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MICROBIOLOGICAL CONTROL IN AN INDUSTRIAL COPPER DUMP LEACHING OPERATION

Direct microscopic counting was the most suitable method to enumerate Thiobacillus ferrooxidans in solutions and ores under industrial dump leaching conditions. Determinations of the bacterial activity in situ were necessary, since there was no regular correlation between the number and activity of these bacteria. Measurements of the bacterial iron oxidation rates as well as of the bacterial $^{14}CO_2$- fixation were the most suitable ways to determine this activity.

Introduction

The ore piles in the vicinity of the Vlaikov vrah mine, Bulgaria, contain about thirty million tons of waste low-grade copper sulphide and mixed ores from open cut mining. The initial copper content was about 0.05 - 0.10%, but some parts of the dumps had a higher copper content - up to 0.20%. The main copper-bearing minerals are chalcopyrite, covellite and chalcocite. Pyrite is well represented, too, where quartz and feldspars are the basic minerals of the host rock.

The observations conducted in that area during 1986 showed that after rainfall, acid drainage waters with a high content of copper and iron ions (over 0.5 and 2 g/l, respectively) and of bacteria related to the species Thiobacillus ferrooxidans and T. thiooxidans flow out of

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the dumps. Detailed information about the climate at Vlaikov vrah was obtained, and the geological, mineralogical and hydrogeological characteristics of the ore and dumps were investigated. The leachability of ore samples taken from the orebody and dumps was studied under laboratory conditions by using the shake-flask technique and large column tests. Large column tests were also carried out to determine the optimum parameters of the leaching process.

The favourable character of the data resulting from these studies served as an argument for the construction of an industrial plant for biological leaching, which was commissioned in 1972. In operation, a solution containing bacteria, dissolved oxygen, sulphuric acid and iron ions was pumped to the top of the dumps. The solution percolated through the dump and dissolved copper. Dump effluents were sent to the precipitation unit where copper was removed by cementation with iron. The tailing solution was recycled to the dumps. Detailed information about this industrial copper dump leaching operation has been published elsewhere [1].

Since 1968 there have been regular checks of the microflora in the dumps. Since 1972, these checks have also covered the separate stages of the industrial plant. The microbial ecology was investigated to determine the nature and extent of microbial populations occuring in these ecosystems. The activities of these populations depended upon, and also affected, the physicochemical parameters of their micro--environments. Information from such studies is of great importance to the understanding of the mechanisms of the leaching of sulphide minerals under industrial conditions. However, their final purpose is to establish the possibilities of increasing the leaching rate and extent. Some data in this respect have already been published [2]. This paper emphasizes the different methods which were used for direct and indirect enumeration or measurement of the activity of T. ferroxidans in circulating solutions and ore dumps from the industrial plant at Vlaikov vrah. This bacterium is the most important microorganism taking part in the leaching of copper. The character of the microbiological control in such systems is also presented.

Materials and Methods

The procedures for collecting liquid or solid samples for microbiological analysis have already been described [3]. Regular sampling was carried out at the following points of the industrial plant: the four main effluents from the dumps, the collection pond in front of the

cementation unit, the entrance and outlet of the cementation unit, the regeneration pond and several points in the dump area. Other points were assayed only occasionally. Solid samples were collected with a calibrated stainless-steal hand corer not only from (or near) the surface of the dumps, but also at various levels by cutting sampling trenches with a backhoe.

The following methods of enumerating T. ferrooxidans were used in the test work: direct microscopic counting, the most probable number (MPN) method, culturing on membrane filters [4], culturing on solid agar [3,5,6] and silica gel [3,7] media, the determination of bacterial nitrogen [8], the determination of organic carbon by an organic carbon analyzer, the determination of $^{14}CO_2$- fixation [3], counting by Coulter Counter [9] and the fluorescent antibody (FA) staining technique (IO). For enumeration of the cells attached to the solid samples, accurately--weighed amounts of ground solid samples (1 to 20 g) were transferred to 300 ml sterile Erlenmeyer flasks containing 50 ml of sterile acidified (pH 2.0) distilled water. The slurry was agitated for 15 - 30 min.on a rotary shaker and allowed to settle for 1 - 2 hours. The supernatant from this treatment was decanted aseptically. The solid residue was further disrupted by ultrasound to release bacteria that may have been firmly attached in the ore pores. The powders that resulted after ultrasound treatment were washed with acidified distilled water and decanted. The decanted liquid was mixed with the first supernatant and the mixture was used for cell enumeration.

