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TITLE

Microbial oxidation and reduction of inorganic sulphur compounds in relation to the development and control of microorganisms active in leaching operations, (part of a coordinated prpgramme on bacterial leaching of uranium ores)

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AUTHOR(S)

O.H.Tuovinen

INSTITUTE

University of Adelaide
Waite Agricultural Research Institute
Glen Osmond
Australia

INTERNATIONAL ATOMIC ENERGY AGENCY

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FINAL REPORT TO THE INTERNATIONAL ATOMIC ENERGY AGENCY

Project: Microbial oxidation and reduction of inorganic sulphur compounds in relation to the development and control of micro-organisms active in leaching operations

Part of the IAEA Coordination Programme on 'Bacterial Leaching of Uranium Ores'

Department of Agricultural Biochemistry and Soil Science,
Waite Agricultural Research Institute,
The University of Adelaide,
Glen Osmond,
SOUTH AUSTRALIA 5064

Chief Scientific Investigator: Dr. Olli H. Tuovinen

Period of contract:

1470/RB	1 April 1974 - 31 March 1975
1470/R1/RB	1 April 1975 - 31 March 1976
1470/R2/RB	1 April 1976 - 31 October 1976

Present address: Department of General Microbiology,
University of Helsinki,
Malmiukatu 20,
SF-00100 Helsinki 10,
Finland

Background and scope of the project

Applied microbiological studies clearly demonstrate the potential and feasibility of the use of Thiobacillus ferrooxidans-type of bacteria for the leaching of metals from ores and regenerating acidic ferric sulphate solutions for the dissolution of uranium ores. However, the biochemical and physiological regulation and control of bacterial activities in leaching operations remains to be elucidated in detail. The project described in this report was initiated to provide further information on the various pathways by which T. ferrooxidans bacteria utilize inorganic sulphur compounds for oxidation, energy, growth and synthesis of cellular material. The type of the sulphur metabolism - oxidative, or assimilative and reductive - depends on the growth conditions of the bacterium. A detailed biochemical and physiological study of the various energy-related pathways is of importance to applied leaching operations involving bacterial action, where the same bacteria may have either sulphur compounds or ferrous-iron as an oxidizable substrate. Thus the bacteria are able to shift the type of their metabolism in relation to the availability of the substrate in their environment.

With ferrous-iron as a source of energy, sulphate is often the only source of sulphur in the medium and therefore it has to be assimilated and reduced to sulphide before its incorporation into cellular materials and sulphur-containing amino acids. During growth on reduced sulphur compounds, e.g. thiosulphate, reduced sulphur is also available for the cellular synthesis and thus the energetically expensive sulphate reduction is not required provided that the bacterium can derive the sulphur requirement from the oxidizable sulphur compound.

In this project, the thiosulphate-oxidizing strain of T. ferrooxidans was originally derived from an iron-oxidizing culture. In addition, the study included a heterotrophic glucose-growing strain of T. ferrooxidans obtained from Dr. F. Shafia. This particular strain (KG-4) had reputedly lost its ability to grow autotrophically on ferrous-iron. It grows on a number of organic compounds and is supposed to be an obligate organotrophic bacterium. Parallel with these studies, several analytical methods were developed and further improved.

Experimental methods

Experimental methods and research materials are described in detail in the enclosed publications.

Results obtained under the Research Contract

The results are described in full in the publications attached with this report, as follows:

(1) The uptake and assimilation of sulphate by Thiobacillus ferrooxidans. Archives of Microbiology 105, 123-127, 1975.
By O.H. TUOVINEN, B.C. KELLEY, and D.J.D. NICHOLAS

(2) A bioluminescence method for determining adenosine 3'-phosphate 5'-phosphate (PAP) and adenosine 3'-phosphate 5'-sulphatophosphate (PAPS) in biological materials.
Analytical Biochemistry 67, 540-551, 1975.
By P.E. STANLEY, B.C. KELLEY, O.H. TUOVINEN, and D.J.D. NICHOLAS

(3) Patent protection of microorganisms with special reference to ferrous-iron and sulfur oxidizing bacteria.
Biotechnology and Bioengineering 17, 1853-1857, 1975.
By O.H. TUOVINEN and D.J.D. NICHOLAS

(4) Fate of adenosine 5'-sulphatophosphate (APS) and adenosine 3'-phosphate 5'-sulphatophosphate (PAPS) in various biological materials.
Proceedings of the Australian Biochemical Society 8, 34, 1975.
By B.C. KELLEY, O.H. TUOVINEN, S.K. SAWHNEY, and D.J.D. NICHOLAS

(5) The use of a bioluminescence method to study the metabolism of adenosine 3'-phosphate 5'-sulphatophosphate (PAPS) in microorganisms and plants.
Proceedings of the Australian Biochemical Society 8, 39, 1975.

