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Leaching Uranium from Carbonate Ore Using *Thiobacillus thiooxidans* (S–V)

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ABSTRACT

By appropriate enrichment of solid and liquid samples collected from mining, processing, overburden and process waste of uranium ore, a number of acidophilic, chemoautotrophic sulphur oxidizers resembling *Thiobacillus thiooxidans* have been isolated. These were compared for their rate of sulphur oxidation and the best strain, *T. thiooxidans* (S-V) was used for leaching studies. Carbonate bearing sandstone uranium ore found in Pakistan requires 10–13 g H_2SO_4 for every 100 g ore to solubilize above 90%. When the selected strain, *T. thiooxidans* (S-V) was grown on mineral salt medium supplied with sulphur as the source of energy, it could produce 25 g/l H_2SO_4 in the medium dropping the pH from 2.5 to 0.87 in 18 days.

A suspension of 5% w/v slurry of uranium ore in mineral salt medium supplied with elemental sulphur (1%) and sodium thiosulphate (1%) resulted in a pH of 5.9. Using controlled conditions in a bioreactor (Biostat E), the pH dropped to 2.1 within three days after inoculation of the bacteria and resulted in 90% solubilization of the uranium. Incremental increase of slurry raised the pH which was then lowered by the action of bacteria. In this way up to 20% ore slurry could be achieved without inactivating the culture. At this stage more than 85% uranium was solubilized. These bacteria could be adapted to grow in a mineral medium containing 10% w/v ore slurry (pH 8.3) when supplied with sulphur and sodium thiosulphate as the energy source. Acid production resulted in the pH dropping below 1.3 within 8 days. When the ore slurry was increased to 15, 20 and 30% after the pH dropped below 2 in each case it was found that the bacteria could decrease the pH to 1.2 and cause an 85% solubilization of uranium.

INTRODUCTION

Uranium ore commonly occurs in tetravalent form and its solubilization involves oxidation to hexavalent uranium in the form of uranyl ions.

$$2U^{4+} + O_2 + 2H_2O \text{ ---- } 2UO_2^{2+} + 4H^+$$

This process can be carried out by bacteria either directly by their oxidation (DiSpirito and Tuovinen, 1981; 1982; 1984) or indirectly by ferric iron

MATERIALS AND METHODS

Media

9 K mineral salt medium at pH 2.5 without ferrous sulphate (Silverman and Lundgren, 1959) was supplied with either elemental sulphur (2% w/v, 9 KS^0) or ferrous sulphate (5% w/v, 9 KFe) for the enrichment and purification studies. Microscopic examination, using a bacteria counting chamber with circular cell depth of 0.02 mm and Thoma ruling on a 1 mm thick slide (Weber, England) and dark ground illumination was carried out to find the bacterial population. The biochemical changes i.e., production of ferric iron (in case of *Thiobacillus ferrooxidans*) and sulphuric acid (in case of both *T. thiooxidans* and *T. ferrooxidans*) were estimated and compared with zero day observations and uninoculated controls. Changes in these metabolites were taken as criteria for growth. For adaptation studies and fermentor runs, 9 K medium, without ferrous sulphate, was supplied with 1% w/v elemental sulphur and 1% w/v sodium thiosulphate (9KS^0 + ST). 9 K medium with or without sodium thiosulphate was autoclaved at 121°C for 20 min. (flasks) and 25 min. (fermentor) whereas steam sterilized sulphur or filter sterilized ferrous sulphate solution was added later on. Ore used for increasing slurry percentage in the fermentors was also steam sterilized.

Ore

A single lot of 40 Kg low grade uranium ore (0.038% U) of less than 1 mm particle size (as received from a mining area) was thoroughly mixed and used throughout the study.

Enrichment and isolation

Solid and liquid samples from mining, overburden, processing and process waste of a uranium ecosystem were used for enrichment of acidophilic chemoautotrophs using 9 KS^0 and 9 KFe media. For enrichment of *T. thiooxidans*, samples showing growth ($pH < 1$) on 9 KS^0 and no growth on 9 KFe were successively inoculated to fresh medium three times (to $pH < 1$). Cells were separated by centrifugation, checked for purity (no growth on 9 KFe), and diluted to 10^{-8}, 10^{-9}, 10^{-10} using pH 2.5 sulphuric acid solution. 1 ml from these dilutions was inoculated to 25 ml fresh 9 KS^0 medium (10 flasks for each dilution). Flasks having growth ($pH < 1$) at

RESULTS AND DISCUSSION

As a result of enrichment and purification for acidophilic chemoautotrophic sulphur oxidizers (*T. thiooxidans*), twelve bacterial strains were isolated from different solid and liquid samples. The were compared for the efficiency of oxidizing sulphur and *T. thiooxidans* (S-V) was selected as the best among them. The oxidation of sulphur in 9 KS^0 medium is shown in Figure 1. The drop in pH from 2.5 to 0.87 with 25 g/l sulphuric acid production in 18 days was observed at 30°C with shaking at 100 rpm.

