

Short Communication

Effect of pH on Metal Extraction from Bauxite Ore by *Thiobacillus Ferrooxidans*

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Abstract

The extremophile, *Thiobacillus ferrooxidans* was selected to study the effect of pH on metal extraction from bauxite ore. This bacterium was inoculated in 9K medium having different pH, along with the bauxite ore, as metal source. After one month of incubation the extraction of metals aluminum and iron was measured by spectrophotometric methods. It was found that the extraction of aluminum was found better as compare to iron from bauxite at pH 2.

**Keywords:** Aluminum; iron; metal extraction; pH; *Thiobacillus ferrooxidans*.

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1. Introduction

The pH of the solution is a critical factor in metal extraction because metals dissolve in acids [1]. The metals are extracted by using microbes which secrete acids. These microbes may reside in the extreme conditions like low pH therefore these are termed as extremophiles [2]. The bacterium, *T. ferrooxidans* is metal extracting bacterium and grown on 9K medium which contains ferrous sulphate as major constituent from which sulphuric acid is produced [1]. The metals are dissolved in the sulphuric acid. In addition this bacterium undergo oxidize the  $Fe^{2+}$  to  $Fe^{3+}$  which results into the reduction of other metal ions. It is also reported that, *Thiobacillus ferrooxidans* have the iron reducing activity therefore it can extract iron and other metals too [3]. In the present investigation, the effect of pH on metal extraction was studied.

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## 2. Materials and Methods

To study the effect of pH on bioextraction of metals from bauxite ore the experiment was carried out by shake flask method [4]. *Thiobacillus ferrooxidans* was cultivated in 9K medium [Composition g/l as ammonium sulphate  $(\text{NH}_4)_2\text{SO}_4$  3.0, magnesium sulphate  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  - 0.5, potassium hydrogen phosphate  $\text{K}_2\text{HPO}_4$  - 0.5, potassium chloride KCl - 0.1, calcium nitrate  $\text{Ca}(\text{NO}_3)_2$  - 0.01, ferrous sulphate  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  - 21.00 [5]. The medium was autoclaved at 121 °C for 15 minutes to prevent interference of other microorganisms.

The pH of the medium was adjusted to 1, 2, 3, 4, 5, 6, 7, and 8, with 10 N  $\text{H}_2\text{SO}_4$ . The medium was inoculated with approximately  $5 \times 10^6$  cells / ml (maximum cell biomass concentration). The culture was grown under sterile condition in 250 ml Erlenmeyer flasks on a rotary shaker (140 rpm) and incubated at  $36 \text{ }^\circ\text{C} \pm 2 \text{ }^\circ\text{C}$ . Pre-weighed 2 g bauxite ore was added in each Erlenmeyer flask. In control flask, inoculum was replaced by 40 ml of 9K medium along with 60 ml of sterile distilled water. The aerobic condition was maintained by applying non absorbent cotton to the mouth of the flasks [6].

The experiment was conducted for one month and the final pH was measured. The contents of each Erlenmeyer flask were filtered through Whatman number 41 filter paper. Aluminum and iron extracted were measured according to the spectrophotometric methods developed by [7] and [8] for aluminum and iron estimation respectively.

Aluminum was analyzed by taking 20  $\mu\text{l}$  leached samples from the filtrate and added with 20  $\mu\text{l}$  of  $\text{H}_2\text{SO}_4$ . The volume was made upto 3 ml with double distilled water. 500  $\mu\text{l}$  of 15 % sodium acetate was added to it for adjusting reaction pH. Then 200  $\mu\text{l}$  of 0.1 % ascorbic acid was added to overcome the interference of iron. Lastly 200  $\mu\text{l}$  of chrome azurol-s (0.04 % working solution diluted from stock solution of 0.1 %) was added to it. The whole assembly was incubated for 10 min for violate colour development. The absorbance was measured at 545 nm. For standard curve, aluminum potassium sulphate was used.

The iron was detected from the filtrate by taking 20  $\mu\text{l}$  leached sample and digested with 20  $\mu\text{l}$  10 N sulfuric acid. The volume was made upto 3 ml with doubled distilled water. Afterwards, 1 ml of hydroxylamine hydrochloride (10 %) and 8 ml of sodium acetate (10%) and 10 ml of 1, 10 phenanthroline solution (0.1 %) were added to it. The whole mixture was diluted to 100 ml with distilled water and allowed to stand for 10 min till brick red colour form. The absorbance was measured at 510 nm. The standard curve was prepared with ferrous ammonium sulphate.

## 3. Results and Discussion

The aluminum extraction ranged from 40.12 to 81.13 % (Table 1). The pH 2.0 gives higher extraction as compared to other pH. It was observed that with the increase of pH (3.0, 4.0, 5.0, 6.0, 7.0 and 8.0) the metal extraction was decreased. Same thing was occurred with iron extraction and bioextraction efficiency together. The bacterium, *T.*

*ferrooxidans* is an extremophile therefore it is able to grow at very low pH [9]. The bacterium extract metal aluminum by donating the electron from  $Fe^{2+}$  and  $Fe^{3+}$  is formed. At the same time, it is able to reduce the iron ions which results into the extraction of iron. At extreme low pH, solubilization of metals was carried out at optimal level; hence *T. ferrooxidans* served the purpose. With the onset of pH stabilization, at an optimum 2.3, *T. ferrooxidans* cells entered the exponential phase of growth and were metabolically highly active during this period, leading to further generation of biologically synthesized sulfuric acid [10]. The increase in the pH leads to the increasing negative charges on the bacterial cell surface with the subsequent attraction with positively charged metal ions [11]. The fastest leaching rates was achieved under oxidized conditions and at low pH values [12], indicating bacterial activity is responsible for the oxidative leaching of sulphide minerals.

Table 1. Effect of pH on extraction of metals from bauxite ore by *T. ferrooxidans*.

pH	Bauxite ore (g)		Metal extracted (mg)		Metal extracted (%)		Bioextraction Efficiency of Al and Fe (%)
	Initial wt	Final wt	Aluminum	Iron	Aluminum	Iron	
1.0	2	2.789	482.20±3.43	262.45±1.78	66.20	80.01	70.49
2.0	2	2.896	590.66±2.78	278.23±1.26	81.13	84.82	82.28
3.0	2	2.846	564.87±2.45	264.53±1.68	77.59	80.64	78.54
4.0	2	2.856	562.10±2.56	246.67±1.45	68.95	75.20	70.89
5.0	2	2.734	465.00±1.78	216.23±1.83	63.87	65.92	64.51
6.0	2	2.72	405.33±2.67	196.43±1.64	55.67	59.88	56.98
7.0	2	2.679	384.00±2.56	178.67±2.45	52.74	54.47	53.28
8.0	2	2.523	298.08±2.43	162.00±2.34	40.12	49.39	43.56
C.D. ( $p=0.05$ )			97.57	39.97			

C.D.= Critical difference and  $p$  = probability.

#### 4. Conclusion

At the acidic pH range from 2-4 there was significant increase in aluminum extraction while at pH 2, maximum iron extraction was carried out by *T. ferrooxidans*. Hence the media with this pH are optimum for better aluminum and iron extraction from bauxite ore.

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