards the end.

Keywords: Microbes; Culture; Biodegradation; Trace; Metal; Biomass

1. Introduction

The Nigeria's Agbaja iron ore reserve with its rich iron content 47.5% - 51.50% Fe [1] is the country's largest but had long been abandoned due to its high phosphorrus status which researchers variously estimated at about 0.76 - 0.89 wt% [2,3]. The mineralogy of Agbaja iron ore revealed abundant goethite with minute magnetite and some hematite, pyrite, siderite and chlorite also identified Uwadiale [4]. A successful removal of phosphorus to a metallurgical acceptable level, therefore, from the over 1.2 billion tonnes of ore reserve Uwadiale [5] can mean an economic boom for the country with quite substantial multiplier effects in the areas of job and wealth creation for the downstream sectors of iron and steel industry.

The negative effects of phosphorus in high quality steels namely: the effect of steel brittleness coupled with the effect of strong primary segregation during solidification of castings and the formation of high phosphorus brittle streaks between metal grains which impede plastic deformation are undesirable and therefore should be minimized as much as possible. Thus, for high quality steels, the phosphorus acceptable level is in the range of 0.020 - 0.030 wt% or even less Kudrin [6].

A flurry of research activities into the removal of phosphorus from the Nigeria's Agbaja iron ore commenced in the eighties. Researchers predominantly applied the conventional froth flotation technique for the removal of phosphorus but failed because the phosphorus is not associated with the gangue but is in bonding with the iron [3,7]. An evolving trend in mineral processing currently gaining popularity is the use of microbes and the works of researchers in this regards are well documented [8-10].

The current work therefore intends to adopt the microbial degradation approach for comparative investigations of the phosphorus removal capabilities of *Eurotium herbarorium* and *Clostridium* species on Nigeria's Agbaja iron ore. This approach is cheap, environmental friendly and has a potential for easy incorporation into existing iron and steel making technologies.

2. Materials and Methods

The major raw material, iron ore, was obtained from Agbaja in Lokoja area of Kogi State of Nigeria. A sizable piece of the iron ore was crushed in the laboratory using hammer and anvil and then a standard laboratory Shital Test Kits was employed to sieve 0.50 mm/0.25 mm particle size distribution. This was analyzed for composition. The microbes for the experiment were those native to the iron ore environment and were obtained from the crushed ore sample. In order to generate sufficient amount of microbes necessary to cause degradation of phosphorus in the ore, the microbes were initially cultured in mineral oil medium (MOM). 10 g of the particle size of ore was serially diluted up to 10^{-6} (6 times by 10 folds dilution). The final dilution was taken to seed sterile petri dishes and about 20 ml of MOM at 45˚C was added to each seeded petri dish, swirled and allowed to set and thereafter incubated for 14 days. Some petri dishes were incubated aerobically and while others anaerobically. Bacteria and fungi colonies were counted and recorded in colony forming unit per milli-litre (cfu/ml). The fungi colonies were sub-cultured into Sabouard dextrose agar (SDA) and the bacterial colonies into Nutrient agar (NA). The growth colonies were characterized and identified using standard manuals for fungal and bacterial identification [11,12]. The isolates with stronger survival chances were *Eurotium herbarorium* and *Clostridium* species.

In order to allow biodegradation of phosphorus in the iron ore using the test organisms to take place, malt extract broth (MEB) and nutrient broth (NB) were prepared to standard and 100 ml of each dispensed into 250 ml conical flasks. The conical flasks were sterilized by autoclaving at 121˚C at 15 psi for 15 minutes. On cooling, 1 g of an already sterilized ore was mixed with each of the 100 ml MEB and NB media. 1 g wet weight of *Eurotium herbarorium* spores from the culture in petri dishes was used to inoculate each of the MEB, and 1ml of the *Clostridium* species broth culture was used to inoculate each of the NB aseptically. Some conical flasks of each of the media were not inoculated with the test organisms, some were inoculated with test organisms but without ore samples and they served as the control.

