Environmental Monitoring and Biodiagnostics of Hazardous Contaminants

Edited by
Michael Healy, Donald L. Wise
and Murray Moo-Young

Kluwer Academic Publishers

Environmental Monitoring and Biodiagnostics of Hazardous Contaminants

Edited by

Michael Healy
Department of Chemical Engineering,
The Queen's University of Belfast,
Belfast, Northern Ireland

Donald L. Wise
Department of Chemical Engineering,
Northeastern University,
Boston, MA, U.S.A.

and

Murray Moo-Young
Department of Chemical Engineering,
University of Waterloo,
Waterloo, Ontario, Canada

KLUWER ACADEMIC PUBLISHERS

DORDRECHT / BOSTON / LONDON

Library of Congress Cataloging-in-Publication Data

ISBN 0-7923-6869-X

Published by Kluwer Academic Publishers,
P.O. Box 17, 3300 AA Dordrecht, The Netherlands.

Sold and distributed in North, Central and South America
by Kluwer Academic Publishers,
101 Philip Drive, Norwell, MA 02061, U.S.A.

In all other countries, sold and distributed
by Kluwer Academic Publishers,
P.O. Box 322, 3300 AH Dordrecht, The Netherlands.

Printed on acid-free paper

Printed in the Netherlands.

ENVIRONMENTAL MONITORING AND BIODIAGNOSTICS OF HAZARDOUS CONTAMINANTS

List of Contributors

Abasaeed, A.E., Chemical Engineering Department, King Saud University, P.O. Box 800, Riyadh 11421, Saudi Arabia

Adrian, P., National Institute of Chemical & Pharmaceutical Research, Calea Vitan 112, sector 3, Bucharest 75593, Romania

Alfafara, C.G., National Institute of Molecular Biology and Biotechnology, University of the Philippines Los Baños College, Laguna 4031, Philippines

Allen, S.J., Department of Chemical Engineering, The Queen's University of Belfast, Stranmillis Road, Belfast BT9 5AG, Northern Ireland, U.K.

Al-Masry, W.A., Chemical Engineering Department, King Saud University, P.O. Box 800, Riyadh 11421, Saudi Arabia

Anderson, B.N., Department of Chemical & Metallurgical Engineering, RMIT University, GPO Box 2476V, Melbourne, Victoria 3001, Australia

Anderson, J.E., College of William and Mary, School of Marine Science, Virginia Institute of Marine Science, Gloucester Point, VA 23062, U.S.A.

Anderson, W.A., Department of Chemical Engineering, University of Waterloo Waterloo, Ontario, Canada N2L 3G1

Appanna, V.D., Department of Chemistry and Biochemistry, Laurentian University, Sudbury, Ontario, Canada P3E 2C6

Bae, H.K., Water Environment Research Center, Korea Institute of Science and Technology, 130-650, P.O. Box 131, Cheongryang, Seoul, Korea

Bugante, E.C., National Institute of Molecular Biology and Biotechnology, University of the Philippines Los Baños College, Laguna 4031, Philippines

Cain, R.B., Department of Biological and Nutritional Sciences, Kings Walk, The University of Newcastle upon Tyne, NE1 7RU, U.K.

Chandrasekaran, M., Center for Biotechnology, Cochin University of Science and Technology, Cochin 682022, India

Cheung, C.W., Department of Chemical Engineering, The Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong, S.A.R.

Davidson, C.A.B., Institute of Biotechnology, University of Cambridge, Tennis Court Road, Cambridge CB2 1QT, U.K.

Deng, S., Guangdong Institute of Microbiology, Guangzhou, P.R. China

Dick, R.E., Department of Chemical Engineering/QUESTOR Centre, The Queen's University of Belfast, David Keir Building, Belfast BT9 5AG, Northern Ireland, U.K.

Duddy, A.-M., Department of Environmental Science, Sligo Institute of Technology, Ballinode, Sligo, Republic of Ireland

Endo, G., Faculty of Engineering, Tohoku Gakuin University, Tagajo, Miyaga 985-8537, Japan

Ewell, M., Center of Marine Biotechnology, University of Maryland Biotechnology Institute, Baltimore, MD 21202, U.S.A.

Farrell, A., School of Biological Sciences, Dublin City University, Dublin 9, Ireland

Ferguson, J.A.E., Department of Chemistry/QUESTOR Centre, The Queen's University of Belfast, David Keir Building, Belfast BT9 5AG, Northern Ireland, U.K.