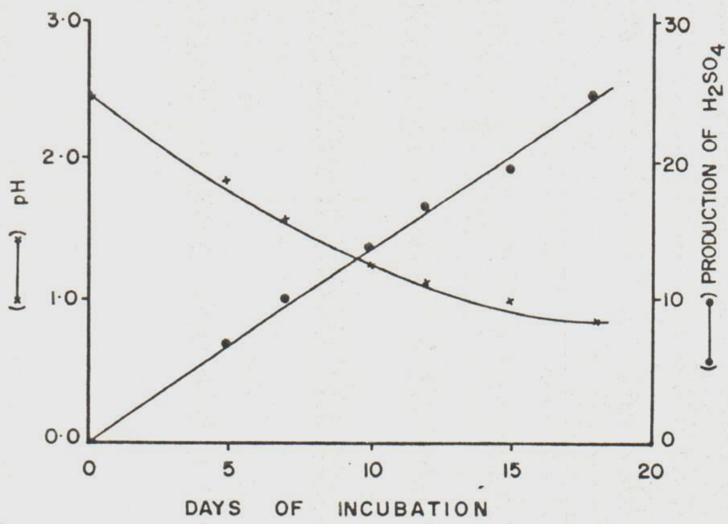

FIGURE 1 Production of sulphuric acid and pH change as a measure of oxidation activity of *Thiobacillus thiooxidans* (S-V) on 9 K mineral salt medium substituted with 2.0% w/v sulphur for ferrous sulphate, incubated at 30°C, 100 rpm.

These bacteria were then adapted to different slurry concentrations in 9 KS^0 + ST medium in shake flasks. Growth (pH < 1) was observed up to 5% w/v slurry in two weeks while higher concentrations required 5 weeks to show growth, pH 1.9 compared to 8.4 in uninoculated 10% ore slurry (Figure 2).

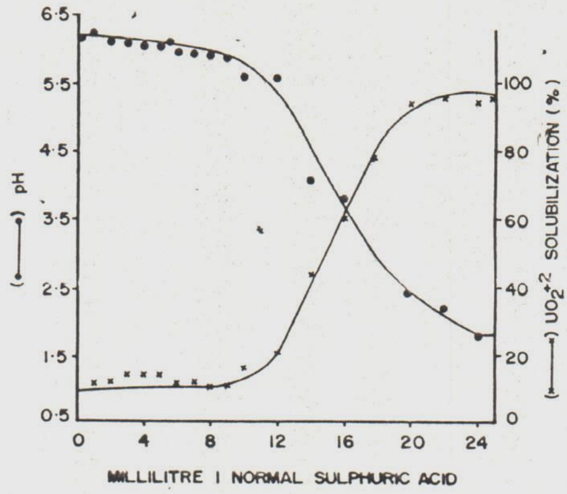

FIGURE 3 Changes in pH and percent solubilization of low grade uranium ore (0.038% U) with different amounts of acid (ml N H_2SO_4) in 10% w/v slurry in mineral salt medium at 100 rpm for 24 hours.

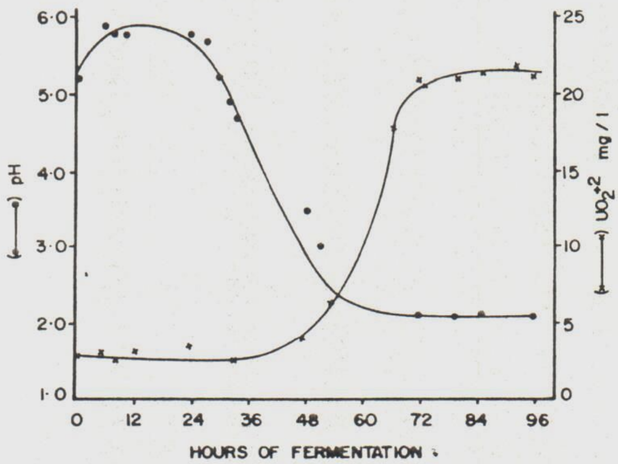

FIGURE 4 Bacterial leching of low grade uranium ore (0.038% U) and pH change as a result of growth of *T. thiooxidans* (S-V) inoculated to 8 l of mineral salt medium having 5% w/v ore slurry, 1% w/v elemental sulphur and 1% w/v sodium thiosulphate as the energy source. Batch fermentation at 300 rpm, 35°C and 60% pO_2 in a bioreactor.

