

This project was only possible with the co-operation of many individuals and institutions. The authors therefore wish to record their sincere thanks to the following:

The Water Research Commission personnel for administering and co-ordinating the project, in particular Dr. M.J. Pieterse, former Deputy Executive Director of the Water Research Commission, for his part in the initiation of the project and Mr. H.M. du Plessis, Research Manager, for his part in the administration and coordination of the latter part of the project.

Consulting engineers Wates, Meiring and Barnard, in particular Mr. J.A. Wates and Mr. E.M. Rykaart, for their roles in designing and establishing the pilot scale experiment at the Kilbarchan Mine, and supplying engineering, physical and technical data in connection with the project.

The Department of Water Affairs and Forestry, in particular Mr. S.A.P. Brown, Mr. J. Maré and Mrs. M.C. Eksteen, Water Quality Management Office, Pretoria, as well as Mr. J.F. Rothman of the Dundee Office and his personnel, for their roles in planning, constructing and maintaining the pilot scale experiment at the Kilbarchan Mine. Particular thanks are due to Mr. Y. Nehro and Mr. R. Luckan of the Dundee Office, for their dedicated monitoring of the rainfall, oxygen and carbon dioxide on or in the pilot scale coal waste dumps, as well as assistance with the sampling of the coal waste.

Mr. J. Purchase of Bateman Instruments, Benoni (formerly S.I. Analytics) for designing and organizing the manufacture of the highly successful probes for the determination of oxygen and carbon dioxide in the pilot scale coal waste dumps.

Mrs. L. Strydom of the Department of Metallurgical Engineering, University of Stellenbosch, Prof. F.D.I. Hodgson and colleagues of the Institute for Ground Water Studies, University of the Orange Free State, Bloemfontein, for chemical analyses of coal waste from the experimental site.

Dr. J.H. Randall, formerly Biometrician, Faculty of Agricultural Sciences, University of Stellenbosch, for invaluable assistance with statistical aspects of the most probable number (MPN) determinations of bacteria.

Dr. D.B. Johnson of the University of Wales, Bangor, U.K., for valuable technical advice and cultures.

Miss. M.S. Venter for competent laboratory assistance during the initial stages of the project.

Ms. L. Bekker, Ms. M. Veenstra and Ms. D. van der Merwe, formerly or presently in the Department of Microbiology, University of Stellenbosch, for competent typing of the manuscript and production of the tables and figures of this report.

CONTENTS

	Page
A. GENERAL INTRODUCTION	1
A.1. PROJECT MOTIVATION	1
A.2. PROJECT OBJECTIVES	3
A.3. THE PROJECT AS PART OF A COLLABORATIVE INVESTIGATION	3
A.4. STRUCTURE OF THE PROJECT AND REPORT	4
B. LITERATURE SURVEY	7
B.1. CHEMICAL AND MICROBIAL PROCESSES INVOLVED IN ACID MINE DRAINAGE FORMATION	7
B.1.1. Oxidation of Pyrite and Other Metal Sulphides	7
B.1.1.1. Overall pyrite oxidation processes	7
B.1.1.2. Abiotic oxidation of pyrite	8
B.1.1.3. Abiotic oxidation of pyrite oxidation products	10
B.1.1.4. Biotic oxidation of pyrite oxidation products	13
B.1.1.5. Oxidation of other metal sulphides	17
B.2. MICROORGANISMS INVOLVED IN ACIDIFICATION OF METAL SULPHIDES	18
B.2.1. Acidophilic Iron-oxidizing Bacteria	18
B.2.1.1. <i>Thiobacillus ferrooxidans</i>	18
B.2.1.2. <i>Leptospirillum ferrooxidans</i>	20
B.2.1.3. Moderately acidophilic very low ferrous iron-oxidizing bacteria (genus <i>Metallogenium</i>)	21
B.2.1.4. Moderately thermophilic, mixotrophic/facultatively lithotrophic iron-oxidizing bacteria	22
B.2.1.5. Mesophilic heterotrophic iron-oxidizing bacteria	23
B.2.1.6. Thermophilic iron-oxidizing archaea	23
B.2.2. Acidophilic Sulphur-oxidizing Bacteria	24
B.2.2.1. Mesophilic obligately lithotrophic <i>Thiobacillus</i> species	24
B.2.2.2. Mesophilic facultatively lithotrophic <i>Thiobacillus</i> species	25
B.2.2.3. Moderately thermophilic <i>Thiobacillus</i> species	26
B.2.2.4. Thermophilic sulphur-oxidizing archaea	26
B.2.3. Acidophilic Heterotrophic Bacteria	26
B.2.3.1. Mesophilic acidophilic heterotrophic bacteria	27
B.2.3.2. Thermophilic acidophilic heterotrophic bacteria	28
B.2.3.3. Thermophilic acidophilic heterotrophic archaea	28
B.3. ECOLOGICAL FACTORS INFLUENCING BACTERIAL ACID MINE DRAINAGE FORMATION IN COAL WASTE	29
B.3.1. Physical Factors	29

