Salinity, Sanitation and Sustainability

A Study in Environmental Biotechnology and Integrated Wastewater Beneficiation in South Africa

Volume 3

INTEGRATED ALGAL PONDING SYSTEMS AND THE TREATMENT OF DOMESTIC AND INDUSTRIAL WASTEWATERS

Part 3: Mine Drainage Wastewaters
The ASPAM Model

P D Rose, G E Boshoff, N P Molipane

WRC Report No: TT 192/02

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Water Research Commission

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Cover Photograph:

Flamingoes on tannery wastewater ponds at Mossop Western Leathers Co., Wellington, South Africa. The presence of Phoenicopteridae, including both the Greater and Lesser Flamingo, is an important indicator of healthy and naturally functioning saline aquatic ecosystems. This flock occupied the ponding system shortly after commissioning the novel Spirulina-based Integrated Algal Ponding System which had been developed for the treatment of tannery wastewaters. This apparent seal of environmental approval became an icon for the studies which followed in this series.

Photograph by Roger Rowswell, whose observation of this system, over a number of years, was instrumental in the initiation of these studies.

Salinity, Sanitation and Sustainability:
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Integrated Wastewater Beneficiation in South Africa

Volume 3

INTEGRATED ALGAL PONDING SYSTEMS AND THE TREATMENT OF DOMESTIC AND INDUSTRIAL WASTEWATERS

PART 3: MINE DRAINAGE WASTEWATERS - THE ASPAM MODEL

Report to the Water Research Commission
By

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Grahamstown

JULY 2002
WRC Report No: TT 192/02

Obtainable from:

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0031

Report to the Water Research Commission on the Project K5/656, 'Appropriate Low-cost Treatment of Sewage Reticulated in Saline Water Using the Algal High Rate Oxidation Ponding System'

Project Leader: Prof P.D. Rose

ISBN No: 1 86845 889
ISBN Set No: 1 86845 853 9
Printed in the Republic of South Africa

FOREWORD

The work presented in this series covers a decade of concerted research into critical sustainability issues in the water-scarce Southern African situation. The provision of safe and adequate drinking water and sanitation services to all our people remains a challenge. Pervasive salination from a range of mining, industrial and agricultural activities threatens the quality of our water resources. Simultaneously, the complex ecological needs of the aquatic environment are being understood with ever-increasing clarity.

Significant progress has been made in meeting some of these challenges. In the years since the democratic elections of 1994, millions of previously unserviced South Africans have been supplied with safe drinking water and sanitation services. The problem of increasing salinity of our water resources, with its direct economic impacts and future threat to sustainability, is being addressed at policy and implementation levels, for example by reduction-at-source measures. The ecological needs of the aquatic environment have been recognised by the provision in our water law of a prioritised ecological reserve, to be managed by the catchment management agencies being formed.

Such promising developments notwithstanding, ultimately sustainable resolution of these issues depends crucially also on acquiring appropriate and affordable technologies that provide physical solutions to our water-related challenges. It is in this context that the research described in this series deserves special commendation for the highly innovative biotechnological linkage developed between the treatment of saline wastewaters on one hand and domestic sewage and sludges on the other.

In the novel approach followed, salinity and sanitation issues are each viewed essentially as a resource base (rather than simply as "waste problems") in a suite of integrated process schemes which can be variously manipulated to deliver products of treated water, recovered nutrients and metals, and algal biomass. The paradigm is consequently changed from one of "managing problems" to one of "engineering opportunities", with the potential of offering a major contribution towards the management of water and sanitation in the RSA - some applications have already been taken to full scale implementation, for example in the accelerated digestion of sewage sludge. Significantly, the achievements of this research add weight to biotechnology as "the" technology of the 21st century.

So, as we approach the World Summit on Sustainable Development, we can reflect on the provisions of Agenda 21 adopted after the Earth Summit some 10 years ago, and note that in this time we have ourselves in various ways "done something" about our own situation. And we can therefore point with a justifiable sense of pride and achievement to the body of work presented here as being "Made in South Africa", at a time when social, environmental, political and economic calls are being made to all of Africa to stand up in the continental and global communities of nations.

My deep thanks and appreciation go to the Water Research Commission for the foresight in funding this work, and, in particular, to Prof Peter Rose and his research team at Rhodes University, for the vision, purposefulness, innovation and application with which this work has been conceived and executed.

Ronnie Kasrils

Minister of Water Affairs and Forestry
Pretoria
31 July 2002

EDITOR'S NOTE

In 1990 the Water Research Commission, under the (then) Executive Director Dr Piet Odendaal, appointed the Environmental Biotechnology Group at Rhodes University, led by Prof Peter Rose, to carry out a one-year feasibility study to evaluate the potential of a biotechnological approach to the linked treatment and management of saline and sanitation wastewaters with recovery of useful components such as nutrient bio- products.

In the intervening years, this seminal project has resulted in a rich research programme, managed initially by Dr Oliver Hart, subsequently by Zola Ngcakani, and latterly (since 1997) by myself. The progression of the research programme is reflected in this series of reports. Report 1 critically reviews the main arguments considered in the sustainability discourse and their relation to salinity and sanitation, and presents an overview of the work covered in the individual Reports 2 – 12, each of which deals with specific aspects of the research programme. The reports are also to be issued on CD.

The research period concerned spans approximately the decade between the Rio Earth Summit in 1992 and the imminent World Summit for Sustainable Development in Johannesburg. During this time, international concern has been expressed about the limited extent to which the sustainability objectives formulated at Rio, as captured for example in Agenda 21, have been followed through to implementation.

By contrast, it is a noteworthy achievement of this research programme that the "sustainable biotechnology" originally conceptualised by the researchers has in fact, by dint of rigorous research development, experimentation and testing, been translated into a suite of practicable processes for delivering treated water as well as value-adding organic and inorganic co-products. In some applications, full-scale plants are already being installed, fulfilling the cycle of research → development → implementation.

It is probably fair to say that the full potential of the original work initiated twelve years ago, with its various applications as they have been developed since then, could at inception only have been dimly foreseen – which, with hindsight, underscores the clarity, breadth and depth of the originators' vision.

It has been a pleasure and a privilege to be involved with this work, as Research Manager and now as Editor of this series. I am confident that you, the reader, will find the contents both informative and as stimulating as I have.

Greg Steenveld
Water Research Commission
Pretoria
31 July 2002

PREFACE

This report is one of a series of twelve Water Research Commission studies undertaken by the Environmental Biotechnology Group at Rhodes University, on biotechnology and integration in the management of saline and sanitation wastewater systems. Environmental problems in these areas are reckoned to be responsible for six of the seven priority pollution issues undermining the sustainable development project in Southern Africa. While both salinity and sanitation have separately been the subject of quite extensive investigation, relatively little has been reported on the potential linkage of these systems in meeting sustainable development objectives.

At the time these studies commenced, in 1990, focus on the operationalisation of the sustainability idea had identified 'integrated waste resource management' as a key requirement for progress towards 'closed systems' production. Here human activities, and the associated technological environment, would be detached as far as possible from the bio-physical environment related to natural systems. Waste recovery, recycle and reuse had emerged as major strategies for achieving the radical shift to new technologies which would enable societies to live off nature's income, rather than consuming its capital. Waste beneficiation (a term still more common in the traditional resources sector, and referring to operations that add value by transforming raw material into finished products), was seen as a means of placing treatment operations on an economic footing, with value added in the form of products and services accrued in the waste management operation.

To meet the time-scale of the sustainability agenda, the breakthroughs in technology required would have to be initiated now to guarantee their availability in the next 2 to 4 decades. This led to widespread use of technology-push approaches in sustainable technologies research.

The principal aim of this programme was thus to investigate potential in environmental biotechnology for the development of technological enablement in the linkage of saline and sanitation wastewater management. This involved initial studies in the biology of organic saline wastewater impoundments and an evaluation of the recovery of nutrient values in these wastes in the form of high-value bio-products produced by halophilic micro-organisms. Integrated Algal Ponding Systems were investigated as a 'core technology' in delivering these objectives.

A critical path research methodology was used to identify technological constraints in the organic saline wastewater treatment operation and served to prioritise the research inputs required to underpin bioprocess development. Studies in the microbial ecology and environmental biotechnology of these systems provided the basis for bio-process innovation, and the subsequent development of treatment processes to full-scale engineered applications.

This series includes an introductory volume which provides an overview of the twelve-year programme to date. The reports are listed inside the front cover, and each study in the series is identified by a 'racing flamingo' number, which also appears on the outside cover. This relates to the appearance of a large flock of flamingoes, which took up residence on tannery wastewater ponds following the installation of the *Spirulina*-based Integrated Algal Ponding System developed in the initial studies in this series. The development of the 'Salinity, Sanitation and Sustainability' programme is outlined below in Figure P1, and shows studies

in the integrated algal ponding of saline, and domestic and industrial wastewaters, leading to the Rhodes BioSURE Process®, which provides linkage in the treatment of sulphate saline wastewaters and sewage sludge disposal.

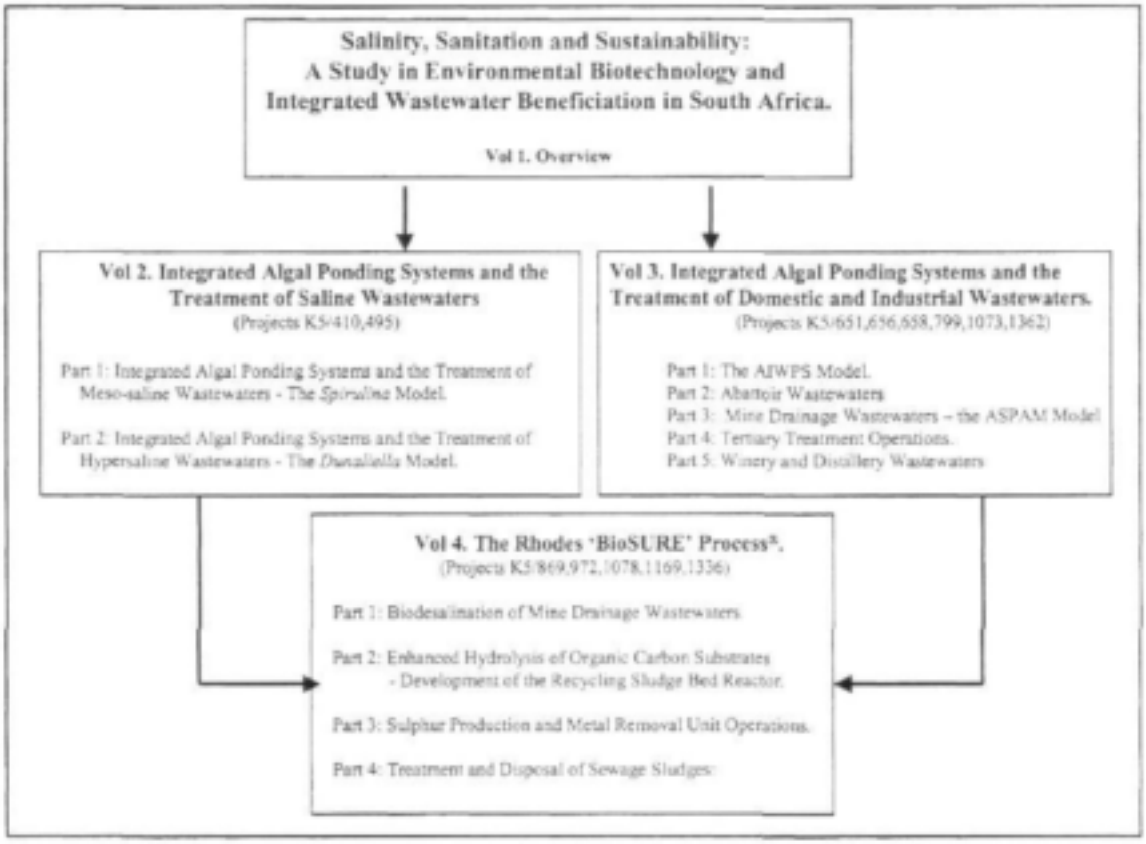

Figure P1. Research projects undertaken as components of the Water Research Commission study 'Salinity, Sanitation and Sustainability'.

A large number of people have assisted generously in many ways in the development of these studies, and are thanked under Acknowledgments. The support of former Water Research Commission Executive Director, Dr Piet Odendaal, is noted in particular. His vision of research needs in water resource sustainability, in the period leading to the Rio Earth Summit in 1992, not only contributed to this study, but also initiated early contributions to sustainable development research in water and sanitation service provision to developing communities. His inputs, together with Research Managers Dr Oliver Hart, Mr Zola Ngcakani, and Mr Greg Steenveld, have made substantial contributions to the development of the ideas investigated in these studies. The contribution and enthusiasm of my post-graduate research students is beyond measure.

Peter Rose
Environmental Biotechnology Group
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CONTENTS

EXECUTIVE SUMMARY

1 BACKGROUND

Mine drainage wastewaters are identified among the principal pollutants responsible for a progressive salinisation and an ongoing deterioration of the fresh water resource in South Africa (Walmsley *et al.*, 1999; DEAT, 2000). The physico-chemical and biological processes giving rise to pyrite oxidation, acid formation and heavy metal solubilisation, associated with acid mine drainage (AMD) formation, are now quite well known (Johnson, 1995), and occur in many mining environments worldwide. It is only relatively recently that the long-term impact of the problem in South Africa has become apparent. Previous assumptions that, on mine closure, the shut-down of active mine dewatering would result in the establishment of a final sub-surface hydraulic resting level have been challenged by Scott (1995). In a seminal WRC study he has indicated that abandoned mines in the Witwatersrand gold fields will fill within 10 years, and will then decant large volumes of low-grade saline and heavy metal-polluted water. Apart from contaminating the dolomitic water table, this water would find its way ultimately into the Vaal River system. He has estimated that the flow from the East Rand Basin alone will be more than 30 $ML.day^{-1}$. Coal mines face a similar situation and the long-term nature of the problem has been noted by Younger *et al.*, (1997), whose study of abandoned British mines showed that AMD flows may be anticipated to last anywhere from many decades to centuries.

A considerable research effort has been directed at the AMD problem by the mining industry over a period of many years (Pulles *et al.*, 1995), with treatment strategies focusing on both physico-chemical and biological processes. With the anticipated long-term post-closure treatment requirement, increasing interest has focused on the low-cost sustainability advantages of biological processes. Notwithstanding substantial research in this area, the singular factors constraining the widespread application of biological treatment approach have been reactor configuration, the cost of reactor construction and operation, and the availability and cost of the carbon source and electron donor for the sulphate reduction processes (Rose *et al.*, 1998).

2 WRC PROJECT K5/656 - TREATMENT OF SALINE WASTEWATERS

The project reported here developed around the concept of linkage between saline wastewater treatment and the co-disposal of organic wastes using Integrated Algal Ponding Systems (IAPS) technology. Applications of IAPS had been extended in a number of novel respects in the course of preceding WRC Project K5/495 'A biotechnological approach to the removal of organics from saline effluents' (Appendix 1), undertaken by the Rhodes University Environmental Biotechnology Group (EBG). In particular the treatment of high sulphate-saline tannery wastewaters had been demonstrated in both technical- and full-scale installations (Rose *et al.*, 2002a). The engineering of IAPS applications in domestic wastewater treatment has also been demonstrated in Project K5/651 (Rose *et al.*, 2002b), and for abattoir wastewaters in Project K5/658 (Rose *et al.*, 2002c).

Although the application of IAPS in AMD treatment has not been previously

reported, a number of factors emerged from these studies which indicated an apparent potential in the IAPS design to handle various complex issues related to the treatment of mine drainage wastewaters. These included observations in a tannery wastewater IAPS application developed by the EBG of efficient rates of sulphate reduction, the mobilisation of complex organic carbon compounds as electron donor sources, alkalisation reactions and the effective precipitation and removal of heavy metals. It was against this background that the current WRC Project K5/656, 'Appropriate low-cost treatment of sewage reticulated in saline water using the algal high rate oxidation ponding system', took shape.

2.1 Aims

The initial research objectives for this project focussed broadly on IAPS technology in the treatment of saline wastewaters and the linkage of the saline and sanitation wastewater systems. The original aims were identified as follows:

1. To evaluate the use of HRAP for the treatment of sewage reticulated in saline water, in a laboratory study;

2. To compare sources of saline water, including sea-water and saline groundwater, for the treatment of saline reticulated sewage effluent;

3. To evaluate the scale-up of the HRAP to a demonstration plant;

4. To evaluate the suitability of returning purified water to inland aquifers or the ocean, from which the reticulated waters were derived;

5. To investigate the recovery and value of the biomass obtained during the treatment of the above wastewaters.

Despite the initial intention to investigate a range of saline wastewater types, project budgetary restrictions at the time, and increasing awareness of the AMD problem, highlighted by the Grootvlei Mine dewatering environmental incident in the Blesbokspruit in 1996, resulted in a decision to focus the study on sulphate-saline wastewaters only.

The study aims were thus amended as follows:

1. To investigate the application of IAPS technology in the treatment of acidic and metal contaminated sulphate-saline wastewaters;

2. To evaluate waste organic carbon sources as electron donor substrates in biological sulphate reduction;

3. To investigate neutralisation and metal removal operations in AMD treatment with application of IAPS technology.

3 INTEGRATED ALGAL PONDING SYSTEMS IN AMD TREATMENT

The decision to investigate an IAPS application in AMD treatment was based on a number of general observations:

- The linkage between the treatment of large volumes of wastewaters and mass algal production has been firmly established as a mature and widely utilised operational technology (Oswald, 1988a; Mara *et al.*, 1996);
- The earthwork pond provides a reactor at least an order of magnitude less costly to construct than steel-reinforced vessels (Oswald 1995);
- Renewable algal biomass (as a potential carbon source for sulphate reduction activity mediated by sulphate reducing bacteria -SRB) may be reliably produced in large amounts (up to 50 tons.ha^{-1}.yr^{-1}), in separately optimised HRAP systems (Oswald 1988b);
- The ability of algal biomass to adsorb and sequester heavy metals, and thereby reduce metal concentrations to residuals in the ppb range, has been demonstrated and commercialised (Wilde & Benneman 1993);
- IAPS had been investigated in the treatment of sulphate-saline tannery wastewaters and shown to support high levels of SRB activity using complex organic carbon electron donor sources (Rose *et al.*, 2002a);
- A technology transfer exercise in collaboration with Professor William Oswald (University of California, Berkley, USA) had established an engineering base for IAPS technology development in South Africa;
- Studies had commenced in the EBG on development of an IAPS application in hypersaline wastewater treatment of soda ash production brines in Botswana.

Given the substantial progress already made in technical- and full-scale studies on the use of IAPS noted above, sufficient information seemed to be available to propose a core IAPS configuration as a model system for AMD treatment. However, a number of important issues would need to be clarified to demonstrate feasibility, before proceeding with any confidence to pilot-scale studies. A critical path research methodology was used to identify these areas and this formed the substance of the investigation which followed.

4 THE ASPAM MODEL

The process model which emerged, and was to be tested in these studies, became known as the Integrated Algal Sulphate Reducing Ponding Process for Acidic Metal Wastewater Treatment (ASPAM). The individual unit operations of the ASPAM process were conceived to operate as outlined in Figure 1 and described below.

