UPTAKE OF METAL IONS BY PHOTOTROPHIC BACTERIUM

T. M. VATSALA
Shri AMM Murugappa Chettiar Research Centre, Tharamani, Madras 600 113, India.

ABSTRACT

The phototrophic bacterium (PTB) *Rhodospirillum* sp. was found to extract a variety of metal ions. Nearly 80% of cadmium, 60% of nickel and 100% of lead were absorbed. Older cells were able to remove these metals faster.

INTRODUCTION

In addition to their usefulness in metal extraction from ores[1–5], microbes have proved to be excellent tools for recovering accumulated metals from solutions[6–8]. These procedures may have overlapping functions. The use of microbial cells as biosorbents for heavy metals offers a potential alternative to the existing methods for decontamination or recovery or both of heavy metals from a variety of industrial process streams[9,10]. Microbes are useful in extracting metals from seawater also[11]. In the present work, we describe the removal of lead (Pb), nickel (Ni) and cadmium (Cd) ions from solutions by a phototrophic bacterium (PTB) *Rhodospirillum* sp. isolated from the shoot apex of water hyacinth[12]. Preliminary results were reported earlier[13,14].

MATERIALS AND METHODS

Cultures of *Rhodospirillum* sp. were maintained on a medium described by Kobayashi *et al*[15].

For the metal uptake studies, experiments were carried out in 30 ml screw-cap bottles completely filled to maintain anaerobicity. Metal ions (Pb:25–75 ppm; Cd:15–30 ppm; Ni:15–30 ppm and Hg 1–5 ppm) were added to the medium individually and sterilized. Ten-day-old cultures were inoculated at 1% level (O.D. 0.30), controls were maintained without addition of any metal ions. All the tubes were incubated at 25°C and illumination was provided from 3 × 100 W incandescent lamps (3000 lux at the vessel surface) for 8 hr a day with occasional shaking. After 10 days of incubation, the growth was measured at 665 nm in a Systronic 106 spectrophotometer. The cells were centrifuged, washed, dried, weighed and digested. The digested cells, culture filtrate and washing were analysed for metals by using an atomic absorption spectrophotometer[16].

To test the ability of older cells in the uptake of metals, cells grown in control medium for 10 days were centrifuged, washed and reintroduced into tubes containing the metals and further incubated for 3 days under conditions as mentioned above. After incubation, the cells were centrifuged, washed, dried, weighed, digested and tested for the metals. In each study, 10 tubes were kept for each metal at various levels as mentioned and the experiments were repeated 6 times.

RESULTS

The uptake of various metals is given in table 1. Hg even at 1 ppm level was toxic to the bacteria. Pb promoted the growth of bacteria as shown by increased biomass at 25, 50 and 75 ppm. Nearly 100% of the Pb added was taken up when added at 25 and 50 ppm levels initially. Only 42% of the added Pb was taken up by the cells at 75 ppm level.

Ni promoted the growth to an insignificant level and nearly 60% was taken by the cells. Cd did not enhance the growth of the bacteria but nearly 80% uptake of the added Cd was observed. Above 30 ppm levels Cd and Ni were lethal to the bacteria.

Table 1 *Absorption of various metals by Rhodospirillum sp.*

Metals added (ppm)		Dry biomass (mg/l of culture	Levels of metal (ppm) absorbed in filtrate washing	cells	% absorption by cells
Control		333.0	–	–	–
Lead	25	625.0	N.D	N.D	24.8	100.0
	50	768.0	N.D	N.D	49.8	100.0
	75	750.0	46.0	N.D	28.0	42.0
Nickel	15	340.0	1.5	N.D	10.5	70.0
	25	495.0	3.3	3.0	18.7	74 0
	30	253.8	3.3	0.4	25.6	85.3
Cadmium	15	370.0	0.7	1.4	13.0	86.6
	25	330.0	N.D	3.7	21.3	85 2
	30	348.0	0.9	1.4	26.3	87 6

N.D: Not detectable.