Gallagher, K.A., 15 Briar Hill, Greysteel, Co. Derry, Northern Ireland BT47 3DE, U.K.

Garcia, C., Department of Soil and Water Conservation and Organic Wastes Management, Centro de Edafologia y Biologia Aplicada del Segura (CEBAS-CSIC), P.O. Box 4195, 30080 Murcia, Spain

Gheorghiu, E., National Institute of Chemical & Pharmaceutical Research, Calea Vitan 112, sector 3, Bucharest 75593, Romania

Hamel, R., Department of Chemistry and Biochemistry, Laurentian University, Sudbury, Ontario, Canada P3E 2C6

Healy, M.G., Department of Chemical Engineering, The Queen's University of Belfast, Stranmillis Road, Belfast BT9 5AG, Northern Ireland, U.K.

Hernandez, T., Department of Soil and Water Conservation and Organic Wastes Management, Centro de Edafologia y Biologia Aplicada del Segura (CEBAS-CSIC), P.O. Box 4195, 30080 Murcia, Spain

Hilmi, A., Biotechnology Research Institute, 6100 Royalmount Avenue, Montreal, Canada

Hind, J.S., Center of Marine Biotechnology, University of Maryland Biotechnology Institute, Baltimore, MD 21202, U.S.A.

Ichigo, H., Hiroshima Municipal Industrial Technology Center, Sendamachi 3-8-24, Naka-ku, Hiroshima 730-0052, Japan

Ikeda, T., Department of Fermentation Technology, Hiroshima University, Higashi-Hiroshima, Hiroshima 739-8527, Japan

Ishida, T., Department of Environmental Sciences, Hiroshima Institute of Technology, Miyake 2-1-1, Saeki-ku, Hiroshima 731-5193, Japan

Jian, H., Guangdong Institute of Microbiology, Guangzhou, P.R. China

Jones, W.R., Center of Marine Biotechnology, University of Maryland Biotechnology Institute, Baltimore, MD 21202, U.S.A.

Jones-Meehan, J., Naval Research Laboratory, Environmental Quality Sciences Section, Washington, DC 20735-5348, U.S.A.

Juhasz, A.L., CSIRO, Land and Water, Private Bag No. 2, Glen Osmond, Adelaide, S.A. 5064, Australia

Kato, J., Department of Fermentation Technology, Hiroshima University, Higashi-Hiroshima, Hiroshima 739-8527, Japan

Kibazohi, O., Department of Chemical Engineering, University of Waterloo Waterloo, Ontario, Canada N2L 3G1

Kiely, D., Jennings O'Donovan & Partners, Consulting Engineers, Finisklin, Sligo, Republic of Ireland

Kuroda, A., Department of Fermentation Technology, Hiroshima University, Higashi-Hiroshima, Hiroshima 739-8527, Japan

Luong, J.H., Biotechnology Research Institute, 6100 Royalmount Avenue, Montreal, Canada

Lynch, J., School of Biological Science, University of Surrey, Guildford GU2 5XH Surrey, United Kingdom

Magbanua, J.P., National Institute of Molecular Biology and Biotechnology, University of the Philippines Los Baños College, Laguna 4031, Philippines

McCarroll, S.C., Department of Chemical Engineering, The Queen's University of Belfast, Belfast BT9 5AG, Northern Ireland, U.K.

McKay, G., Department of Chemical Engineering, The Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong, S.A.R.

Mc Veigh, R.J., Pfizer Pharmaceuticals, Ringaskiddy, County Cork, Ireland

Migo, V.P., National Institute of Molecular Biology and Biotechnology, University of the Philippines Los Baños College, Laguna 4031, Philippines

Mol, S.N., Department of Chemical & Metallurgical Engineering, RMIT University, GPO Box 2476V, Melbourne, Victoria 3001, Australia

Moo-Young, M., Department of Chemical Engineering, University of Waterloo Waterloo, Ontario, Canada N2L 3G1

Morohoshi, T., Department of Fermentation Technology, Hiroshima University, Higashi-Hiroshima, Hiroshima 739-8527, Japan

Naidu, R., CSIRO, Land and Water, Private Bag No. 2, Glen Osmond, Adelaide, S.A. 5064, Australia

Narita, M., Faculty of Engineering, Tohoku Gakuin University, Tagajo, Miyaga 985-8537, Japan

Nguyen, A.-L., Biotechnology Research Institute, 6100 Royalmount Avenue, Montreal, Canada

Nord, Jr., G.L., U.S. Geological Survey, 956 National Center, Reston, VA 20192, U.S.A.