4.1 Advanced Facultative Pond

At (1) a portion of the treated water flows into the Advanced Facultative Pond (AFP) together with the carbon source which may include a range of organic wastes including tannery wastewaters, sewage sludges and agro-industrial organic waste products. Sulphide produced in this unit passes forward to a metal precipitation unit

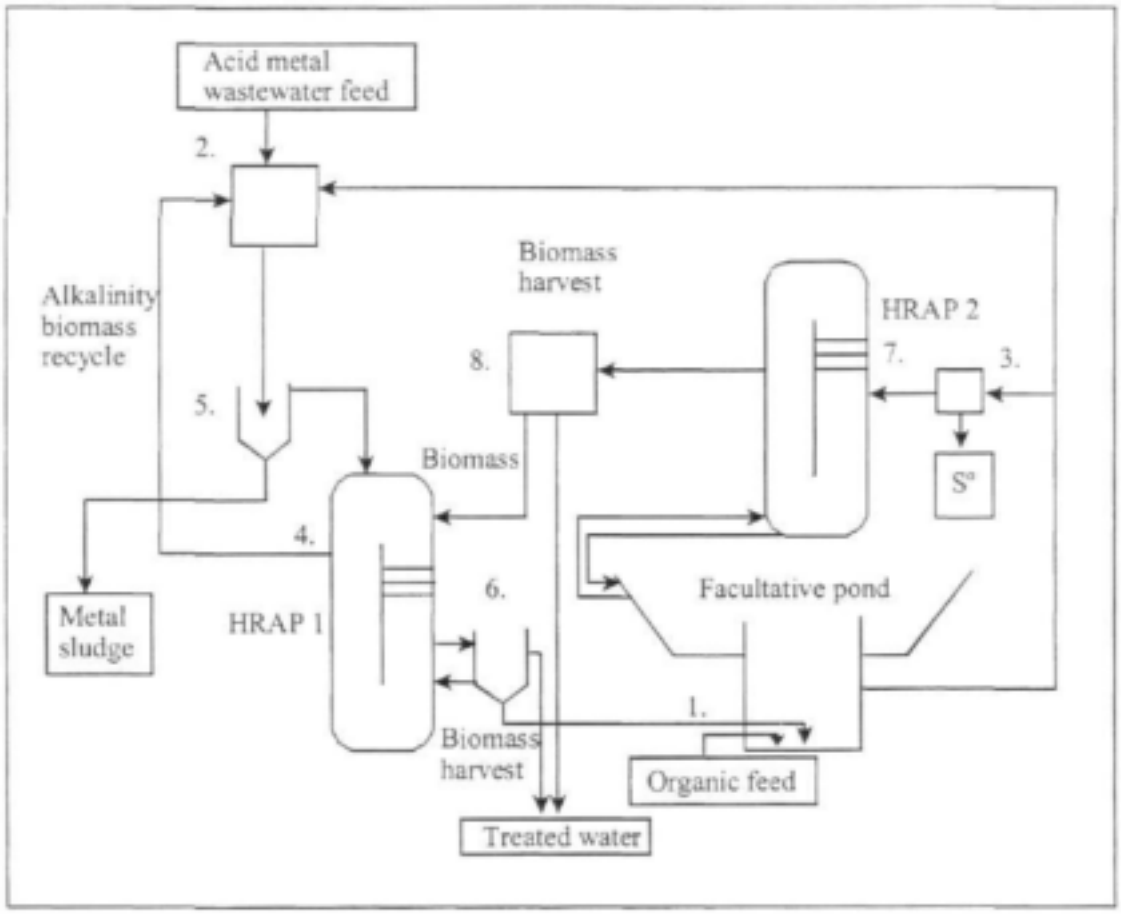

Figure 1. Flow diagram of the individual unit operations of the Integrated Algal Sulphate Reducing Ponding Process for Acid Metal Wastewater Treatment (ASPAM). Organic feed enters at 1= Facultative pond with anaerobic upflow digester compartment; Feed water enters at 2 = Inlet and metal precipitation unit; Sulphide is recycled to metal precipitation unit from 3 = sulphide recycle and sulphur recovery unit; Alkalinity and algal biomass generated in High Rate Algal Pond (HRAP)1 recirculated via 4; 5 = Metal sludge settler; 6 = Algal biomass settler; 7 = High Rate Algal Pond (HRAP)2 for capping the Facultative Pond and seeding HRAP 1 with fresh biomass; 8 = Algal biomass harvester.

operation (2). Recirculation of algal-laden waters from HRAP 2 is used to maintain an oxygenated layer on the surface of the AFP and thus control sulphide gas release.

The AFP described by Oswald (1998 a&b), provides for the inclusion of one or more Upflow Digesters (UD) in the base of the pond, enabling optimum anaerobic digestion function. Tannery pond studies, in Wellington, South Africa, had shown that sulphidogenic anaerobic digestion will perform efficiently utilising tannery waste as an electron donor source. High rates of sulphate reduction were observed to be associated with an accelerated hydrolysis and solubilisation of particulate organic matter fed to the system. An associated precipitation of heavy metals was also observed in this unit with low residual metal levels in the effluent (Dunn, 1998).

The maintenance of an aerobic compartment above the anaerobic layer was shown in the tannery IAPS not only to provide for odour control, with the scrubbing of released gasses, but also ensured that the sulphur cycle was completed above the

oxypause, with a nearly full oxidation of sulphide back to sulphate (Rose *et al.*, 1996).

Objectives identified for the current study included an evaluation of the performance of a sulphate reducing anaerobic pit in the AFP, operating as an Upflow Digester (UD), and also the performance of a number of organic carbon sources, other than tannery waste, as electron donors for sulphate reduction.

4.2 Metal Precipitation Operation

Sulphide-rich waters pass from (1) to the metal precipitation unit operation (2) where, in mixing with the influent AMD stream, a combination of metal complex formation reactions would occur. Effective precipitation of heavy metals in the sulphidogenic tannery wastewater AFP, had been reported by Boshoff (1998) and Dunn (1998), and the relative advantages of metal precipitation as the sulphide compared to metal hydroxide forms has been widely noted (Peters and Ku, 1985; Singh, 1992 and Hammack *et al.*, 1994). Dunn (1998) had reported preliminary observations on both the *ex situ* precipitation of metals utilising the sulphide-rich AFP liquors in the tannery IAPS, and also their removal in the presence of algae in the HRAP. Both systems demonstrated strong alkalising properties with the influent pH elevated to values between 9 and 10, at the end of the ponding cascade.

The objective of this component of the study was to investigate the feasibility of utilising both the micro-algal and sulphidic AFP streams in a metal precipitation unit operation, providing primary treatment of the influent AMD stream. Following the formation of a metal complex precipitate, and the removal of the major portion of the metal contaminants from the influent stream, the metal residuals would be stripped and the effluent finally polished during the subsequent stages of HRAP operation .

The stripping of sulphide gas from the pond liquors, and its use directly for metal removal in the precipitation unit, is an added option, but has not been further considered in the study reported here.

4.3 High Rate Algal Pond 1

Following the removal of metal sludges, the partly neutralised AMD passes to HRAP 1 (4). Here photosynthetically-driven alkalisation would be optimised and final metal removal effected by micro-algal biosorption.

The observations of the alkalising and metal binding properties of micro-algae in the tannery IAPS had been investigated by Boshoff (1998) and Dunn (1998). These had indicated the need for the inclusion of HRAP 1, following preliminary metal removal and prior to the stream entering the AFP. The relatively high pH minima of the bio-sulphidogenic system in the FP makes the neutralisation of the acid stream essential to the operation of this unit. Neutralisation may be handled by both feeding HRAP 1 liquors forward to the precipitation unit, and through *in situ* alkalisation in the raceway itself. Physiological stress in this unit may be expected to depress micro-algal growth rates. In this regard it would be important to maintain biomass against washout by replacement with algae recovered from HRAP 2, prior to discharge of the

final treated stream.

4.4 High Rate Algal Pond 2

At (7) a portion of the AFP surface water circulates through HRAP 2 where a high concentration of algal biomass is maintained. This is used for recirculation and capping of the surface of the AFP to contain sulphide release, and for constant biomass replenishment to HRAP 1, in which a stress reduction of growth rates would be anticipated.

The performance of the biomass recycling and pond capping operation has been investigated in the tannery IAPS (Rose *et al.*, 2002a) and the Grahamstown AIWPS Plant studies (Rose *et al.*, 2002b).

Issues which needed to be clarified in this study included an evaluation of the potential toxicity of sulphide production in the AFP on the micro-algal biomass circulated from HRAP 2. The Wellington study had shown that the HRAP following the Facultative Pond in that system had been able to handle a limited level of sulphide throughput (Dunn, 1998), but this had not been quantified.

4.5 Treated Water

At (6) the main flow of treated water exits the system from HRAP 1. This follows harvesting of the algal biomass, and together with a portion of treated water the biomass passes into the AFP where the organic carbon feed requirement is supplemented.

The use of algal biomass as a carbon source for sulphate reduction in AMD treatment was studied by Boshoff (1998). Photosynthetic production within the system would not only contribute to the organic carbon requirement, but in small installations might provide an independence from external carbon sources.

4.6 Sulphur Recovery and Biodesalination

A portion of the total sulphur load entering the system would be removed with the metal sulphide sludge. Where a more complete sulphate removal is required a biodesalination of the AMD may be achieved by passing the reduced AFP waters through a sulphur recovery unit (3). Here elemental sulphur (S^o) will be a final by-product of the process. Sulphide oxidation and sulphur recovery is the subject of separate WRC Projects K5/1078 and K5/1336 (Appendix 1).

5 RESULTS

An experimental program was set up to investigate aspects of the questions identified above. The results of these studies are summarised here:

5.1 Electron Donor Sources and the Pond as Bioreactor

Algal biomass, tannery wastes and sewage sludge solids were investigated as potential electron donors for sulphate reduction in separate studies utilising the UD bioreactor configuration. In addition, preliminary observations made during the tannery IAPS study, of an accelerated hydrolysis of complex organic carbon substrates within the biosulphidogenic environment, were confirmed in this investigation. The manner in which complex carbon substrates are made available as electron donors for sulphate reduction in these systems had become the subject of a subsequent follow-up study of the Rhodes BioSURE Process for AMD treatment (WRC Projects K5/869 and 972).

Sewage sludges and tannery wastes both provide useful opportunities for co-disposal together with AMD treatment. Where long-term treatment will be required, as is likely to be the case with the closure of the Witwatersrand gold mines, the low-cost integration of sewage and AMD management offers substantial advantages in the sustainability of the treatment operation.

Micro-algal biomass was shown to provide an electron donor source for sulphate reduction comparable with both sewage sludges and tannery wastewaters. While this application was not evaluated at pilot-scale in this study, a number of reports on feeding algal biomass to fresh water methanogenic UD-type digesters has been documented (Gerhardt *et al.*, 1994). While micro-algal production in the ASPAM ponding system provides independence from external electron donor sources, the practicality of this option will depend on the volume flow to be treated and the surface areas available for pond construction. Clearly, remotely operated systems treating small volume seeps could conceivably be managed utilising only the micro-algal biomass production of the system.

In both remotely-located applications, and those treating large volume flows, the pond provides an effective choice of bioreactor. Oswald (1995) has noted that ponds are at least an order of magnitude cheaper to construct than conventional concrete reactors, and considerably more so than highly-engineered steel reactors. In long-term AMD treatment applications, the lower operational costs associated with pond management versus constructed bioreactor controls would assume major significance over time.

5.2 Heavy Metal Removal

The studies reported here showed that metal sulphide complexation, formed during the recycling of AFP anaerobic liquors to the AMD influent stream, was not the only mechanism involved in precipitate formation. It was evident that metal carbonate and/or hydroxide formation might also be involved, as well as the adsorption of metal complexes to particulate organic carbon remaining in the AFP stream.

In addition to the adsorption properties of the micro-algal biomass itself, or its partly degraded particulate residues in the AFP stream, the production of extracellular polymeric substances (EPS) by these organisms may play an important role in adsorbing and removing metals, prior to the neutralisation reactions and the

formation of alkaline metal sludges. Studies undertaken here showed that EPS binding was most efficient at acidic pH values, and also that the production of EPS may be maximised under conditions of physiological stress pertaining in the HRAP 1 unit. While growth in this unit may be slow, provision may be made to feed biomass forward from HRAP 2 where cell growth may be managed at optimised rates.

The HRAP 2 unit also provides a final polishing step where remaining metal contamination may be reduced to very low residuals, within the range of surface water discharge standards.

5.3 Neutralisation of the Acid Stream

The effective neutralisation of the acid stream is critical before it reaches the anaerobic compartment of the FP. Although the SRB have quite a wide pH range compared to MPB, this does not generally fall much within the acid range. However, where acidotolerant or acidophilic SRB have been identified, their growth rate has been generally slow (Johnson, pers.comm. 2000).

While the results reported here indicate that micro-algal growth in HRAP 1 may be relied on to provide an impressive elevation of the pH of the influent stream, this only occurs reliably above pH 4, the crossover point where HCO_3^- species exceed the H^+ concentration (Stumm and Morgan, 1981). However, AMD is also generally low in carbonate, and the system is thus dependent on the SRB reaction in the AFP for net addition of carbonate to the system. It seems therefore that a preliminary alkalisation step is required, and coincides with the addition of AFP effluent to the influent AMD, in the metal precipitation step.

6 CONCLUSIONS

These studies answered a number of questions relating to the feasibility of ASPAM in AMD treatment and enabled a number of conclusions to be drawn about the performance of the proposed unit operations:

- The anaerobic pit in the AFP may be applied as an UD reactor, suitable for the biosulphidogenic digestion of a range of complex organic carbon sources, and their use as electron donors for the production of sulphide, alkalinity and particulate organic carbon fractions;
- A sulphide toxicity tolerance was shown for certain micro-algal species, and also that algal-enriched oxygenated water from the HRAP 2 may be passed to the surface of the AFP to control odour and reoxidise residual sulphide levels in the outflow;
- In addition to providing actively growing micro-algal biomass for supplementing algal production in the HRAP 1, the HRAP 2 unit would provide final polishing and residual metal removal from the treated stream;
- AFP contents may be cycled directly to the metal precipitation step, where

a number of mechanisms would be involved in the precipitation of heavy metals contaminating the influent AMD stream. The provision of carbonate to subsequent algal acid consumption reactions would be an important component of the recycle operation in carbon deficient AMD streams;

- Following the metal precipitation operation, the HRAP 1 unit would complete the neutralisation of the stream prior to its passing to the AFP. Production of EPS as a result of physiological stress in this unit would provide a metal binding function under the more acidic conditions prevailing, and together with alkalisation (acid consumption) in HRAP 1, these liquors would contribute to metal removal in the precipitation unit.

The studies reported here indicated a preliminary feasibility for the ASPAM model in certain important respects, as an IAPS configuration to be applied in AMD treatment.

7 RECOMMENDATIONS

It is apparent that the functionality of an IAPS approach in the treatment of AMD would depend on a wide range of factors. While certain additional factors have been clarified here, at both laboratory and pilot-scale, these results relate to the evaluation of independent free-standing unit operations. The results of the study indicate that sufficient information may now be at hand to warrant proceeding to the next stage of investigation. This should allow the various unit operations comprising the system to be integrated into the ASPAM process, with process piloting at technical-scale to determine the mass balances and flow regulation required between the various units.

Given the anticipated growth in the AMD problem nationally, with pending closure of increasing numbers of both coal and gold mines, the long-term nature of the problem, and the relatively high cost of existing treatment technologies, it is recommended that the WRC consider proceeding to scale-up evaluation of the ASPAM system. Particular areas of application to be investigated should include treatment of large volume AMD flows, and also quasi-passive applications where the generation of algal biomass would provide some independence from external carbon sources.

8 RESEARCH PRODUCTS

The studies reported here led to a number of research spin-off developments in follow-up studies based on the above recommendations. These are the subject of separate project reports which are listed in Appendices 1 and 3.

Student training in IAPS treatment of saline wastewaters has included 3 PhD and 2 MSc students. The results of these studies contributed to the publication of 1 patent, 9 papers in international journals and 6 articles of general scientific interest. Publication in conference proceedings includes 5 plenary and key note papers, 17 international and 29 local conference presentations. The student training and publication outputs are reported in Appendix 2.

9 FOLLOW-UP ACTIONS

The observation, in the tannery pond studies (K5/495), of enhanced hydrolysis of complex organic carbon compounds in the sulphidogenic environment, and the preliminary investigation in this study of their use as electron donor substrates in sulphate reduction activity, had indicated a useful linkage of waste organic carbon and sulphate-saline wastewater treatment. A more rigorous study of the mechanisms involved in the enhanced hydrolysis process was undertaken in follow-up WRC Projects K/5 869 and K/5 972 (Appendix 1). These studies have led to a number of follow-up actions which are noted here, and are the subject of subsequent WRC reports in the 'Salinity, Sanitation and Sustainability' series (Appendices 1 and 3):

- The follow-up investigation on the use of sewage sludge as a carbon source in AMD treatment resulted in the development of the Rhodes BioSURE Process® and was scaled up from laboratory studies to pilot-scale evaluation at the Grootvlei Mine, Springs (Reports 9 and 10);
- The effective solubilisation of sewage sludge observed in the above study led to the evaluation of the BioSURE Process® in sewage sludge disposal. This process has been the subject of technical-scale evaluation in a joint WRC/EBG project with Erwat Co., at the Ancor Works in Springs, South Africa (Report 12);
- Evaluation of alternative carbon sources has led to an investigation of maize lignocellulose wastes in collaboration with Eskom. In this sustainable development programme, the BioSURE Process® provides the initial operation in an AMD beneficiation process whereby treated water is used in downstream agro-industrial job creation. Providing economic sustainability for communities following mine closure provides a basis for long-term environmentally sustainable AMD management;
- The studies in sulphidogenic enhanced hydrolysis were applied to the development of AMD treatment in passive systems in a Department of Arts, Culture Science and Technology Innovation Fund programme in collaboration with Pulles Howard and de Lange;
- The sulphur recovery unit operation to effect a biodesalination of the AMD was developed in follow-up WRC Projects K5/1078 and K5/1336;
- Collaborative studies on the enzymology of sewage sludge solubilisation have been undertaken in the Department of Biochemistry Microbiology and Biotechnology at Rhodes University with Professor Chris Whiteley and Dr Bret Pletschke. These studies have led to a separate WRC programme in environmental enzymology;
- Collaborative studies on the modelling of the BioSURE Process®, and the aqueous chemistry of the metal precipitation operations, has been undertaken with the Departments of Chemical and Civil Engineering at the University of Cape Town, involving Professors Geoff Hansford and Dick Loewenthal and Dr Alison Lewis.

ACKNOWLEDGMENTS

The authors wish to express their thanks and appreciation to the WRC for finance and support throughout the project. A wide range of people and organisations have been involved in the provision of generous support, advice, encouragement and other inputs associated with the execution of this study.