The oxidation rates of bacteria in situ versus ferrous iron were determined by using 300 ml Erlenmeyer flasks containing 100 ml of solution. Solutions from different points in the industrial plant as well as supernatants containing cells dislodged from solid samples were used in these experiments. Controls containing synthetic nutrient media were also set up. Five ml of a methanol solution containing 2% of thymol were added to the sterile control flasks. Some flasks were incubated in situ in the industrial plant at 9 - 15°C, while other flasks were incubated in thermostat at desired temperatures. The flasks were incubated for 5 days without agitation.

The technique described by Karavaiko and Moshniakova [11] was used with some modifications to determine the $^{14}CO_2$- fixation in situ. Ten ml of solutions containing bacteria were added to sterile 16 ml glass bottles. Controls containing synthetic nutrient media were also used. These synthetic nutrient media were inocculated with local bacterial strains. The bottles were capped with rubber serum stoppers. Using a syringe, 0.1 ml of $Na^{14}CO_3$ solution releasing 3 x 10^6 counts/min. ml was injected through the serum caps into the bottles. The bottles were

agitated for several minutes, after which, some of them were incubated in situ at 9 - 15°C, while the others were incubated in thermostat at desired temperatures. The incubation time varied from 3 to 10 days. After incubation, 1 ml of 40% formalin was added to each bottle to stop the bacterial activity. The solutions were filtered through membrane filters, which were then treated with 2% hydrochloric acid and dried, and the radioactivity of the bacteria was measured, using a scintillation counter.

Results and Discussion

Practically all the known methods for enumerating T. ferrooxidans were used in this study. Most of them have been critically evaluated in an excellent review [12]. However, only direct microscopic counting, the MPN method using serial dilutions in liquid medium, and culturing on agar or silica gel plates seemed to be feasible as reglular methods under industrial plant conditions.

Direct counting of T. ferroxidans by means of a phase-contrast microscope and a counting chamber appeared to be the most suitable method for enumerating these bacteria in solutions under such conditions. The method is a tedious procedure but its accuracy (Table 1) and duration compare well with those of the remaining methods. Direct microscopic counting does not differentiate between T. ferrooxidans and T. thiooxidans, and Leptospirillum ferrooxidans is also poorly distinguishable from these bacteria. However, mixed populations of these three species make up the most active biological oxidizer of sulphide minerals [13], so a separate determination of each of these species is unnecessary in common practice under such conditions. Some acidophilic heterotrophs are also included in the total number of bacteria found by this method. However, the number of such heterotrophs is usually much lower than that of the chemoautotrophic bacteria.

The MPN method does not differentiate between T. ferrooxidans and L. ferrooxidans, and the results are obtained only after several days of incubation. Culturing on agar media did not prove suitable, since most of the local strains of T. ferrooxidans had no a reproducible growth on such media. The silica gel media were more suitable, but the incubation period for colony production on them was long (15 days, at least).