Ohtake, H., Department of Fermentation Technology, Hiroshima University, Higashi-Hiroshima, Hiroshima 739-8527, Japan

O'Sullivan, M., School of Biological Sciences, Dublin City University, Dublin 9, Ireland

Paje, M.L., National Institute of Molecular Biology and Biotechnology, University of the Philippines Los Baños College, Laguna 4031, Philippines

Parker, E., Department of Chemistry and Biochemistry, Laurentian University, Sudbury, Ontario, Canada P3E 2C6

Pascual, J.A., Department of Soil and Water Conservation and Organic Wastes Management, Centro de Edafologia y Biologia Aplicada del Segura (CEBAS-CSIC), P.O. Box 4195, 30080 Murcia, Spain

Peberdy, J.F., School of Biological Sciences, Microbiology Division, University Park, University of Nottingham, Nottingham NG7 2RD, U.K.

Petre, M., National Institute for Biological Sciences, 296 Splaiul Independentei, sector 6, P.O. Box 17-16, Bucharest 77748, Romania

Podwysocki, M.H., U.S. Geological Survey, 927 National Center, Reston, VA 20192, U.S.A.

Porter, J.F., Department of Chemical Engineering, The Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong, S.A.R.

Quilty, B., School of Biological Sciences, Dublin City University, Dublin 9, Ireland

Robbins, E.I., U.S. Geological Survey, 956 National Center, Reston, VA 20192, U.S.A.

Roddick, F.A., Department of Chemical & Metallurgical Engineering, RMIT University, GPO Box 2476V, Melbourne, Victoria 3001, Australia

Schmidt, S., Abteilung für Mikrobiologie, Institut für Allgemeine Botanik der Universität Hamburg, Ohnhorstraße 18, D-22609 Hamburg, Germany

Shin, P.K., Water Environment Research Center, Korea Institute of Science and Technology, 130-650, P.O. Box 131, Cheongryang, Seoul, Korea

Spedding, P.L., Department of Chemical Engineering, The Queen's University of Belfast, Belfast BT9 5AG, Northern Ireland, U.K.

Sularia, M., Polytechnic University of Bucharest, Faculty of Automatics and Computer Science, 313 Splaiul Independentei, Bucharest 77206, Romania

Sun, G.-P., Guangdong Institute of Microbiology, Guangzhou, P.R. China

Takiguchi, N., Department of Fermentation Technology, Hiroshima University, Higashi-Hiroshima, Hiroshima 739-8527, Japan

Tanaka, S., Department of Fermentation Technology, Hiroshima University, Higashi-Hiroshima, Hiroshima 739-8527, Japan

Todd, S.J., Department of Molecular Biology and Biotechnology, The University of Sheffield, Firth Court, Western Bank, Sheffield S10 2TN, U.K.

Tso, W.-W., Chinese University of Hong Kong, Hong Kong

Wang, D., Department of Chemical & Metallurgical Engineering, RMIT University, GPO Box 2476V, Melbourne, Victoria 3001, Australia

Weatherley, L.R., Department of Chemical and Process Engineering, University of Canterbury, Private Bag 4800, Christchurch 1, New Zealand

Wong Tso, M.-Y., Hong Kong University, Hong Kong

Wu, H., Department of Fermentation Technology, Hiroshima University, Higashi-Hiroshima, Hiroshima 739-8527, Japan

Xu, M., Guangdong Institute of Microbiology, Guangzhou, P.R. China

Yabes, M.P., National Institute of Molecular Biology and Biotechnology, University of the Philippines Los Baños College, Laguna 4031, Philippines

Zarnea, G., Romanian Academy, 117 Calea Victoriei, sector 1, Bucharest 79717, Romania

Zhang, X., Guangdong Institute of Microbiology, Guangzhou, P.R. China

Table of Contents

1. Bioaccumulation of Yttrium: A Microbial Model for the Management of Nuclear Wastes

VASU D. APPANNA, EMMANUEL PANKAR and ROBERT HAMEL

Abstract. *Pseudomonas fluorescens* was found to multiply readily in a minimal mineral medium supplemented with millimolar amounts of yttrium complexed to citrate, the sole carbon source. At stationary phase of growth, the microbe accumulated 65% of the trivalent metal originally found in the growth medium. The examination of cell fractions revealed that most of the metal was associated with the outer membranes. Subsequent exposure of these membranes to yttrium pointed to their ability to further accumulate the metal. Electrophoresis of the membranes isolated from the yttrium stressed cells revealed the presence of numerous polypeptide bands that were absent in the membranes from the control cells. Transmission electron microscopy aided in the identification of yttrium in the membrane components. This model system has the potential of removing yttrium from contaminated sites.