- Prof William Oswald without whose pioneering work in the field over many years, and kind and generous support, none of this would have been possible. Also his colleague Dr Bailey Green who both visited the Wellington site in 1994, and made valuable contributions to the study in a number of ways;

- Dr Oliver Hart who has contributed to much of this work as WRC Research Manager, and following his retirement from the WRC as a Fellow of Rhodes University;

- The previous Executive and Deputy Directors of the WRC, Messrs. P Odendaal and D van der Merwe, who have provided substantial advisory inputs, moral support and encouragement throughout;

- The WRC Research Managers who followed Dr Hart's portfolio at the WRC and oversaw the projects at different stages, including Mr Zola Ngcakani, and Mr Greg Steenveld;

- Rhodes University and the City of Grahamstown who have generously provided facilities for much of the work undertaken;

- The Project Team consisting of:

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Dr O.O. Hart	Researcher
Dr G. Boshoff	Researcher
Dr K. Dunn	Researcher
Ms P. Molepane	Researcher

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Dr H.G. Snyman	Erwat
Mr W. Pulles	Pulles Howard & de Lange
Prof P.D. Rose	Rhodes University
Dr O.O. Hart	Rhodes University
Prof J.R. Duncan	Rhodes University

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ABBREVIATIONS

AFP	Advanced Facultative Pond
AIWPS	Advanced Integrated Wastewater Ponding Systems
ALBAZOD	Algal bacterial zoogloeal detritus
AMD	Acid mine drainage
ASPAM	Algal Sulphate Reducing Ponding Process for Acidic Metal Wastewater Treatment
ATP	Adenosine triphosphate
AZ	Acetozolamide
CA	Carbonic anhydrase
Ci	Inorganic carbon
COD	Chemical oxygen demand
DIC	Dissolved inorganic carbon
EBG	Environmental Biotechnology Group
EDR	Electrodialysis reversal
EPS	Extracellular polymeric substances
HRAP	High rate algal pond
K_{sp}	Solubility product
MPB	Methane producing bacteria
PSS	Primary sewage sludge
SPARRO	Slurry precipitation and recycle reverse osmosis
SRB	Sulphate reducing bacteria
SS	Settleable solids
STR	Stirred tank reactor
TOC	Total organic carbon
TP	Trench reactor
TRO	Tubular reverse osmosis
TS	Total solids
UD	Upflow digester
WRC	Water Research Commission
WSP	Waste stabilisation pond

NOTATION

A wide range of terms has been used over the years by different authors to describe various configurations of ponding systems used in wastewater treatment and in algal biotechnology applications. This has created a certain confusion in the literature, and to avoid possible further confusion the following usage has been followed in this study:

- The term Advanced Integrated Wastewater Ponding System (AIWPS) refers to a specific trade-marked process application design. This ownership of name has been respected and care has been taken throughout not to use the term in a generic sense to cover the many forms of integrated ponding systems involving the use of algal photosynthesis. The term Algal Integrated Ponding Systems (AIPS), and Integrated Algal High Rate Oxidation Ponding Process (IAHROP) which was used in this sense in the earlier part of this study to describe the hybrid algal ponding systems, the development of which was under consideration in this programme, has been changed to Integrated Algal Ponding Systems (IAPS) to avoid confusion;

- The IAPS is used here to refer generically to combinations of ponding system unit operations involving an algal component in their operation;

- The term High Rate Algal Pond (HRAP) has been used here and replaces High Rate Oxidation Pond (HROP) used in some literature references, as it is not necessarily inclusive of the algal component;

- The terms algae or micro-algae are used for convenience in the more traditional sense broadly covering both the eucaryotic algae as well as the cyanobacteria.

1 MINE DRAINAGE WASTEWATERS AND INTEGRATED ALGAL PONDING SYSTEMS

1.1 INTRODUCTION

The pollution of surface waters with acid mine drainage (AMD), also known as acid rock drainage, generally follows geochemical trauma induced by mining or quarrying operations involving sulphide-containing ore bodies. The effects of this biologically-driven process, including the formation of ferric oxide 'Yellow Boy' precipitates, have been known since at least Roman times (Wildeman *et al.*, 1991), and may have far reaching impacts on the environment. Both the South African National State of the Environment Report (DEAT, 1999), and the White Paper on Integrated Pollution and Waste Management (DEAT, 2000) have identified AMD as one of the priority pollutants influencing the quality and availability of water in South Africa, particularly in the inland regions served by the Vaal River catchment system. Walmsley *et al.*, (1999) have noted concerns regarding the long-term sustainability of the national water resource, and that water will "increasingly become a major restriction to the future socio-economic development of the country".

Although the problems of AMD production and treatment relate to most of the country's gold and coal mines, the issue has come to the fore, particularly in recent years, with the decline and closure of the Witwatersrand gold mines. By 1995 only four mines remained actively working underground on the Witwatersrand, compared to 39 in the 1940's (Scott, 1995). Previous assumptions that the cessation of active dewatering, on mine closure, would result in the establishment of a final subsurface hydraulic resting level have been challenged by Scott (1995). In a seminal Water Research Commission (WRC) study, he has indicated that abandoned mines will fill within 10 years, and will then decant large volumes of low-grade saline and heavy metal-polluted water which will find its way ultimately into the Vaal River system. He has estimated that the flow from the East Rand Basin alone would be over 30 $ML.day^{-1}$. The probable long-term nature of the problem has been noted by Younger *et al.*, (1997) whose study of abandoned British mines showed that AMD flows may be anticipated to last anywhere from many decades to centuries.

The biological and physico-chemical processes giving rise to pyrite oxidation, acid formation and heavy metal solubilisation have been well described and are the subject of a number of extensive reviews by Andrews (1989), Silver (1989), Kuenen and Robertson (1992), Pronk and Johnson (1992), Robb (1994) and Johnson (1995).

1.2 TREATMENT

A considerable research effort has been directed at the AMD problem by the mining industry over a period of many years (Pulles *et al.*, 1995), with treatment strategies focusing on both physico-chemical and biological processes.

1.2.1 Physico-chemical Treatment

A number of physico-chemical processes are available for the treatment of mine drainage wastewaters. The most conventional involve chemical precipitation of some form using either hydroxide, carbonate or sulphide treatment, or a combination of these. The most commonly used method is hydroxide precipitation as it is simple and inexpensive, and addresses both the metal and the acidity problems associated with AMD. Those effluents resulting from the mining of arsenopyrite ores can be treated by either ferric sulphate or ferric chloride precipitation (Pulles *et al.*, 1995).

Other chemical methods utilised for mine water remediation include coagulation and flocculation, flotation, ion-exchange, complexation and sequestration, electrochemical reactions, evaporation and distillation and adsorption using activated carbon (Peters and Ku, 1985; Pulles *et al.*, 1995; Saha, 1993). A number of membrane processes are also available for the treatment of wastewater containing heavy metals (Peters and Ku, 1985; Kim 1984; Neytzell-de Wilde, 1992). Electrodialysis reversal (EDR) and tubular reverse osmosis (TRO) have been shown to be technically feasible for desalting non-scaling mine waters with high salinity (Juby, 1992; Juby and Pulles, 1990). However, the majority of mine service waters tend to be, or to become, sodium sulphate scaling waters, leading to scale formation on the filtration membrane surface. More recently the SPARRO (Slurry Precipitation and Recycle Reverse Osmosis) process has been developed to overcome membrane scaling and high operation costs (Juby *et al.*, 1996).

These treatment processes tend to be expensive as they require skilled operators and the installation of plant with agitated reactors, precipitators, clarifiers and thickeners (Gazea *et al.*, 1996). They may also be inefficient, especially when the metals are present in relatively low concentrations in large volumes of water. Thus biological methods for effluent treatment have become an area of increasing focus.

1.2.2 Biological Treatment

Biological approaches to AMD treatment have been reviewed by Kuenen & Robertson (1992); Gadd & White (1993); Barton (1995) and Johnson (1995). Applications of the sulphate reducing bacteria (SRB) have been of particular interest, given their role in the generation of insoluble metal sulphides and the neutralising effect of the sulphate reducing reaction (Barton & Tomei 1995). The biology of these organisms has been the subject of comprehensive treatment by Postgate (1984), Widdel & Hansen (1992), Odom & Singleton (1993) and Barton (1995).

Notwithstanding the type of biological AMD process technology used, the singular factors constraining the biological treatment approach are the reactor configuration used, the cost of construction and the availability and cost of the carbon source and electron donor for the microbial reduction processes (Rose *et al.*, 1998).

Active biological AMD treatment systems rely in the main on SRB production in high rate bioreactors and the precipitation of metal sulphides. Numerous SRB reactor design studies have been reported, including anaerobic filters (De Walle *et al.*, 1979; Chian & De Walle 1983), mixed reactors (Maree & Hill 1989), packed bed

anaerobic bioreactors (Riviera 1983; Maree *et al.*, 1987) fluidised bed systems (Umita *et al.*, 1988; Van Houten *et al.*, 1994), sequencing batch reactors (Herrera *et al.*, 1991), the upflow anaerobic sludge bed (Buisman *et al.*, 1989; Barnes *et al.*, 1991) and the baffle reactor (Grobicki & Stuckey 1992). Scaled-up applications of active AMD treatment technologies have been limited, but the successful operation of an SRB process effecting a geohydrological containment function at the Budelco zinc refinery, in the Netherlands, has been reported (Scheeren *et al.*, 1993).

The use of wetlands for the treatment of AMD is a so-called 'passive technology' which has developed rapidly in recent years (Johnson 1995; Robinson & Robb 1995; Van Zyl 1996; Younger *et al.*, 1997) and provides a low operational cost approach to long-term management of the problem. Drawbacks include the large surface area requirement for higher AMD flows and concerns relating to the diffuse spread and long-term stability of the metals deposited.

Among the complex biota which establish in wetlands, plantings of *Sphagnum sp.*, *Typha latifolia* and *Phragmites australis* have been used and may provide a carbon source for the system of up to 40 $ton.ha^{-1}.yr^{-1}$ (Wieder 1993). Among many other carbon sources which have been evaluated for active SRB production are sewage sludge (Butlin *et al.*, 1956; Pipes 1960; Burgess & Wood 1961; Conradie and Grutz 1973), animal waste slurries (Ueki *et al.*,1988); straw, hay and lucerne (Bechard *et al.*, 1993); lactate and cheese whey (Olezkiewicz and Hilton 1986; Herrera *et al.*, 1991) molasses (Maree and Hill 1989), ethanol and methanol (Postgate 1984; Braun & Stolp 1985) and producer gas (Du Preez *et al.*, 1992; Van Houten *et al.*, 1994).

Boshoff *et al.* (1996) have investigated the anaerobic fermentation of waste-grown microalgal biomass produced in waste stabilisation ponds and the linked production of sulphide by SRB. Early suggestions for engineering algal removal systems include the description of meanders treating AMD in which algal growth occurs together with benthic flora and other vegetation (Gale and Wixson 1979; Jennet *et al.*, 1979; Mann and Fyfe 1988). Filip *et al.* (1979) have reported metal removal by sand filter immobilised algae, and Brady *et al.* (1994) have described a membrane immobilization process for removing metals by filtration. Immobilisation of algal biosorbants has been commercialised as a silica bead preparation marketed as AlgaSORB™ (Greene and Bedell 1990).

1.3 INTEGRATION OF ALGAL AND SRB PROCESSES

While waste stabilisation pond (WSP) technology has been developed over the past 50 years in a wide range of wastewater treatment applications (Mara & Marecos Do Monte, 1987; Mara *et al.*, 1996), and SRB activity has been noted in their anaerobic compartments (Pescod, 1996), little attention has apparently focussed on the use of these systems for AMD remediation. Early studies on these systems identified the role of microalgae in their successful operation (Gotaas and Oswald 1954), and the High Rate Algal Ponding (HRAP) concept developed from attempts to optimise, and intensify, the function of the micro-algal components. The detailed theory of the HRAP, and its incorporation into Integrated Algal Ponding Systems (IAPS), has been reviewed by Shelef *et al.* (1980); Abeliovich (1986); Oswald (1988 a&b) and De

Pauw & Salomoni (1991), and mainly as applied to the treatment of domestic wastewaters.

Oswald (1988a) has described the HRAP as the most efficient way known to fix solar energy in the form of biomass. Soeder (1986) saw the HRAP, as applied to waste water treatment, offering the greatest potential of all microalgal biotechnologies to be exploited as a multi-purpose system. A number of other authors have drawn attention to the cost credits that are available where algal production is coupled to waste treatment. This can justify the use of otherwise expensive cell harvesting technology for the recovery of useful products. The incorporation of algal production into an already funded waste treatment process can deal decisively with the three factors that have been identified by Richmond (1986) as most limiting in the development of algal biotechnology - the costs of production media, construction of ponds and the harvesting and recovery of micro-algae.

The overall yield of algal-bacterial-zoogloeal detritus (ALBAZOD) biomass from the domestic wastewater IAPS can be around 150 tons.ha.$^{-1}$ year^{-1} dry weight, of which the algal component accounts for 60%. This is close to the theoretical photosynthetic productivity limits for the system (Oswald 1988b).

1.4 THE IAPS AND SALINE WASTEWATER TREATMENT

An investigation of IAPS-based developments in domestic and industrial wastewater treatment commenced in the Rhodes University Environmental Biotechnology Group (EBG) in 1990 with the WRC Project K5/410 'A biotechnological approach to the removal of organics from saline effluents' (Appendix 1). An important objective of the study was to investigate how far the IAPS concept could be pushed as both a 'core technology', for use in a range of wastewater treatment applications, and as a source of innovation within broader sustainability and 'integrated waste resource management' strategies. The study was underpinned by the following:

- Algal biotechnology had made rapid advances, particularly in the 1970s and 1980s, and a basis for sustainability had been demonstrated for saline wastewater treatments in IAPS applications (Rose *et al.*, 2002a);

- Sustainability potential in the IAPS application was identified in terms of technical sustainability, as technology-that-lasts (low-construction costs, low-operator skills requirement, reliability, flexibility and upgradeability); resource sustainability (potential to segregate saline wastes, closed system operation; recovery of nutrients and resources, integrated resource management); economic sustainability (value-adding opportunities in the form of high-value bioproducts); social sustainability (service provision, job creation) Rose (2002);

- While engineering design of IAPS had reached an advanced stage at this time, principally as applied to sewage treatment, the concept needed to be subjected to a concerted process of 'technology-push' in order to adequately demonstrate the viability of the potential linkages between wastewater

treatment and value recovery in 'integrated waste resource management' strategies. Few commercially successful examples were available, and little had been reported on saline wastewater applications;

- Integrated algal ponding systems appeared to offer advantages as appropriate technology in the developing world context, given low-cost, ease of operation and apparent flexibility in the range of possible wastewater treatment applications. Here also the technical possibilities needed to be pushed to demonstrate the wider application potential, specifically in the context of saline and sanitation wastewaters. These systems appeared to offer potential for bioprocess development leading to improved treatment technology for saline wastewaters, and specifically related to pretreatments prior to the segregation of these wastes in saline water impoundments;

- Linkage between saline and sanitation wastes appeared to extend beyond the common use of a 'core' IAPS technology for the treatment of a variety of different waste types. The potential for saline wastewater use in sewage reticulation, sewage sludge disposal in low-grade saline wastewaters, and specifically also its use as a carbon source in the treatment of high-sulphate saline wastewaters, such as AMD and tannery wastewaters, emerged from the EBG study;

- In addition to salinity, the IAPS system offered opportunities for dealing with other priority sanitation pollution issues, especially in the development context, including nutrient enrichment, microbiological contamination and disinfection, removal of organic and inorganic pollutants, and especially heavy metal contamination.

It was evident that the choice of an appropriate model system would be necessary to develop the above objectives, and to subject the broad potential of these ideas to critical examination. Tannery wastewater treatment appeared to provide certain of the key requirements for such a model system. Apart from high organic loads, these wastewaters also contain both sodium chloride and sulphate/sulphide salinities in high concentrations, a range of heavy metals, and numerous other organic and inorganic contaminants. Conventional WSP have been used in the treatment of tannery wastewaters and, in dealing with the salinity component, have been operated as zero emission terminal evaporation ponding cascades (Shuttleworth, 1978; Rowswell *et al.*, 1984).

The tannery system was used in the preliminary investigations undertaken as part of WRC Project K5/495 ('A Biotechnological Approach to the Removal of Organics from Saline Effluents'), and included a study of the microbial ecology of a tannery WSP at Mossop-Western Leathers Co. Wellington, South Africa (Figure 1.1). This showed the presence, at times, of massive micro-algal blooms of near monocultures of both *Spirulina spp.* and *Dunaliella salina* in the meso-saline (<40 $g.L^{-1}$) and hyper-saline (>40 $g.L^{-1}$) compartments respectively, occurring across the well-established salinity gradients in these systems (Rose *et al.*,1996; Dunn, 1998). Laboratory studies followed and showed the potential use of both micro-algal species in the treatment of these complex wastewaters. Apart from effective organic load

reductions which were demonstrated in the algal-based treatments, enhanced micro-algal growth in the presence of organic substrates and the recovery potential for micro-algal biomass and β-carotene, as a fine chemical product, was demonstrated in the study (Laubscher, 1992; Rose, 1992; Rose *et al.*, 1992; Maart, 1993; Phillips, 1994; Rose *et al.*, 2002a).

The digestion of organic compounds occurring in the saline and hypersaline anaerobic compartments of these systems was noted, and both efficient sulphate reduction, and also the removal of heavy metals from the water column in the form of metal sulphide, and possibly also as carbonate and hydroxide precipitates, was demonstrated (Dunn, 1998). Given the high levels of sulphates in these wastewaters, the potential for follow-up investigation of biodesalination opportunities, using complex organic carbon as the electron donor, was indicated (Laubscher, 1992; Rose *et al.*, 1998; Molepane, 1999).

Figure 1.1. The Waste Stabilisation Pond evaporative disposal system treating tannery wastewaters at the Mossop-Western Leathers Co. Wellington. This unit was used as a model for the investigations on ponding systems reported in this study.

It was also evident from the initial studies that development of 'closed system' and 'integrated resource management' applications, based on a process of environmental and algal biotechnology-driven innovation, was dependent on the development of an indigenous capacity with respect to the engineering requirements for the design, construction and operation of IAPS. Best studied at this time was the trade marked Advanced Integrated Wastewater Ponding Systems (AIWPS) process, developed for

domestic sewage treatment by Prof William Oswald in California, USA.

Following visits to Professor Oswald at the University of California, Berkeley, USA, and a consultancy visit to South Africa by Prof Oswald in 1993, WRC Project K5/651, 'Appropriate low-cost sewage treatment using the advanced algal high rate oxidation pond', commenced in 1994. This project undertook the technology transfer exercise, in collaboration with Prof Oswald and Dr Bailey Greene, whereby an AIWPS Plant was constructed in Grahamstown.

1.5 WRC PROJECT K5/656 - TREATMENT OF SALINE WASTEWATERS

It was against the above background that WRC Project K5/656 'Appropriate Low-cost Treatment of Sewage Reticulated in Saline Water Using the Algal High Rate Oxidation Ponding System'was formulated. The research objectives for the project were identified initially as follows:

1. To evaluate the use of HRAP for the treatment of sewage reticulated in saline water, in a laboratory study;

2. To compare sources of saline water, including sea-water and saline groundwater, for the treatment of saline reticulated sewage effluent;

3. To evaluate the scale-up of the HRAP to a demonstration plant;

4. To evaluate the suitability of returning purified water to inland aquifers or the ocean, from which the reticulated waters were derived;

5. To investigate the recovery and value of the biomass obtained during the treatment of above wastewaters.

Prior to its commencement, this project was included in a wide range of budget cuts by the WRC. At the inaugural meeting of the project Steering Committee in Grahamstown, 22 November 1995, it was agreed that the all project objectives could not be met on the basis of funds then available and that focus should fall, in the first instance, on research objectives 1 and 2 as priority items.

