MICROBIAL AND GEOCHEMICAL ASPECTS OF SELENIUM CYCLING IN AN ESTUARINE SYSTEM - LAKE MACQUARIE, NSW

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A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy

Department of Chemical Engineering
University of Sydney
July 1999

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DECLARATION

I hereby certify that this thesis is my original work, and that it has not been submitted to any other University or Institution for the award of a degree.

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Brett Ian Carroll

July 19, 1999
ABSTRACT

This work examined the role of micro-organisms in the biogeochemical cycling of selenium within the benthic ecosystem of Lake Macquarie, a coastal lake in New South Wales with a history of anthropogenic heavy metal contamination. Certain micro-organisms possess the ability to oxidise or reduce selenium (Fleming and Alexander, 1973; Doran and Alexander, 1977), and microbial volatilisation of selenium from contaminated sediments and soils utilising naturally-occurring microflora has been shown in overseas research (Thompson-Eagle and Frankenberger, 1992) to be a potentially effective remediation strategy. In examining the impact of micro-organisms upon the oxidation state of selenium in Lake Macquarie sediments, this work also investigated and characterised selenium (and heavy metal) concentrations, speciation and geochemical phase associations (an indicator of potential bioavailability) in the sediments.

Seven distinct bacterial species indigenous to Lake Macquarie were identified in this work with the ability to reduce selenium as selenite to elemental selenium, and selenium as selenate to organic forms of selenium, including volatile methylated selenium compounds. Metabolic parameters calculated for these organisms compared favourably with those reported in the literature by other researchers. Mixed populations of sediment micro-organisms were also isolated and studied in this work for their selenite and selenate reduction abilities.

Total reduction of added selenite at levels up to 100 mg/L was recorded for a number of the organisms studied in this work. A maximum specific uptake rate for selenite of 3040 µgSe(IV).(gcells)^-1.(h)^-1 for one isolate (Shewanella putrefaciens) was determined, exceeding rates reported in the literature by other authors. Use of the indigenous micro-organisms from Lake Macquarie for the bioremediation of selenium containing waste streams was also examined in this work and selenium reduction in an immobilised cell reactor was demonstrated with such organisms.
Concentrations, speciation, sediment core profiles and geochemical phase associations for selenium were determined for sediment samples collected at a variety of sites throughout Lake Macquarie and from Wyee Creek, a selenium-impacted fluvial input to the lake. The maximum concentration of selenium obtained in this work for the lake proper was 4.04 mg/kg, considerably lower than values reported over a decade ago (Batley, 1987) but consistent with reported reductions of selenium input into the lake from the lead-zinc smelter. Selective extraction methodology (Tessier et al. and BCR methods) studied geochemical phase association of selenium in Lake Macquarie sediments and found up to 44% of selenium was in bioavailable forms. Of interest and environmental concern was levels of selenium found in sediments of Wyee Creek, which previously received overflows from the ash dam associated with the Vales Point Power Station. Sediment selenium levels of up to 300 mg/kg were determined for this creek. These were an order of magnitude or more greater than those recorded for the lake itself and are of concern as to the potential impact on benthic organisms and those animals, including humans, who consume them.

While this work can only provide a “snapshot” of conditions within Lake Macquarie at the time of the sampling events recorded herein, it does make several important contributions to the understanding of selenium biogeochemistry in Lake Macquarie. These include:

- presentation of the hypothesis that selenium levels in surficial sediments being deposited in the north of the lake have decreased in recent years as a result of selenium reduction measures undertaken by the lead-zinc smelter;

- determination that up to 44% of selenium in surficial sediments from the lake is associated with sediment phases in which selenium has the potential to become remobilized and hence possibly bioavailable; and

- documentation of selenium concentrations in Wyee Creek, identifying the area as having selenium concentrations an order of magnitude or more greater than the lake itself.
Concerning the role played by microorganisms in the biogeochemical cycling of selenium in Lake Macquarie, this work has:

- identified individual isolated and mixed cultures of bacteria that can reduce selenium as selenite to lower oxidation states;
- identified individual isolated and mixed cultures of bacteria that can reduce selenium as selenate to lower oxidation states;
- identified volatile methylated selenium compounds in the headspace gases of microorganisms reducing selenate;
- determined Minimum Inhibitory Concentrations for selenate and selenite for organisms isolated from Lake Macquarie;
- identified casein hydrolysate as a preferred carbon source for selenium reducing microorganisms from Lake Macquarie; and
- demonstrated that bioremediation of selenium contaminated waste streams using indigenous organisms from Lake Macquarie is feasible on the laboratory scale.

Further research areas suggested by this work include:

- additional investigations of elevated selenium levels in Wyee Creek sediments;
- determination of the role of microbes in *in-situ* selenium reduction; and
- optimisation of selenium biotreatment/bioremediation of selenium-containing waste streams and sediments.

