Bacillus Cereus Biodegradability of Phosphorus and other Heavy Metals in Nigeria's Agbaja Iron Ore

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***Abstract*—An investigation into the capacity of Bacillus cereus to degrade some of the heavy metals found in Nigeria's Agbaja iron ore was carried out in the laboratory for 10 weeks. As obtained iron ore from Agbaja community in Kogi State of Nigeria was crushed and sieved to select 0.50/0.25mm particle size. Bacillus cereus was one of the microorganisms identified after culturing a portion of the crushed iron ore in suitable media overtime. Some control samples were also set up that did not contain either the microbes and/or ore samples. At weekly interval, samples were removed and analyzed to obtain ammonium phospho-molybdate precipitate which was titrated with 0.1 N-HCl to determine the amount of phosphorus (P). Liquid samples were analyzed for heavy metals concentration using UniCam Solaar 969 AA spectrophotometer. Findings reveal that the microbes degraded 58.18% P and in the process accumulated some of the heavy metals: 82.17% Cu, 100% Cd and Fe, 8.12% Zn and 68.42% Pb. The pH of the media remained entirely basic attaining 9.63 at the end of the investigation. The log of microbial density which maximized in the 3rd week at 7.498 cfu/ml (colony forming unit/milliliter) showed serious decline by the 10th week to 4.763 cfu/ml. It is possible the microorganisms may have had a low degradation capacity as a result of heavy metals over-accumulation, especially that of Pb, known to be highly poisonous above certain concentrations. Recommendations include a further study of the broth media mixture with samples and microorganisms as findings may enhance an efficient metabolic wastes management and a sustainable microbial degradation of iron ore samples.**

***Index Terms*—Beneficiation; Iron ore; Microorganism; Phosphorus Accumulation**

I. Introduction

Nigeria falls among the richest countries of the world as far as human and natural resources are concerned. In mineral resources alone, the estimates of workable iron ore deposits stand in excess of 2.5 billion tones, most of which belong to hematite, hematite-magnetite, hematite-geothite and siderite-geothitie [1]. More than twenty iron ore deposits have been documented and reported in Nigeria [2]. The Agbaja iron ore deposit alone accounts for over 1.2 billion tones [3]. The Agbaja iron ore has been reported as high-phosphorus and high-alumina [4] and that the beneficiation attempts on the ore did not offer much hope of finding suitable beneficiation techniques because magnetic separation, froth flotation and gravity concentration failed [5], [6].

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In view of the above background therefore, a search for other possible beneficiation approaches have become necessary to see whether or not the Agbaja iron ore still has a chance at beneficiation. The current investigation is aimed at using the bio-sorption approach with Bacillus cereus as the degrading agent.

II. Materials and Methods

The major raw material for this research is the as-obtained and crushed iron ore, Figs. 1 and 2, respectively, which was obtained from Agbaja community, near Lokoja in Kogi State of Nigeria. The other materials like standard glass wares, chemicals, reagents and equipment were sourced locally.

Fig. 1. Agbaja Iron Ore (as obtained)

Fig. 2. Ore Sample 0.50/0.25mm

The ore was initially analyzed for compositional elements and compounds. Ore crushing was carried out with hammer and anvil and then sieved with Shital test kits to select 0.50/0.25mm particle size. 10g of it was serially diluted to 10-6 in 90ml of distilled water. The final dilution was taken to seed sterile petri dishes and 20ml of mineral oil medium (MOM) at 45 OC was added to each seeded petri dish, swirled and allowed to set and thereafter incubated for 14 days. The bacterial colonies were sub-cultured into nutrient agar (NA). The growth colonies were characterized and identified using standard manual for bacterial identification [7]. The isolate was Bacillus cereus.