Finally, a batch of conical flasks representing both MEB and NB media were removed for phosphorus analysis weekly for 10 weeks. The treated ore samples were digested through the standard volumetric ammonium phosphomolybdate method [13] and the precipitate back titrated with 0.1 N-HCl solutions using phenolphthalein as indicator. The fermentation broth media were analyzed for iron (Fe), copper (Cu), cadmium (Cd), zinc (Zn), nickel (Ni), manganese (Mn) and lead (Pb) using UNICAM solaar 969 atomic absorption spectrophotometer (AAS). The pH of the fermentation broth media was monitored using pH meter (EIL 7020, Kent Industrial Measurement Ltd.). The population of the microbes was carefully monitored also within the period by harvesting the fungal mycelia in each MEB flask and drying on filter paper in the oven at 50˚C for 12 hours. Each dried mass was recorded. Bacterial count in colony forming unit per millilitre involved culturing 1 ml of fermented NB in fresh nutrient agar to allow the growth of *Clostridium* species which was estimated using the colony counter. The data obtained from the experiment are presented in **Figures 1**-**8**.

3. Results and Discussion

The result of iron ore compositional analysis is presented in **Table 1**. LOI (Loss on ignition) was obtained at 939˚C. The analysis of Fe, Mg, Cu, Zn and Mn was carried out using AAS (Unicam 939). P was analyzed by colorimetry using ammonium vanadate. S was analyzed by Eschka method. Al was analyzed by Titrimetry while SiO_2 by colorimetry using ammonium molybdate.

Table 1. Result of iron ore compositional analysis.

Mineral	$Fe_{(T)}$	SiO_2	P_2O_5	MgO	Cu_2O	ZnO	LOI	S	MnO_2	Al_2O_3
Fe_2O_3	51.50	0.57	1.25	0.08	0.005	0.091	8.01	3.25	0.001	34.77

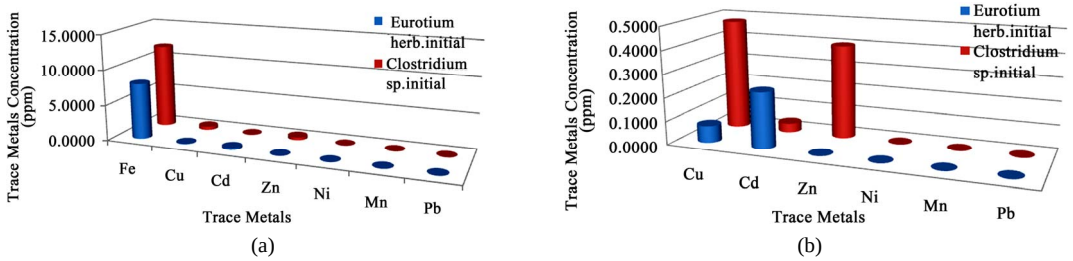

Figure 1. (a) MEB + *Eurotium herbarorium* and NB + *Clostridium* species Initial Analytical Results without Nigeria's Agbaja iron ore 0.50/0.25 mm samples; (b) MEB + *Eurotium herbarorium* and NB + *Clostridium* species initial analytical results without Nigeria's Agbaja iron ore 0.50/0.25 mm samples.

Figure 1(a) presents the result of initial analyses of MEB + *Eurotium herbarorium* and NB + *Clostridium* species without the iron ore samples. It shows the concentration of the trace metals in the broth cultures in the presence of the microorganisms. The MEB does not contain Zn, Ni, MN and Pb but has some amounts of Fe, Cu and Cd ions. On the other hand, NB contains Fe, Cu, Cd and Zn but no Ni, Mn and Pb ions. **Figure 1(b)** is a scale up modification of **Figure 1(a)** without values for Fe ions concentration to give better visualization of amounts of the other very insignificant trace metals.

