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Phosphorus Degradation Capability of *Aspergillus terreus* on Nigeria's Agbaja Iron Ore

C.N. Anyakwo[1] and O.W. Obot[2]

[1]Department of Metallurgical and Materials Engineering, Federal University of Technology, Owerri, Nigeria. Email: charlesanyakwo@yahoo.com

[2]Department of Mechanical Engineering, Faculty of Engineering, University of Uyo, Nigeria. Email: obotowo2004@yahoo.com

ABSTRACT

A microbial fungus - Aspergillus terreus was used to degrade phosphorus in Nigeria's Agbaja iron ore in the laboratory. The ore was first crushed to very tiny particles, screened using Shital test kits and 1.00/0.50mm, 0.50/0.25mm and 0.25/0.125mm particle size fractions were selected for the experiment. The microbes, obtained from the nascent remains on the ore environment, were cultured, used to inoculate 1g of sterile ore samples in 250ml conical flasks containing 100ml of equally sterile malt extract broth media and left to stand. At weekly interval, the samples were removed, treated through series of chemical reactions and ammonium phospho-molybdate precipitate was obtained. This was back-titrated with 0.1 N-HCl to determine the amount of phosphorus left in samples and consequently, the amount removed. Findings reveal that A. terreus is capable of degrading the ore samples. pH monitoring reveals that the P degradation process proceeded in a culture media of increasing acidity. It is recommended to further study the chemistry of the mixture of culture media, ore samples and microbes to find parameters that favour the degradation process.

Key words: *Ore, microbes, screening, broth, degradation, accumulation, inoculation*

1. INTRODUCTION

The Nigeria's Agbaja iron ore reserve analyzed 47.5%Fe [1]. It remains the country's largest reserve [2] but had long been abandoned due to the fact of very high phosphorus content variously estimated by researchers as 0.76 – 2.13% [1, 2, 3]. The mineralogy of Agbaja iron ore revealed abundant goethite with minute magnetite. Hematite, pyrite, siderite and chlorite were also identified [4, 5]. Any successful degradation of phosphorus in the ore to a metallurgical acceptable level of 0.03 - 0.02% [6] for high quality steels, will have the potential of transforming the national economy into a boom with quite substantial multiplier effects in the areas of job creation, wealth creation generally and much specifically in the downstream sector of iron and steel industry where the global demand for highest quality products cannot be compromised.

Phosphorus is a deleterious inclusion in steel that is responsible for steel brittleness causing it to fracture and snap at very low stress values, also associated with phosphorus are the problems of strong primary segregation during solidification of castings and the formation of high phosphorus brittle streaks between metal grains thereby impeding plastic deformation [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, 8]. The effort of researchers in the use of microbes in mineral processing with its promises of ecologically friendlier and cleaner environment is widely reported in recent time. For example, using the bacterium *Burkholderia caribensis* isolated from a Brazilian high-phosphorus iron ore, Delvasto et al [9] were able to mobilize between 5-20% of the phosphorus originally contained in the ore in 21 days of treatment in shake-flask cultures. Similarly in another work, *Aspergillus niger* also isolated from the same Brazilian high-phosphorus ore was employed to remove phosphorus from the same ore and again in 21 days between 13.8-33.2% was achieved [10]. In the beneficiation of iron ore slime *Aspergillus niger* and *Bacillus circulans* were able to remove alumina with *B. circulans* and *A. niger* showing 39 and 38 percents, respectively of alumina removal after 6 and 15 days, respectively of in situ leaching at 10% pulp density. Also a culture filtrate leaching with *A. niger* removed 20% alumina at 2% pulp density with 13 day old culture filtrate. *B. circulans* was more efficient than *A. niger* for selective removal of alumina [11]. Earlier on *A. niger* isolated from Nigeria's Agbaja iron ore was used to mobilize phosphorus from the same ore and in 49 days of leaching, 81%, 63% and 68% efficiencies for Mesh 5, Mesh 100 and Mesh 250 grain sizes, respectively were achieved [12]. In this present research, *Aspergillus terreus* – a microbial fungus was mobilized to degrade phosphorus in Nigeria's Agbaja iron ore for 10 weeks. During this period the pH of the broth media and the microbial population were monitored on weekly basis.

2. MATERIALS AND METHODS

The iron ore was collected from Agbaja, near Lokoja in Kogi State of Nigeria. Standard glass wares, heavy equipment, chemicals and disposables used in the degradation of phosphorus were sourced locally.