In order to allow biodegradation of heavy metals in the iron ore using the test organism to take place, 100ml of nutrient broth (NB) was prepared and dispensed into sterilized 250ml conical flasks and autoclaved at 121 OC at 10 psi. On cooling, 1g of sterilized ore was mixed with each of the 100ml NB media. 1ml of Bacillus cereus broth culture was used to inoculate each of the NB aseptically. Some conical flasks were not inoculated with the test organism, and others were without samples and microbes and served as the controls.

Weekly, a set of samples were removed and analyzed through standard methods [8] and the obtained ammonium phospho-molybdate precipitate was back-titrated with 0.1 N-HCl to determine the quantity of P in the ore. Liquid samples were analyzed using UniCam Solaar 969 AA spectrophotometer with readability 0.0001 mg/l.

III. Results and Discussion

The ore elemental and compounds compositional analysis data are presented in Table I. Loss on ignition was taken at 939 OC. This result reveals the very nature of this particular ore as containing very high phosphorus and alumina, a position shared by earlier researchers.

TABLE I: Compositional Analysis of Nigeria's Agbaja Iron Ore

Entries	%, Content
Fe_T (total iron)	51.50
SiO_2	0.57
P_2O_5	1.25
MgO	0.08
Cu_2O	0.005
ZnO	0.091
S	3.25
MnO_2	0.001
Al_2O_3	34.77
Miscellaneous	8.483

In Fig. 3 a curve of weight % phosphorus (P) content for 10 weeks is shown with an initial 0.805 Wt. % P for Week 1. The degradation of P was progressive such that by the end of the 7th week 0.376 Wt. % P was achieved and by the 10th week the P content in the ore was as low as 0.367 Wt. %.

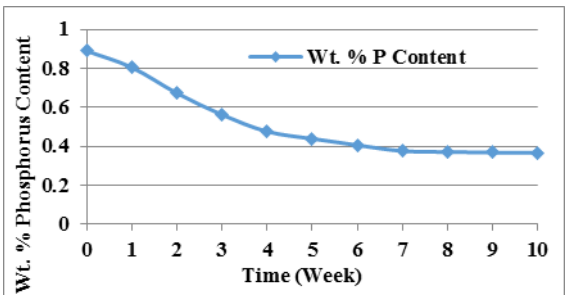

Fig. 3. Curve of Weight Percent Phosphorus Content vs. Time during Phosphorus Degradation in Nigeria's Agbaja Iron Ore. 0.50/0.25 mm by *Bacillus Cereus* for 10 Weeks

Fig. 4 presents the growth of Bacillus cereus during P and other heavy metals degradation for 10 weeks. The log of the initial cells population was 5.531 cfu/ml. This maximized to 7.835 cfu/ml by the 4th week and thereafter, dwindled to 4.763 cfu/ml by the 10th week. The reduction in cells population towards the end of degradation process may explain the microbial over-metabolization of heavy metals in the ore which, consequently, reduced their degradability capacity within the same period.

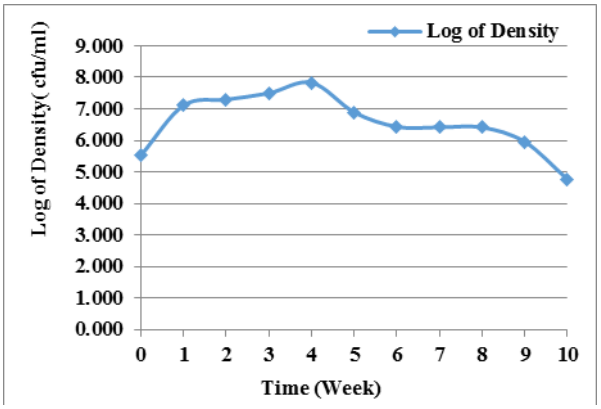

Fig. 4. Growth of *Bacillus Cereus* in NB Medium during Phosphorus Degradation in Nigeria's Agbaja Iron Ore. 0.50/0.25 mm for 10 Weeks

Fig. 5 presents the substrates pH. It is observed that the initial pH 7.18 decreased to 8.03 by the end of Week 1 and remained within the basic region coming down to 8.99 by the end of the 10th week. These data confirm the microbial functionability in an entirely basic media throughout the period of investigation which goes further to confirm the natural behavior of bacteria generally.