Given the increasing focus at the time on mine drainage problems experienced in the Witwatersrand gold mines, and a number of parallel studies undertaken by the WRC on the broad implications of the AMD problem (Pulles *et al.*, 1995; Scott, 1995), it was decided to confine the saline context of the study to the high-sulphate salinities generated in these environments. With the development by the EBG, also at that time, of the IAPS in the treatment of meso-saline and hyper-saline wastewaters (Rose *et al.*, 2002a), it was decided, in discussion with the WRC, and at the subsequent meeting of the Project Steering Committee, to constrain the investigation to the use of IAPS as appropriate reactor design for the study in hand.

Thus a number of key questions relating to the feasibility of the IAPS in AMD treatment to be investigated and would be tested initially in laboratory and then in

pilot-scale studies. Technical- and full-scale development would depend on the outcome of this investigation, and would need to be dealt with at a later stage, and under a different project allocation.

The study aims were thus amended as follows:

1. To investigate the application of IAPS technology in the treatment of acidic and metal-contaminated sulphate-saline wastewaters;

2. To evaluate waste organic carbon sources as electron donor substrates in biological sulphate reduction;

3. To investigate neutralisation and metal removal operations in AMD treatment with application of IAPS technology.

1.6 INTEGRATED ALGAL PONDING SYSTEMS IN AMD TREATMENT

Although IAPS had not been previously reported for AMD treatment, the decision to investigate this application was based on a number of general observations:

- The linkage between the treatment of large volumes of wastewaters and mass algal production has been firmly established as a mature and widely utilised operational technology (Oswald, 1988a; Mara *et al.*, 1996);
- The earthwork pond provides a reactor at least an order of magnitude less costly to construct than steel-reinforced vessels (Oswald 1995);
- Renewable algal biomass (as a potential carbon source for sulphate reducing bacteria-mediated sulphate reduction activity) may be reliably produced in large amounts (up to 50 tons.ha^{-1}.yr^{-1}), in separately optimised HRAP systems (Oswald 1988b);
- The ability of algal biomass to adsorb and sequester heavy metals, and thereby reduce metal concentrations to residuals in the ppb range, has been demonstrated and commercialised (Wilde & Benneman 1993);
- IAPS had been investigated in the treatment of sulphate-saline tannery wastewaters and shown to support high levels of SRB activity using complex organic carbon electron donor sources (Rose *et al.*, 2002a);
- A technology transfer exercise in collaboration with Professor William Oswald (University of California, Berkley) had established an engineering base for IAPS technology development in South Africa;
- Studies had commenced in the EBG on development of an IAPS application in hypersaline wastewater treatment of soda ash production brines in Botswana.

Given the substantial progress that had already been made in technical- and full-scale studies on the use of IAPS noted above, sufficient information seemed to be available to propose a core IAPS configuration as a model system for AMD treatment. However, a number of important issues would need to be clarified to demonstrate feasibility, before proceeding with any confidence to pilot-scale studies. A critical path research methodology was used to identify these areas and this formed the substance of the investigation which followed.

1.7 THE ASPAM MODEL

The process model which emerged, and was to be tested in these studies, became known as the Integrated Algal Sulphate Reducing Ponding Process for Acidic Metal Wastewater Treatment (ASPAM). The individual unit operations of the ASPAM process were conceived to operate as described in Figure 1.

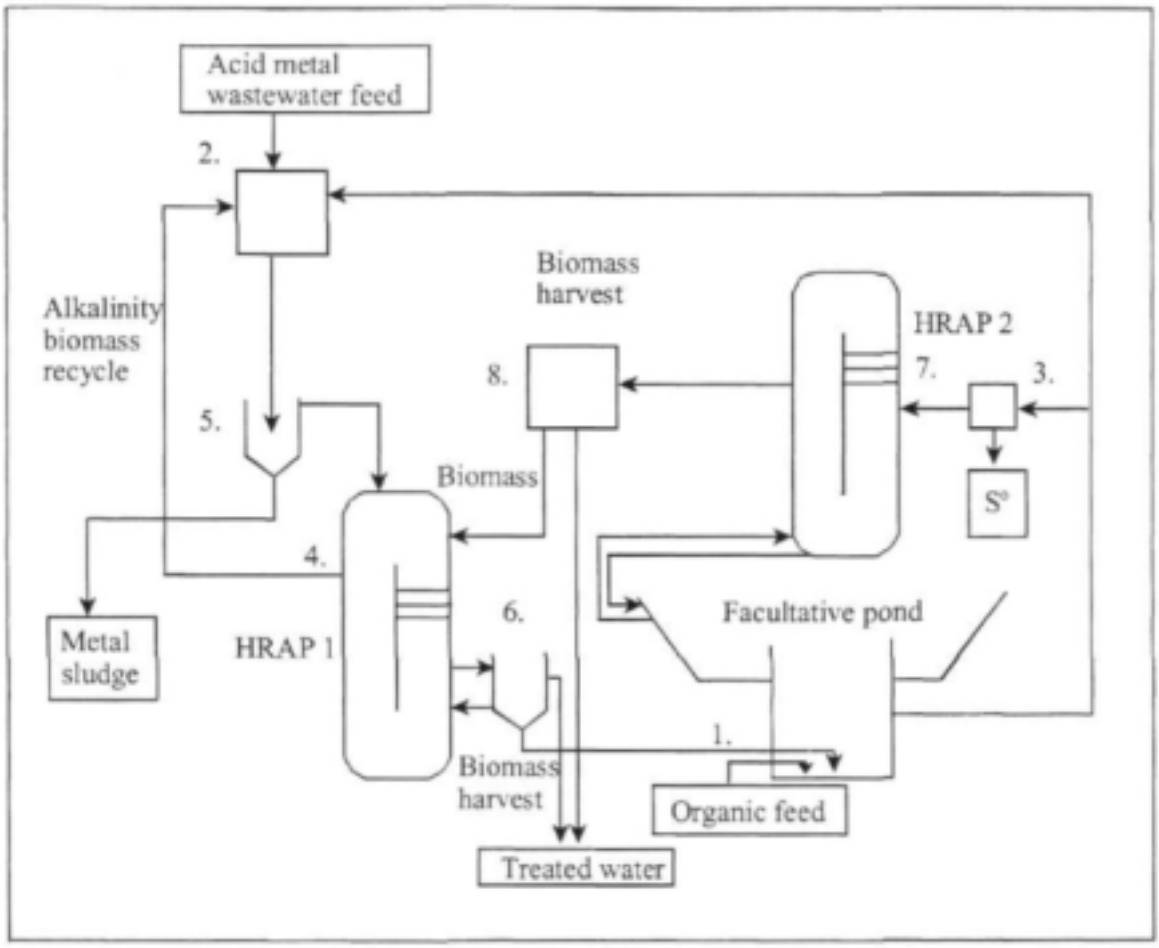


Figure 1.2. Flow diagram of the individual unit operations of the Integrated Algal Sulphate Reducing Ponding Process for Acid Metal Wastewater Treatment (ASPAM). Organic feed enters at 1= Facultative pond with anaerobic upflow digester compartment; Feed water enters at 2 = Inlet and metal precipitation unit; Sulphide is recycled to metal precipitation unit from 3 = sulphide recycle and sulphur recovery unit; Alkalinity and algal biomass generated in High Rate Algal Pond (HRAP)1 recirculated via 4; 5 = Metal sludge settler; 6 = Algal biomass settler; 7 = High Rate Algal Pond (HRAP)2 for capping the Facultative Pond and seeding HRAP 1 with fresh biomass; 8 = Algal biomass harvester.

1.7.1 Advanced Facultative Pond

At (1) a portion of the treated water flows into the Advanced Facultative Pond (AFP) together with the carbon source which may include a range of organic wastes such as tannery wastewaters, sewage sludges and agro-industrial organic waste products. Sulphide produced in this unit passes forward to a metal precipitation unit operation (2). Recirculation of algal-laden waters from HRAP 2 is used to maintain an oxygenated layer on the surface of the AFP and thus control sulphide gas release.

The AFP, provides for the inclusion of one or more Upflow Digesters (UD) in the base of the pond, enabling optimum anaerobic digestion function (Oswald, 1998 a&b). Tannery pond studies, in Wellington, South Africa, had shown that sulphidogenic anaerobic digestion will perform efficiently utilising tannery waste as an electron donor source. High rates of sulphate reduction were observed to be associated with an accelerated hydrolysis and solubilisation of particulate organic matter fed to the system. An associated precipitation of heavy metals was also observed in this unit with low residual metal levels in the effluent (Dunn, 1998).

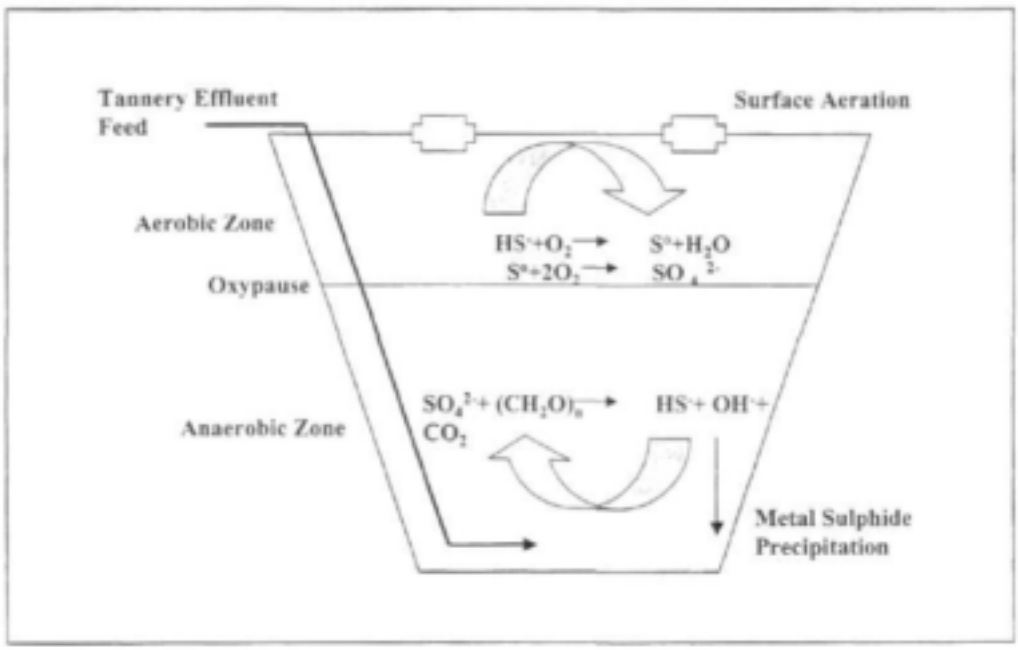


Figure 1.3. A crossectional illustration of the sulphuretum-type behaviour of the Advanced Facultative Pond treating tannery wastewaters at the Mossop Western Leathers Co. in Wellington, RSA.

The maintenance of an aerobic compartment above the anaerobic layer was shown in the tannery IAPS not only to provide for odour control, with the scrubbing of released gasses, but also ensuring the sulphur cycle was completed above the oxypause, with a nearly full oxidation of sulphide back to sulphate (Rose *et al.*, 1996). This sulphuretum-type behaviour is shown in Figure 1.3.

Objectives identified for the current study included an evaluation of the performance of the sulphate reducing anaerobic pit in the AFP, operating as an UD, and also the performance of a number of organic carbon sources, other than tannery waste, as electron donors for sulphate reduction.

1.7.2 Metal Precipitation Operation

Sulphide-rich waters pass from (1) to the metal precipitation unit operation (2) where, in mixing with the influent AMD stream, a combination of metal complex

formation reactions would occur. Effective precipitation of heavy metals in the sulphidogenic tannery wastewater AFP, had been reported by Boshoff (1998) and Dunn (1998), and the relative advantages of metal precipitation as the sulphide compared to metal hydroxide forms have been widely noted (Peters and Ku, 1985; Singh, 1992 and Hammack *et al.*, 1994). Dunn (1998) had reported preliminary observations on both the *ex situ* precipitation of metals utilising the sulphide-rich AFP liquors in the tannery IAPS, and also their removal in the presence of algae in the HRAP. Both systems demonstrated strong alkalising properties with the influent pH elevated to values between 9 and 10, at the end of the ponding cascade.

The objective of this component of the study was to investigate the feasibility of utilising both the micro-algal and sulphidic AFP streams in a metal precipitation unit operation, providing primary treatment of the influent AMD stream. Following the formation of a metal complex precipitate, and the removal of the major portion of the metal contaminants from the influent stream, the metal residuals would be stripped, and the effluent finally polished, during the subsequent stages of HRAP operation.

The stripping of sulphide gas from the pond liquors, and its use directly for metal removal in the precipitation unit, is an added option, but has not been further considered in the study reported here.

1.7.3 High Rate Algal Pond 1

Following the removal of metal sludges, the partly neutralised AMD passes to HRAP 1 (4). Here photosynthetically-driven alkalisation would be optimised and final metal removal effected by micro-algal biosorption.

The observations of the alkalising and metal binding properties of micro-algae in the tannery IAPS had been investigated by Boshoff (1998) and Dunn (1998). These had indicated the need for the inclusion of HRAP 1, following preliminary metal removal and prior to the stream entering the AFP. The relatively high pH minima of the bio-sulphidogenic system in the FP makes the neutralisation of the acid stream essential to the operation of this unit. Neutralisation may be handled both by feeding HRAP 1 liquors forward to the precipitation unit, and through *in situ* alkalisation in the raceway itself. Physiological stress in this unit may be expected to depress micro-algal growth rates. In this regard it would be important to maintain biomass against washout by replacement with algae recovered from HRAP 2, prior to discharge of the final treated stream.

1.7.4 High Rate Algal Pond 2

At (7) a portion of the AFP surface water circulates through HRAP 2 where a high concentration of algal biomass is maintained. This is used for recirculation and capping of the surface of the AFP to contain sulphide release, and for constant biomass replenishment to HRAP 1, in which a stress reduction of growth rates would be anticipated.

The performance of the biomass recycling and pond capping operation has been investigated in the tannery IAPS (Rose *et al.*, 2002a) and the Grahamstown AIWPS

Plant studies (Rose *et al.*, 2002b).

Issues which needed to be clarified in this study included an evaluation of the potential toxicity of sulphide production in the AFP on the micro-algal biomass circulated from HRAP 2. The Wellington study had shown that the HRAP following the Facultative Pond in that system had been able to handle a limited level of sulphide throughput (Dunn, 1998), but this had not been quantified.

1.7.5 Treated Water

At (6) the main flow of treated water exits the system from HRAP 1. This follows harvesting of the algal biomass, and together with a portion of treated water the biomass passes into the AFP where the organic carbon feed requirement is supplemented.

The use of algal biomass as a carbon source for sulphate reduction in AMD treatment was studied by Boshoff (1998). Photosynthetic production within the system would not only contribute to the organic carbon requirement, but in small installations might provide an independence from external carbon sources.

1.7.6 Sulphur Recovery and Biodesalination

A portion of the total sulphur load entering the system would be removed with the metal sulphide sludge. Where a more complete sulphate removal is required a biodesalination of the AMD may be achieved by passing the reduced AFP waters through a sulphur recovery unit (3). Here elemental sulphur (S^o) will be a final by-product of the process. Sulphide oxidation and sulphur recovery is the subject of separate WRC Projects K5/1078 and K5/1336 (Appendix 1).

1.8 EXPERIMENTAL PROGRAMME

While the ASPAM process had been conceptualised on the basis of experience derived from the various research studies, the development and full-sale implementation of the IAPS treating tannery wastewaters (Rose *et al.*, 2002a), numerous issues remained to be clarified before proceeding, with any confidence, to a process of scale-up evaluation of the system in AMD treatment. The primary objective of the research programme described here was to establish clarity on certain of the most important potential constraints to which the process might be subject. The main objectives of the research programme were identified in a critical path analysis and are outlined below:

1.8.1 Electron Donor Source

While the preliminary process observations on which this study was to be based had been made on the treatment of tannery wastewater, it was clearly important for the AMD application to test these under experimental conditions, including preferably a number of alternative electron donor sources. In addition to tannery waste, sewage sludge was included given general availability, and the obvious cost-benefits of co-disposal. The successful use of micro-algal biomass as an electron donor source would offer advantages of independence from an external supply of organic carbon, which would be particularly valuable where remotely located treatment was required.

The study therefore undertook the evaluation of tannery wastewater, sewage sludges and micro-algal biomass as electron donors for sulphate reduction in AMD treatment. The UD reactor configuration was to be evaluated throughout in order to indicate transferability of the results to an AFP design.

1.8.2 Effect of Sulphide on Micro-algal Growth

Sulphide, particularly in the un-ionised H_2S form, is generally toxic to most forms of life, both aquatic and terrestrial. While Dunn (1998) had found quite a wide sulphide tolerance in a tannery pond-adapted *Spirulina* sp., it was uncertain to what levels this could be pushed where maximum sulphide conversion was desired. The effective capping of the AFP to contain odour release would not be the only requirement in the ASPAM system, and the re-oxidation of residual sulphide in the aerobic compartment of the AFP would be necessary before final discharge of treated wastewater from the system.

The study therefore undertook an investigation of the effects of sulphide on a number of micro-algal species which might be considered for use in the ASPAM system.

1.8.3 Metal Removal Operation

The implications for metal removal in the ASPAM system have been noted above. The study thus undertook an investigation of both the sulphide and micro-algal assisted metal precipitation reactions which are likely to occur in the system.

1.8.4 Neutralisation of the Acid Stream

The neutralisation function has also been noted above and the study undertook an investigation of micro-algal assisted alkalisation of AMD-type streams.

1.8.5 Integration of ASPAM Unit Operations

While the above did not constitute an exhaustive list of aspects of the ASPAM hypothesis which required some form of preliminary investigation, it was considered that with the successful outcome of these enquiries it would be possible to commence follow-up planning of a programme of pilot plant and technical-scale evaluation studies. The final product of this study was therefore to provide informed comment on the feasibility of the process, and on the practicality of proceeding to scale-up

studies.

Clearly sulphate removal would be an important advantage for the process, especially once all influent sulphate had been completely reduced to the sulphide form. While some sulphate would be removed as metal sulphide precipitate, the residual sulphide would need to be removed. Biological sulphide oxidation to elemental sulphur has been investigated, as noted above, and will be reported separately in Report 11 of the 'Salinity, Sanitation and Sustainability' series.

2 MICRO-ALGAL BIOMASS AS AN ELECTRON DONOR SOURCE IN BIOLOGICAL SULPHATE REDUCTION

2.1 INTRODUCTION

The availability of appropriate sources of organic carbon has been one of the principal limitations in the development and implementation of full-scale SRB-based systems for sulphate wastewater treatment. A variety of external sources of organic carbon have been evaluated in this role (Rose *et al.*, 1998), and in addition to micro-algal biomass, both tannery wastes and sewage will be discussed further here. Where ponding systems are to be used as the reactor vessel for treating large volume flows, the production of substantial amounts of micro-algal biomass, which may be engineered in IAPS, offers a possible independence from the constraint of external carbon supply.