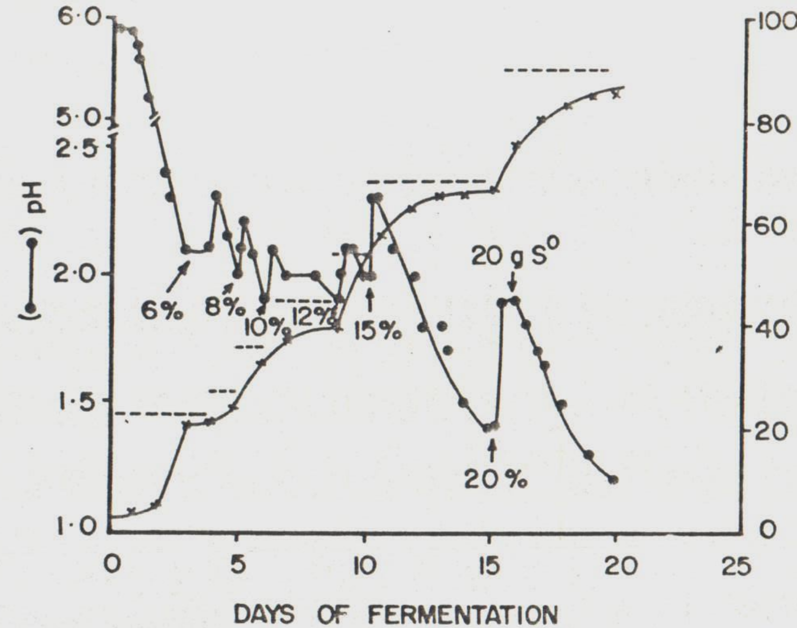

FIGURE 5 pH change and solubilization of uranium by *T. thiooxidans* (S-V) inoculated to 5% ore slurry in 8 l of mineral salt medium having 1% w/v elemental sulphur and 1% w/v sodium thiosulphate at 35°C, 300 rpm and 6 l/min aeration in bioreactor. Batch increase of ore slurry to 6, 8, 10, 12, 15 and 20%, additional energy source (20 g S^0) was supplied on 16th day. Lines (----) indicate the maximum UO_2^{+2} concentration at 100% solubilization of uranium from ore.

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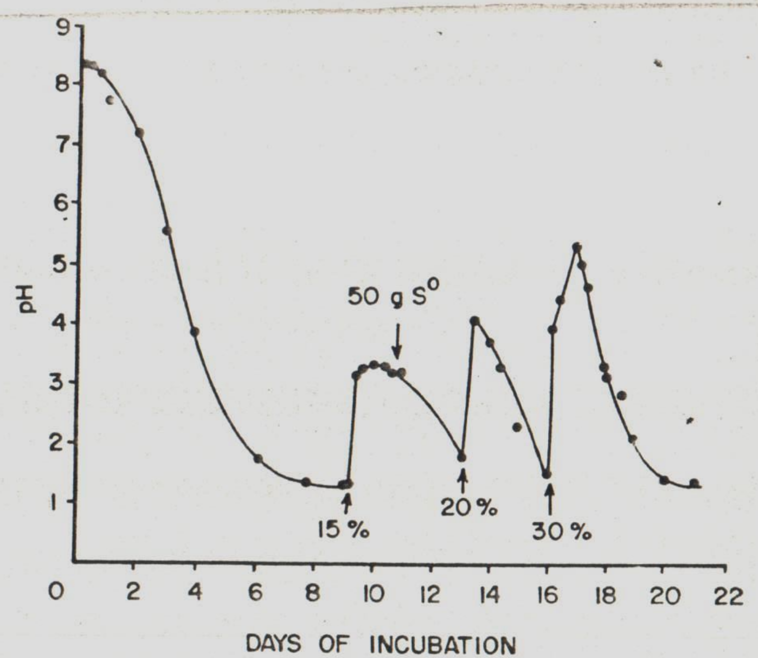

FIGURE 6 Production of acid (pH drop) by *T. thiooxidans* (S-V) inoculated to 10% ore slurry in 10 l mineral salt medium (without pH adjustment) having 1% w/v elemental sulphur and 1% w/v sodium thiosulphate as energy sources, in a bioreactor operated at 35°C, 300 rpm and 60% pO_2. Additional 50 g S^0 was supplied on day 11.