Direct microscopic couting was also employed to enumerate bacteria in washings of ground and sonicated ore samples. However, the values obtained in such cases were significantly lower than the values obtained

Table 1

Enumeration of Thiobacillus ferrooxidans in Solutions from the Copper Dump Leaching Operation at Vlaikov vrah by Means of Different Methods

Method	Solution No. 1[a]	Solution No. 2[a]	Solution No. 3[a]	Solution No. 4[4]
	Cells/ml[c]			
Direct microscopic counting	5.1×10^7	8.0×10^6	4.4×10^5	2.3×10^4
MPN method	4.5×10^7	6.8×10^6	3.5×10^5	1.8×10^4
Culturing on membrane filters	4.1×10^7	6.6×10^6	3.3×10^5	1.7×10^4
Culturing on solid media:				
- on Manning's medium	3.5×10^7	5.9×10^6	3.0×10^5	1.5×10^4
- on 9K silica gel medium	3.8×10^7	5.6×10^6	3.3×10^5	1.8×10^4
Bacterial N determination	5.3×10^7	8.8×10^6	5.0×10^5	2.7×10^4
Organic C determination	5.5×10^7	9.1×10^6	5.3×10^5	2.7×10^4
$^{14}CO_2$-fixation determination	4.7×10^7	8.6×10^6	4.1×10^5	2.5×10^4
Counting by Coulter Counting	5.3×10^7	8.6×10^6	5.9×10^5	2.8×10^4
FA staining method	4.1×10^7	7.3×10^6	3.0×10^5	1.4×10^4

[a] - Dump effluent, [b] - Cementation unit effluent, [c] - Pregnant solution from the dumps with pH 2.3, T. ferrooxidans 10^7 cells/ml, NH_4^+ 1 mg/l, PO_4^{3-} 1 mg/l, Cu^{2+} 0.5 g/l.

Table 2

Enumeration of Thiobacillus ferrooxidans in Ores from the Copper Dump Leaching Operation at Vlaikov vrah by Means by Different Methods

Method	Ore No. 1	Ore No. 2	Ore No. 3
	Cells/g[a]		
Direct microscopic counting	1.2×10^8	2.3×10^6	4.8×10^4
Bacterial N determination	1.8×10^8	3.5×10^6	8.1×10^4
Organic C determination	2.1×10^8	3.9×10^6	9.0×10^4
$^{14}CO_2$-fixation determination	1.7×10^8	3.2×10^6	8.0×10^4

[a] - Each value represents the mean of ten determinations

Table 3

Oxidation of Ferrous Iron by Means of Bacteria from the Vlaikov vrah Copper Dump Leaching Operation

Test conditions[a]	Fe^{2+} oxidized, g/l	
	12 - 15°C	27°C
Solution No 1[b]	0.51	1.49
Solution No 1 + thymol	0.01	0.03
Solution No 1 + $(NH_4)_2SO_4$ + KH_2PO_4	0.86	2.24
Solution No 2[c] + Fe^{2+}	1.87	4.40
Solution No 2 + thymol + Fe^{2+}	0.01	0.03
Solution No 2 acidified with H_2SO_4 to a pH of 2.0 + Fe^{2+}	1.22	2.71
Solution No 2 + $(NH_4)_2SO_4$ + KH_2PO_4 + Fe^{2+}	2.35	5.58
Solution No 2 but containing 1.5 g/l Cu^{2+} + Fe^{2+}	1.29	2.64
9K nutrient medium without bacteria	0.02	0.03
9K nutrient medium + ore from the dumps (10^7 cells/g of T. ferrooxidans)	4.85	9 (for 104 h)
9K nutrient medium + the same ore + thymol	0.01	0.03
9K nutrient medium + local strain of T. ferrooxidans	5.01	9 (for 100 h)

[a] - $(NH_4)_2SO_4$, KH_2PO_4 and Fe^{2+} (as $FeSO_4 \cdot 7H_2O$) were added in concentrations of 1.0, 0.5 and 6.0 g/l, respectively.

[b] - Barren solution from the cementation unit with pH 3.5, Fe^{2+} 3.7 g/l, T. ferrooxidans 10^5 cells/ml, NH_4^+ non detected, PO_4^{3-} 1.4 mg/l.

[c] - Each value represents the mean of ten determinations.

by means of other methods (Table 2).

