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Research Article

Potentiality of *Acidithiobacillus thiooxidans* in microbial solubilization of phosphate mine tailings

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Abstract: This paper deals with the solubilization behaviour of the tailings produced by the floatation of a complex low-grade phosphate ore. The composition of the tailings was essentially dolomite (52.04%) with minor amounts of phosphate, iron and aluminium oxides (10.4 and 0.5% respectively).The presence of these products created uncontrolled land pollution and severely affected groundwater. An initiative has been taken up for utilization of this waste to generate an eco-friendly product. The first step towards this panorama is an incorporation of suitable microorganisms for the effluent's biodegradation. Sulphur oxidizing bacteria *Acidithiobacillus thiooxidans* produces sulphuric acid which neutralizes the dolomitic tailings and converts it into available plant forms. The solubilization activity was tested in sulphur medium with 5, 10, 15 and 20% concentration of tailings. The solubilization was graded on the basis of pH, Electrical conductivity (EC), soluble calcium and magnesium and soluble phosphate. The results from ex-situ experiments showed that the treatment with 15% tailings ended with highest solubilization. The values of pH, EC, soluble calcium and magnesium and soluble phosphate for this treatment were 4.92, 31.6dS/m, 10.8 mL EDTA and 17.24 µg/mL respectively. Also, the results proved that sulphur oxidizing bacteria *Acidithiobacillus thiooxidans* was capable of solubilizing dolomitic tailings from the Jhamarkotra mines. Finally, an important factor taken into account was the solubilization of residual phosphate along with dolomite in the tailings. This combined action affected the solubilization behaviour of the residue, which was also showed successfully with the assayed laboratory studies.

***Keywords:** Acidithiobacillus thiooxidans, dolomite, mine tailings, solubilization, sulphur oxidation*

Introduction

Phosphate tailings pose significant risk to the environment as point sources of basic, carbonate-rich effluents (Babel et al., 2015). These highly alkaline sandy tailings are produced as the result of beneficiation of low grade ore of rock phosphate. Several countries, notably Australia, have banned beneficiation of low-grade (phosphatic) ores due to this reason (Ranawat et al., 2009). But countries which have limited resources of high-grade ore cannot meet out their P-needs without beneficiation, as they have to provide P-fertilizer for their agriculture. India is a fitting example for this situation with meager resource of rock phosphate (only 159 mt. compared to the world's reserve of 33 billion tones) (Ranawat et al., 2009).These dolomitic tailings contain 6-10% P_2O_5, 16-18% MgO and 32-36% CaO. It is therefore necessary to utilize these tailings in a judicious manner. Microorganisms from genus *Thiobacilli* play an important role in sulphur oxidation in soil and mine deposits. Sulphur oxidation results in the formation of sulphate (acid) which helps to solubilize plant nutrients and improves alkali soils. The accelerated oxidative dissolution of dolomite and other phosphatic minerals in tailings by acidophilic chemolithotrophic prokaryotes like *Acidithiobacillus* has been widely reported (Nancucheo et al., 2011). Hortenstine and Rothwell (1972) studied tailings produced in phosphate mines that were nearly sterile and almost void of plant nutrients (except for relatively insoluble P). In the experiments performed by Findley et al. (1974), the use of sulphur-oxidizing bacteria was judged by the kerogen and bitumen release, the predecessors of shale oil, from oil shale. Dolomite in oil shale has been solubilized by the formation of sulphuric acid from added sulphur, leaving a complex of the silica and kerogen. *A. thiooxidans* decreases the pH of mineral salt medium from 3.5 to approximately 1.9 in the lightly buffered medium. Dolomite consists of fine grain mixture of MgO and CaO. Fruehan and Brabie (2003) found that in solution when the CaO dissolves rapidly the MgO grains may simply fall off the dolomite and dissolve quickly because of their large surface area. Conversely, when MgO dissolves quickly the CaO grains may go into the slag and dissolve. Sulphur oxidizing bacteria has been employed for remediation of metal contaminated soil by metal mobilization by various researchers (Gomez and Bosecker, 1999). It was investigated that by manipulating microbial communities in tailings (sulfur-oxidizing acidophilic bacteria) it is possible to mitigate the impact of tailing deposits in the environmental concern.