resulted in a slight increase (5.2 to 5.8) in pH which on bacterial growth, dropped during 24–60 h to nearly 2.0 resulting in solubilization of 90% uranium during subsequent 48 to 72 h, after which it was stabilized (Figure 4). At this stage the ore slurry was increased (Figure 5) to 6% thus resulting in pH increase. As a result of acid production by the bacteria pH of the medium again dropped. Similarly stepwise increase of ore slurry to 8, 10, 12, 15 and 20% increased the pH which again was dropped by bacterial acid production. Addition of ore was only carried out when the pH of the suspension had come down to 2.0. On day 15, (at 20% ore slurry) 20 g sterilized sulphur was added to supply additional source of energy for growing bacteria. On day 20, when pH was 1.2, 90% of uranium was recovered in solution form.

Fermentor run-II

Having an initial ore slurry of 10% w/v in 10 1 (9 KS^0 + ST) working volume, under the same fermentor conditions as above, the adapted bacteria (growing in a shake flask on 10% w/v slurry) were inoculated to observe their pH behaviour. It was observed (fig. 6) that bacteria could produce acid to drop the pH to 1.3 in 8 days. At this stage, the uranium solubilization was also monitored and was above 90% of total uranium load. Increasing the ore slurry to 15% by adding 500 g sterilized ore resulted in a rise of the pH to 3.25. On day 11, 50 g sulphur was added as an additional source of energy and acid. The bacteria by producing acid could drop the pH to 1.9 on day 13 when ore slurry was increased to 20% resulting in a pH of 4.0. The bacteria by their activity could drop the pH to 1.4 resulting in 85% of total uranium solubilized. At this stage, the slurry was increased to 30% causing a pH increase to 5.3. The bacteria were still active which resulted in acid production to drop the pH to 1.2, resulting in uranium solubilization above 85% of total load.

CONCLUSIONS

Uranium ore which requires 10–13 g H_2SO_4/100 g ore for solubilization of uranium can be bacterially leached. The bacteria which showed tolerance up to 30% ore slurry (maximum tried) by acid production from sulphur and thiosulphate dropping the pH below 1.2 could solubilize above 85% of uranium in 20 days by stepwise increase in ore slurry. Further experiments to optimize the nutrient requirements and multistage leaching will be carried out based on these results.

From 10 days old culture broth (pH < 1) of *Thiobacillus thiooxidans* (S-V) grown on 9 K mineral medium (with sulphur 2.0% instead of ferrous sulphate), the cells were harvested by centrifugation at 12,000 rpm, 4°C for 30 minutes. The cell pellet was resuspended in diluted H_2SO_4 of pH 2.5. One ml suspension (10^8–10^9 cells/ml) was inoculated to 100 ml of mineral medium 9K, (without $FeSO_4$) supplied with sodium thiosulphate (1% w/v) and sulphur (1% w/v), pH 4.5, in 250 ml Erlenmeyer flasks having varying amounts of ore. Changes in pH after 2 and 5 week incubation at 30°C, 100 rpm were recorded:

Ore	1 g		2 g		3 g		4 g		5 g	
	U	I	U	I	U	I	U	I	U	I
2 wk pH	4.7	< 1	5.0	< 1	5.1	< 1	5.4	< 1	5.9	< 1

	6 g		7 g		8 g		9 g		10 g	
	U	I	U	I	U	I	U	I	U	I
2 wk pH	6.3	5.4	6.7	6.5	7.3	6.9	7.9	7.2	8.5	8.3
5 wk pH	6.3	1.5	6.6	1.5	7.1	1.7	7.7	1.7	8.4	1.9

U = Uninoculated I = Inoculated

FIGURE 2 Adaptation procedure.

Acid leaching in shake flasks

From double strength 9 K medium, without ferrous iron, and 1 N H_2SO_4, 10% ore slurries having different amounts (0–24 ml) of acid in 100 ml final volume were made. The flasks were shaken at 100 rpm and were kept at 30°C (conditions used for bacterial incubation) for 24 hours. This incubation period was sufficient for chemical reactions, before estimating the pH and soluble uranium contents (Figure 3). It is clear that initially 10 ml acid was used to neutralize the ore without solubilization of uranium. Solubilization of uranium by acid occurred below pH 5 and was maximum (> 90%) below pH 2. Further addition of acid had no additional effect. Thus, it is calculated that 10–13 g H_2SO_4 are required for every 100 g ore.

