



Bioleaching and beneficiation of Agbaja iron ore using *Providencia vermicola* KUBT-1 under varying process conditions

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Abstract

In this study, the efficacy of *Providencia vermicola* KUBT-1 in the leaching and beneficiation of Agbaja iron ore was evaluated under varying conditions of temperature, ore particle size, and pH. Agbaja iron ore is a Nigerian low-grade ore containing about 43%–52% iron and a high amount of gangue materials, including phosphorus (1.4%–2.0%) and sulphur (0.12%). The study was organised in triplicate in shake flasks with 48 samples. One millilitre of washed cells of *Providencia vermicola* KUBT-1 suspended in normal saline at 0.5 McFarland standard (10^8 cells ml⁻¹) was introduced into each flask containing 50 g of sterilised ore in 100 ml of bioleaching medium, and the samples incubated at room temperature for eight weeks. The levels of upgraded iron, mobilised sulphur, and phosphorus were determined by atomic absorption and ultraviolet spectrophotometric analyses, respectively. Results showed that a particle size of 0.8 mm was optimal for iron upgrading and sulphur mobilisation while 1.0 mm ore size was optimal for phosphorus mobilisation. Up to 89.60% of the iron was upgraded while phosphorus and sulphur were mobilised, leaving residual values of 0.223% and 0.02%, in the ore, respectively. The organism produced best beneficiation results at a pH of 2.5 and a temperature of 3°C. Correlation analysis with the Pearson's correlation model showed a positive correlation between iron upgrading and sulphur-phosphorus removal ($r = 1.000$, and $r = 0.967$), respectively. These results present *Providencia vermicola* KUBT-1 as a promising candidate for large scale leaching and beneficiation of Agbaja iron ore.

Keywords

bioleaching, beneficiation, iron ore, gangue, mobilisation

Introduction

Agbaja iron ore is one of Nigeria's iron (Fe) ore reserves. Nigeria is rich in natural resources, including iron ore reserves, and estimates of iron ore deposits stand in excess of 2.5 billion tonnes (Alafara et al., 2005). Agbaja iron ore is a low-grade ore containing approximately 47.50% Fe content and is located on a plateau at Agbaja, Kogi State. The ore has unusually high levels of phosphorus (1.66%) and sulphur (0.12%), which are significant setbacks to its utilisation in the blast furnace for steel production. Phosphorus and sulphur are responsible for steel brittleness, causing it to fracture at very low stress values. The conventional froth flotation technique for removing gangue from iron ore has not succeeded with phosphorus because phosphorus is bonded with iron (Uwadiale, 1983; Anyakwo, Obot 2010). Phosphorus and sulphur removal is essential to get high-quality steel with phosphorus and sulphur levels within the acceptable ranges of 0.010–0.020 wt% and ≤ 0.03 wt%, respectively (Kudrin, 1985).

For many years, certain chemolithotrophic bacteria (particularly the chemolithotroph, *Acidithiobacillus ferrooxidans*) have been used commercially to extract some metals from low-grade ores (Singleton, 1998). This process, also called biomining or bioleaching, attracts greater attention today because many of the sources of richer ore have been exhausted. High-grade ores are more amenable to extraction through conventional hydrometallurgical and pyrometallurgical procedures; for low grade ores, these techniques provide low metal extraction due to high process and energy costs. Such mining techniques also cause serious air, water, and land pollution. Water drainage from abandoned mines is acidic and has soluble metals contaminating waterbodies and land. In low grade ore mining, industries focus on cost-effective, low energy consuming and environment-friendly technology, one of which is bioleaching (Wasim, 2019).

Biohydrometallurgy or bioleaching uses microorganisms to extract metals from sulfides and/or iron-bearing ores. Here, either the metabolic activities of the microbe or its metabolic products are

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utilised to convert insoluble metal sulfides to soluble metallic forms, which can then be recovered later through electrolytic and other processes (Singleton, 1998). Presently, bioleaching is widely applied to recover metals from low-grade ores as well as from tailings because it is simple, cost-effective, and ecofriendly (Tao et al., 2021). However, the shortcoming associated with bioleaching is its slow rate compared to chemical leaching. However, waste consumption as substrate by bioleaching organisms during biooxidation is an additional process benefit.

Microbes produce organic and inorganic acids that extract metals by deriving energy from this. Bioleaching effectively reduces capital costs and ecological pollution as it precludes the need for supplementation with toxic substances.