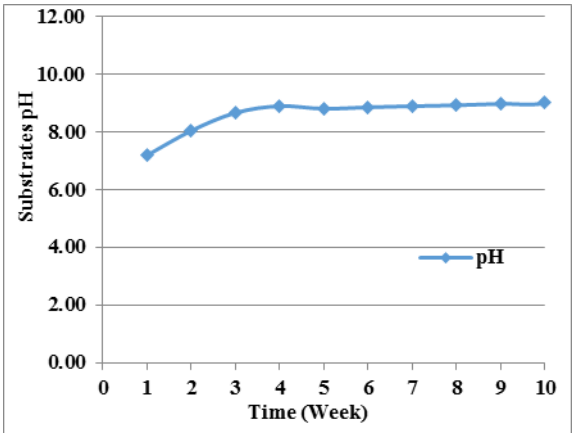

Fig. 5. Curve of Substrates pH vs. Time for Nigeria's Agbaja Iron Ore. 0.50/0.25 mm by Bacillus Cereus for 10 Weeks

Fig. 6 presents the trend of % P degraded, the substrates pH and the log of density of Bacillus cereus for the 10 weeks of investigation with ore samples. As is evident from the curves, it stands to reason that the optimum bio-degradation took place from about the 3rd week till the last week. Within this interval the microbial population which had reached its climax of 7.8325 cfu/ml between Weeks 3 and 5, thereafter averaged out with two compensating population troughs at 6.4220 cfu/ml and did not decrease significantly till the end of the investigation, thus corresponding to near stagnation in P and other heavy metals degradation from the 7th week till the end. This very significant period in the biodegradation process remained predominantly in the basic region pH 7.18 – 9.63. It satisfied the degrading microbes – a bacterial natural medium.

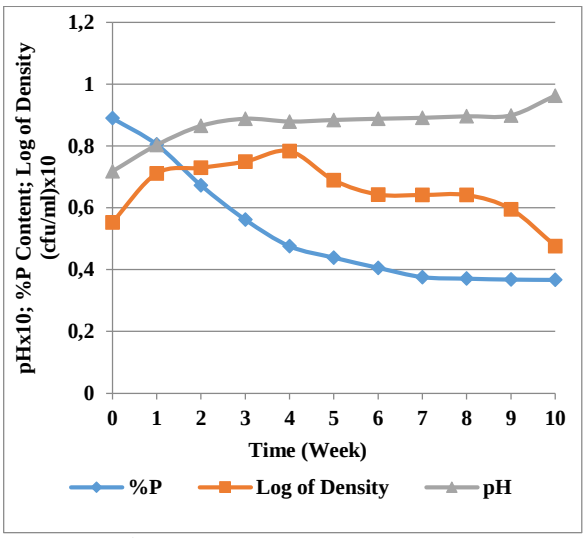

Fig. 6. Variation of Weight Percent Phosphorus Content, Substrates pH and Log of Density of *Bacillus cereus* during Phosphorus Degradation in Nigeria's Agbaja Iron Ore 0.50/0.25mm for 10 Weeks

Fig. 7 presents the % P degraded in the course of 10 weeks. It is observed that 57.27 % P was degraded by the end of Week 8 and thereafter the amount of P degraded proceeded very slowly such that by the 9th and 10th weeks respectively, the % P degraded by the microbes were 57.84 and 58.18, almost stagnated. It is observed that the metabolic activity of the microbes beginning from around the 6th week did not demonstrate the capacity for much more degradation as was the case from the outset (due to over-accumulation of heavy metals). One possible explanation for this noticeable tendency could be alluded to the fact of a microbial declining population in the submerged culture technique.