Materials and Methods

Various tailing treatments were set up by using microbial inoculation variants, and analyzed at regular intervals for changes in physicochemical and microbiological parameters for 3 days. The experiment was set to determine whether the in-situ sulphuric acid exudated by *Acidithiobacillus* was able to solubilize phosphate tailings and the extent of solubilization of tailings by the bacteria at different concentration levels of tailings. Differences between treatment protocols were most apparent between inoculated and uninoculated tailings. Ten microbial variants were set up. They were as follows:

- A Control treatment; only sterilized broth without microbe and tailings
- B Treatment with broth and tailings at 5% concentration.
- C Treatment with broth and tailings at 10% concentration.
- D Treatment with broth and tailings at 15% concentration.
- E Treatment with broth and tailings at 20% concentration.
- A' Treatment with broth, no tailings & *Acidithiobacillus thiooxidans*
- B' Treatment with broth, tailings at 5% concentration & *Acidithiobacillus thiooxidans*
- C' Treatment with broth, tailings at 10% concentration & *Acidithiobacillus thiooxidans*
- D' Treatment with broth, tailings at 15% concentration & *Acidithiobacillus thiooxidans*
- E' Treatment with broth, tailings at 20% concentration & *Acidithiobacillus thiooxidans*

Acidithiobacillus thiooxidans was directly obtained from Agrilife, Hyderabad in the form of talc-based culture. The bacterium was assessed in elemental sulphur medium. The CFU count of talc-based culture was 10^7per g. Composition of Sulphur Medium (Waksman and Joffe, 1925). Part A -KH_2PO_4 (3.0 g), $MgSO_4.7H_2O$ (0.5 g), $(NH_4)_2SO_4$ (0.3 g), $CaCl_2.2H_20$ (0.25 g), $FeCl_3.6H_2O$ (0.02 g), Part B -Elemental Sulphur (10.0 g) / Both the parts should be separately sterilized and mixed just before inoculation. Final pH (at 25°C)- 4.8±0.2

Results and Discussion

The pH was determined by calibrated pH meter (Systronics-335). The electrical conductivity was estimated by calibrated EC meter (Systronics-304). The soluble Ca and Mg were analyzed by complexometric method and soluble phosphorus by vanadomolybdate (Olsenet al., 1954) method. The data was statistically analyzed to determine the significance of results.

Effect on pH

A data perusal (Figure 1) indicated that pH increased both in the inoculated and uninoculated treatments with an increase in the percentage of phosphate tailings. However, it was higher in all inoculated treatments as compared to their respective uninoculated compliments (Table 1). The final pH denoted the extent of solubilization in the medium. Stamford et al. (2002) used sulphur inoculated with Thiobacillus in amendment of saline and sodic soils to observe reduction in soil pH occurring until the total consumption of the added sulphur, promoting soil acidification varying from initial pH 8.2 up to pH 4.5 applying 1.8 t/ha of sulphur. The sulphuric acid produced by the microbial inoculums is used up by the insoluble inorganic feed and converted into soluble forms. Thus, when the solubilization is higher, pH goes on increasing since the H^+ ions produced by microbes are being utilized for the solubilization of salts. In the inoculated treatments, with 5 to 20% phosphate tailings, the degree of pH change was not as significant as expected. Thus, there was significant effect of inoculation on the solubilization of tailings but no effect of concentration of tailings was noticed.

Effect on electrical conductivity

Electrical conductivity showed varied trend in inoculated and uninoculated treatments (Table 1). A close examination of the data presented in the Figure 2 depicted that there was slow decrease in EC in the uninoculated treatments. This may be due to the saturation of the solution by dissolved salts. In the inoculated treatments, EC increased with the increase in the tailings percentage but not very significant. Anandham et al. (2007) reported that an inoculation of sulphur oxidizing bacteria increased EC from 0.20 to 0.25 dS/m. The treatment E' with highest concentration of tailings possessed highest EC (32.1 dS/m). The lowest EC was reported in control treatment (A').

Table 1. Inoculation's effect of *Acidithiobacillus* on the solubilization of tailings

Treatment	pH	EC (dS/m)	Soluble Ca + Mg (mL of 1M EDTA per 100 mL)	Soluble PO_4^{2-} (µg/mL)
A	2.18	24.0	2.0	0.9
B	3.31	22.6	4.4	2.25
C	3.29	22.5	5.7	4.56
D	3.45	21.4	7.6	5.17
E	3.38	21.5	10.9*	6.13
A'	2.08	5.2	2.1	0.4
B'	4.54	28.7	9.5	8.14
C'	4.82	30.1	10.1	12.49
D'	4.92*	31.6*	10.8*	17.24
E'	4.98*	32.1*	9.2	10.96
SEm±	0.0739	0.4870	0.1503	0.1474
CD@5%	0.2181	1.4367	0.4435	0.4350