	Page
B.3.1.1. Moisture	29
B.3.1.2. Temperature	30
B.3.2. Chemical Factors	31
B.3.2.1. pH	31
B.3.2.2. Oxygen and carbon dioxide	31
B.3.2.3. Substrate availability	33
B.4. AN ECOLOGICAL APPROACH TO CONTROL OF ACID DRAINAGE FROM COAL WASTE DUMPS: REDUCTION OF WATER AND OXYGEN INFILTRATION BY MEANS OF SOIL AND/OR CLAY LINERS AND COVERS	34
C. RESEARCH: PART 1. ABIOTIC ECOLOGICAL DETERMI- NANTS (TEMPERATURE, MOISTURE, OXYGEN, CARBON DIOXIDE AND pH) AND ACID-PRODUCING BACTERIA IN PILOT SCALE COAL WASTE DUMPS IN RELATION TO COVERS USED FOR DUMP REHABILITATION	37
C.1. INTRODUCTION	37
C.2. MATERIALS AND METHODS	37
C.2.1. Materials and Construction of Pilot Scale Dumps	37
C.2.1.1. Coal waste	37
C.2.1.2. Soil cover materials	39
C.2.1.3. Construction of pilot scale dumps	41
C.2.1.4. Vegetation of dumps	45
C.2.2. Studies of Abiotic Ecological Determinants	46
C.2.2.1. Temperature measurements	46
C.2.2.1.1. Atmospheric temperature	46
C.2.2.1.2. Soil and coal waste temperatures	46
C.2.2.2. Rainfall	46
C.2.2.3. Oxygen and carbon dioxide in the coal waste	46
C.2.2.4. Sampling and analysis of coal waste for moisture and pH	46
C.2.2.4.1. Sampling	46
C.2.2.4.2. Moisture analyses	47
C.2.2.4.3. pH analyses	47
C.2.3. Microbiological Studies	47
C.2.3.1. Experimental approach	47
C.2.3.2. Media	48
C.2.3.2.1. HJJ medium	48
C.2.3.2.2. Starkey's medium	48
C.2.3.2.3. Beijerinck's medium	48
C.2.3.2.4. <i>Metallogenium</i> medium	49
C.2.3.2.5. L medium	49
C.2.3.2.6. JLFe medium	49
C.2.3.2.7. S ⁰ medium	49
C.2.3.3. Preliminary studies on the coal waste	50