Figures 2(a) and **(b)** present the initial analytical results of MEB + *Eurotium herbarorium* and NB + *Clostridium* species cultures with the ore sample. **Figure 2(b)** is a scale up modification of **Figure 2(a)** without values for Fe concentration, again for visualization effect. Comparing **Figures 1** and **2** shows that the introduction of iron ore sample into both MEB and NB cultures, resulted in increase in the trace metals concentration. For MEB inoculated with *Eurotium herbarorium*, Zn, Mn and Pb ions increased 31%, 317% and 105%, respectively. NB inoculated with *Clostridium* species during the same interval recorded 5.5% and 424% increase for Ni and Pb, respectively. The trace metals increase is expected with the introduction of the ore as this has increased whatever concentration of trace metals that came with the MEB medium. However, it is observed that in the MEB culture, the concentration of Fe and Cd decreased by 11% and 80% respectively. It is possible that the decrease in trace metals is due to bio-accumulation by the microbes.

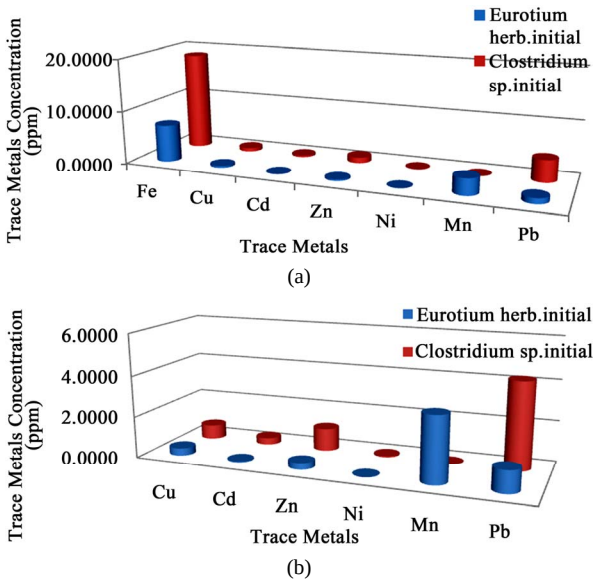

Figure 2. (a) Concentration of trace metals in broth media during phosphorus removal from Nigeria's Agbaja iron ore by *Eurotium herbarorium* and *Clostridiumn* species for 10 weeks; (b) Initial concentration of trace metals in broth media during phosphorus removal from Nigeria's Agbaja iron ore by *Eurotium herbarorium* and *Clostridium* species.

Comparing **Figures 2** and **3** shows trace metals sharp decrease. It is possible that in the MEB culture *Eurotium herbarorium* accumulated 100% of Cu, Cd and Pb; 68% Fe, 49% Zn and 89% Mn ions at the end of 10 weeks experimentation. During the same period *Clostridium* species in NB culture possibly also accumulated 100% Fe and Cd, also 60% Zn and 79% Pb. In NB culture 87% Cu, 92% Ni and 38% Mn ions were released back. It is confirmed in literature that the microorganisms can accumulate trace metals under favourable conditions and also release same back to the environment if the conditions change. It is equally known that the over-accumulation of trace metals disrupt the normal functioning cells due to toxicity [14]. The reduction in cells population observed in **Figure 5**, especially towards the end of experimentation with its concomitant reduction in phosphorus removal capability of the microbes about the same period, may be facts to show why the stagnation noticed from the 7th week till the end of the experiment occurred. It is most probable that the microbes having over-accumulated trace metals especially Pb, simply died and this could have affected this route of processing iron ore to reduce phosphorus.

The removal of phosphorus in the ore by *Eurotium herbarorium* and *Clostridium* species for 10 weeks is presented in **Figure 4**. During this period, the initial phosphorus content in the ore 0.89 wt% reduced to 0.339 wt% and 0.271 wt%, respectively.

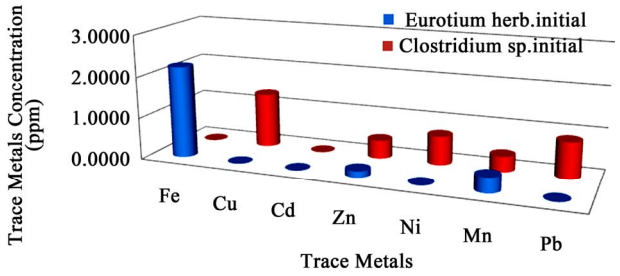

Figure 3. Concentration of trace metals in broth media during phosphorus removal from Nigeria's Agbaja iron ore by *Eurotium herbarorium* and *Clostridium* species at end of 10 weeks.