*Not significant

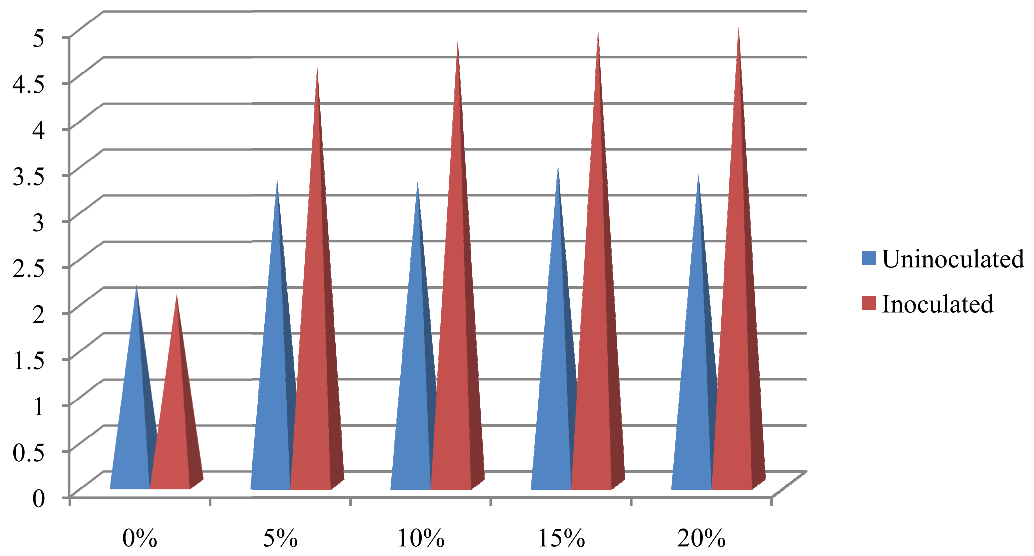

Figure 1. Effect of microbial inoculation on pH at different concentration of phosphate tailings

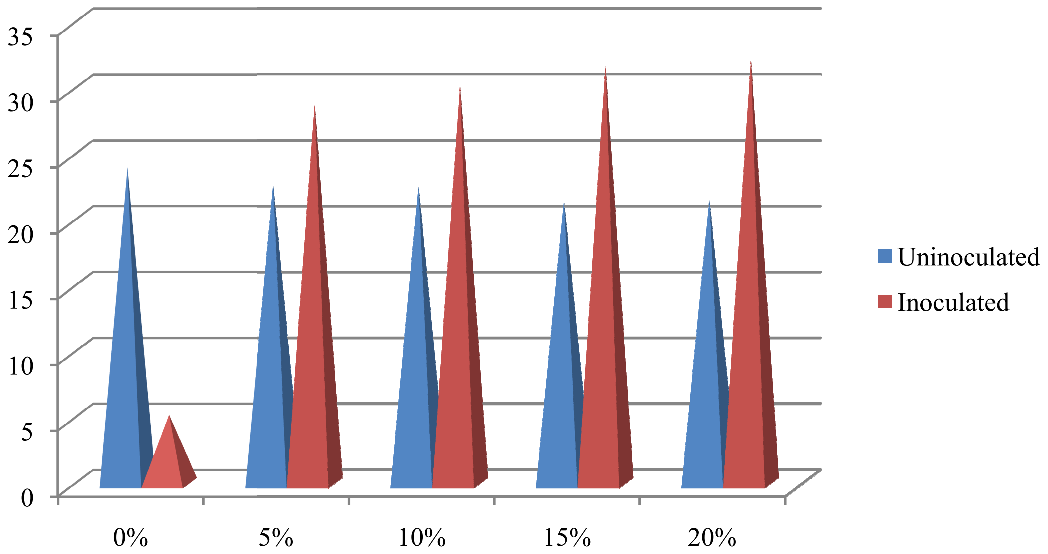

Figure 2. Effect of microbial inoculation on EC at different concentrations of phosphate tailings

Effect on concentration of soluble calcium and magnesium

The effect of inoculation was found quite prominent in variation of soluble calcium and magnesium (Figure 3). The inoculated treatment always reported higher calcium and magnesium than uninoculated one except in the treatments containing 20% tailings, where some poisonous effects of tailings were being seen among the inoculated microbial population, due to the decrease of soluble Ca and Mg in the inoculated treatment. However, the value of uninoculated treatments was also higher than expected. This trend might be justified by the activity of native bacteria present in the tailings (unsterilized) which were well adapted to the environment. The highest soluble Ca and Mg were reported in E treatment with 20% tailings (10.9 mL) and the lowest one was in the control (2.0 mL).

