



<http://researchspace.auckland.ac.nz>

ResearchSpace@Auckland

Copyright Statement

The digital copy of this thesis is protected by the Copyright Act 1994 (New Zealand).

This thesis may be consulted by you, provided you comply with the provisions of the Act and the following conditions of use:

- Any use you make of these documents or images must be for research or private study purposes only, and you may not make them available to any other person.
- Authors control the copyright of their thesis. You will recognise the author's right to be identified as the author of this thesis, and due acknowledgement will be made to the author where appropriate.
- You will obtain the author's permission before publishing any material from their thesis.

To request permissions please use the Feedback form on our webpage.

<http://researchspace.auckland.ac.nz/feedback>

General copyright and disclaimer

In addition to the above conditions, authors give their consent for the digital copy of their work to be used subject to the conditions specified on the Library Thesis Consent Form.

SOME ASPECTS OF THE MICROBIOLOGICAL ACTIVITY OF THE
MANGERE OXIDATION PONDS.

by

O.D. BROCKETT

Thesis submitted for the degree of Doctor of Philosophy, University
of Auckland, New Zealand, May 1971.

ACKNOWLEDGEMENTS

I wish to thank Prof. V.J. Chapman, Head, for allowing me to work in the Botany Department, also Prof. F.J. Newhook for helpful discussion and constructive criticism particularly in the preparation of the manuscript.

The study was carried out with financial assistance from the Research Advisory Sub Committee of the Auckland Regional Authority, and this is gratefully acknowledged.

I would also like to thank Mr R. Hicks, Works Superintendent and Chief Chemist (retd), and Mr P. Welsby, Assistant Works Superintendent and Plant Chemist, for stimulating discussion, provision of data, and the setting up and maintenance of redox potential equipment essential for my studies. Mr C.C. Collom for permission to use the map in Fig. 1. and for provision of facilities for the publication of the thesis. Mr J. Bryce, Office Manager who undertook supervision of the publication. Mr R. Murphy, Chief Draughtsman whose division prepared the graphs and diagrams, and Miss G. Morgan who typed the manuscript.

Finally I would like to thank my husband Neville, who, with patience and understanding, has given me every encouragement to undertake this thesis.

CONTENTS

	Page
SUMMARY	I
GENERAL INTRODUCTION	1
SECTION I. THE OCCURRENCE AND ROLE OF FUNGI IN THE MANGERE OXIDATION PONDS.	
INTRODUCTION	5
ENUMERATION AND IDENTIFICATION OF FUNGI IN OXIDATION POND SLUDGE AND SLUDGE/WATER INTERFACE.	7
Introduction	7
Collection of Samples	7
Materials and Methods used for the Isolation, Purification and Identification of Fungi.	8
Results and Discussion	9
GROWTH OF FUNGI UNDER LOW REDOX POTENTIAL CONDITIONS.	15
Introduction	15
Materials and Methods.	15
Results and Discussion	17

	Page
THE ACTIVITY OF FUNGI IN SCUM FORMATION	18
Introduction	18
Materials and Methods	18
Results and Discussion	18
The Reduction of Light Transmittancy by Filamentous yeasts.	23
Control of filamentous yeast scums	26
Results and Discussion	27
CONCLUSIONS AND DISCUSSION OF SECTION I	29
SECTION II. SOME ASPECTS OF THE NITROGEN CYCLE IN THE MANGERE OXIDATION PONDS.	
INTRODUCTION	32
DECOMPOSITION OF PROTEIN	34
Introduction	34
Proteolytic Activity of Sludge	35
Materials and Method.	35
Results and Discussion	35
Protein Content of Sludge	40
Materials and Method	40
Results and Discussion	40
NITRIFICATION	42
Introduction	42
Materials and Methods	43
The Most - Probable - Number Method for Population Estimates	43
Enrichment Studies	45
Materials and Methods	45

	Page
Results and Discussion	46
Enumeration of the Nitrifying Bacteria	47
Introduction	47
Materials and Methods	47
Results and Discussion	47
Nitrification in other biological systems at	
Mangere.	52
Introduction	52
Results and Discussion	53
Conclusions on Nitrification	56
 DENITRIFICATION	 57
Introduction	57
Nitrate Metabolism in oxidation ponds	60
Enumeration of denitrifying bacteria	61
Introduction	61
Materials and Methods	62
Results and Discussion	62
Denitrifying Population size under unusual	
conditions in the Mangere oxidation ponds.	66
Denitrification and Eutrophication	67
Isolation and Identification of Denitrifying	
Bacteria from the Mangere oxidation ponds.	69
Introduction	69
Materials and Methods	69
Identification of pure cultures of	
Denitrifying Bacteria.	70
Isolation and Identification of a denitrifying	
<u>Thiobacillus</u> .	76