Also associated with phosphorus are the problems of strong primary segregation during the solidification of castings and the formation of high phosphorus brittle streaks between metal grains, impeding plastic deformation (Anyakwo, Obot 2010). Numerous research activities on the bio-beneficiation of low grade iron ores have been going on for decades now. Delvasto et al. in 2005 used the bacterium *Burkholderia caribensis* isolated from Brazilian high-phosphorus iron ore. They mobilised between 5%–20% of the phosphorus initially contained in the ore in 21 days of treatment in shake flask cultures. In 2008, *Aspergillus niger*, isolated from Nigeria's Agbaja iron ore, was used to remove phosphorus from the same ore and in 49 days of leaching, up to 81% removal was achieved (Anyakwo, Obot, 2008). Agbaja iron ore needs to be upgraded in terms of increasing the weight per cent of iron per unit gramme of the ore as well as the removal of sulphur and phosphorus, which, due to their presence, led to the iron ore being abandoned in the past. For these reasons, this work has been designed to study the bacterial leaching and beneficiation of Agbaja iron ore under varying process conditions.

Materials and method

Bioleaching organism

The bioleaching and beneficiation organism used in this study was isolated from the iron ore. The use of organisms indigenous to the ore has some advantages as it precludes delays in the adaptation time of the organism to the system.

Iron ore

The Agbaja iron ore sample was collected from the National Development Centre in Jos, Nigeria. The iron ore was pulverised, sieved and separated into five different grain sizes: 0.2 mm, 0.4 mm, 0.6 mm, 0.8 mm and 1.0 mm.

Media

Two media, designated A and B, were mixed in a ratio of 1:0.5 for bacterial isolation and bioleaching experiments. Medium A was a 9K medium (Zhang et al., 2020), composed in g l⁻¹ of (NH₄)₂SO₄ 3 ml, K₂HPO₄ 0.5 ml, MgSO₄·7H₂O 0.5 ml, KCl 0.1, Ca(NO₃)₂ 0.014 ml, and de-ionised H₂O 1000 ml. Medium B was nutrient broth.

Analytical equipment

Atomic absorption spectrophotometer (AA 7000) and UV spectrophotometer were used to evaluate residual iron and phosphorus/sulphur, respectively. The analyses were conducted at the Energy Centre, University of Nigeria, Nsukka.

Isolation and identification of bioleaching organism

Two hundred grammes (200 g) of pulverised Agbaja iron ore sample

was dispensed into each of three 250 ml conical flasks containing sterilised mineral salt medium, as described earlier, and nutrient broth. The pH was adjusted to 2.5 using 0.4 ml of concentrated sulphuric acid (H₂SO₄). After incubation for 7 days at room temperature (25°C–28°C), 0.1 ml of the supernatant from each flask was used to inoculate triplicate petri dishes of nutrient agar by the spread plate method. The inoculated plates were incubated for 24h under room temperature. The organisms that grew were subcultured on a freshly prepared nutrient agar and later transferred to nutrient agar slants. The bioleaching heterotrophic bacterium (a chemoorganotroph) was identified by 16S rDNA gene sequencing (Delvasto et al., 2006b) as *Providencia vermicola* strain KUBT-1.

Confirmation of Fe, P, and S levels in the ore

Analysis to confirm the already documented levels of Fe, P, and S in Agbaja iron ore was carried out using atomic absorption (AA7000) and UV spectrophotometers.

Bioleaching and beneficiation experiments

Experiments to determine the effects of particle size, pH, and temperature on the rates of biobeneficiation and upgrading of Agbaja iron ore lasted for eight weeks. The analyses were performed every two weeks. Parameters of particle size (0.2 mm, 0.4 mm, 0.6 mm, 0.8 mm, and 1 mm), pH (2.5, 5, 7, 9, and 11) and temperature (20°C, 25°C, 30°C, 35°C, and 45°C) were used in triplicates. Fifty grammes (50 g) of Agbaja iron ore was weighed into three 250 ml conical flasks for each particle size, pH and temperature. The ore samples were sterilised at 121°C for 15 minutes for three consecutive days. One hundred millilitres of sterilised mineral salt medium and 50 ml of nutrient broth were poured into each flask containing the iron ore. One millilitre (1 ml) of the washed cells of *Providencia* species suspended in normal saline to a level of 0.5 McFarland standard was introduced into each flask. A total of 48 flasks were used, and they were loosely covered with cotton wool and set in an orbital shaker operated at 150 rpm. Samples were collected every two weeks for analysis using atomic absorption and ultraviolet spectrophotometers to determine iron, phosphorus, and sulphur levels.