In 1956, Oswald first noted that the settling of micro-algae in ponds led to active fermentation, with the release of toxic substances such as hydrogen sulphide into the water (Oswald *et al.*, 1957). Sulphide generation in natural systems, due to the degradation of micro-algal biomass, has also been reported by Brierley and Brierley (1983). IAPS have been developed and applied in South Africa under a variety of conditions and using a number of different effluents (Rose *et al.*, 1996; Rose, 2002). Dunn (1998) reported the growth of *Spirulina* sp. in tannery facultative ponds with an average productivity of 7.48 g $C.m^{-2}.day^{-1}$, which compared well to the 8-12 g $C.m^{-2}.day^{-1}$ cited for outdoor culture basins by Fox (1983). This translated into an estimated biomass production of 109 $tons.annum^{-1}$ from a 197 000 m^3 ponding system (Dunn, 1998). Maximum rates of micro-algal biomass production have been recorded for a variety of systems at around 30 $g.m^{-2}.day^{-1}$. Mixed biomass production from sewage HRAP may reach 150 $tons.ha.^{-1}\ yr^{-1}$ (Oswald, 1998b). Micro-algal biomass has also reportedly been used as an energy source for the biodegradation of manganese dioxide (Hart and Madgwick, 1987), and also the bacterial reduction of nitrate, selenate and selenite (Gerhardt and Oswald, 1990 a&b). However, the literature contains little comment on the use of micro-algal biomass in engineering SRB-based water treatment systems.

2.2 OBJECTIVES

The following objectives were established for this component of the study:

1. To investigate the feasibility of micro-algal biomass used as the sole carbon source for biological sulphate reduction;

2. To determine the rates of sulphate reduction using different feed concentrations of micro-algal biomass;

3. To determine the distribution of substrate and products in a micro-algal biomass fed laboratory-scale UD reactor.

2.3 MATERIALS AND METHODS

2.3.1 Reactor Operation

A bench scale anaerobic UD reactor with a volume of 6 litres and a height of 40 cm was used to study the growth of a mixed culture of SRB (Figure 2.1). The reactor was seeded with sludge from a methanogenic reactor treating raw sewage. The reactor was initially fed on a weekly basis with lactate until the presence of a population of SRB was demonstrated by the production of sulphide. Thereafter it was fed on a continuous basis using a Watson Marlow peristaltic pump, at a rate of 3 $L.day^{-1}$, i.e. a hydraulic retention time (HRT) of 2 days, with media of the following composition: NH_4Cl 0.5 $g.L^{-1}$; K_2HPO_4 1.0 $g.L^{-1}$; $MgSO_4.7H_2O$ 0.2 $g.L^{-1}$; $CaCl_2.2H_2O$ 0.1 $g.L^{-1}$; $FeSO_4.7H_2O$ 0.1 $g.L^{-1}$; Na_2SO_4 0.5 $g.L^{-1}$. Dried *Spirulina* sp. biomass (produced in a tannery wastewater IAPS), was used as the organic carbon source and fed consecutively to the reactor at concentrations levels around 4, 8 and 10 $g.L^{-1}$, giving organic loading rates of 4.5 (±0.59), 3.4 (±0.45) and 2.5 (±0.26) g $COD.L^{-1}.day^{-1}$ respectively. Samples were removed and analysed for sulphate, total and dissolved sulphide, COD and ATP. ATP levels were used as an indicator of microbial activity. Results reflect the mean of three readings.

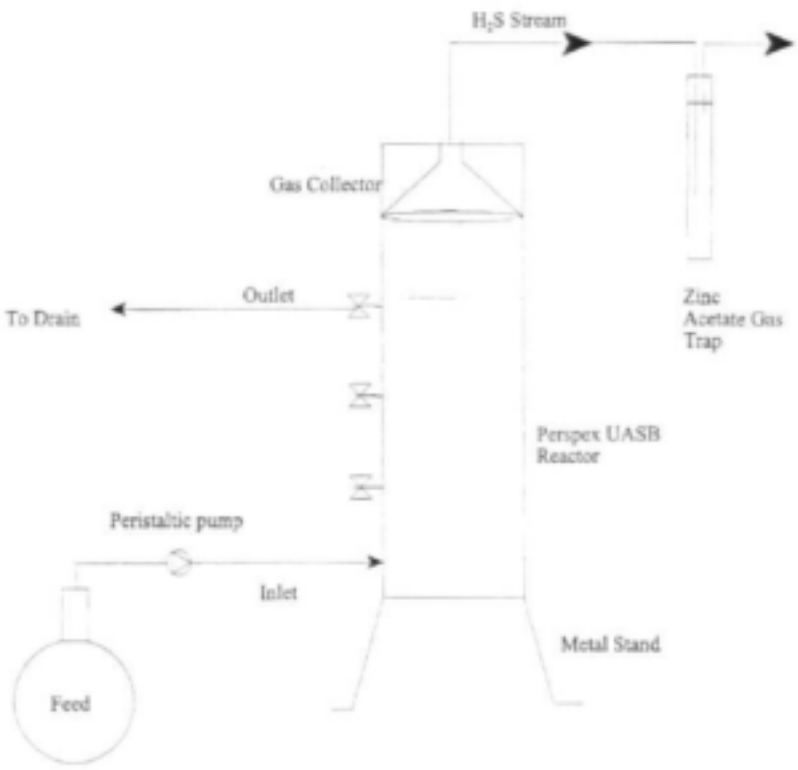


Figure 2.1. Schematic diagram of the reactor used in this study.

2.3.2 Analysis

Sulphate was measured turbidometrically and sulphides by the methylene blue method according to Standard Methods (APHA, 1989). COD was measured using the Merck Spectroquant system. ATP was extracted into Tris buffer and assayed using a Bio-Orbit Luminometer. Metals were assayed on a Varian Techtronic Atomic Absorption Spectrophotometer.

The percentage of dissolved sulphide in the free sulphide form was calculated from the equation according to Oleskiewicz *et al.* (1989):

$$H_2S = [1+1.02 * 10^{-(pH-pKa)}]^{-1} \quad (1)$$

2.4 RESULTS AND DISCUSSION

The effect of the COD:SO_4 ratio on substrate consumption and removal efficiencies was assessed by varying the organic carbon content of the medium. Li *et al.* (1996) have shown that the ratio of organic feed to sulphate feed is important in controlling the relative growth of SRB and methane producing bacteria (MPB) populations, which in turn determines the measure of sulphate reduction and COD removal. The reactor studies reported here showed quite similar COD removal efficiencies: 32.4 % (±17.4), 38.6% (±18.0) and 34.5% (±15.3), irrespective of the influent COD:SO_4 ratio, which was varied from 8.1, through 11.2 and to 15.0 respectively.

However a decrease in sulphate removal efficiency was seen with an increase in the COD:SO_4 ratio of the influent medium (Figure 2.2). The highest removal, 90.3% (±10.0), was measured in the reactor fed medium with an influent COD:SO_4 ratio of 8.1, followed by 74.5% (±17.0) and 70.8% (±17.2) in the reactors fed medium with an influent COD:SO_4 ratio of 11.2 and 15 respectively. This conversion rate is high compared to results reported for simple organic carbon sources, which suggests a COD:SO_4 influent ratio of between 0.66 and 2 to obtain above 80% SO_4 conversion (Table 2.1). However, results obtained using settled sewage sludge as a carbon source showed that a COD:SO_4 ratio of at least 2 is required for efficient SO_4 reduction as not all carbon present is readily degradable (results not shown).

Table 2.1. Effect of COD:SO_4 ratio on sulphate reduction.

COD:SO_4 ratio	% SO_4 reduction	Substrate	Reference
1.5	67%	butyrate	Mizuno and Noike, 1994
0.66	90%	acetate	Bhattacharya *et al.*, 1996
2.0	90%	glutamic acid	Choi and Rim, 1991
0.44	60%	propionate	Uberoi and Bhattacharya, 1995
1.37	95%	propionate	Uberoi and Bhattacharya, 1995

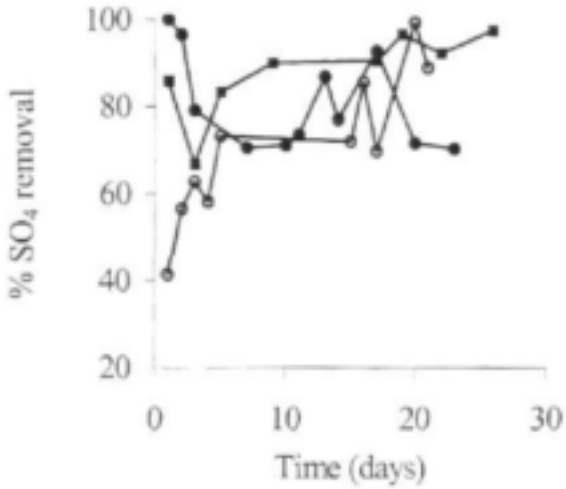


Figure 2.2. Percentage sulphate removal over time in reactors fed medium with a COD:SO_4 ratio of 8.1 (○), 11.2 (■) and 15 (♦).

If the partitioning of substrate electrons (in terms of COD) is examined, only between 11% and 14% of total COD removal in the reactors fed 10 $g.L^{-1}$ and 8 $g.L^{-1}$ algal biomass can be attributed to use in SO_4 reduction. This is in comparison to the reactor fed 4 $g.L^{-1}$ algal biomass, where 31% of COD removal can be attributed to use in SO_4 reduction. The COD removal achieved at the higher COD:SO_4 ratio may be due to processes other than sulphate reduction, possibly including re-oxidation of sulphide at the reactor outlet. Methane production was not observed.

Within the reactors, a spatial distribution of organics was observed for both substrate and product (Figure 2.3). The occurrence of zones of differing COD:SO_4 ratios within the reactors may have caused the microbial species composition in the reactor to change. The organics, sulphide and intracellular ATP were concentrated mainly at the base of the reactor closest to the inlet. Chen *et al.* (1994), in an examination of sulphate reduction in an anaerobic upflow bioreactor, also found an accumulation of biomass in the initial part of the column where the substrate concentration was highest.

As can be seen in Table 2.2, the major part of sulphate reduction occurs at the base of the reactor, with very little decrease in sulphate levels between the base and outlet. Thus one could assume that the SRB activity is concentrated in the lower region of the reactor. The COD levels decreased by between 80 to 90% between the base of the reactor and the outlet. Levels also fluctuated with time, but in general did not show a significant increase. Thus even though the reduction in COD can be attributed to the settling of the heavier particulate organics in the base of the reactor, it would appear that there is an active breakdown of these organics by the microbial consortia present. However, unlike sulphate reduction which appears to be limited mainly to the base of the reactor, COD reduction takes place throughout the length of the

reactor. The high concentration of COD in both the outflow and base of the reactor, suggests that the system is not carbon limited, thus partially allowing for the non-competitive growth of MPB (Choi and Rim, 1991).

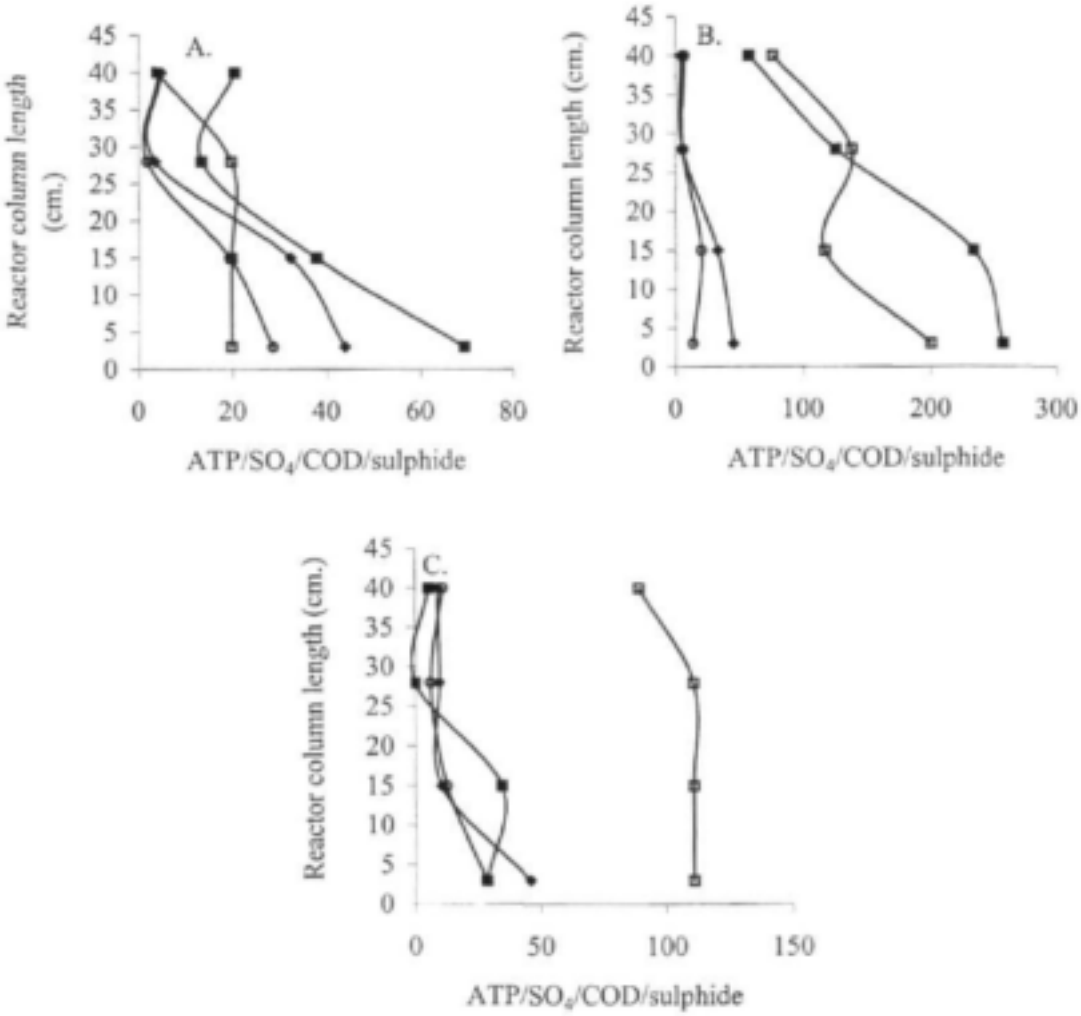


Figure 2.3. ATP (○), SO_4 (▪), COD (♦) and total sulphide (▫) profile up the column of the reactor fed medium with a COD:SO_4 ratio of A) 8.1, B) 11.2 and C)15 at steady state. Units for measured variables are as follows: SO_4= mg.L^{-1}, ATP = µmoles, COD = x10^2 mg.L^{-1}, sulphide = mg.L^{-1}.

Table 2.2. Percentage of total sulphate removed up the length of the column.

	Feed COD:SO_4 ratio		
Distance up reactor (cm)	8.1	11.2	15
31528	93.6	64.4	96.3
	1.3	7.02	0
	2.8	10.6	0.34

Fallon and Brock (1979) concluded that the decomposition of blue-green algal biomass is a rapid process that can occur under both aerobic and anaerobic conditions. Even though the loss of chlorophyll under anaerobic conditions is considered delayed or completely inhibited (Fallon and Brock, 1979), Gunnison and Alexander (1975) showed that the cell walls of a number of species of cyanobacteria were amongst the least resistant to decomposition of the phototrophic organisms tested. Uziel (1978) found the fermentability of *Spirulina maxima* to be significantly higher than that of other micro-algae. The chemical composition of *Spirulina* sp. biomass has been documented and it is known that 50% to 70% of its dry weight is composed of protein. Lipids also constitute a fraction of the dry weight, with numerous values reported: 16.6% (Tornabene *et al.*, 1985), 11% (Hudson and Karis, 1974) and even as low as 5% (Switzer, 1980). The biomass is also said to contain a number of carbohydrates such as glucose, levulose, heptose, sucrose, glycerol and several polyols (Quillet, 1975). The microbial population involved in the degradation of the algal biomass is composed of a wide consortia of bacteria: fermenters, acetogens, MPB and SRB. The proteins are degraded to organic acids, amines, CO_2 and ammonia (Almasi and Pescod, 1996).

The COD:SO_4 ratio and ATP profiles also follow each other, with high intracellular ATP levels corresponding to high COD:SO_4 ratios. These fluctuations with time up the length of the reactor column, as well as the cyclical pattern of the ATP and COD levels (Figure 2.4) in the outflow, may have been the result of variations in substrate utilisation and product formation taking place in the base of the reactor. As a result the medium which passed up the reactor column would also have differing substrate levels, thus affecting population dynamics along its length. It would be expected that ATP levels would decrease as the microbial population declines along the length of the reactor. This was, however, not observed and, although the ATP and COD results are not clear cut, high levels of ATP were found to be associated with high COD and SO_4 levels.

These high ATP levels may have been the result of an increase in the intracellular ATP concentration of SRB as their maintenance energy increased in response to the sulphide toxicity. However, the levels may also be attributed to the growth of a population of bacteria which was not previously present, either because they were out-competed by the SRB, or because the substrate which they grew on was not present in high enough concentrations lower down.

The influence of ammonia on anaerobic digestion has also been reported (De Baere *et al.*, 1984), and considering the high protein content of the algal biomass used in this study (70%), as well as the amount of biomass used, one could expect a large amount of ammonia production. However, as ammonia and acetate were not measured in this study, these suggestions are speculative and warrant further investigation.

The sulphide levels in the overflow also fluctuated over time, though more so in the reactor fed 8 $g.L^{-1}$ algal biomass (Figure 2.5). Although sulphides have been reported to be toxic to both SRB (Reis *et al.*,1992) and MPB (Isa *et al.*, 1986), this does not appear to be the case in the digesters used in this study. High levels of sulphide in

the overflow from the digester corresponded to high ATP levels. The chemical equilibrium of sulphide species and thus its toxicity is pH dependent (Okabe *et* al., 1995) and in most cases H_2S appears to be the toxic component (Kroiss and Wabnegg, 1983; Speece, 1983; Parkin *et al.*, 1990).