(6) Utilization of thiosulphate by Thiobacillus ferrooxidans. Proceedings of the Australian Biochemical Society 9, 30, 1976.
By B.C. KELLEY, O.H. TUOVINEN, and D.J.D. NICHOLAS

(7) Enzymic comparisons of the inorganic sulfur metabolism in autotrophic and heterotrophic Thiobacillus ferrooxidans.
Canadian Journal of Microbiology 22, 109-113, 1976.
By O.H. TUOVINEN, B.C. KELLEY, and D.J.D. NICHOLAS

(8) The degradation of sulphur nucleotides in cell-free extracts of Thiobacillus ferrooxidans.
Zeitschrift für Allgemeine Mikrobiologie 16, 551-555, 1976.
By O.H. TUOVINEN, B.C. KELLEY, and D.J.D. NICHOLAS

(9) Utilization of ^{35}S-thiosulphate and an appraisal of the role of ATP-sulphurylase in chemolithotrophic Thiobacillus ferrooxidans.
Archives of Microbiology 109, 205-208, 1976.
By B.C. KELLEY, O.H. TUOVINEN, and D.J.D. NICHOLAS

(10) Oxidation and reduction of inorganic sulphur compounds by Thiobacillus ferrooxidans.
IAEA Coordination Meeting on "Bacterial Leaching of Uranium Ores", University of Warwick, U.K., December 1976.
By O.H. TUOVINEN

(11) Bacterial oxidation of polythionates: determination of tetrathionate with an ion-selective electrode.
Applied and Environmental Microbiology 33, in press, 1977.
By O.H. TUOVINEN and D.J.D. NICHOLAS

(12) Proton translocation in intact cells of Thiobacillus denitrificans.
Archives of Microbiology, in press, 1977.
By O.H. TUOVINEN, D.J.D. NICHOLAS, and M.I.H. ALEEM

In addition, a very detailed account of some of the results is included in a dissertation submitted by Mr. B.C. Kelley to the University of Adelaide in partial fulfilment for a Ph.D. degree. A copy of this thesis will be forwarded to the Agency as soon as the Council of Research Studies of the University of Adelaide has conferred the degree to Mr. Kelley. It is anticipated that a copy will be available to the Agency within the next three months.

The unpublished work commenced during the Research Contract period includes three major projects as follows:

(1) Transition of Thiobacillus ferrooxidans from heterotrophic growth on glucose to autotrophic growth on ferrous-iron.
The reputedly obligate organotrophic cultures of T. ferrooxidans KG-4 (Shafia) which had been subcultured on glucose contained a small number of cells which grew autotrophically when transferred to media containing

ferrous-iron. The ratio of ferrous-iron oxidizers to glucose-grown bacteria (0.06-13.81 iron-oxidizers per 10^4 glucose-grown bacteria) was greatest during the mid-logarithmic growth phase. The ability to oxidize ferrous-iron was tested with plate counts and in dense cell suspensions and was related to the increase in the cellular ATP-content. Similar results were obtained using the glucose-grown strain of T. acidophilus (Silver) which is another bacterium reputed to have lost its ability to couple the energy from ferrous-iron oxidation to growth and carbon dioxide fixation. It is suggested that the enzymes mediating the autotrophic type of metabolism, namely the "ferrous-iron oxidase" and those of the Calvin cycle, may be repressed during growth on glucose. These enzymes may be partially derepressed when the bacteria are cultured in conditions which only permit the autotrophic mode of growth. The study will be shortly written up for publication.

(2) The development of a method to determine the ATP-content of bacteria attached to ore particles.

There is currently a lack of reliable methods to measure bacterial numbers and activities in environments where the bacterial cells may be partly attached to ore surfaces. The present study indicates that the bacterial ATP-content may be used as an index, alternative to or parallel with bacterial enumeration techniques, to follow bacterial activities in leaching environments. The study involves the development of a method for quantitative extraction of ATP from bacterial cells in samples containing mineral particles to which the bacterial cells may be attached. The extraction method also dissolves metals from ore particles and an additional procedure is required for their removal by precipitation. It is emphasized that the determination of the bacterial ATP content requires long series of comparative samples but has the advantage of indicating the total bacterial content irrespective of whether the cells are free-swimming or attached to ore particles. The work is planned to be continued under the Research Agreement proposed for 1977.

(3) Microbiological and chemical interactions of inorganic sulphur compounds.

The work initiated towards the end of the research period indicated that several chemical reactions take place during the bacterial oxidation

of inorganic sulphur compounds. The two important reactions, with respect to the regulation of the pathway of sulphur compound oxidation, are as follows:

tetrathionate + sulphide ⟶ thiosulphate + sulphur

tetrathionate + sulphite ⟶ thiosulphate + sulphate

Thiosulphate oxidation by T.ferrooxidans may proceed via two pathways:

either to form tetrathionate;

or to cleave to sulphide and sulphite.

Thus the chemical reaction between tetrathionate and sulphide/sulphite may regulate the relative contributions of the two routes of thiosulphate oxidation. In addition, these reactions can be employed to determine tetrathionate by a simple and fast titrimetric method. This work will be continued under Research Agreement proposed for 1977.

Conclusions

The results described in this report indicate that T.ferrooxidans-type of bacteria have a very versatile metabolism, ranging from the ability to assimilate sulphate during growth on ferrous-iron to that to oxidize inorganic sulphur compounds and organic matter for energy. These bacteria can adapt to various growth conditions which is of great importance to the microbiological leaching processes where a wide range of oxidizable substrates is available to support the bacterial solubilization of ores. A unique feature to these bacteria is their ability to maintain, in heterotrophic growth conditions, the enzymes required for the autotrophic mode of growth. This ability may offer a further industrial application for T.ferrooxidans: the bacteria can be grown up in large quantities on organic (waste) materials for use in the oxidation of metal sulphides and ferrous-iron in metallurgical processes.

Financial statement on the project expenditure

A financial (interim) statement prepared on 1st November 1976 is enclosed with this report.

NOTE: The balance of funds due under the Research Contract No. 1470/R2/RB should be directed to the University of Adelaide.
Any other correspondence relating to these research contracts or this report should be addressed to Dr. O.H. Tuovinen at his present address in Finland.

29th December 1976 [redacted]

O.H. Tuovinen

O.H. TUOVINEN