	Page
C.2.3.4. Coal waste samples from pilot scale dumps	50
C.2.3.5. MPN determinations: General procedures	50
C.2.3.6. MPN determinations: Specific procedures for different bacterial groups	51
C.2.3.6.1. Acidophilic high ferrous iron-oxidizing bacteria	51
C.2.3.6.2. Thiosulphate-oxidizing bacteria	51
C.2.3.6.3. Moderately acidophilic very low ferrous iron-oxidizing bacteria (presumptive <i>Metallogenium</i> count)	51
C.2.3.6.4. Acidophilic relatively high temperature high ferrous iron-oxidizing bacteria	52
C.2.3.6.5. Acidophilic moderate ferrous iron-oxidizing bacteria (count in JLFe medium)	52
C.2.3.6.6. Iron- and sulphur-oxidizing bacteria able to grow in both JLFe and S ⁰ medium	52
C.2.3.6.7. Iron-, sulphur- and thiosulphate-oxidizing bacteria able to grow in JLFe, S ⁰ and Starkey's medium	52
C.3. RESULTS	52
C.3.1. Abiotic Ecological Determinants in Pilot Scale Coal Waste Dumps	52
C.3.1.1. Temperature conditions	52
C.3.1.1.1. Atmospheric temperatures	52
C.3.1.1.2. Soil and coal waste temperatures	53
C.3.1.2. Moisture conditions	53
C.3.1.2.1. Rainfall	53
C.3.1.2.2. Moisture content of coal waste	53
C.3.1.3. Oxygen and carbon dioxide concentrations in coal waste	56
C.3.1.3.1. Uncovered cells (1, 2 and 3)	56
C.3.1.3.2. Avalon soil-covered cells (4, 5 and 7)	60
C.3.1.3.3. Estcourt and Avalon soil-covered cells (6, 8, 9 and 10)	60
C.3.1.4. pH of coal waste	60
C.3.1.4.1. Uncovered cells (1, 2 and 3)	62
C.3.1.4.2. Avalon soil-covered cells (4, 5 and 7)	62
C.3.1.4.3. Estcourt and Avalon soil-covered cells (6, 8, 9 and 10)	62
C.3.2. Microbial Populations in Coal Waste of the Pilot Scale Dumps	63
C.3.2.1. Preliminary studies on coal waste used for dump construction	63
C.3.2.2. Acidophilic high ferrous iron-oxidizing bacteria	63
C.3.2.3. Acidophilic and non-acidophilic thiosulphate-oxidizing bacteria	68
C.3.2.4. Moderately acidophilic very low ferrous iron-oxidizing bacteria (presumptive <i>Metallogenium</i>)	68
C.3.2.5. Highly acidophilic relatively high temperature high ferrous iron-oxidizing bacteria	68
C.3.2.6. Acidophilic moderate ferrous iron-oxidizing bacteria	73
C.3.2.7. Acidophilic moderate ferrous iron- and sulphur (S⁰)-oxidizing bacteria	73
C.3.2.8. Acidophilic moderate ferrous iron-, sulphur- and thiosulphate-oxidizing bacteria	77

	Page
C.4. DISCUSSION	80
C.4.1. Characteristics of Coal Waste Used in Construction of Pilot Scale Dumps	80
C.4.1.1. Physicochemical characteristics	80
C.4.1.2. Microbiological characteristics	81
C.4.2. Characteristics of Soil Cover Materials	81
C.4.3. Abiotic Ecological Determinants in Pilot Scale Dumps	82
C.4.3.1. Temperature conditions	82
C.4.3.1.1. Atmospheric temperatures	82
C.4.3.1.2. Soil and coal waste temperatures	83
C.4.3.2. Moisture conditions	83
C.4.3.2.1. Rainfall	83
C.4.3.2.2. Moisture content of coal waste	84
C.4.3.3. Oxygen and carbon dioxide concentrations in coal waste	84
C.4.3.4. pH of coal waste	85
C.4.4. Microbial Populations in Coal Waste of Pilot Scale Dumps	87
C.4.4.1. Acidophilic high ferrous iron-oxidizing bacteria	87
C.4.4.2. Acidophilic and non-acidophilic thiosulphate-oxidizing bacteria	88
C.4.4.3. Moderately acidophilic very low ferrous iron-oxidizing bacteria (presumptive <i>Metallogenium</i>)	88
C.4.4.4. Highly acidophilic relatively high temperature high ferrous iron-oxidizing bacteria	89
C.4.4.5. Acidophilic moderate ferrous iron-oxidizing bacteria	89
C.4.4.6. Acidophilic moderate ferrous iron- and sulphur-oxidizing bacteria	90
C.4.4.7. Acidophilic moderate iron-, sulphur- and thiosulphate-oxidizing bacteria	90
D. RESEARCH: PART 2. MICROORGANISMS OF IRON-OXIDIZING CONSORTIA INVOLVED IN THE GENERATION OF ACID MINE DRAINAGE IN NORTHERN KWAZULU-NATAL	91
D.1. INTRODUCTION	91
D.2. MATERIALS AND METHODS	91
D.2.1. Microbiological Media	91
D.2.1.1. HJJ, 9K, L and S ⁰ media	91
D.2.1.2. H medium	91
D.2.1.3. <i>Thiobacillus</i> solid medium (TSM)	92
D.2.1.4. <i>Acidiphilium</i> solid medium (ASM)	92
D.2.1.5. FeSo medium	92
D.2.2. Enrichment Cultures	93
D.2.2.1. Cultures from coal waste	93
D.2.2.2. Cultures from mine dump drainage water	95