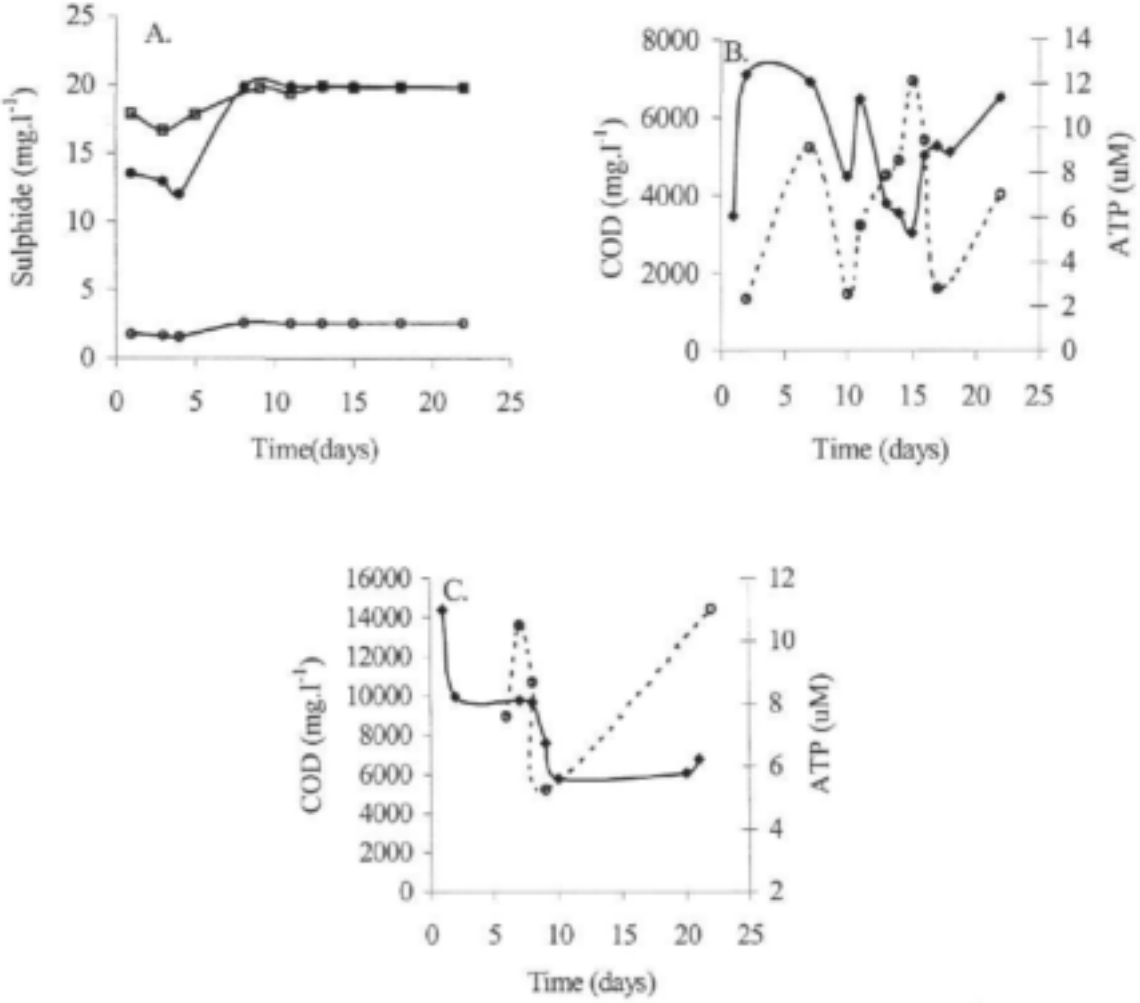


Figure 2.4. COD(♦) and ATP(○) in the outflow from reactors fed medium with a COD:SO_4 ratio of A) 8.1, B) 11.2 and C) 15.

Sulphide levels were highest in the reactor fed 8 $g.L^{-1}$ algal biomass and lowest in that fed 4 $g.L^{-1}$. These levels compare favourably to those reported from compost studies (Hammack and Edenborn, 1992). The pH of the reactors was approximately 7.4, at which only about 13% of the dissolved sulphide is in the free sulphide form thus being below the levels which are considered toxic to MPB.

Substantially lower total sulphide levels were measured than were anticipated from the amount of sulphate reduced. The visual observation of white sulphur in the outflow indicated that at least a part of the sulphide was oxidised to elemental sulphur, thus accounting for part of the discrepancy.

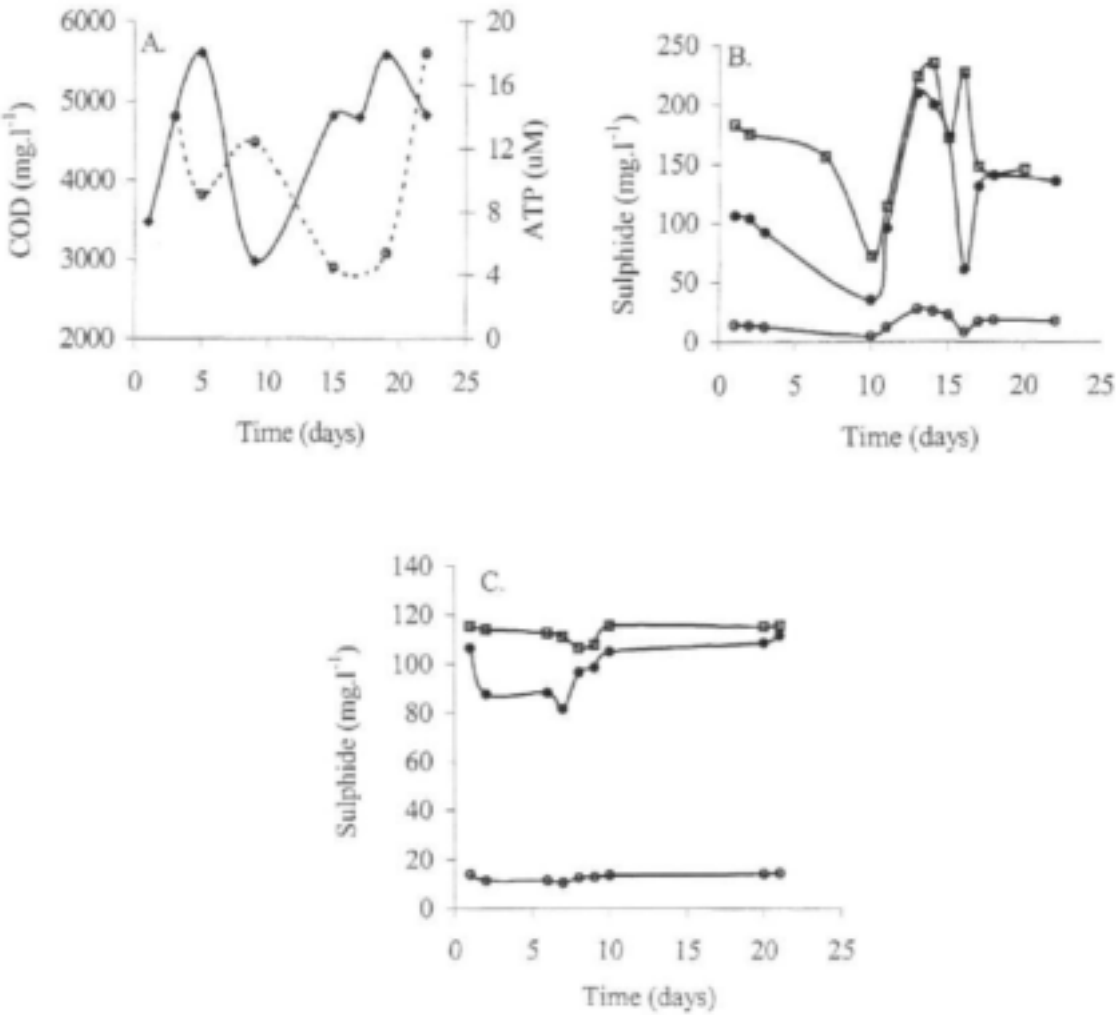


Figure 2.5. Total (¤), dissolved (•) and free (◦) sulphide concentration in the outflow from reactors fed medium with a COD:SO_4 concentration of A) 8.1, B) 11.2 and C)15.

2.5 CONCLUSIONS

This study demonstrated that micro-algal biomass can function as an effective carbon source for biological sulphate reduction. However, the load ratio is an important factor in the operation of the system. The results indicate that at lower organic loading rates, sulphate reduction is more efficient, with an average removal of 150 mg $SO_4.g^{-1}$ algal biomass.day^{-1} recorded. However, considering that not all of the algal biomass was broken down and, of that component which was digested, only 31% was used by SRB, this feed rate could clearly be further reduced.

The results obtained also furnished an insight into the operation of an algal biomass-fed UD reactor and stressed the importance of biomass hydrolysis in the process.

Sulphate reduction was most prevalent in the base of the reactors, with less occurring as the effluent moved up the reactor column length. COD reduction, on the other hand, took place throughout the length of the reactor.

Despite the absence of process optimization studies it may be concluded that the growth of algal biomass, as a component product of wastewater treatment operations (tannery effluent in this case), may be used to generate a sustainable carbon source for sulphate reduction operations.

3 TANNERY WASTEWATER AS AN ELECTRON DONOR SOURCE IN BIOLOGICAL SULPHATE REDUCTION

3.1 INTRODUCTION

In reports on the investigation and development of IAPS applications in the treatment of tannery wastewaters (Rose *et al.*, 1996, 1998, 2002a; Dunn, 1998), it was noted that not only high rates of sulphate reduction occurred in these systems, but also that organic solids were apparently solubilised at enhanced rates under biosulphidogenic conditions. Tannery wastewater is characterised by a high COD, originating from the hide and skin, as well as from chemicals added during the tanning process (Table 3.1). Solids levels are high, and the effluent may also contain high levels of salts including sulphates, chlorides, chromium, and a range of other heavy metals in lesser amounts (Genschow *et al.*, 1996).

Apart from the apparent advantages of configuring ponding systems for the maximum consumptive removal of organic solids, the possibility emerged in these studies that this waste might serve as an effective electron donor and carbon source for a biological systems-based approach in the treatment of a range of other high sulphate wastewaters such as AMD. A number of issues would need to be addressed including the availability of tannery organic carbon as a feed for SRB activity under controlled conditions. The reactor vessel is an important determinant of both technical and financial constraints, and the critical question to be addressed here was whether the anaerobic compartments of ponding systems would provide a reaction environment in any way comparable to conventional stirred tank reactor (STR) vessel systems.

Two possible pond reactor configurations were investigated, including the UD reactor - comparable to the anaerobic pit in the Advanced Facultative Pond (Oswald, 1988a), and the Trench Reactor (TR) which would simulate the direct discharge of untreated effluent into an anaerobic pond.

3.2 OBJECTIVES

The following objectives were established for this component of the study:

1. The evaluation of tannery wastewater as an electron donor source for biological sulphate reduction;

2. The experimental evaluation of two simulated pond reactor environments compared to a conventional STR design.

3.3 MATERIALS AND METHODS

3.3.1 The Tannery

Seeton Leathers (Nigel, South Africa) is a tannery producing leather for the automotive industry. During full production the factory processes between 1000 and 1200 local hides daily as well as imported wet-blue and wet-white hides. Of these hides 350 to 500 are green hides, thus requiring pickling and resulting in the generation of saline effluents. Effluent from the different areas of the tannery is combined in a mixing tank from where it is transferred to a Silflo unit where organic precipitation and flocculation occur. The sludge generated in this process is allowed to dry in drying beds after which it is removed to a landfill site. The effluent is discharged to drain and to the municipal sewage treatment works for final treatment.

3.3.2 Pilot Plant

The performance of a UD, an STR with a volume of 1.5 m^3 (Figure 3.1) and a TR with a volume of 2 m^3 (Figure 3.2) were compared on site at the tannery. Effluent was fed to the digesters from the mixing tank, receiving wastewater streams from all parts of the factory, by submersible pumps (Rule 360). A submersible pump (Rule 500) in the mixing feed tank served to keep the feed mixed. The rate at which the reactors were fed was under the control of ST203 220V timers. The chemical composition of the wastewater varied from day to day and an average composition is shown in Table 3.1. The effluent fed to the digesters from the feed tank was composed of tannery effluent diluted 50% with tap water.

3.3.3 Experimental

The reactors were operated in continuous mode with an HRT of 4 days. The UD and STRs were fed at a rate of 375 $L.day^{-1}$. The UD was operated as an upflow system for a period of 33 days, with the overflow passing directly to drain. Thereafter, and for a period of 18 days, the reactor was operated as a STR. The reactor contents were mixed using a recirculation pump, and the overflow passed to a 450 L settling cone from which settled sludge was recycled to the reactor. The TR was fed at a rate of 400 $L.day^{-1}$ with outflow passing directly to drain. The temperature of the digesters was not controlled.

3.3.4 Analysis

Sulphate, sulphide and total settleable solids were measured according to Standard Methods (APHA, 1989). COD was measured using the Merck Spectroquant 1800 system. The pH was measured using a Cyberscan 2500 pH meter.

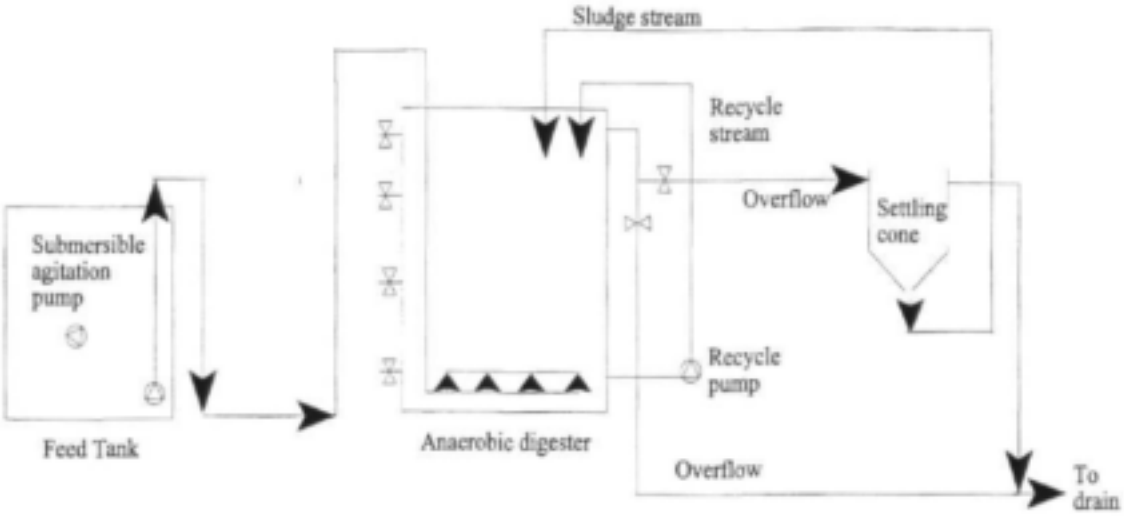


Figure 3.1. Treatment scheme for the Upflow Digester and Stirred Tank Reactor. The latter configuration was initiated with the activation of the recycle pump.

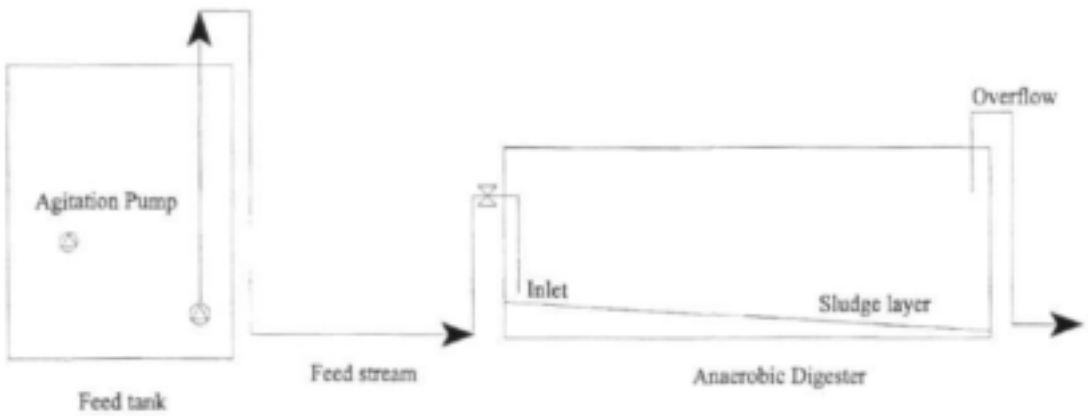


Figure 3.2. Treatment scheme for the Trench Reactor.

Table 3.1. Representative analysis of the tannery effluent used in this study

Water Quality Parameter	Measured
pH	7.5
Conductivity (μS)	1850
Chemical Oxygen Demand (COD) ($mg.L^{-1}$)	5324
Permanganate Value (PV) ($mg.L^{-1}$)	1194
Suspended Solids (SS) ($mg.L^{-1}$)	5664
Total Dissolved Inorganic Solids (TDIS) ($mg.L^{-1}$)	2914
Total Dissolved Solids (TDS) ($mg.L^{-1}$)	9760
Total Kjedahl Nitrogen (TKN) ($mg.L^{-1}$)	969
Ammonia ($mg.L^{-1}$)	567
Alkalinity - Bicarbonate ($mg.L^{-1}$)	1080
- Carbonate ($mg.L^{-1}$)	120
Sodium ($mg.L^{-1}$)	2010
Chlorine ($mg.L^{-1}$)	3180
Sulphate ($mg.L^{-1}$)	3190
Sulphide ($mg.L^{-1}$)	1354
Chromium ($mg.L^{-1}$)	49
Boron ($mg.L^{-1}$)	<1
Phosphate ($mg.L^{-1}$)	<1
Iron ($mg.L^{-1}$)	<1

3.4 RESULTS AND DISCUSSION

Figures 3.3 and 3.4 show the performance of the UD up to day 33, and thereafter for the STR, following the reactor's conversion to completely mixed operation. Figures 3.5 and 3.6 show the performance of the TR. Organic loading rates were not constant during operation due to fluctuations in the composition of the wastewater stream derived from the tannery. The UD was operated at an organic loading rate of 0.4-1 g $COD.L^{-1}.day^{-1}$, the STR at 0.2-1g $COD.L^{-1}.day^{-1}$ and the TR at 0.15–0.7 g $COD.L^{-1}.day^{-1}$. The three different reactor configurations were compared in terms of substrate utilisation and removal efficiency rate as well as sulphate reduction activity. In this case sulphate removal refers to the difference in sulphate levels between the feed and outflow and thus takes into account sulphate reduction as well as sulphide re-oxidation.

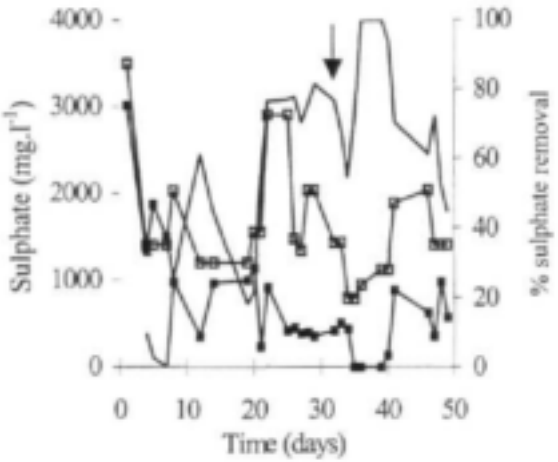


Figure 3.3. Sulphate removal in tannery effluent in the UD (days 1 to 33) and in the STR (day 33 to 50). Effluent sulphate (▫), outflow sulphate (▪), percentage sulphate removal (—). Arrow marks transition from UD to STR.

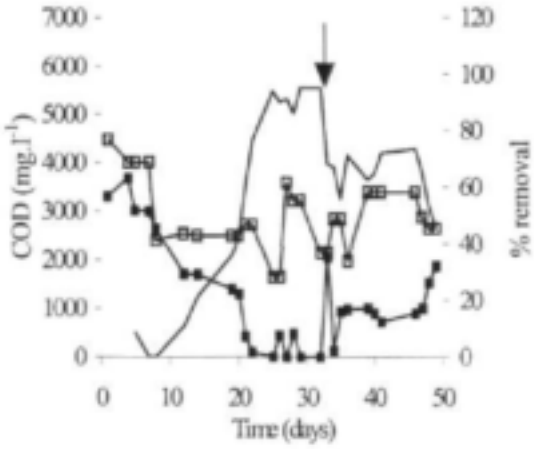


Figure 3.4. Tannery effluent COD removal in the UD (days 1 to 33) and the STR (days 34 to 50). Effluent COD (▫), outflow COD (▪), percentage COD removal (—). Arrow marks transition from UD to STR.