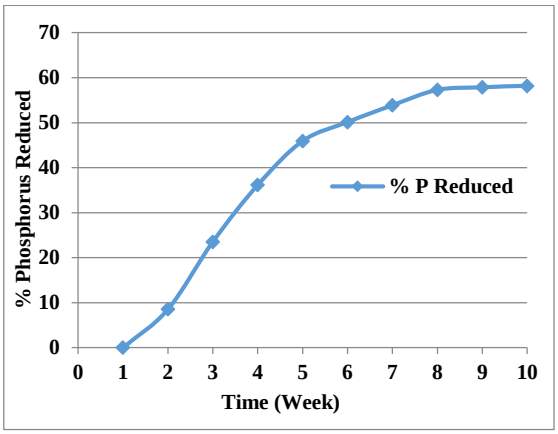

Fig. 7. Curve of Percent Phosphorus Degraded vs Time for Processed Nigeria's Agbaja Iron Ore for 10 Weeks by Bacillus cereus

The concentration of other heavy metals in the broth media at the initial instance and at the end of 10 weeks of investigation is presented in Fig. 8. The fluctuation of heavy metals concentration in the broth media during the degradation process clearly shows that Bacillus cereus has a higher affinity for some metals than others. At the end of 10 weeks it is observed that the microbes had actively accumulated all of Fe and Cd ions in the broth media in spite of their initial concentrations. They probably metabolized ferrous ions before any other ions as Fig. 8 shows. Cu ions became selectively and preferentially accumulated in the presence of more other ions apparently released as a consequence of solubilization of the ore samples. Zn ions were moderately metabolized, 8.12%. Pb ions which were absent in the pure NB and even after the NB was inoculated with the agent, suddenly became evident as the ore sample became introduced into the culture and amounted to 2.8228ppm. B. cereus, however, accumulated these ions and by the end of 10 weeks left behind a mere 0.8913ppm. Ni and Mn ions were permanently absent in the broth media.

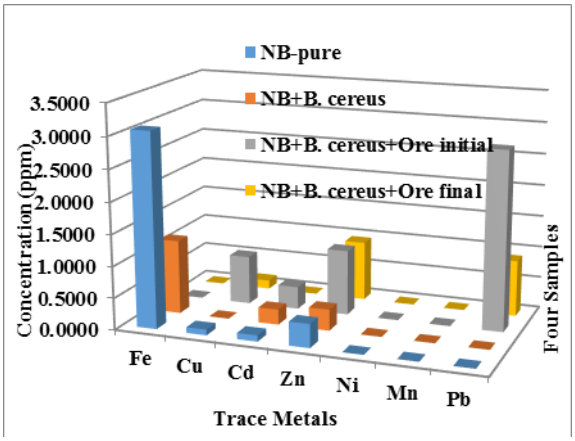

Fig. 8. Analytical Results of NB Medium Cultures during Phosphorus Degradation in Nigeria's Agbaja Iron Ore 0.50/0.25mm by *Bacillus cereus* for 10 Weeks

The biodegradability of phosphorus and other heavy metals in Nigeria's Agbaja iron ore by Bacillus cereus in the course of 10 weeks has been investigated. Findings reveal that the micro-organism has the capability to degrade 58.18% P, 100% Fe and Cd, 68.42% Pb, 8.12% Zn and 82.17% Cu found in the iron ore sample provided the metabolic wastes especially, those associated with Pb, Fe (possibly in the form of poisonous pyrites) and Cd are completely removed or passivated such that they become less harmful to the microbes. Furthermore, it is found that the pH of the sub-merged culture remained in the basic region throughout the period of investigation, pH 7.18 – 9.63. The micro-organism having actively accumulated some of these heavy metals especially Pb, may have probably depopulated as a consequence of over-accumulation, which apparently resulted in the stagnated P degradation noticed towards the end of the investigation.

It is recommended that further investigation on metabolic wastes management and full chemistry of the broth, ore samples and microorganisms mixture be undertaken, as knowledge gained may open up the optimal conditions for sustained microbial degradation of iron ores.

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