1. Introduction

Metal pollution is a major environmental concern due to its negative impact on most living systems. This problem has been further exacerbated as a result of acid rain and industrial wastes. The bioavailability of toxic metals is on the rise (Lewis, 1989). Although higher organisms are more susceptible to the harmful effects of metals, numerous microbes are known to have acquired elaborate strategies to circumvent the occurrence of elevated levels of metallic elements in their environment. Biotransformation, reduced uptake and intracellular sequestration are among some of the mechanisms that enable microorganisms to combat increased concentrations of metals (Silver et al., 1989). These metal-resistant properties have made microorganisms a very important tool in environmental bioremediation.

Today, bioremediation technology is routinely applied to soils, sludges, ground water, surface waters, etc., contaminated with organic chemicals ranging from crude oil to industrial solvents. Toxic metals pose a new challenge to scientists working in the field of bioremediation. While biological methods may help minimize or reduce organic pollutants, inorganic contaminants have to be either physically removed from polluted sites or converted into biologically inert forms (Cunningham and Ow, 1996). Both living cells (typically microbes) and non-living biomaterials can function in metal recovery and remediation. Removal can be accomplished by removing the biomass or, with certain metal pollutants by metal insolubilization (Summers, 1992). Production of hydrogen sulphide, or fixation of carbon dioxide as bicarbonate may allow the precipitation of metals as insoluble

Michael Healy et al. (eds.), Environmental Monitoring and Biodiagnostics of Hazardous Contaminants, 1–9.

sulphides or carbonates, thus combatting the toxic effects of some metals (Appanna and Anderson, 1997; Silver, 1998). Many heavy metals may be removed by metal phosphate precipitation via the release of phosphate ligands (Macaskie et al., 1994) and the sequestration of metals in phospholipid moieties (Appanna and Hamel, 1997).

Due to their extreme nutritional versatility and their ability to produce a wide variety of products from simple and usually cheap carbon sources, pseudomonads are organisms of choice in various food, agricultural and medical industries. These microbes are common in numerous biotechnological processes. The discovery, in our laboratory, of a minimal mineral medium with citrate as the sole carbon source on which *Pseudomonas fluorescens* proliferates readily, provided a unique vehicle to probe cellular interactions in response to metal stress. Citrate, a naturally occurring ligand, is an excellent metal chelator and hence the microbe has no alternative but to deal with the metal if it wishes to multiply. Consequently, the organism must either adapt to the metal stress or risk death.

In this study we have examined the ability of the soil microbe *Pseudomonas fluorescens* ATCC 13525 to accumulate yttrium, a pollutant from nuclear industries. Radionuclides are usually retained in the soil complexed to mineral or organic matter and eventually absorbed by plants. ^{90}Y and ^{91}Y products of radioactive uranium and strontium are known to be present in the environment for many years (Kathren, 1984). In the present report, we describe the ability of *Pseudomonas fluorescens* to concentrate yttrium. The role of the outer membranes in the accumulation of this metal is explained and the potential of this finding in the decontamination of yttrium is also discussed.

2. Material and Methods

All chemicals were reagent grade. Folin Ciocalteu's phenol reagent, serum albumin, Victoria Blue reagent and phosphatidylethanolamine (PE) were from Sigma Company. The Bradford assay kit and citrate determination kit were from Biorad and Boehringer respectively.