1kg of the iron ore was crushed to tiny particles and then screened using Shital Test sieves to obtain 1.00/0.50mm, 0.50/0.25mm and 0.25/0.125mm particle size fractions.

10 g of each of the iron ore particle size fractions was weighed using Adventurer-AR3130 (with readability 0.001g), placed in 90 ml of sterile water in 250 ml conical flask and then serially diluted to 10^{-6}. Using a pipette, 1 ml from each final dilution was taken to seed 10 sterile Petri dishes and about 20 ml of mineral oil medium at 45 ^{O}C was added to each seeded Petri dish, swirled and allowed to stand for 14 days. The growth of colony of microbes was observed and later sub-cultured in suitable media and then identified as *Aspergillus terreus* using the standard manual for fungal identification [13]. These microbes were preserved for subsequent use in the experiment.

Malt extract broth (MEB) powder was mixed with distilled water in accordance with standard procedure. 100ml of this was dispensed into 250ml conical flasks. The conical flasks were sterilized using autoclave at 121 ^{O}C at 10 psi for 15 minutes. On cooling, 1g of the already sterilized set of samples was accurately weighed and mixed with each of the 100ml MEB

medium earlier dispensed into the 250ml flasks. A loopful of the broth culture was used to inoculate each of the flasks aseptically. 2 conical flasks were not inoculated with the test organisms, and they served as the control.

Finally, a batch of 3 conical flasks representing each of the 3 particle size fractions was removed for phosphorus analysis weekly. The pH and cells count of the microbes were noted on weekly basis as well. The samples were treated through series of standard chemical reactions [14] to obtain ammonium phospho-molybdate precipitate which was back-titrated with 0.1 N-HCl to determine the amount of phosphorus left in samples and consequently, the amount removed. The data obtained from above procedure were organized and presented in Figures 1 – 5.

3. RESULTS AND DISCUSSION

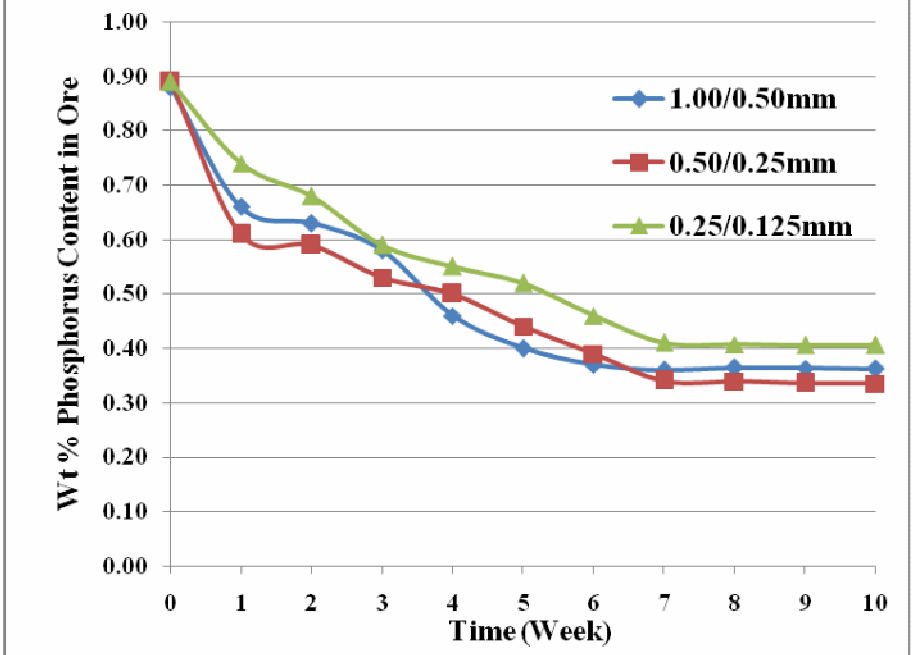

Figure 1 Curve of Weight Percent Phosphorus Content vs Time for Nigeria's Agbaja Iron Ore Degraded by *Aspergillus terreus* for 10 weeks

The result of P degradation activities by *A. terreus* in MEB medium of Nigeria's Abaja iron ore 1.00/0.50mm, 0.50/0.25mm and 0.25/0.125mm in the course of 10 weeks is presented in Figure 1. The above curve presents a progressive P degradation process from Week 1 to Week 7 and thereafter the process stagnated from Weeks 7 to 10. In all cases the initial phosphorus content for all the particle size fractions was 0.89 wt. %. The minimum phosphorus concentration attained for the particle size fractions of 1.00/0.50mm, 0.50/0.25mm and 0.25/0.125mm used for the experiments are respectively 0.362 wt. %, 0.335 wt. % and 0.406 wt. % at the end of 10 weeks experimental period. As the result shows, the best degradation took place in 0.50/0.25mm particle size fraction.

