University of Canberra

METAL-REDUCING MICROORGANISMS

IN

PETROLEUM RESERVOIRS

SHAHRAKBAH YACOB

MASTER OF APPLIED SCIENCE

UNIVERSITY OF CANBERRA

2000

ACKNOWLEDGEMENTS

I am deeply indebted to a number of people with whom I have worked during my project and from whom, by association and discussion, I have learnt so much that has helped me in my task.

First of all, I am very grateful to Land & General (Malaysia) Bhd. for sponsoring the research project. In this assignment, much support was received from Dr. Baharuddin Abd Ghani and Mr. Zainol Alang, Chief Operating Officer of Research & Development and Azerbaijan Operations, respectively.

Special word of thanks to the Director of MRU, who is also my primary supervisor, Professor Alan Sheehy, for his excellent editorial and guidance and for the time and trouble taken to read through the manuscript. I wish most particularly to express my gratitude to Dr. Tony Greene, who is my co-supervisor, and gave me a basic training, the value of which I have appreciated more and more with the passing years, also for his invaluable criticisms, suggestions and demonstrated great patience while waiting for the thesis to be completed.

I am very much indebted to three wonderful people, Dr. Gino Grassia, Dr. Gavin Rees, and Dr. Tony Dimichel, who gave valuable technical and moral support when things did not develop as they were expected to. Special thanks are also due to staff of Faculty of Applied Science and to those who experienced the MRU, Monica Cole, Tony Hancock, Kathryn Russel, Karyn Wilde, Claire Batum, Elaine Wyatt and Kathryn Smith.

Many thanks are forwarded to Dr. Bharat Patel of Griffith University, Roger Heady and Lily Shen of Australian National University for their collaboration and significant contribution to the thesis.

Most of all I am indebted to my wife for her unfailing patience, encouragement and immeasurable support.

S. Yacob, Malaysia.

ABSTRACT

Metal-reducing microorganisms reduce a variety of metals in metabolic processes coupled to the oxidation of organic compounds. These bacteria play an important role in the biogeochemical cycling of metals and organic matter in anaerobic aquatic and sediment ecosystems. It has been proposed recently that metal-reducing microorganisms also are active in deep subsurface environments such as petroleum reservoirs. Only two metal-reducing bacteria have been isolated from petroleum reservoir fluids, *Shewanella putrefaciens* and *Deferribacter thermophilus*. This project studied the occurrence and distribution of metal-reducing microorganisms in petroleum reservoirs. The research focused on the isolation, characterisation and identification of anaerobic bacteria from petroleum reservoirs that were capable of reducing metals and the potential roles of these isolates in the microbial ecology and biogeochemical cycling of petroleum reservoirs.

Petroleum reservoirs were selected for this study on the basis of physio-chemical conditions such as temperature, salinity, pH and the presence of organic and inorganic compounds, that were likely to provide a suitable environment for anaerobic bacteria capable of reducing metals. Factors such as the stratigraphic features of the sedimentary basin, age of reservoir and past oil field practices also were considered in choosing the reservoir for study. Seven petroleum reservoirs in the USA and Azerbaijan were chosen for extensive investigations. The physico-chemical conditions in these reservoirs varied substantially.

A systematic study of the production water from these petroleum reservoirs revealed a consistent presence of iron- and manganese-reducing microorganisms. It was found that salinity and temperature play a significant and defining role in the occurrence and distribution of these metal-reducing microorganisms. Biotic metal reduction was detected from production waters from all but one of the oil wells sampled. It was significant that the water from this well (Neftcala #1074) was the most saline (78 g/l NaCl). Metal-reducing activity was detected at temperatures up to 70°C.

Two pure cultures, strains RED1 for Redwash petroleum reservoir (USA) and NEF1 from the Neftcala petroleum reservoir (Azerbaijan) were isolated and characterized.

The strains had diverse physiological and metabolic properties including the ability to oxidize a wide range of carbon compounds and reduce a variety of metals. Their temperature, salinity and pH optima varied markedly. Phylogenetic analyses of the 16S rRNA of strain RED1 showed that the strain represented a new species of a new genus in the domain *Bacteria*. The bacterium most closely related to strain RED1 is the fermentative Fe(III)-reducer, *Pelobacter acetylenicus* (similarity value, 92.8%). Strain NEF1 possesses a unique combination of phenotypic traits and a low mol % G+C. From preliminary analyses and comparative biochemistry, NEF1 appears to be a novel metal-reducing bacterium of the Flexistipes group.

The bacteria isolated in this study were able to grow at temperatures and salinities consistent with the reservoir from which they were isolated. This indicated that petroleum reservoirs are a new source of physiologically diverse, novel, metal-reducing microorganisms. The bacteria isolated also demonstrated a number of characteristics that would enable them to survive and persist in extreme subsurface conditions and develop a selective ecological advantage in petroleum reservoir environments. Significantly, the metal-reducing bacteria isolated were able to utilize an array of metabolic products produced by bacteria indigenous to petroleum reservoirs. This has resulted in a new proposed model for the ecological succession of bacteria in petroleum reservoirs.

CONTENTS

LIST OF TABLES

LIST OF FIGURES