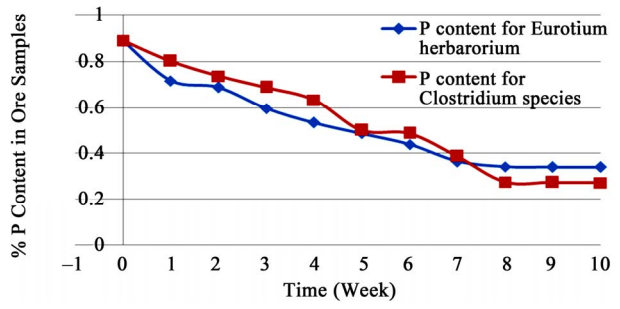

Figure 4. Variation of wt% *P* content during phosphorus removal by *Eurotium herbarorium* and *Clostridium* species from Nigeria's Agbaja iron ore 0.50/0.25 mm for 10 weeks.

The variation of the biomass weight of *Eurotium herbarorium* in MEB and the log of the density of *Clostridium* species in NB submerged media bearing the iron ore with time is shown in **Figure 5**.

The variation of the pH of the MEB and NB cultures with time during the experimentation period is shown in **Figure 6**.

Figure 7 presents the percent phosphorus reduced by *Eurotium herbarorium* and *Clostridium* species in 10 weeks. The percent phosphorus reduced from the ore was calculated using Equation (1):

$$\%P = \frac{\%P_{\text{initial}} - \%P_{\text{final}}}{\%P_{\text{initial}}} \times 100 \quad (1)$$

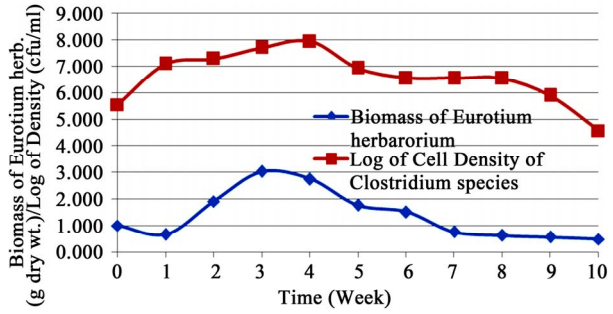

Figure 5. Growth of *Enrotitum herharorium* and *Clostridium* species during phosphorus removal from Nigeria's Agbaja iron ore 0.50/0.25 mm for 10 weeks.

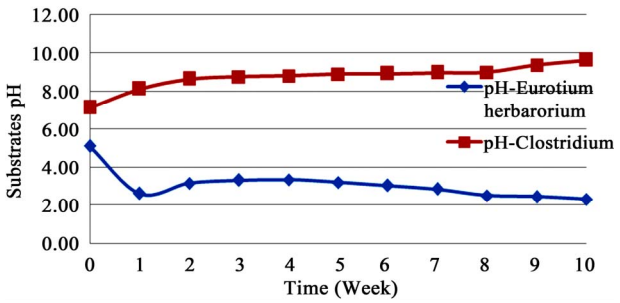

Figure 6. Curve of subsrates pH vs time during phosphorus removal from Nigeria's Agbaja iron ore 0.50/0.25 mm by *Eurotium herbarorium* and *Clostridium* species for 10 weeks.

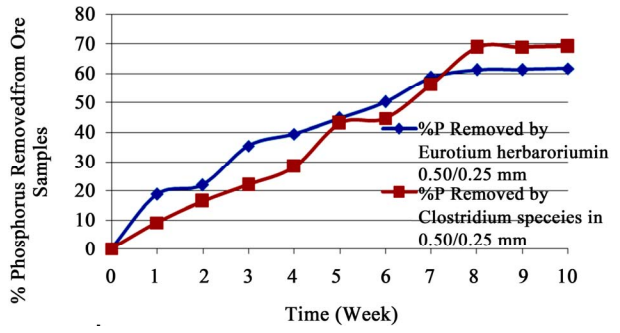

Figure 7. Curve of % phosphorus removed vs time for Nigeria's Agbaja iron ore by *Eurotium herbarorium* and *Clostridium* species for 10 weeks.