2.1. Microbial Culture Conditions and Growth Measurement

The bacterial strain *Pseudomonas fluorescens* ATCC 13525 was obtained from American Type Culture Collection (Rockville Maryland, USA). It was maintained and grown in a mineral medium that contained Na_2HPO_4 (2.3 mg), KH_2PO_4 (1.134 mg), NH_4Cl (0.8 g), $MgSO_4 \cdot 7H_2O$ (0.2 g) and citric acid (4.0 g) per liter of deionized distilled water. Trace elements were present as described in Anderson et

al. (1992). Yttrium chloride (0.5–15 mM) was complexed to the tricarboxylic acid prior to sterilization. The pH of the medium was adjusted to 6.8 with dilute NaOH. The media were dispensed in 200 ml amounts in 500 ml Erlenmeyer flasks and inoculated with 1 ml of stationary phase cells grown in a medium unamended with the test metal and the phosphate concentrations were 6 and 3 g/l for Na_2HPO_4 and KH_2PO_4, respectively. The cultures were aerated on a gyratory water bath shaker model G76 (New Brunswick Scientific) at 26°C at 140 rev. min^{-1}. At various growth intervals, microbial multiplication was measured by monitoring solubilized bacterial protein by the method of Bradford (1976). The harvested cells were treated with 0.5 M NaOH and bovine serum albumin was used as the standard. Citrate was assayed enzymatically (Moellering and Gruber, 1966).

2.2. Measurement of Yttrium Bioaccumulation

At various timed intervals, aliquots of 20 ml of culture were centrifuged at 10 000 xg in order to afford a bacterial pellet and a supernatant. Following the washing of cells with 1 mM EDTA and acid digestion, yttrium was analyzed by (ICP) induction coupled plasma atomic emission spectrophotometry (Perkin-Elmer ICP/5500). The cells obtained at stationary phase of growth were fractionated into outer membranes, inner membranes and soluble components and yttrium was monitored as described above. Experiments were repeated three times and the mean values are reported.

The outer membranes and inner membranes obtained from cultures grown in 0.5 mM of yttrium enriched medium for 45 h, were evaluated for their yttrium binding capacity. 1 mg of protein equivalent of these components were incubated with either yttrium chloride or/and yttrium citrate for 1 h and the trivalent metal content was monitored following washing with EDTA and acid digestion.

2.3. Lipid Extraction and Analyses

The bacterial cells were harvested at 40 h incubation. The pelletized product was extracted with a mixture of CH_3OH-$CHCl_3$-H_2O (2:1:0.8). The lipids were placed as spots on thin layer silica gel plates (Whatman, Germany) and resolved by ascending chromatography using $CHCl_3$-CH_3OH-28% NH_4OH (65:25:5 vol/vol) mixture. The lipids were visualized with I_2 vapor and ninhydrin (Kates, 1988). Phosphatidylethanolamine and phosphotidylcholine were used as standards.

2.4. Determination of Uronic Acids

Bacterial cells from both control and yttrium stressed cultures were obtained after 40 h of incubation and uronic acids were quantified by the method of Blumenkrantz and Asboe-Hansen (1973).

2.5. Electron Microscopic Studies

Bacteria harvested at various incubation periods were washed twice with 0.85% NaCl solution and were fixed with 3% glutaraldehyde in 0.1 M sodium phosphate buffer pH 7.2. Post fixation was achieved in 1% osmium tetroxide dissolved in 0.1 M phosphate buffer for 1.5 h. Following washing with double distilled water and staining in 2% uranyl acetate, the cells were embedded in 2% agar. Thin sections were examined with the aid of a Zeiss 902A transmission electron microscope.

2.6. SDS-PolyAcrylamide Gel Electrophoresis (SDS-PAGE)

Both yttrium stressed and control cells were collected after 40 h growth and the various cellular fractions were isolated (Schnaitman, 1981). SDS-PAGE was performed according to the method by Laemmli (1970).

3. Results and Discussion

The presence of millimolar amounts of yttrium, complexed to citrate the sole source of carbon had disparate effects on *Pseudomonas fluorescens*. While 0.5 mM yttrium has a stimulatory effect on cell yield, the inclusion of 15 mM yttrium triggered a marked decrease in cellular yield. In this instance , bacterial multiplication was observed only after 48 h of incubation (Figure 1). No significant change in protein and carbohydrate contents of the spent fluid was observed in control and metal-rich cultures. The lipid profile, the uronic acid content and carbohydrate content of control and metal-stressed cells did not show any marked variation.

The accumulation of the trivalent metal in the bacterial cells was also monitored. Yttrium was initially associated with the supernatant. However, as growth progressed the test metal was localized within the cells. At 72 h of incubation 65 to 70% of the test metal was associated with the cells grown in 1 and 0.5 mM yttrium. The amounts of yttrium in the cells isolated from 3 mM yttrium cultures was markedly lower (Figure 2).

The various cellular fractions were isolated and these components were analyzed for their yttrium contents. It was determined that of the total amount of