The weight percentage of mobilised phosphorus and sulphur was determined as follows in Formulae 1 and 2:

$$\text{Phosphate mobilisation (\%)} = \frac{\text{Wt\% P}_{\text{initial}} - \text{W\%P}_{\text{final}}}{\text{Wt\% P}_{\text{initial}}} \times 100 \quad [1]$$

$$\text{Sulphur mobilisation (\%)} = \frac{\text{W\% S}_{\text{initial}} - \text{W\%S}_{\text{final}}}{\text{Wt\%S}_{\text{initial}}} \times 100 \quad [2]$$

The result of the 16S rDNA sequencing (Devasto et al., 2006b) identified the isolate as having 95.20% pairwise identity with *Providencia vermicola* KUBT – 1 with NCBI accession number KX098543.

Results and discussion

Bioleaching isolate

The bioleaching isolate identified as *Providencia vermicola* KUBT-1 through 16S rDNA sequencing analysis (Devasto et al., 2006b) is a chemoorganotroph (heterotroph) and its use in bioleaching adds novelty to this work since its leaching potentials have not been harnessed or reported in previous studies. Previous works over the years have focused on the use of chemolithotrophs such as *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans* in metal leaching works both in the laboratory and open field (Di

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Spirito, Tuovinen 1982; Konishi et al., 1992; Choi et al., 1993; Hang et al., 1994; Garcia et al., 1995; Schnell, 1997; Fowler, Crundwell 1998; Singleton, 1998; Fowler, Crundwell 1999; Kelly, Wood, 2000; Brierly, Brierly, 2001; Jain, Sharma 2004; Ponce et al., 2012; Yang et al., 2013; Gao et al., 2020; Zhang et al., 2020).

Biobeneficiation of Agbaja iron ore

Results of the biobeneficiation experiments (dephosphorisation and desulphurisation) are depicted in Figures 1 to 6. Figure 1 shows the effects of ore grain size on the rate of phosphorus mobilisation by *Providencia vermicola* KUBT-1. There was a consistent increase in the percentage of mobilised phosphorus as the ore size increased until it reached an optimum at an ore size of 1.0 mm. The pH and temperature were kept at constant levels of 2.5 and 30°C, respectively. When other factors were kept constant and the pH

varied, a pH of 2.5 was optimal for phosphorus mobilisation (Figure 2) throughout the eight-week beneficiation experiment. A slightly different trend was noted for sulphur mobilisation (Figures 4 and 5) with respect to the effect of ore grain size. A particle size of 0.8 mm and pH of 2.5 were best for desulphurisation by the bioleaching bacterium.

The effects of temperature on biobeneficiation (biological removal of impurities) are reported in Figures 3 and 6. A temperature of 30°C is seen to be optimal for dephosphorisation and desulphurisation. The optimal mobilisations of phosphorus (P) and sulphur (S) from the ore occurred at ore grain sizes of 1.0 mm and 0.8 mm, respectively (Figures 1 and 4), a pH of 2.5 (Figures 2 and 5), and temperature of 30°C (Figures 3 and 6), portray the organism as an acidophilic mesophile. Reports from previous studies show that most microorganisms used in biohydrometallurgical processes

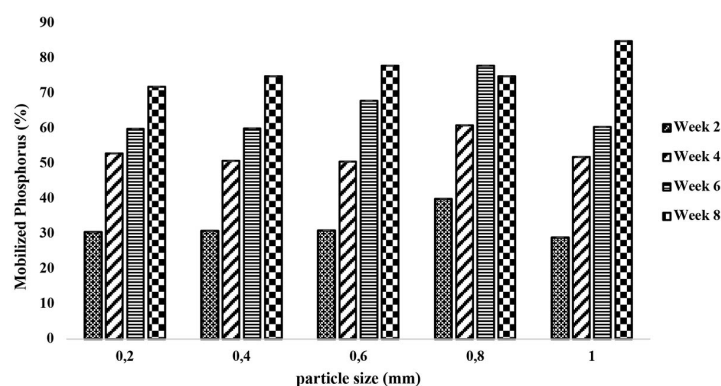


Figure 1—Percentage of phosphorus mobilised by *Providencia vermicola* KUBT-1 at varying ore grain sizes