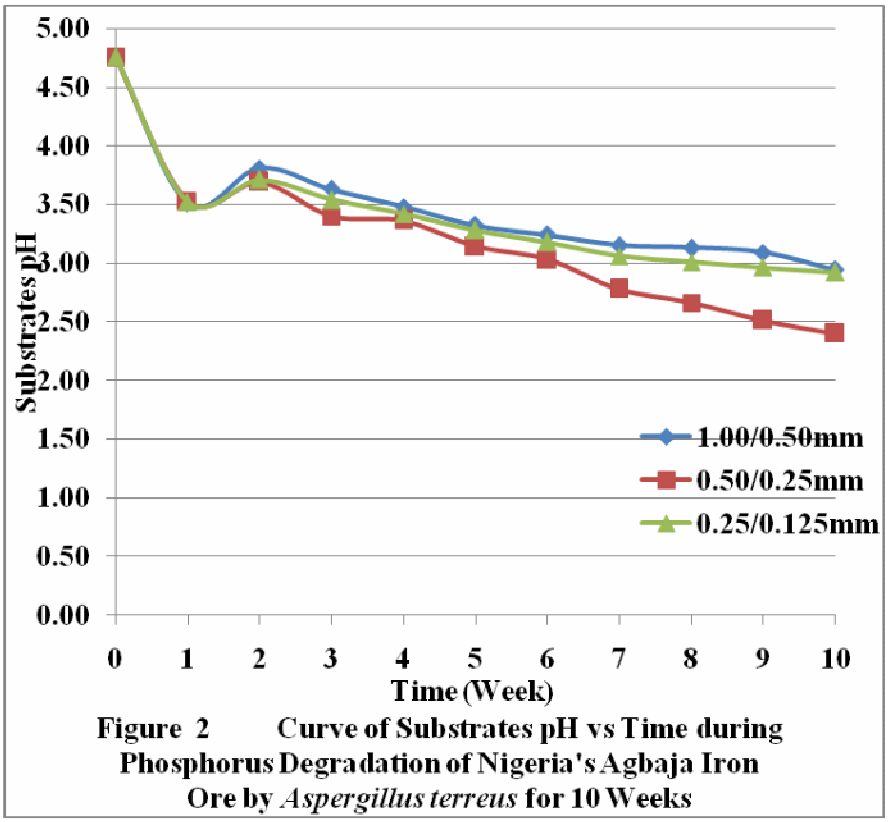

Figure 2 Curve of Substrates pH vs Time during Phosphorus Degradation of Nigeria's Agbaja Iron Ore by *Aspergillus terreus* for 10 Weeks

Figure 2 presents the pH changes during the process of P degradation by *A. terreus* for 10 weeks across the three particle size fractions. It is observed that the longer the P degradation duration the more acidic the broth culture in which *A. terreus* accumulated phosphorus and other metabolic wastes became. This observation is in consonance with the observation made by Delvasto et al, 2007 when they dephosphorized a Brazilian iron ore using acidophilic *Aspergillus niger*. The reducing pH however, seems to be an advantage as it facilitates the microbial ability to continuously solublize the ore samples by advancing the leaching frontiers inwards from the ore periphery. Figure 2 shows that the initial recorded pH values 4.76, 4.75 and 4.76, respectively for the substrates in which *A. terreus* was steadily degrading P in the iron ore, which were taken less than an hour after inoculation, quickly reduced to 3.51, 3.53 and 3.52, respectively at the end of Week 1. This observed tendency continued till the end of Week 10 when the corresponding pH readings recorded 2.94, 2.40 and 2.92, respectively for the 3 particle size fractions.