Regardless of the fact that most T. ferrooxidans bacteria are located in the dumps and not in the circulating solutions [2], the enumeration of bacteria attached to ore particles is unnecessary in common practice under industrial dump leaching conditions. Such systems can be regarded as chemostat cultures in a steady state, and the bacteria may not show much change in population level over long periods [14]. An equilibrium exists between the cells attached to the ore particles and the cells in solutions [3]. This equilibrium is not stable and may be sharply changed, e.g., after rainfall. Nevertheless, the number of cells in the solutions reflected the character of the leaching process. A higher number of T. ferrooxidans in dump effluents usually

Table 4

$^{14}CO_2$-fixation Activity of Bacteria from the Vlaikov vrah Copper Dump Leaching Operation

Test conditions[a]	Temperature, °C	Radioactivity of bacteria, counts/min.ml(g)
Solution No 1[b]	9 - 11	800
Solution No 1	12 - 14	1 400
Solution No 1	27	4 800
Solution No 1 + $(NH_4)_2SO_4$ + KH_2PO_4	11 - 14	1 800
Solution No 2[c] + Fe^{2+}	12 - 15	11 800
Solution No 2 acidified with H_2SO_4 to a pH of 2.0 + Fe^{2+}	11 - 15	6 800
Solution No 3[d] + Fe^{2+}	12 - 14	4 200
Ore suspension in 9K nutrient medium (10^7 cells/ml of T. ferrooxidans)	11 - 14	7 000
The above-mentioned ore suspension	27	21 700
9K nutrient medium + local strain of T. ferrooxidans	12 - 14	12 300
9K nutrient medium + the same strain of T. ferrooxidans	27	27 500

[a] - See footnote a to Table 3; The incubation period was 5 days.
[b] - See footnote b to Table 3. [c] - See footnote c to Table 3.
[d] - Pregnant solution from the dumps with 10^6 cells/ml of T.ferrooxidans.

denoted better leaching. Furthermore, in each case where there was evidence of leaching, T. ferrooxidans was present.

However, more important than the determination of the number of cells was the determination of the level of bacterial activity in situ in ores and natural solutions and at natural temperatures. This was due to the fact that in some cases no correlation was observed between the number of bacteria and their activity. Measurements of the bacterial iron oxidation rates (Table 3) and of the bacterial $^{14}CO_2$-fixation (Table 4) were the most suitable ways to determine the bacterial activity in situ. It is known that in such systems bacterial activity does not depend upon any single factor, but rather upon a complex of factors [15]. Factors such as temperature, pH concentrations of copper ions and some essential nutrients, etc., markedly affected the bacterial activity. The magnitudes of some of these factors in situ differed from optimum physiological magnitudes. Such a finding is of great importance since bacterial activity can be enhanced by the artificial improvement of some of the rate-limiting environmental factors. Nowadays, this is

the one and only applicable way to increase dump bioleaching under industrial conditions.

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STRESZCZENIE

Groudeva V.I., Groudev S.N., 1987. Mikrobiologiczna kontrola przemysłowego procesu ługowania miedzi na hałdach, Fizykochemiczne Problemy Mineralurgii, 19; 283-291.

W warunkach przemysłowego ługowania hałd, prosta metoda mikrosko-

powa była najbardziej odpowiednia do zliczania bakterii Thiobacillus ferrooxidans w roztworach i rudach. Ponieważ nie ma prostej zależności pomiędzy liczbą i aktywnością tych bakterii było konieczne oznaczenie aktywności bakterii in situ. Najbardziej przydatną metodą oznaczania tych aktywności okazały się pomiary szybkości utleniania żelaza II oraz wiązania $^{14}CO_2$ - bakteriami.

СОДЕРЖАНИЕ

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В условиях промышленного выщелачивания отвалов простой микроскопический метод наиболее соответствовал считыванию бактерий Thiobacilus ferrooxidans в растворах и рудах. Так как нет простой зависимости между числом и активностью этих бактерий необходимо обозначить активность бактерий в месте образования. Наиболее пригодным методом обозначения этих активностей оказались измерения скорости окисления железа III, а также вязание $^{14}CO_2$ бактериями.