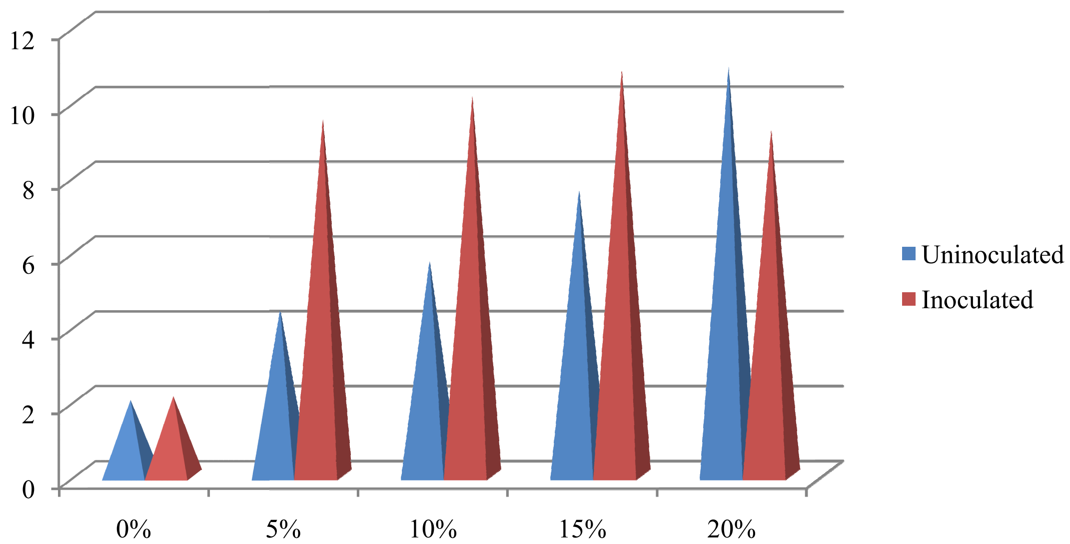

Figure 3. Effect of microbial inoculation on soluble calcium and magnesium at different concentrations of phosphate tailings

Effect on concentration of orthophosphate

As mentioned earlier, the orthophosphate was the total amount of soluble phosphate in the medium. The orthophosphate content first increased up to 15% concentration (17.24 µg/mL) in inoculated treatment but decreased sarcastically to 10.96 µg/mL when the concentration was raised to 20% (Figure 4). The higher concentration of tailings might have caused an inhibitory effect on the bacterial activity and then the subsequent reduction in the orthophosphate content. However, no such drift was seen in uninoculated treatments where the soluble phosphate was always lower than inoculated treatments and increased gradually to 6.13 µg/mL in E' with 20% concentration. The highest quantity of orthophosphate was found in D' with 15% tailings and the lowest one was in A' with no tailings.

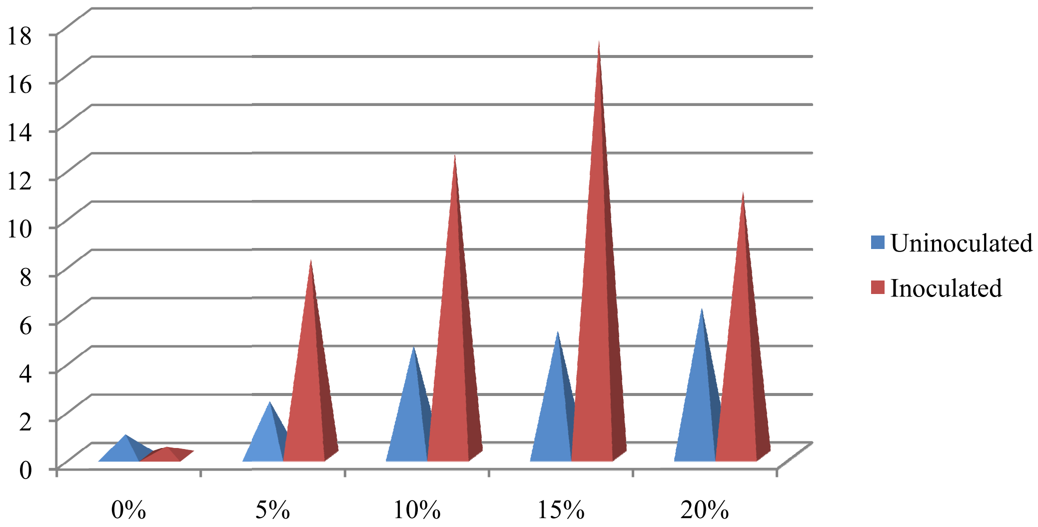

Figure 4. Effect of microbial inoculation on soluble phosphate at different concentrations of phosphate tailings

Conclusions

From the foregoing discussion, it can be concluded that sulphur oxidizing bacteria *Acidithiobacillus thiooxidans* could solubilize phosphate tailings to calcium, magnesium as well as phosphate. But an effective solubilization was noticed only up to 15% concentration, above where microbial destruction occurs since poisoning effect of tailings on the microbial population and quite non-significant results were obtained. Therefore, it may be surmised that the treatment up to 15% tailings may supply positive results for further studies.

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