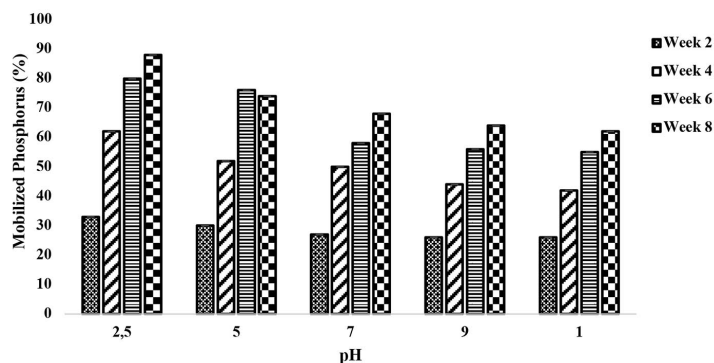


Figure 2—Percentage of phosphorus mobilised by *Providencia vermicola* KUBT-1 at varying pH levels

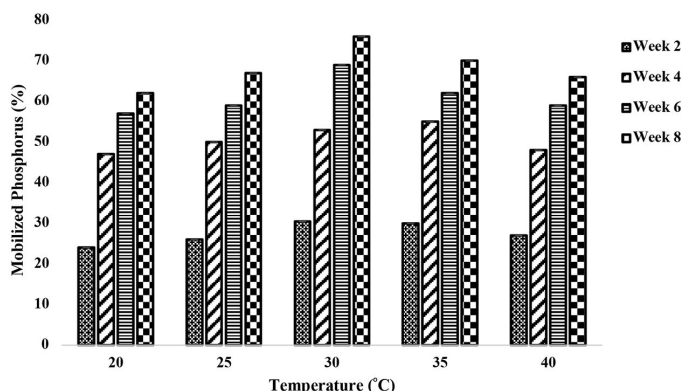


Figure 3—Percentage of phosphorus mobilised by *Providencia vermicola* KUBT-1 at varying temperature levels

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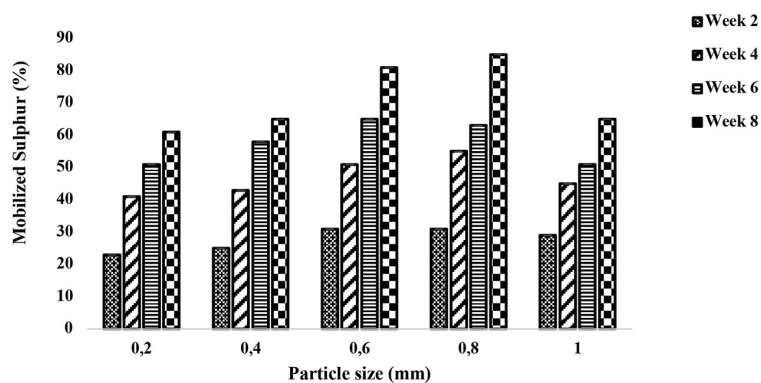


Figure 4—Percentage of sulphur mobilised by *Providencia vermicola* KUBT-1 at varying ore grain sizes

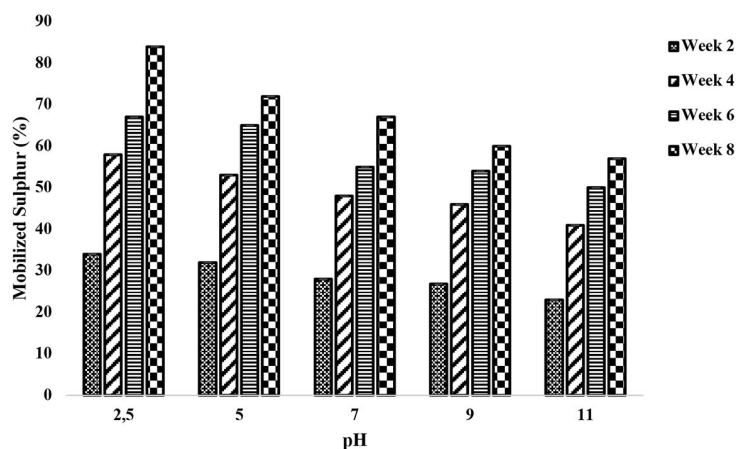


Figure 5—Percentage of sulphur mobilised by *Providencia vermicola* KUBT-1 at varying pH levels