	Page
D.2.2.3. Cultures for microbial isolations	95
D.2.3. Isolation of Iron-oxidizing Bacteria	95
D.2.3.1. Plating procedures	95
D.2.3.2. Test for heterotrophic associates	95
D.2.3.3. Culture maintenance	96
D.2.4. Isolation of Heterotrophic Organisms	96
D.2.4.1. Plating procedures	96
D.2.4.2. Culture maintenance	96
D.2.5. Characterization of Iron-oxidizing Bacteria	96
D.2.6. Characterization of Heterotrophic Organisms	97
D.2.6.1. Bacteria	97
D.2.6.2. Yeasts	97
D.2.6.3. Filamentous fungi	98
D.2.7. Studies of Interactions between <i>T. ferrooxidans</i> and Fungi from Enrichment Cultures	98
D.3. RESULTS	99
D.3.1. Enrichment Cultures	99
D.3.1.1. Cultures from coal waste	99
D.3.1.2. Cultures from mine dump drainage water	99
D.3.2. Isolation and Identification of Iron-oxidizing Bacteria from Enrichment Cultures	99
D.3.2.1. Isolation	99
D.3.2.2. Identification	100
D.3.3. Isolation and Identification of Heterotrophic Organisms from Enrichment Cultures	100
D.3.3.1. Isolation	100
D.3.3.2. Identification	100
D.3.3.2.1. Bacteria	100
D.3.3.2.2. Yeast	100
D.3.3.2.3. Filamentous fungi	101
D.3.4. Distribution of Isolated Organisms in Enrichment Cultures from Coal Waste or Mine Drainage Water	101
D.3.4.1. Organisms in enrichment cultures from coal waste	101
D.3.4.2. Organisms in enrichment cultures from mine dump drainage water	102
D.3.5. Interactions between <i>T. ferrooxidans</i> and Fungi from Enrichment Cultures	102
D.4. DISCUSSION	106
D.4.1. Microbial Consortia in Iron-oxidizing Enrichment Cultures	106
D.4.1.1. Cultures from coal waste	106

	Page
D.4.1.2. Cultures from mine dump drainage water	107
D.4.2. Interaction Studies between <i>T. ferrooxidans</i> and Fungi from Enrichment Cultures	108
E. DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS	109
E.1. DISCUSSION AND CONCLUSIONS	109
E.2. RECOMMENDATIONS	111
E.2.1. Recommendations Concerning Coal Waste Dump Rehabilitation Procedures	111
E.2.2. Recommendations for Further Research	112
F. REFERENCES	113
G. APPENDICES	127
G.1. APPENDIX 1. TABLE OF MPN ESTIMATES WITH 95% CONFIDENCE LIMITS FOR ALL POSSIBLE COMBINATIONS OF FERTILE TUBES INOCULATED IN TRIPLICATE WITH 1, 0.1 AND 0.01 G OR ML OF INOCULUM	127
G.2. APPENDIX 2. DETAILS OF pH, MOISTURE AND MPN DETERMINATIONS OF MICROBIAL POPULATIONS OF COAL WASTE SAMPLES FROM THE TEN PILOT SCALE DUMPS CONSTRUCTED NEAR THE KILBARCHAN MINE	130