Figure 3 presents the growth profile of *A. terreus* from Week 1 to Week 10. According to this figure, it is observed that the longer the P degradation duration the lesser the cells population became in the cultures. It is observed that after the exponential growth period, Weeks 3 - 5, when the average cells population across the three particle size fractions was 2.241 – 1.069 g dry weight, the biomass weight gradually dropped to near stagnation from Weeks 8 – 10 when the average cells was 0.616 – 0.515 g dry weight. It is very probable that the drop in cells population of the microbes is a direct consequence of having accumulated so much phosphorus and other associated wastes especially the heavy metals. Even as older generations die and contaminate the culture with their content, new generations are constantly produced, though in a long standing medium this replication might be taking place howbeit, slowly. The microbes have a unique ability to adapt to aggressive environments [15] while

still carrying out their normal functions as long as nutrients are available – in this case, from the degradation of phosphorus in the ore. It is not surprising therefore that the microbes' population has not diminished completely in the medium but only continues to reduce.

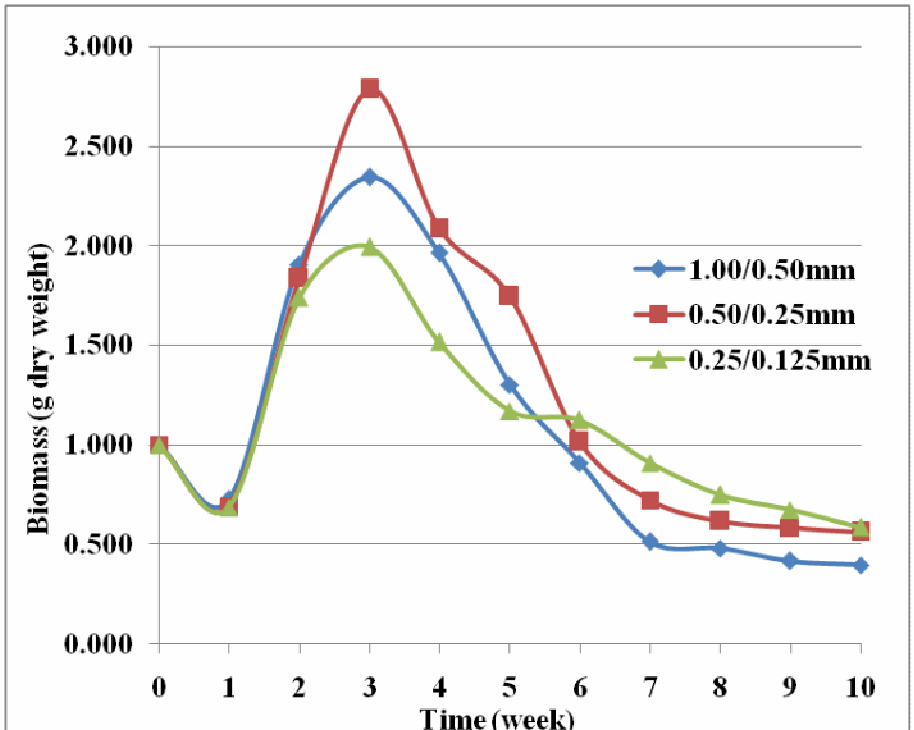

Figure 3 Growth of *Aspergillus terreus* during Phosphorus Degradation of Nigeria's Agbaja Iron Ore Substrates for 10 weeks

Figure 4 presents the % P degradation across the ore fractions by *A. terreus* in malt extract broth medium for 10 weeks. The % P degraded was calculated using the equation:

$$\%P = \frac{\%P_{initial} - \%P_{final}}{\%P_{initial}} \; x\, 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

$\%P_{initial}$ – the initial weight % phosphorus content in ore

$\%P_{final}$ – the final weight % phosphorus in treated ore.

62.36% P degradation is observed in 0.50/0.25mm while 59.33% and 54.38% are seen for 1.00/0.50mm and 0.25/0.125mm, respectively. At about Week 7 the progress in P degradation seems to have peaked and thereafter stagnated till Week 10.