Sulphate reduction rates were not constant throughout operation of the digesters. Sulphate removal in the UD (Figure 3.7) showed an initial decline over the first 20 days of operation during stabilisation. However, thereafter the removal rate increased from 0 mg $SO_4.L^{-1}.day^{-1}$ to 600 mg $SO_4.L^{-1}.day^{-1}$ with an 80% removal efficiency being achieved by day 20. An average COD:SO_4 reduction ratio of around 2:1 was established for the UD and 2.5:1 for STR operation.

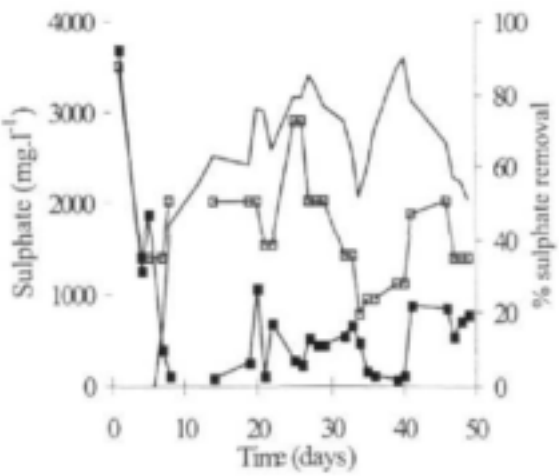


Figure 3.5. Sulphate removal in tannery effluent in the TR. Effluent sulphate (▫), outflow sulphate (▪), percentage sulphate removal (—).

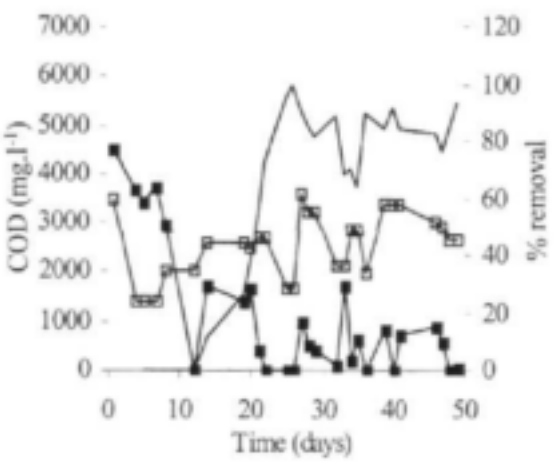


Figure 3.6. Tannery effluent COD removal in the TR reactor. Effluent COD (▫), outflow COD (▪), percentage COD removal (—).

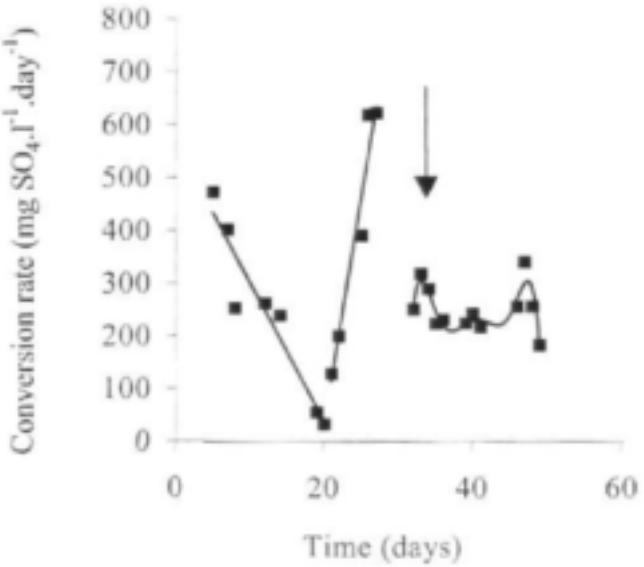


Figure 3.7. Sulphate reduction rate as a function of time for the UD (day 1 to 33) and the STR (day 34 to 50). Arrow marks transition from UD to STR and trends are shown by best-fit line.

The rate of COD removal for the UD (Fig 3.8) on the other hand, as well as COD removal efficiency increased, from the time of commissioning of the reactor from 0 mg $COD.L^{-1}.day^{-1}$ to 500 mg $COD.L^{-1}.day^{-1}$.

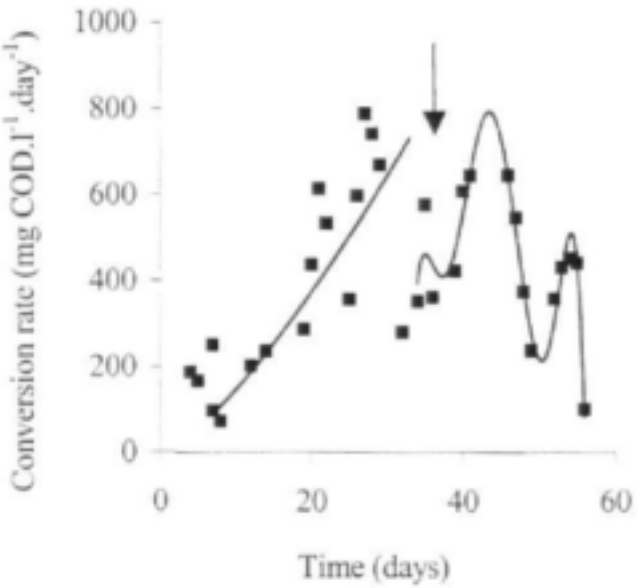


Figure 3.8. COD removal as a function of time for the UD (day 1 to 33) and the STR (day 34 to 50). Arrow marks transition from UD to STR and trends are shown by best-fit line.

Change in operation mode from an up-flow system to a continuously stirred system had a pronounced negative effect on sulphate removal, with rates decreasing to 250 mg $SO_4.L^{-1}.day^{-1}$. However, as can be seen in Figure 3.5, sulphate removal efficiency increased with an almost 100% removal of sulphates, before dropping to 78% removal. COD removal efficiency, as well as the rate of removal, was also affected by the change in operation mode, with removals dropping from 95% to 75% (Figure 3.4). A possible reason for this could have been the disturbance of the sludge bed leading to washout of bacterial cells involved in COD reduction. SRB use a variety of fermentative end products produced by fermenters and acetogens which are present in sulphidogenic reactors (Zhang and Noike, 1994). In the case of tannery effluent these would be the hydrolysis products of protein containing materials such as keratins and collagen, as well as lipids and fats. Lipids would be partially split by enzymatic hydrolysis into long and then short-chain fatty acids, leading to acetate. Proteins are hydrolysed into soluble organics and amino acids, and then into acetate through the intermediate, pyruvic acid (Carre *et al.*, 1983). Although Isa *et al.* (1986) found that acetate alone was not a good substrate for SRB, it was reported to be the major electron donor for sulphate reduction in marine and brackish water environments (Thauer, 1982).

As can be seen in Figures 3.9 and 3.10, settleable solids (SS) were absent in the overflows of the TR and the UD up to day 33. Total solids (TS) in the overflow decreased at a rate of 136 $mg.L^{-1}.day^{-1}$ and 132 $mg.L^{-1}.day^{-1}$ respectively. As anticipated there was a measurable increase in TS and SS in the outflow of the STR, with levels of up to 50 $ml.L^{-1}$ SS being recorded (Figure 3.10). SS were collected and returned to the STR.

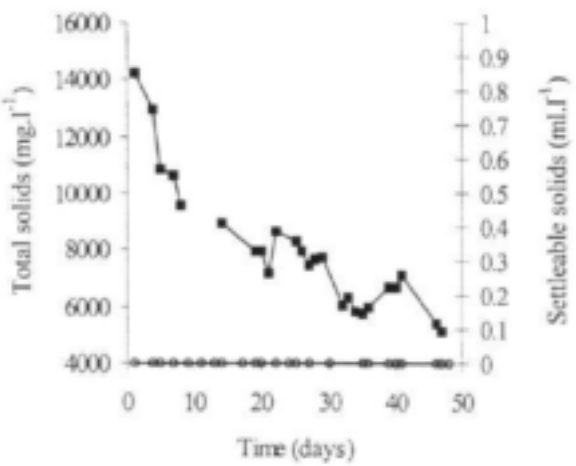


Figure 3.9. Total (▪) and settleable (○) solids in the overflow from the TR.

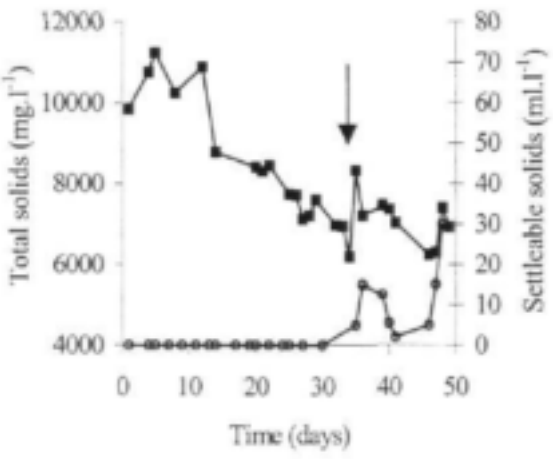


Figure 3.10. Total (▪) and settleable (○) solids in the overflow from the UD (days 1 to 33) and the STR (day 34 to 50). Arrow marks transition from UD to STR.

The rate of sulphate conversion in the TR increased steadily over the first 30 days of operation to reach levels ranging between 400 and 500 mg $SO_4.L^{-1}.day^{-1}$. However, as can be seen in Figure 3.11, the rate declined in the final 30 days of operation to approximately 200 mg $SO_4.L^{-1}.day^{-1}$. Average removal efficiencies of 72.12%

(±11.72) were obtained. COD removal rates also increased with time, reaching 500 *mg COD.L^{-1}.day^{-1} (Figure 3.12). Removal efficiencies were comparable to levels* obtained in the UD. Sulphate removals substantially higher than those reported by Genschow *et al.* (1996), for a two-stage biological sulphate reducing process for the treatment of tannery effluent, were recorded in the UD and TR digester (Figure 3.13).

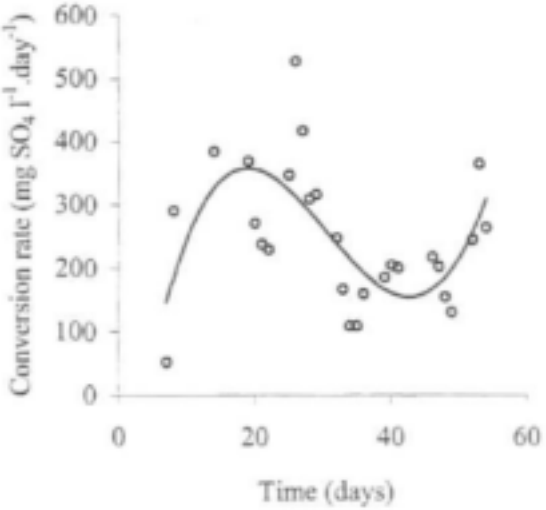


Figure 3.11. Sulphate reduction rate as a function of time in the TR. Trends are shown by best-fit line.

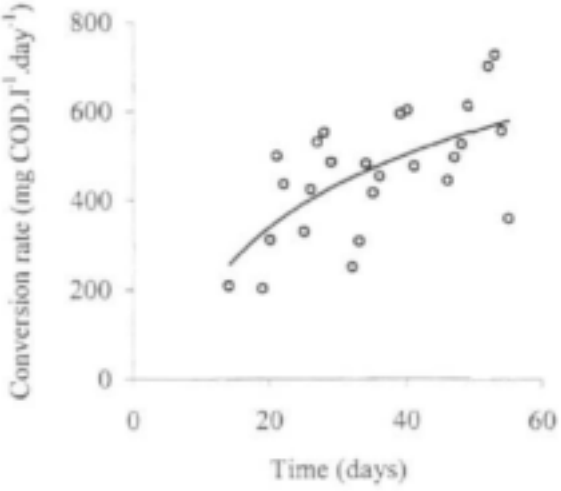


Figure 3.12. COD conversion rate as a function of time in the TR. Trends are shown by best-fit line.

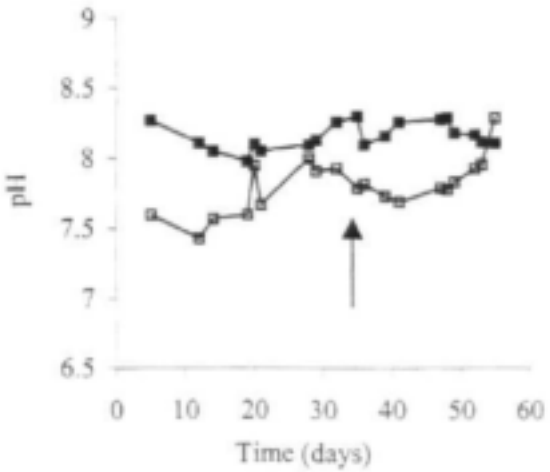


Figure 3.13. The pH of the outflow from the UD (day 1 to 33) and the STR (day 33 to 55) (▫) and the TR (▪). Arrow marks transition from UD to STR

Although the TR and UD digester were comparable in terms of rates of substrate utilisation and COD and SO_4 removal efficiency (COD:SO_4 reduction ratio around 2:1), the time taken for them to attain these levels differs and may be important in choice of reactor design. The rate of microbial population establishment within the

reactors was assessed in terms of the increase in COD and sulphate removal efficiency. The microbial population appeared to establish itself quickest in the TR, with sulphate removal efficiency increasing at a rate of 11.7%.day $^{-1}$, compared to 5.5%.day $^{-1}$ and 6.3%.day $^{-1}$ in the UD and STR respectively. Similar results were obtained for COD removal, with efficiency increasing in the TR at a rate of 11.8%.day^{-1}, compared to 4.6%.day $^{-1}$ in the UD.

Total sulphide levels were highest in the STR (170 mg.L^{-1}) (Figure 3.14), followed by the TR (150 mg.L^{-1}) (Figure 3.15) and the UD (130 mg.L^{-1}). The rate of sulphide production mirrored these results: 80.1 mg.L^{-1}.day $^{-1}$ in the STR, 49.1 mg.L^{-1}.day $^{-1}$ in the TR and 33.2 mg.L^{-1}.day^{-1} in the UD. The concentration of H_2S was calculated from the soluble sulphide concentration and pH of the solution (Oleszkiewicz *et al.*, 1989; APHA, 1989). The percentage of H_2S in the TR was calculated to range from 6.5-7 % of the soluble sulphide concentration, that of the UD 6.9-7.7 % and the STR 6.5-7.4 %. These values, as shown in Figures 3.14 and 3.15, are lower than those reported to be toxic to MPB: 90-250 mg.L^{-1} (Koster *et al.*, 1986) and over 1000 mg.L^{-1} (Isa *et al.*, 1986). No methane production was observed during the course of the study. This suggests that the repression of methanogenesis, if any, would not have been due to sulphide toxicity, but possibly in part to outcompetition by SRB.

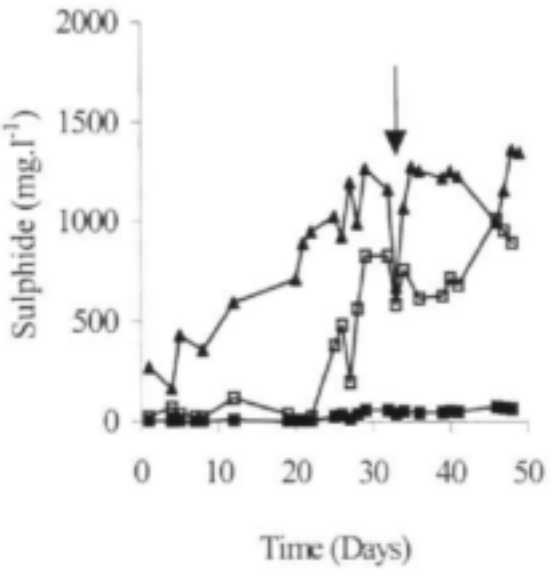


Figure 3.14. Total (▲), dissolved (□) and free (■) sulphide in the outflow from the UD (day 1 to 33) and the STR (day 34 to 50). Arrow marks transition from UD to STR.

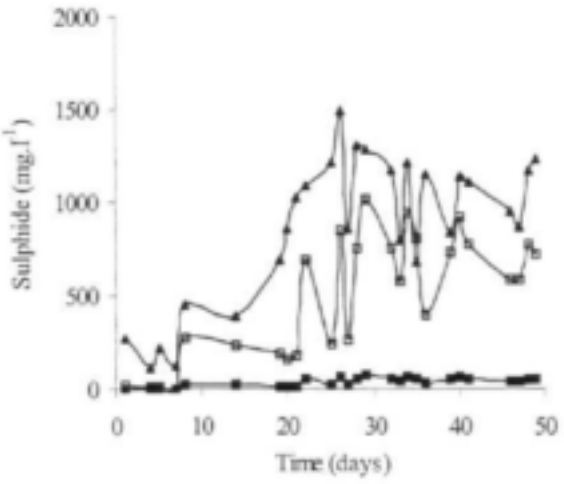


Figure 3.15. Total (▲), dissolved (□) and free (■) sulphide in the outflow from the TR.

3.5 CONCLUSIONS

The study demonstrated that tannery wastewater may be used quite effectively as an electron donor and carbon source for biological sulphate reduction. High levels of organic carbon removal, sulphide production and acid consumption in the system indicated that a linkage of tannery effluent and AMD treatment might be reliably anticipated.

An average sulphate removal of between 60-80% was obtained in all three digesters, and a COD:SO_4 reduction ratio around 2:1. While these rates of removal fluctuated due to changes in the effluent feed values, the system could have established more stable operation if it had run for a longer period of time. However, this was not possible due to time constraints for on-site pilot plant operation. High levels of sulphide were obtained, and although not measured directly, alkalinity production was generated in the sulphate reduction reaction. Both of these products have a role to play in the removal of heavy metals from the AMD effluent, and their use in this application will be discussed later. As the pH in the digesters was >7.4 little sulphide was released in the form of H_2S gas, and the odour problem usually associated with sulphidogenic digesters was absent. The UD and TR performed better than the STR, although longer operating times would have lent increased confidence to this conclusion. The study nevertheless did provide a provisional indication that the pit in the AFP would offer a reactor vessel at least comparable in performance to the substantially more costly STR in this particular application. The TR, while relatively efficient in performance, showed substantial sludge accumulation, and would not be an option without the inclusion of some form of sludge management.

4 PRIMARY SEWAGE SLUDGE AS AN ELECTRON DONOR SOURCE IN BIOLOGICAL SULPHATE REDUCTION

4.1 INTRODUCTION

The association between sulphide production and sewage as an electron donor source for SRB activity had been known for a long time, and especially the relationship in sewer corrosion and odour nuisance at disposal works (Postgate,1984). The first suggestions for SRB process configuration utilising sewage as a carbon source were made by Butlin *et al.* (1956), and Burgess and Wood (1961), who noted the potential for sulphide and sulphur production from sulphate-enriched sewage. Pipes (1961) had suggested that stabilisation of primary sewage sludges (PSS) under sulphate reducing conditions might offer particular advantages for disposal of sewage sludges.

While little development followed these observations, applications in the utilisation of sewage as an electron donor source concentrated mainly on its use in nutrient removal (Bannister and Pretorius, 1998). However the solubilisation rates for PSS are generally slow in conventional anaerobic digestion systems (Pipyn and Verstraete, 1979), with maximum soluble product formation reported between 8 and 20 days (Elefsiniotis and Oldham, 1994). Considerable attention has focused on the rates of degradation of particulate substrates (Wentzel *et al.*, 1995; Vavilin *et al.*, 1996), and Whittington-Jones 2000) has reported on the elevation of hydrolysis rates in the biosulphidogenic environment.