Bacterial leaching

Fermentor run-I

A *Thiobacillus thiooxidans* (S-V) culture adapted to 5% ore slurry in shaken flasks was inoculated into 8 l working volume of 5% ore slurry, which maximum dilution were taken as pure cultures of *Thiobacillus thiooxidans*. The isolates were compared for their sulphur oxidizing enzyme. Equal numbers of bacteria (10^8 cells) of different strains were inoculated to replicate flasks containing 100 ml 9 KS^0 medium. These flasks were incubated at 30°C and 100 rpm for 2 weeks. One flask each from various strains was removed after 5, 10 and 15 days incubation for checking pH and estimating free acid produced using standard methods (given in the 'Estimations' section). The amount of acid produced (or sulphur oxidized) per day was taken as the measure of sulphur oxidizing enzyme. The growth behaviour of selected strains of *T. thiooxidans* (S-V) was checked by shake culture on 9 KS^0 medium.

Adaptation

Actively growing cultures of *T. thiooxidans* (S-V) were inoculated (1 ml) into shake culture flasks containing 100 ml 9 KS^0 + ST medium with 1 g/100 ml ore and the bacterial growth was compared with uninoculated flasks under the same conditions at 30°C.

Estimations

Free acid produced (H_2SO_4) was estimated by titrating a known volume with standard NaOH solution to pH 2.5 and the pH was checked with a Corning 130 pH meter. Soluble uranyl sulphate was determined by the Arsenazo III method after extraction with Tributyl Phosphate in presence of EDTA (Marczenko, 1976).

Fermentor conditions

A B. Braun Biostat·E fermentor was used for leaching of uranium ore at a working volume of 8 1 with 5% initial ore slurry in 9 KS^0 + ST medium in first run, which was incrementally increased to 20%. In a second run 10 1 working volume having 10% ore slurry was incrementally increased to 30% ore slurry. Other conditions were 35°C, 300 rpm, 6 1 air/min. (autocontrol to pO_2 60%). The contents of the vessel were sterilized in-situ for 25 min. Changes in pH were recorded. Samples were withdrawn at intervals and analysed for concentration of soluble uranyl ions.

generation from ferrous iron (Agate, 1983; Guay and Silver, 1981; Vuorinen *et al.*, 1985);

$$UO_2 + 2Fe^{3+} ---- UO_2^{2+} + 2Fe^{2+}$$

and/or sulphuric acid production from elemental sulphur as well as reduced sulphur compounds (Kelly *et al.*, 1979):

$$UO_2 + 0.5O_2 + H_2SO_4 ---- UO_2SO_4 + H_2O.$$

Elemental sulphur and reduced sulphur compounds are oxidized by *T. thiooxidans* producing sulphuric acid efficiently and to greater extent (pH < 0.5) under proper conditions;

$$S^0 + 1.5\ O_2 + H_2O ---- SO_4^{2-} + 2H^+$$

(Bosecker, 1986; Lundgren *et al.*, 1986).

Thiosulphate is oxidized either to tetrathionate by thiosulphate oxidase:

$$2S_2O_3^{2-} ---- S_4O_6^{2-} + 2\ e^-$$

or cleaved to sulphur and sulphite by rhodanase;

$$SSO_3^{2-} ---- S + SO_3^{2-}$$

followed by oxidation of sulphur and sulphite, generating sulphuric acid. Elemental sulphur after conversion to sulphite by sulphur oxidizing enzyme (SOE) which on further oxidation in presence of sulphite oxidase (SO) is converted to sulphate;

$$S + O_2 + H_2O\ \underline{(SOE)}\ SO_3^{2-} + 2H^+,\ 2SO_3^{2-} + O_2\ \underline{(SO)}\ 2SO_4^{2-}$$

(Lundgren *et al.*, 1986)

Thiobacillus thiooxidans has been employed for leaching carbonate rich German copper shale (Bosecker *et al.*, 1978); non-sulphidic industrial waste products (Bosecker, 1983); Vanadium recovery (Sullivan *et al.*, 1980); Chromite (Ehrlich, 1983); Metal sulphides (Groudev, 1983) and copper ores & concentrate (Agate and Khinvasara, 1986).

The uranium minerals found in Pakistan are tyumyunite in the oxidized zone and uraninite or coffinite in the unoxidized zone. These occur in grain binding material and sandstone deposits (having up to 0.5% U_3O_8) mostly containing 5% calcite and minor amounts of pyrite (Moghal, 1974). Feasibility of bacterial leaching of uranium ore in Pakistan was reviewed by Aslam and Aslam (1970). Present studies were aimed to find out a possible role of an active strain of *Thiobacillus thiooxidans* in uranium solubilization through sulphuric acid generation from oxidation of sulphur and thiosulphate.