Table 2 *Absorption of metals by 10-day-old cells at the end of 72 hr after transfer from control culture*

Metals added (ppm)		Dry biomass (mg l of culture) Initial	Final	Levels of metals in cells (ppm)	% absorption by cells
Lead	50	336.4	350.4	45.0	90.0
	75	300.4	313.8	64.0	85.3
	100	360.6	381.2	72.0	72.0
Nickel	15	199.6	206.2	14.5	96.6
	30	287.3	296.4	25.1	83.6

Another significant observation (table 2) was that when a large biomass of older cells was introduced into the media containing these metal ions, the cell took up Pb and Ni at higher levels than when grown entirely on the medium containing respective ions. 80% of Ni and 90% Pb were taken up in 72 hr at 50 ppm level but the uptake declined at higher levels. The older cells were not able to take up Cd.

DISCUSSION

Though the utilization of PTB for H_2 production[17] and in waste-water treatment[18–20] is well known, this is the first report on the capacity of PTB to uptake heavy metals. This study shows that by using solar energy, it is possible to clean up industrial water and recover metals, while a valuable fuel can also be produced.

Since there are many potential ligands including carboxylate, amine, phosphate, hydroxyl, sulphydryl and other functional groups[21–25] on the cell membrane, most cells have a negative charge. These may be the possible sites of uptake of metals, which can attract positively-charged metal ions. These electrostatic forces and other undefined cell surface mechanisms for metal accumulation, are fast, reversible and useful, even if the cells are quiescent or dead. This may be one reason why the absorption of metal ions was more in older cells.

ACKNOWLEDGEMENT

The author thanks Dr C. V. Seshadri for valuable suggestions and discussions and Tata Energy Research Institute, New Delhi, for financial assistance.

19 March 1987

1. Colmer, A. R. and Hinkle, M. R., *Science*, 1947, **106,** 253.
2. Duncan, D. W. and Trussel, P. C., *Can. Metall. Q.*, 1964, **3,** 43.
3. Karvaiko, G. I., *Usp. Mikrobiol., (Prog. Microbiol.)* 1970, **6,** 174.
4. Curtin, M. E., *Biotechnology*, 1983, **1,** 228.
5. Atkin, A. S., Pooley, F. D. and Townsley, C. G., *Process Biochem.*, 1986, **21,** 3.
6. Horikoshi, T., Nakajima, A. and Sakaguchi, T., *Eur. J Appl. Microbiol. Biotechnol.*, 1981, **12,** 90.
7. Stary, J. and Kratzer, K. J., *Environ. Anal. Chem.*, 1982, **12,** 65.
8. Rachlin, J. W., Jonson, T. E. and Warkentine, B., *Arch. Environ. Contam. Toxicol.*, 1984, **13,** 143.
9. Zajic, J. E. and Chiu, Y. S., *Dev. Ind. Microbiol.*, 1972, **13,** 91.
10. Sakaguchi, T., Korikushi, T. and Nakajima, A., *J. Ferment. Technol.*, 1978, **56,** 561.
11. Fisher, N. S. and Frood, D., *Marine Biol.*, 1980, **59,** 85.
12. Seshadri, C. V., Jeeji Bai, N., Monoharan, R. and Bhama S. Rengan, *Microbios Lett.*, 1982, **21,** 25.
13. Seshadri, C. V., Vatsala, T. M. and Monoharan, R., *Proc. 7th Int. Biotech. Symp.*, New Delhi, India, Feb 19–25, 1984, p. 492.
14. Vatsala, T. M. and Balaji, V., *"Book of Abstracts". Int. Conf. on Global Impacts of Applied Microbiology*, Helsinki, Finland, Aug 12–16, 1985, p. 152.
15. Kobayashi, M., Takichomo, N. and Techan, Y. T., *J. Fermen. Technol.*, 1977, **55,** 615.
16. Official Methods of Analysis, Association of Official Anal. Chemists, Washington, 1975, pp. 431, 441, 455.
17. Vatsala, T. M. and Seshadri, C. V., *Proc. Indian Natl. Sci. Acad.*, 1985, **B51,** 282.
18. Kobayashi, M. and Kurata, S., *Process Biochem*, 1978, **13,** 27.
19. Kobayashi, M., Fuji, K., Shimamoto, I. and Maki, T., *Progr. Water Technol.*, 1978, **11,** 279.
20. Balloni, W., Filpi, C. and Florenzano, G., *Algae biomass*, (eds) G. Shelef and J. Soeder, Elsevier North-Holland Biomedical Press, Amsterdam, 1980, p. 217.
21. Bartnicki-Garcia, S. and Nickerson, W. J., *Biochim. Biophys. Acta*, 1962, **58,** 102.
22. Beveridge, T. J. and Koval, S. F., *Appl. Environ. Microbiol.*, 1981, **42,** 325.
23. Crist, R. H., Obserhauser, K., Shank, Ne. and Ng Kuyan, M., *Environ. Sci. Technol.*, 1981, **15,** 1212.