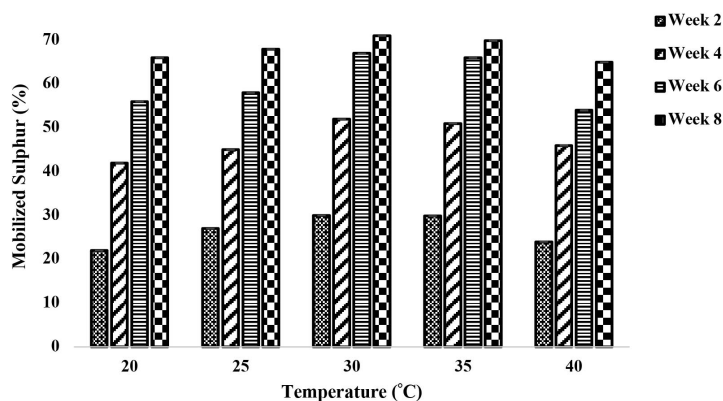


Figure 6—Percentage of sulphur mobilised by *Providencia vermicola* KUBT-1 at varying temperature levels

are acidophiles (Di Spirito, Tuovinen 1982; Hang et al., 1994; Bosecke, 1995; Gehrke et al., 1998; Adeleke et al., 2010; Liao et al., 2019; Liao et al., 2021). Many of them produce organic and inorganic acids during the reaction, and these acids assist the leaching process, leading to mobilisation/removal of the elemental contaminants. Hydrogen ion concentration (pH) and temperature have been reported to be essential factors in the leaching process, as both affect the dissolution rate of the ore (Alafara et al., 2005).

The iron ore originally contained about 1.66 Wt% P and 0.12 Wt% S. At the end of the eight-week beneficiation experiment, the ore was left with residual values of 0.223% P and 0.02% S, amounting to 86.7% and 83.3% of mobilised phosphorus and sulphur, respectively. Particle size affected the reaction rate by

modifying the surface area for the attack of microbial enzymes.

Even though a residual value of 0.223% P is still above the metallurgically acceptable P level for high-quality steels, which is between 0.010–0.020 wt%, it stands out as a rare feat accomplished by this new bioleaching organism, *Providencia vermicola* KUBT-1.

From these results, the three factors tested (ore particle size, pH, and temperature) affected phosphorus and sulphur mobilisations.

Bioleaching/upgrading of Agbaja iron ore

Results of the iron upgrading experiments are reported in Figures 7 to 9. The percentage of upgraded iron was highest at a particle size of 0.8 mm, pH of 2.5, and a temperature of 30°C, which were the same conditions for optimal mobilisation of sulphur

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and phosphorus (Figures 1 to 6). Using the Pearson's correlation model, a positive correlation was noted between iron upgrading, phosphorus, and sulphur mobilisation (Figures 10 and 11).

The bioleaching organism in this study could solubilise up to 89.60% of the iron at the end of the eight-week bioleaching experiment in a liquid medium. According to Wasim et al. (2019), natural ores of several metals, including Ni, Zn, Fe, As, and Cu,

generally exist in metal sulphide forms, which are insoluble in neutral and weak acidic conditions. Oxidation of ferrous to ferric and sulphide into sulphate is required for bioleaching and is carried out by acidophilic microbes.

The leaching mechanism can be illustrated thus:

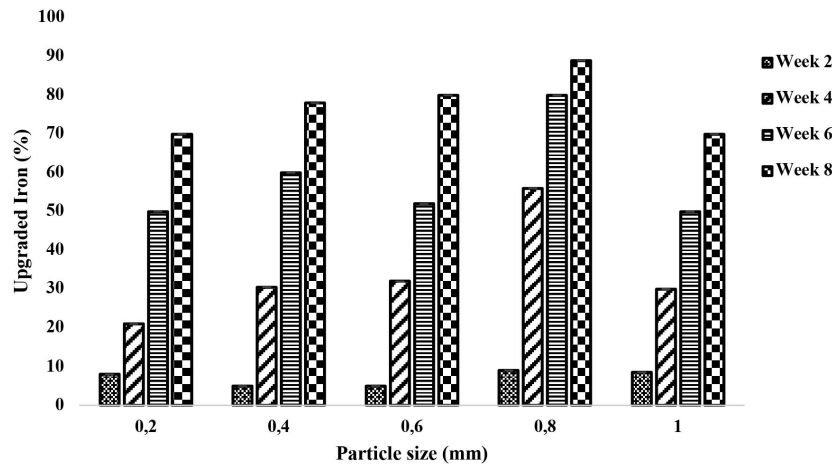


Figure 7—Percentage of iron upgraded by *Providencia vermicola* KUBT-1 at varying ore grain sizes

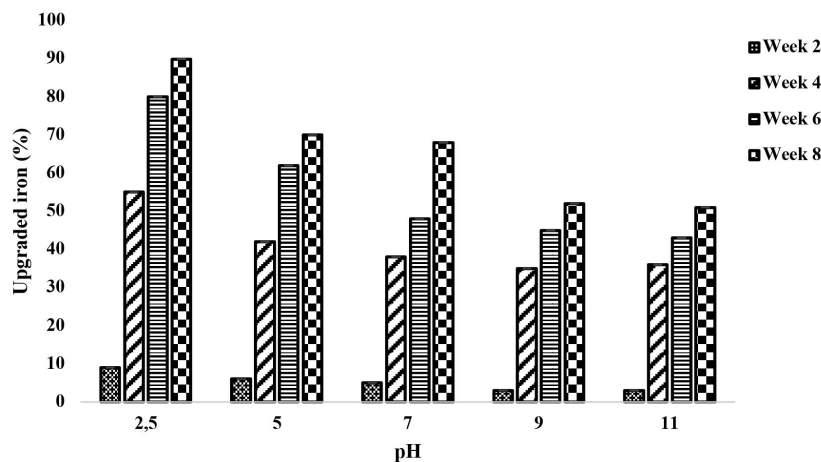


Figure 8—Percentage of iron upgraded by *Providencia vermicola* KUBT-1 at varying pH levels

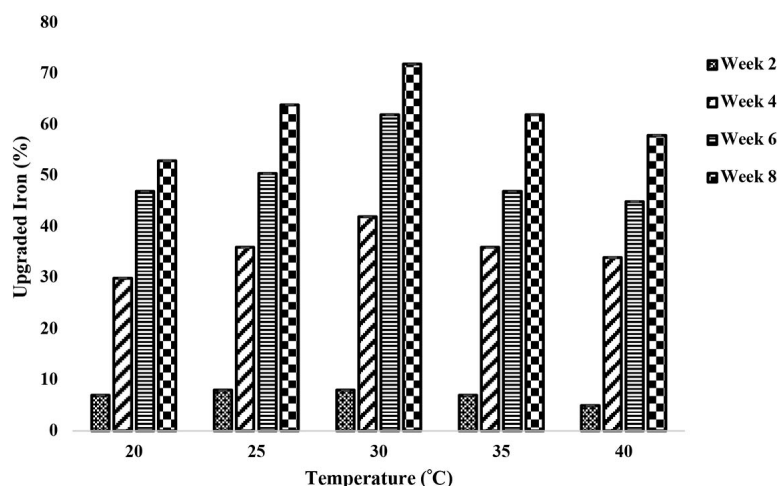


Figure 9—Percentage of upgraded iron by *Providencia vermicola* KUBT-1 at varying temperature levels

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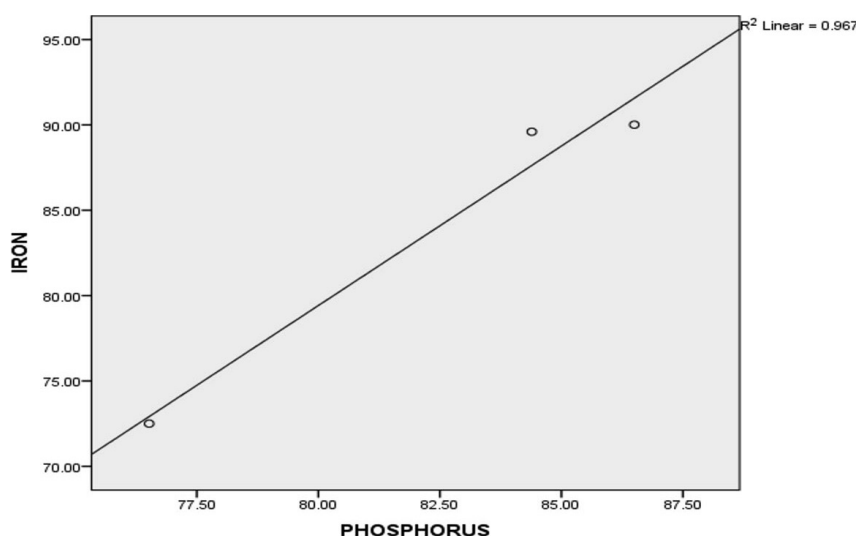