$\%P_{\text{initial}}$ —The initial wt% phosphorus content in ore

$\%P_{\text{final}}$ —The final wt% phosphorus in treated ore.

Figure 8(a) shows that in the first week, an initial rapid drop in biomass weight that also corresponded to a rapid drop in ore's phosphorus concentration occurred. Thereafter for a period of two weeks a marked increase in biomass weight that peaked at the third week took place while phosphorus intake by the fungus continued to take place. Beyond this point the biomass weight began to decrease rapidly while phosphorus intake also continued. It is however seen that when the biomass weight dropped to 1 g dry weight, phosphorus removal attained its lowest value and below this value no further phosphorus removal effectively continued. The highest percent cumulative removal for *Eurotium herbarorium* was 61.48%. Removal occurred in an increasing acidity culture attaining a pH value 2.30 in 10 weeks.

Figure 8(b) shows on the other hand, a gradual phosphorus removal in the ore occurring throughout the period thus lowering its content from an initial value of 0.890 wt% to 0.271 wt%. The same period experienced rapid cells multiplication from an initial log of cfu/ml 5.531 to a climax 7.922 in the 4th week and then a gradual reduction in cells population. During this period the substrates pH value increased from 7.13 to 9.61. The

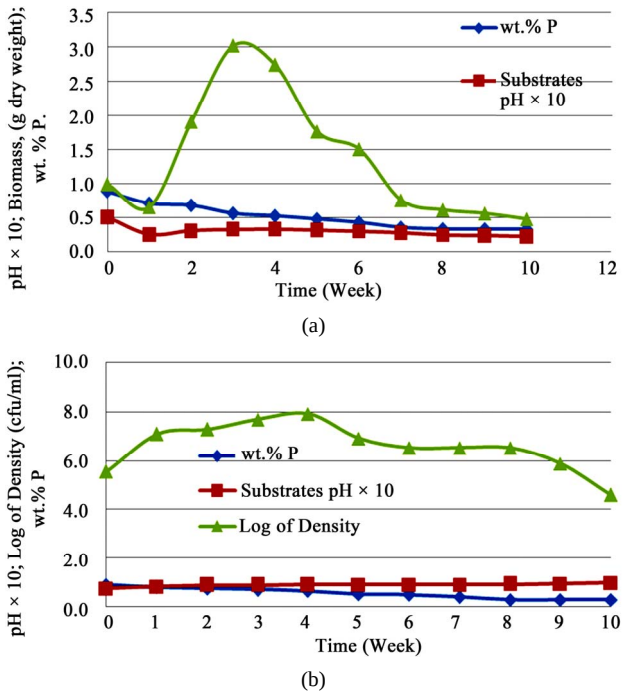

Figure 8. (a) Variation of iron ore phosphorus content, substrates pH and biomass weight during phosphorus removal from Nigeria's Agbaja iron ore 0.50/0.25 mm by *Eurotium herbarorium* for 10 weeks; (b) Variation of iron ore phosphorus content, substrates pH and log of density during phosphorus removal from Nigeria's Agbaja iron ore 0.50/ 0.25 mm by *Clostridium* species for 10 weeks.

period of a steady phosphorus removal was also coincident with the time of maximum cells growth. The highest phosphorus cumulative removal attained was 69.20%.

4. Conclusion and Recommendation

The comparative phosphorus removal capability of *Eurotium herbarorium* and *Clostridium* species on Nigeria's Agbaja iron ore was investigated with 61.48% and 69.20% cumulative removal respectively attained in 10 weeks. The initial rapid removal capacity shown in the fermentation broths till about the 6th and 7th weeks, respectively for the two organisms apparently could not be sustained due to over-accumulation of trace metals and other toxic waste products of biodegradation and this probably adversely affected the further phosphorus removal. It is recommended that further studies on the chemistry of metabolic broth wastes be considered as this may reveal the interacting parameters which can be advantageously harnessed for a continuous bio-degradation that is possible with microorganisms.

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