In summary, this work, in rejecting the null hypothesis that the oxidation states of selenium in sediments from Lake Macquarie, NSW, are independent of microbial
activity and accepting the alternate hypothesis that these oxidation states are not independent of microbial activity, contributes to the understanding of the role of microorganisms in the biogeochemical cycling of selenium, having applicability to both the specific ecosystem of Lake Macquarie, NSW, and also to selenium cycling in the environment in general. In addition, this work has identified selenium contamination in Wyee Creek, one of the fluvial inputs to Lake Macquarie, which was previously been undocumented in the literature and which may pose significant potential risk to humans and the ecosystem due to sediment selenium levels one or more orders of magnitude higher than those recorded in the lake itself. Finally, this work has also identified a number of microorganisms indigenous to Lake Macquarie with the ability to reduce selenium from toxic, mobile forms to less toxic, immobile or volatile forms, and these organisms have been shown to have the potential for use in treatment of selenium contaminated waste streams and also in the bioremediation of selenium-contaminated sediments.
ACKNOWLEDGEMENTS

The work described in this thesis has been supported in part by an ARC Institutional Grant (1995-1997) and by research funds contributed by Lake Macquarie City Council (1995/96). The support of these organisations in the provision of funding is gratefully acknowledged.

The author also acknowledges the endowment of Henry Bertie and Florance Mabel Gritton to the University of Sydney for the establishment of a postgraduate research scholarship designed for the investigation of environmental chemistry, of which this author was a grateful recipient. The Chemical Engineering Foundation and the Department of Chemical Engineering, University of Sydney also provided supplementary scholarships to the author and their support is also gratefully acknowledged.

Notwithstanding declarations elsewhere in respect of the originality of this work, a work such as this cannot ever be said to be truly the work of one individual, and as such I wish to express and offer my thanks to those who have assisted me in various ways throughout my tenure as a postgraduate student and without whom this work would be a pale imitation of what it is. Thanks therefore to:

A/Prof John P Barford, Department of Chemical Engineering, University of Sydney, my supervisor, for his insight, wisdom, knowledge, support and encouragement;

A/Prof Bill Maher, Faculty of Applied Science, University of Canberra, my co-supervisor, for coming on-board when he did, for his great knowledge about things seleniferous, and for his encouragement and support throughout it all;

Mr Denis Nobbs, Department of Chemical Engineering, University of Sydney, my analytical guru, for his generosity and whole-hearted support of my work and myself;
Mr Greg Peters, PhD candidate, Department of Chemical Engineering, University of Sydney, my fellow traveller on the road to understanding selenium in Lake Macquarie, for his lateral thinking, and encouraging me to go that little bit extra and just do one more experiment;

Professor Danny Reible, former Shell Professor of Environmental Engineering in this department, for his support and encouragement during his tenure here;

Dr In Seop Kim, formerly of this Department and now of the University of New South Wales, for taking this work beyond where I left it and for the permission to cite his data concerning identification and experimentation with selenite-reducing bacteria;

The undergraduate students who have worked with me, who have been partners in these investigations and who have contributed to this work in so many ways. Specifically, thanks to: Phyll Chapman, for his assistance in studying selenium volatilisation and identification of volatile selenium compounds; Kait Gotham and Matt Reso, for assistance in my initial studies of the bacterial transformations of selenite, and selenate, respectively; Emily Harston, for assisting with the work on selenium in Wyee Creek; Sally Mitchell, for her tireless efforts with the bioreactors and isolating organisms; Denis Reich, for helping dig-up details of the industrial archaeology of the Lake Macquarie area and for the representation of Lake Macquarie heavy metal data in a Geographic Information Systems form; Geoff Smith, for assisting with the study of bioavailability of sediment-bound selenium; and Kurt Wegenaar, for helping model selenium cycling in the Lake;

Dr Lieke Riadi, University of Surabaya and formerly of this Department, for her work on selenium transformations in ash dam isolates which partly inspired this investigation;

Dr Jeffrey Shi and Mr Adam Lovell, Joint Elemental Analytical Facility, University of Sydney, for grateful assistance with all carbon analyses reported in this study;
The workshop staff, Department of Chemical Engineering, University of Sydney, for building all the strange things I needed built and fixing the broken things;

My fellow postgraduates and other academic members of the Department’s Environmental Research Group, for their encouragement and constructive criticisms of my work;

The other postgraduates in our department, whose conversations about things other than selenium over morning teas have helped keep me sane;

My Friday morning prayer group, Peter Smith and Steve Courtney, for their continual encouragement and banter about all things seleniferous, another contributing factor to the maintenance of my sanity;

To those who laboriously reviewed this manuscript for accuracy, coherence, and typographical and grammatical errors (and any such as remain are the sole responsibility of the author); specifically to John Barford, Denis Nobbs, Bill Maher and Jennifer Wise;