	Page
Denitrification Properties of Bacteria isolated from the Mangere oxidation ponds.	77
Redox Potential	77
Redox Potential in Biological Studies	78
Experimental Procedures for Determining Redox Potential.	79
Materials and Methods	80
Denitrification with various levels of nitrate.	82
Results and Discussion	83
Addition of compounds to Promote Denitrification	84
Effect of Additives on the C:N ratio	85
Materials and Methods	85
Results and Discussion	85
Conclusions on Denitrification	87
 CONTINUOUS MEASUREMENT OF THE REDOX POTENTIAL OF THE MANGERE OXIDATION PONDS.	 89
Introduction	89
Materials and Methods	89
Results and Discussion	90
Redox Potential of Pond top Liquid	90
Redox Potential of Interface	91
Redox Potential of Sludge	91
Changes in Redox Potential of Pond Top Liquid under Abnormal Conditions	92
Conclusions on Redox Potential Measurements.	92
 CONCLUSIONS AND DISCUSSION OF SECTION II	 94
REFERENCES	97
APPENDIXES	(i-xv)

SUMMARY

1. A general introduction to the theory, operation and biological activity of oxidation ponds has been given as well as specific introductions to the two areas of research studied, namely the role of fungi and some aspects of the nitrogen cycle in the Mangere oxidation ponds.
2. The enumeration and identification of fungi present in the bottom layers of the pond showed that the numbers were not sufficiently large to make a valuable contribution to the degradation of organic material in that particular environment. The dominant fungi present were species of the genera Penicillium, Mucor, Trichoderma and Geotrichum.
3. Isolates of some of the fungi most frequently isolated from these lower regions were grown under conditions of low redox potential. It was found that although the fungi did grow over the one month test period, they did not show great metabolic activity.
4. Direct examination of pond water containing a whitish glassy scum showed only the presence of clumps of heterogeneous biomass but no dominant microorganism. Filamentous yeasts isolated from these scum samples formed a surface layer when grown in liquid medium. When inoculated into pond water it appeared that these filamentous yeasts formed a matrix to which the biomass adhered producing a condition similar to that seen in the original samples.
5. Use of ultra thin layers of cetyl alcohol prevented scum formation by these filamentous yeasts but not growth in the medium underneath. Larger scale trials would be required to determine if this method is feasible for reducing scum formation in oxidation ponds.

6. Investigation of the proteolytic activity of pond sludge showed that the part of the nitrogen cycle which concerns the degradation of protein was present and active in the oxidation ponds. Proteolytic activity was found to increase with increasing depth of pond water up to the maximum (220 cm) tested. This suggests that increasing the depth of the ponds to approximately 185 cm (6 ft.) would improve pond efficiency in relation to degradation of protein.

7. The bacteria responsible for nitrification in the oxidation pond surface water were isolated and identified. Nitrosomonas was responsible for the oxidation of ammonia to nitrite and Nitrobacter for the oxidation of nitrite to nitrate. A twelve month survey of these bacteria showed that their numbers increased or decreased in relation to seasonal changes. Nitrite accumulated only when large numbers of Nitrosomonas were present. Statistical analysis of the data obtained indicated that populations of these bacteria were influenced by pH, alkalinity and ammonia.

8. Enumeration of denitrifying bacteria at different pond depths showed that greatest numbers were present in the sludge and interface although they were well distributed through the aqueous phase.

9. Identification of the isolated denitrifying bacteria showed that they were strains of Pseudomonas denitrificans, Micrococcus denitrificans and Bacillus licheniformis. Their identity was confirmed by comparison with standard cultures.

10. The denitrifying properties of these bacteria were studied to compare their ability to remove nitrate, as well as their reaction to additives. All three bacteria were able to remove low levels of nitrate but high nitrate concentrations were shown to be inhibitory. The use of additives to promote denitrification appeared to depend on the additive and the bacterium and not on the carbon nitrogen ratio. Of the materials tested glucose was the most satisfactory and methanol the most inhibitory. Further work is necessary to clarify the role of methanol because in small scale studies with sewage effluents in other laboratories it has been found to be most efficient in promoting denitrification.

11. Continuous measurements of the redox potential interface of sludge, and pond top liquid (10-15 cm. from the surface) revealed that the electronegative potential of the sludge remained remarkably steady. When the electrode was at either the interface or near the surface it recorded a distinct diurnal pattern. In pond top liquid the length of the positive potential period was dependent on day length. A change from the normal redox potential pattern of pond top liquid preceded deterioration of pond stability. The pattern returned to normal after a change in environmental conditions. It appears that continuous redox potential measurements could be valuable in showing changes in pond performance before they can be detected by other methods.