24. Laster, J. N., Sterrott, R. M., Rudd, T. and Brown, M. J., In: *Microbiological methods for environmental biotechnology*, (eds) J. M. Grainger and J. M. Lynchi, Academic Press, New York, 1984, p. 197.
25. Scott, J. A., Sage, G. K., Palmer, S. J. and Powell, D. S., *Biotechnol. Lett.*, 1986, **8**, 711.

ANNOUNCEMENT

INDIAN NATIONAL SCIENCE ACADEMY, BAHADUR SHAH ZAFAR MARG, NEW DELHI 110 002

The Indian National Science Academy invites applications from scientists for partial financial assistance to participate in some important International Conferences abroad during the year 1988–89. Scientists interested to avail of financial support from the Academy for participation in International Conferences to be held during the year 1988–89 are requested to apply in the prescribed proforma. The proforma is available from the Office of the Executive Secretary, Indian National Science Academy, Bahadur Shah Zafar Marg, New Delhi 110 002.

The scheme is operated under three different categories viz. : (1) For International Conferences sponsored by the International Council of Scientific Unions (ICSU) and its affiliated bodies (ICSU Conferences); (2) For International Conferences sponsored by other agencies (Non-ICSU Conferences); (3) For applicants who are interested in partial travel assistance only, for participation in any International Conferences abroad. The applicants must be below 35 years of age. This is categorized as INSA-COSTED Travel Grant.

Category 1: Scientist who has been invited to deliver a plenary lecture/preside over a session of a scientific conference or whose paper has been accepted for presentation, and who will also be provided maintenance allowance during his/her stay abroad and partial travel expenses by some agency, will be given preference over others. INSA's financial support, in case of selection is limited to a maximum of 50% International travel, 50% maintenance allowance for the duration of the conference and registration fee, wherever necessary. The prescribed application form duly completed should be despatched latest by 31st January each year for consideration by a Committee of the Academy during the year.

Category 2: Scientist who has been invited to deliver a plenary lecture/preside over a session or whose paper has been accepted for presentation and who will also be provided maintenance allowance during his/her stay abroad and partial travel expenses by some agency, will be given preference over others. INSA's financial support in case of selection is limited to a maximum of Rs. 5,000/- only. Applications for category 2 should be received in the Academy for consideration three months prior to the date of commencement of the Conference.

Category 3: Under this category the scientist who is interested to avail partial travel assistance only, for participation in any International Conference and is also below 35 years of age may send his/her formal request on a prescribed application form atleast three months prior to the date of commencement of the Conference. The applicant should fulfill the following eligibility criteria before sending his/her application:

(a) His/her age should be below 35 years on the date of commencement of the Conference; (b) He/she should hold a Doctorate degree; (c) His/her paper should have been accepted for presentation; (d) His/her local hospitality and partial travel expenses has to be borne by the organizers or some other agency.

Candidates selected will be supported partially for their travel cost, jointly by INSA and COSTED (Committee on Science and Technology in Developing Countries). The applications will be screened once a quarter during the year.