And finally, but by no means least, to my wife Victoria, who was brave enough to say “yes” to my return to university, who has supported me emotionally and financially throughout it all, who read through all this and who has always been there for me. Thank you Victoria.
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<td>2TY</td>
<td>Tryptone Yeast Media</td>
</tr>
<tr>
<td>AAS</td>
<td>Atomic Absorption Spectroscopy</td>
</tr>
<tr>
<td>AEROCE</td>
<td>Atmosphere/Ocean Chemistry Experiment</td>
</tr>
<tr>
<td>AES</td>
<td>Atomic Emission Spectroscopy</td>
</tr>
<tr>
<td>ANZECC</td>
<td>Australian and New Zealand Environment Conservation Council</td>
</tr>
<tr>
<td>BCR</td>
<td>European Community Bureau of Reference</td>
</tr>
<tr>
<td>CSIRO</td>
<td>Commonwealth Scientific and Industrial Research Organisation</td>
</tr>
<tr>
<td>DMDSe</td>
<td>Dimethyl Diselenide</td>
</tr>
<tr>
<td>DMSe</td>
<td>Dimethyl Selenide</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylenediaminepentaacetic acid</td>
</tr>
<tr>
<td>Eh</td>
<td>Redox Potential</td>
</tr>
<tr>
<td>EPA</td>
<td>Environment Protection Authority (NSW)</td>
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<tr>
<td>FAAS</td>
<td>Flame Atomic Absorption Spectroscopy</td>
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<tr>
<td>GC</td>
<td>Gas Chromatography</td>
</tr>
<tr>
<td>GFAAS</td>
<td>Graphite Furnace Atomic Absorption Spectroscopy</td>
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<tr>
<td>HEPA</td>
<td>High Efficiency Particulate Air</td>
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<tr>
<td>HG</td>
<td>Hydride Generation</td>
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<tr>
<td>HGAAS</td>
<td>Hydride Generation Atomic Absorption Spectroscopy</td>
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<tr>
<td>MIC</td>
<td>Minimum Inhibitory Concentration</td>
</tr>
<tr>
<td>MIS</td>
<td>Microbial Identification System</td>
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<tr>
<td>MS</td>
<td>Mass Spectroscopy</td>
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<tr>
<td>NHMRC</td>
<td>National Health and Medical Research Council</td>
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<tr>
<td>NMR</td>
<td>Nuclear Magnetic Resonance</td>
</tr>
<tr>
<td>pE + pH</td>
<td>log activity-redox</td>
</tr>
<tr>
<td>Q_{substrate}</td>
<td>Specific Uptake Rate of a Substrate/Electron Acceptor</td>
</tr>
<tr>
<td>Q_{Se}</td>
<td>Specific Uptake Rate of Selenium</td>
</tr>
<tr>
<td>RNAA</td>
<td>Radiochemical Neutron Activation Analysis</td>
</tr>
<tr>
<td>RSD</td>
<td>Relative Standard Deviation</td>
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<tr>
<td>Symbol</td>
<td>Description</td>
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<td>--------</td>
<td>-------------</td>
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<tr>
<td>Se(0)</td>
<td>Elemental Selenium</td>
</tr>
<tr>
<td>Se(II)</td>
<td>Selenide</td>
</tr>
<tr>
<td>Se(II + 0), [Se(II) + Se(0)]</td>
<td>“Organic Selenium”</td>
</tr>
<tr>
<td>Se(IV)</td>
<td>Selenite</td>
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<tr>
<td>Se(VI)</td>
<td>Selenate</td>
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<tr>
<td>[Se(VI) + Se(IV)]</td>
<td>Selenate and Selenite Fraction</td>
</tr>
<tr>
<td>SeGSHpx</td>
<td>Glutathione Peroxidase Enzyme System</td>
</tr>
<tr>
<td>[Se(Tot)]</td>
<td>Total Selenium</td>
</tr>
<tr>
<td>SPCC</td>
<td>State Pollution Control Commission</td>
</tr>
<tr>
<td>SRM</td>
<td>Standard Reference Material</td>
</tr>
<tr>
<td>$t$</td>
<td>time</td>
</tr>
<tr>
<td>TC</td>
<td>Total Carbon</td>
</tr>
<tr>
<td>TIC</td>
<td>Total Inorganic Carbon</td>
</tr>
<tr>
<td>TOC</td>
<td>Total Organic Carbon</td>
</tr>
<tr>
<td>TSBA</td>
<td>Tryptophan Soy Base Agar</td>
</tr>
<tr>
<td>$\mu$</td>
<td>Specific Growth Rate</td>
</tr>
<tr>
<td>USEPA</td>
<td>United States Environment Protection Authority</td>
</tr>
<tr>
<td>VWA</td>
<td>Volume Weighted Average</td>
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<tr>
<td>WHO</td>
<td>World Health Organisation</td>
</tr>
<tr>
<td>$[X]$</td>
<td>Concentration of “X”</td>
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<tr>
<td>$x$</td>
<td>Cell Dry Weight</td>
</tr>
<tr>
<td>$Y_{XSe}$</td>
<td>Yield (based on selenium)</td>
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