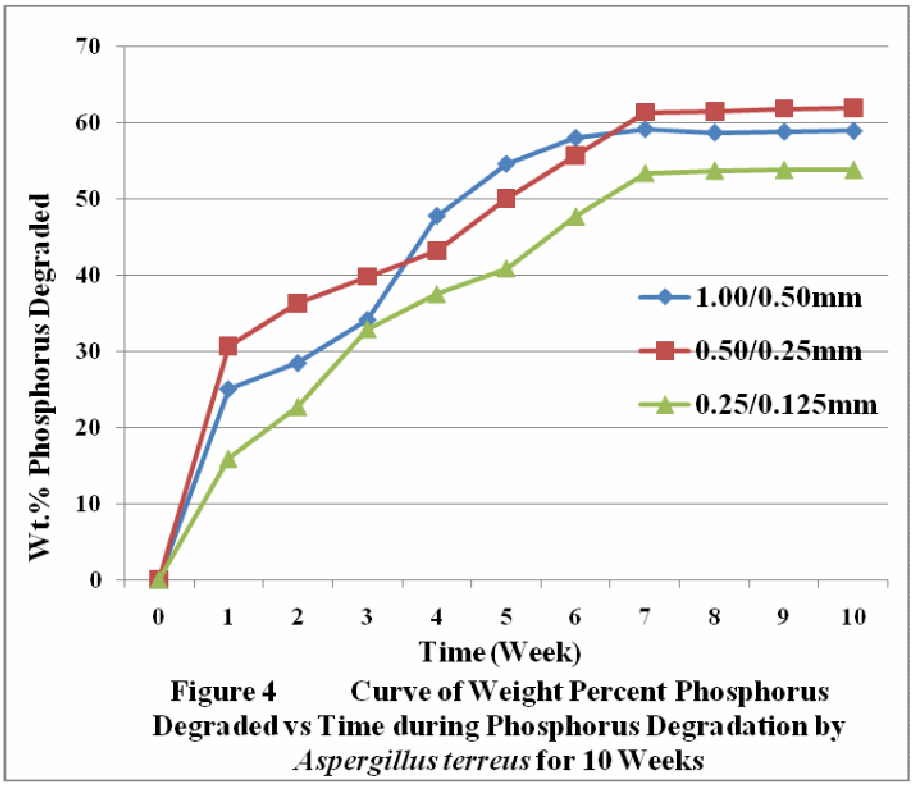

Figure 4 Curve of Weight Percent Phosphorus Degraded vs Time during Phosphorus Degradation by *Aspergillus terreus* for 10 Weeks

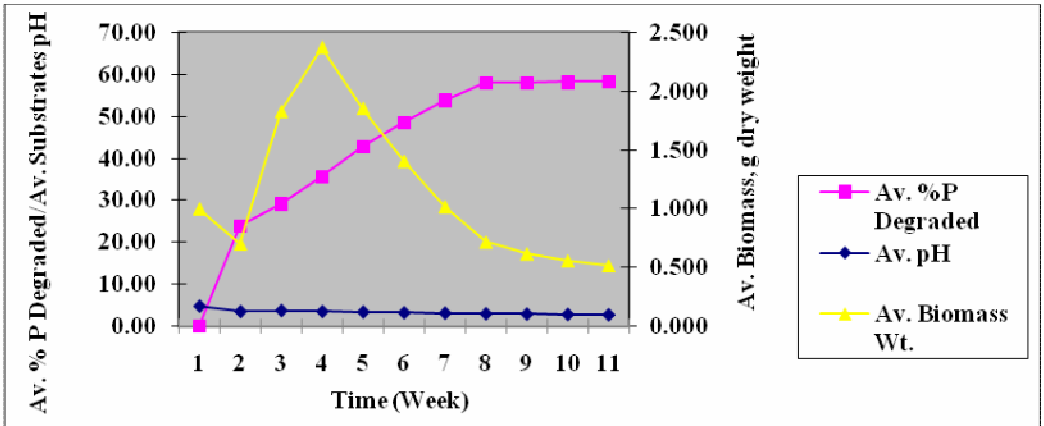

Figure 5 Trend of Av. % P Degraded, Av. Substrates pH and Av. Biomass Weight of A. terreus during Degradation of Nigeria's Agbaja Iron Ore 1.00/0.50mm, 0.50/0.25mm and 0.25/0.125mm for 10 Weeks

Figure 5 presents the average values for % P degraded (Figure 4), the average pH (Figure 2) and the average biomass weight (Figure 3). In Figure 5 it is observed that the period of reduced growth activity more or less corresponded with the period of stagnation observed in the process of P degradation. A noticeable drop in cells population in the lag phase of cell reproduction was quickly followed by the exponential growth phase and then a much gradual death phase indicated that not all microbes died at the end of Week 10. And then, of course, a fungal characteristic reducing pH profile is observed till the end of Week 10. The trend of

activities shows that the biomass curve intersects the % P degraded curve at two points and then the pH curve intersects the same curve once. The probable explanations are that the first indicates the ideal P degradation range when the average cell population is substantial enough to support a maximum degradation. And the second intersection signals the ideal pH point from where the optimum pH for the process actually begins.

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