Figure 10—Graph of correlation showing the relationship between the level of iron upgraded and phosphorus mobilised by *Providencia vermicola* KUBT-1

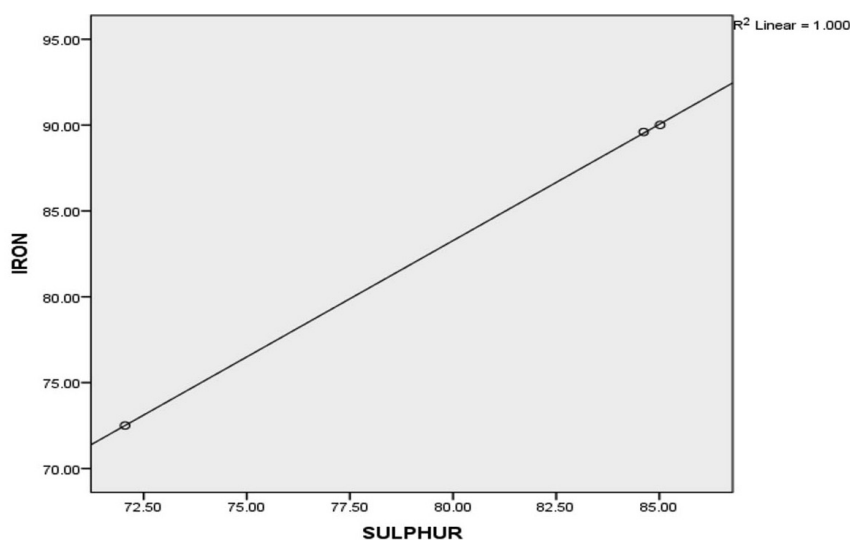


Figure 11—Graph of correlation showing the relationship between the level of iron upgraded and sulphur mobilised by *Providencia vermicola* KUBT-1

This mechanism is direct bioleaching and involves a direct transfer of electrons from iron sulphide to the bacterial cells adhering directly to the metal surface, usually through an extracellular polymeric substance (EPS) produced by the organism (Wang et al., 2018; Wasim et al., 2019; Ye et al., 2021).

From the results of iron bioleaching (upgrading) presented in Figures 7, 8, and 9, optimal solubilisation of the iron sulphide still occurred at a particle size of 0.8 mm, acidic pH (pH 2.5), and mesophilic temperature (30°C). The foregoing has helped to largely delineate the bioleaching capabilities of *Providencia vermicola* KUBT -1, and its potential for use in large-scale leaching and beneficiation of the Nigeria Agbaja iron ore.

Conclusions

Bioleaching and beneficiation of metals is not a new technology. On the other hand, it has existed for many decades, continuously undergoing improvements in both methods and materials. It offers a viable and better alternative for tackling low grade metal ores. In this study, the chemoorganotrophic bacterium *Providencia*

vermicola KUBT-1, isolated from the ore, was found to be endowed with intriguing bioleaching and beneficiation capabilities for the upgrade of the Nigeria Agbaja iron ore laden with high levels of phosphorus and sulphur. Up to 89.60% of Fe was solubilised while the Wt% of phosphorus and sulphur were significantly ($P < 0.05$) reduced from 1.66 Wt % P and 0.12 Wt % S to 0.223 Wt % and 0.02 Wt %, respectively. Factors such as ore grain size, pH, and temperature were found to play important roles in the bioleaching and beneficiation rates of the iron ore.

In the current quest to find a solution to the impurities in the Agbaja iron ore, this study has further highlighted the presence of indigenous microbes in the ore of which their bioleaching and beneficiation potentials can be exploited to upgrade this valuable resource.

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