The purpose of the study reported here was to evaluate the use of PSS as an electron donor source for sulphate reduction from levels between 2000 and 3000 $mg.L^{-1}$ SO_4, which are commonly encountered in AMD streams. While the performance of the UD reactor configuration is well known in the AFP treating sewage wastewaters (Oswald, 1988a), it is primarily the STR which has been used in experimental evaluations of PSS digestion (Nyns *et al.*, 1979; Toerien and Maree, 1987; Elefsiniotis and Oldham, 1994). It was therefore decided to use the STR in this study in determining the sulphate and COD conversions achievable utilising PSS as the electron donor source. The original intention was then to compare these results with a UD operated reactor configuration. However, due to time constraints this was investigated in follow-up studies detailed in Reports 9 and 10 of this series.

4.2 OBJECTIVES

The following objectives were identified for this component of the study:

1. To evaluate the use of PSS as an electron donor source for sulphate reduction, with feed sulphate at levels comparable to that found in AMD;

2. To evaluate sulphate reduction performance using the STR configuration at pilot- scale, both with and without solids recycle.

4.3 MATERIALS AND METHODS

4.3.1 Pilot Plant

The pilot plant is illustrated in Figure 4.1, and consisted of a 1 m^3 plastic tank with three sample ports. It was connected to a feed make-up tank, and the out flow passed through a 400L settling cone from which solids were returned to the reactor. Submersible pumps were used for mixing reactor and feed tank contents. Fresh feed was made up every second day, and fed at a rate equivalent to an hydraulic retention time of 5 days.

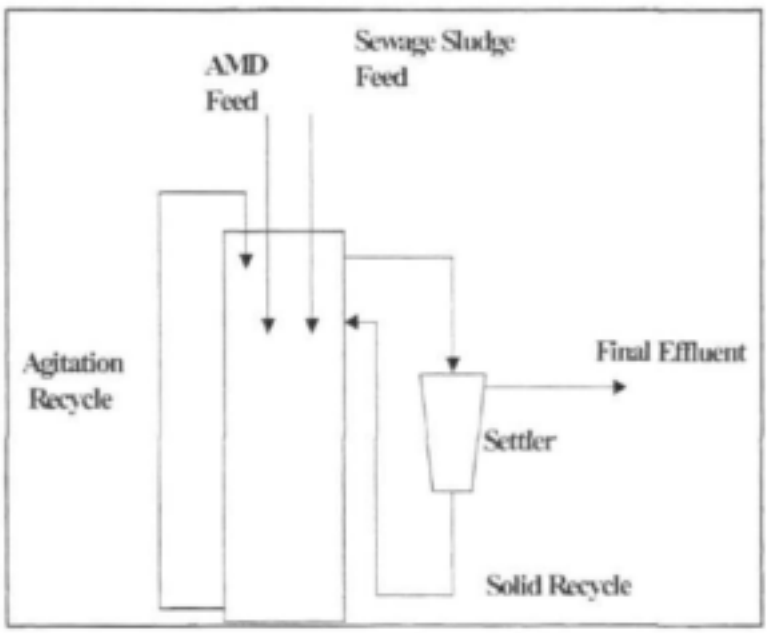


Figure 4.1. Flow diagram of the 1 m^3 pilot plant constructed to evaluate sulphate reduction in a synthetic AMD feed using primary sewage sludge as an electron donor and carbon source.

4.3.2 Analysis

Samples were drawn daily from each port and analysed for total sulphate, total sulphide, COD, total solids and pH.

Analytical methods used are as previously described.

4.4 RESULTS AND DISCUSSION

4.4.1 STR Operation Without Recycle

The STR without recycle was operated for a period of 48 days, and the results reported in Figures 4.2 and 4.3 show COD:SO_4 reduction ratios of around 2:1, after steady state operation had been achieved.

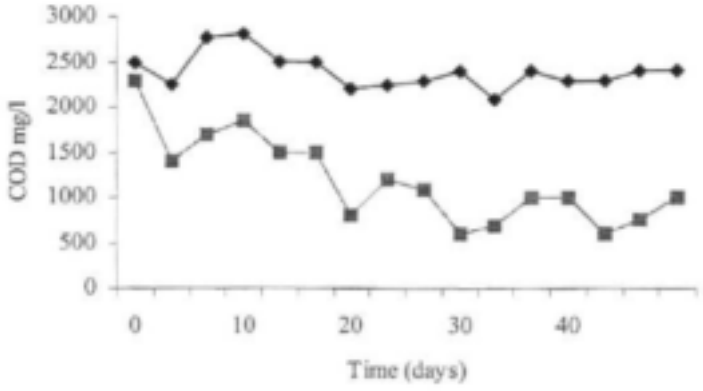


Figure 4.2. Total COD removal in the pilot-scale sulphidogenic STR operated without recycle. COD feed (♦), effluent (▪).

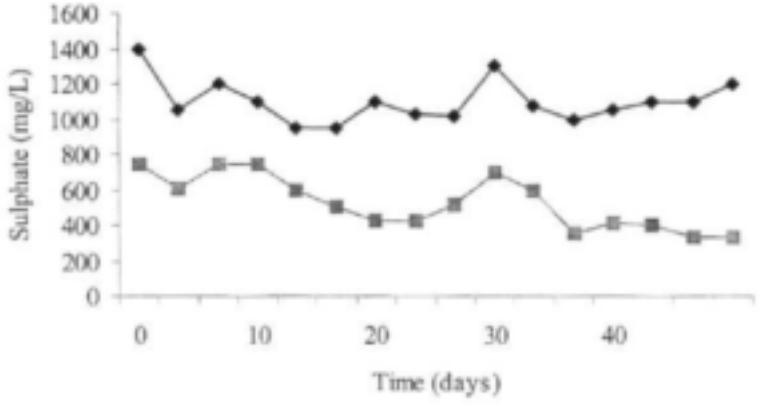


Figure 4.3. Sulphate removal in the pilot-scale sulphidogenic STR operated without recycle. Sulphate feed (♦), effluent (▪).

Sulphate and COD removal efficiencies (Figure 4.4) improved over the course of the study with average values at steady state around 85% and 65% respectively.

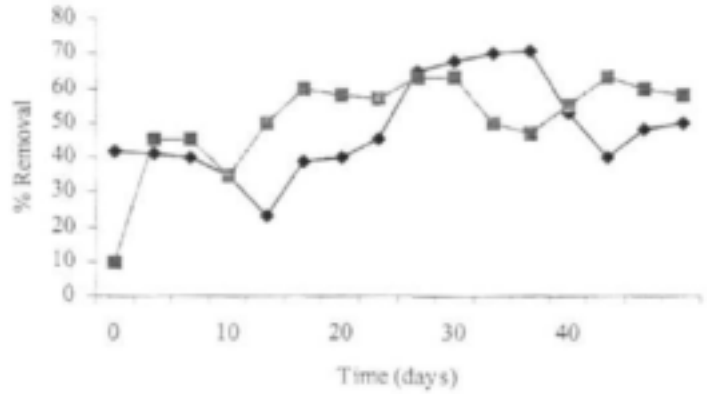


Figure 4.4. Percentage COD and sulphate removal in the pilot-scale sulphidogenic STR operated without recycle. COD (♦), sulphate (▪).

4.4.2 STR Operation With Recycle

Following the study reported above the STR was operated with sludge recycle and the results for COD and sulphate reduction are reported in Figures 4.5 and 4.6. The overall improvement in performance of around 5% was not dramatic, with average removals for COD and sulphate of 60% and 80% observed at steady state operation respectively. However the COD:SO_4 reduction ratio was reduced to 1.5:1 which could have important implications for the efficiency of the process if confirmed in the UD application.

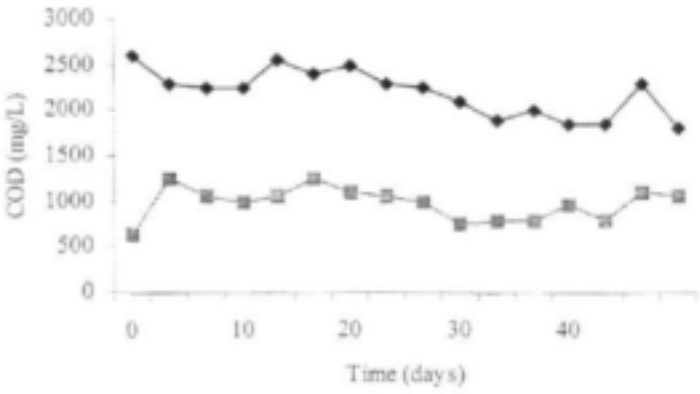


Figure 4.5. Total COD removal in the pilot-scale sulphidogenic STR operated with recycle. COD feed (♦), effluent (▪).

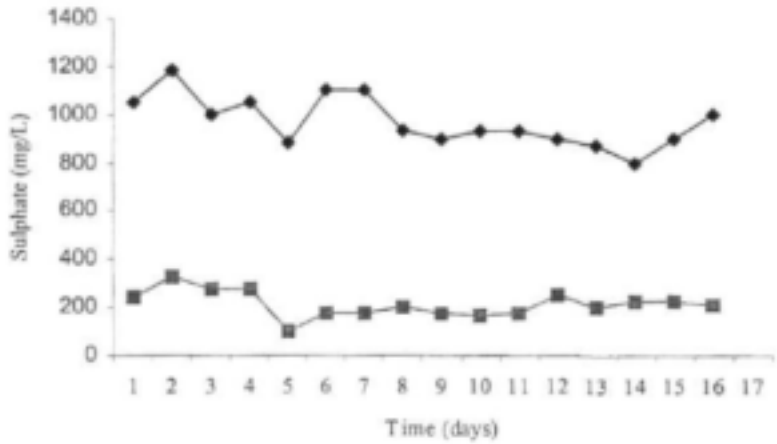


Figure 4.6. Sulphate removal in the pilot-scale sulphidogenic STR operated with recycle. Sulphate feed (♦), effluent (▪).

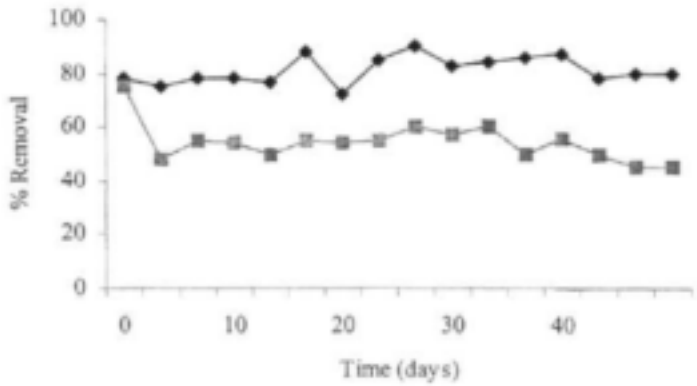


Figure 4.7. Percentage COD and sulphate removal in the pilot-scale sulphidogenic STR operated with recycle. COD (♦), sulphate (▪).

4.5 CONCLUSIONS

The study demonstrated the effective use of sewage sludge as an electron donor source in sulphate reduction, and that an elementary reactor system could be initialised and brought to steady state operation without great difficulty. The relatively minor improvement in process performance with operation of the recycle loop showed that active biomass was not a factor limiting performance. The stability of the process, and the relative ease of start-up were important indicators for follow-up evaluation studies on non-STR systems, which were subsequently undertaken by Whittington-Jones (2000) and Corbett (2001). The successful removal of sulphate at COD:SO_4

reduction ratios of 2:1 was comparable to tannery wastewaters. This was encouraging as it approached the theoretical limits of the system using a complex carbon source as electron donor (Lens *et al.*, 1995).

5 EFFECTS OF SULPHIDE ON MICRO-ALGAL GROWTH

5.1 INTRODUCTION

In the proposed ASPAM system sulphate reduction takes place in the pit of the AFP, generating quantities of sulphide and an organic effluent stream. Part of this stream passes to the metal precipitation unit operation, and the residual feeds to HRAP 2, which serves as a polishing step for the removal of remaining sulphides and organics (see Figure 1.2). An overlay of algal-rich oxygenated water is returned from the HRAP to the surface of the AFP and serves to control both odours and to effect sulphide oxidation as it rises into the upper layers. The potential effects of sulphide on micro-algal growth and productivity would have an important bearing on the operation of the system, and it would be necessary to know under what conditions micro-algal growth may be anticipated at this point in the system.

The growth of a number of cyanobacteria species in sulphate reducing environments has been recorded (Stewart and Pearson, 1970; Garlick *et al.*, 1977; Jorgensen *et al.*, 1986). They are known to occur naturally in anaerobic environments rich in sulphide (Jorgensen *et al.*, 1986), where they may form mats (Anderson *et al.*, 1987; Bender *et al.*, 1995), and are characterised by high primary productivity and rapid recycling of organic matter (Jorgensen *et al.*, 1986). The ability of the cyanobacterium *Oscillatoria limnetica* to use sulphide as an electron donor for the photo assimilation of CO_2 is well documented (Cohen *et al.*, 1975; Garlick *et al.*, 1977; Oren and Padan, 1978). However, this is not a common adaptation amongst all cyanobacteria (Cohen, 1984), and sulphide is known to exert a toxic effect on non-adapted cyanobacteria (Oren and Shilo, 1979) by inhibiting the electron transport chain (Cohen *et al.*, 1986). The species of *Spirulina* used in this study was isolated from WSP treating tannery effluent and thus had been exposed to high sulphide levels over long periods of time.

The tolerance of *Dunaliella salina* to sulphide was also monitored since these micro-algae dominate in hypersaline WSP (> 40 $g.L^{-1}$ NaCl), a situation which could occur in the treatment of concentrated AMD solutions and other high salinity effluents. A change in the predominant micro-algal population from *Spirulina* to *Dunaliella* has also previously been noted across evaporation ponding cascades treating tannery effluent (Dunn, 1998). *Dunaliella* therefore provides a useful indicator of the performance of halophilic populations under the sulphide concentrations likely to be encountered in the latter stages of these systems.

5.2 OBJECTIVES

The following objectives were identified for this component of the study:

1. To assess the sulphide toxicity threshold of *Spirulina* in both batch and continuous culture conditions;
2. To assess the potential for acclimatising *Spirulina* to increasing sulphide concentrations;
3. To assess the sulphide toxicity threshold of *D. salina* in continuous culture conditions.

5.3 MATERIALS AND METHODS

5.3.1 Algal culture and maintenance

A culture of *Spirulina* sp. was sourced from a tannery IAPS operated in Wellington, South Africa. It was maintained in laboratory flask cultures at 27°C in Zarrouk's medium (Zarrouk, 1966), under an 18hr light, 6 hour dark cycle. Illumination was supplied by cold white fluorescent light.

A culture of *D. salina* (var. bardawil Teod.) was obtained from the Culture Collection of Algae and Protozoa (CCAP 19/30). The culture was maintained at 27°C in BAAM medium (Ben Amotz and Avron, 1983), under an 18 hour light, 6 hour dark cycle. Illumination was supplied by cold white fluorescent light.

5.3.2 Experimental

5.3.2.1 Organic-rich medium

Spirulina sp. cells in the logarithmic phase of growth were harvested by filtration through a nylon mesh with a pore size of 100 µm. They were resuspended in 150 mL Zarrouk's medium supplemented with 66% v/v, 33%v/v and 14%v/v sulphide-rich overflow from a sulphate reducing UD reactor treating tannery effluent. A representative analysis of this overflow, which is referred to here as organic-rich medium, is recorded in Table 5.1. Samples were removed daily and analysed for chlorophyll *a*, as an indicator of micro-algal growth. Control cultures were grown in Zarrouk's medium.

A 50 L laboratory-scale HRAP containing Zarrouk's medium was inoculated with a culture of *Spirulina* sp. The HRAP was operated on a continuous basis with organic rich medium being fed into the algal pond daily. Samples were removed and analysed for sulphide and chlorophyll *a*. A control culture was grown in, and fed an equivalent volume of Zarrouk's medium daily.

Table 5.1. Representative analysis of overflow from the sulphate reducing UD reactor treating tannery effluent.

Water Quality Parameter	Measured
pH	7.95
Phosphate ($mg.L^{-1}$)	36.42
Nitrate ($mg.L^{-1}$)	16.18
Ammonia ($mg.L^{-1}$)	0.57
Chemical Oxygen Demand (COD) ($mg.L^{-1}$)	2237
Sulphate ($mg.L^{-1}$)	471
Sulphide ($mg.L^{-1}$)	1029

5.3.2.2 Defined medium

Spirulina sp. cells in the logarithmic phase of growth were harvested by filtration through a nylon mesh with a pore size of 100 µm. They were resuspended in 150 mL Zarrouk's medium supplemented with sodium sulphide. Samples were removed daily

and analysed for chlorophyll *a*. Control cultures were grown in Zarrouk's medium without sulphide. *D. salina* cells in the logarithmic phase of growth were centrifuged at 4420g x 10 minutes. The pellets were resuspended in 100 mL BAAM medium supplemented with increasing concentrations of sodium sulphide. The cultures were operated on a continuous basis and fed either sulphide- supplemented Zarrouk's or BAAM media at rates of 10%, 20% and 30% v/v.day^{-1}. Samples were removed and analysed for sulphide and chlorophyll *a*. Control cultures were grown in Zarrouk's medium and fed daily an equivalent volume of Zarrouk's medium without sulphide.

5.3.4 Analysis

Chlorophyll was extracted into acetone and quantified according to Lichtenhaler (1987). Sulphide was analysed by the Methylene Blue method according to Standard Methods (APHA, 1989).

5.4 RESULTS AND DISCUSSION

5.4.1 *Spirulina* sp.

Initial experiments were carried out to determine if *Spirulina* sp. could grow on sulphide-rich organic medium. As can be seen in Figure 5.1, the chlorophyll *a* content of the alga after a 5 day period was highest in the cultures with the highest initial concentration of organic rich medium.

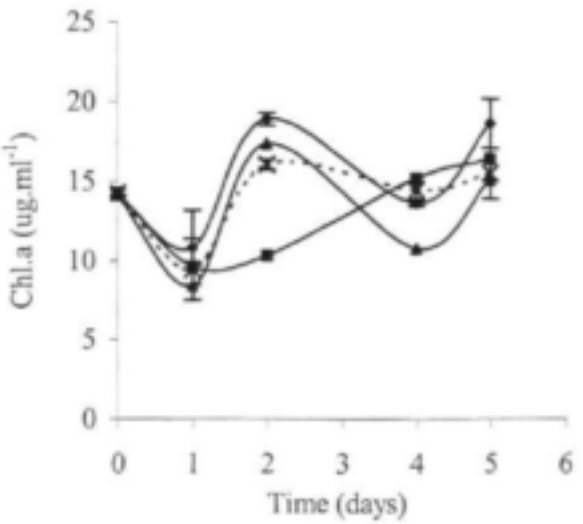


Figure 5.1. Chlorophyll *a* profile of *Spirulina* sp. grown in Zarrouk's medium supplemented with sulphide-rich organic medium so as to give a final sulphide concentration of 2.3 mg.L^{-1} (▪), 0.96 mg.L^{-1} (▲) and 0.616 mg.L^{-1} (♦) as compared to the control (×) grown in Zarrouk's medium without sulphide.

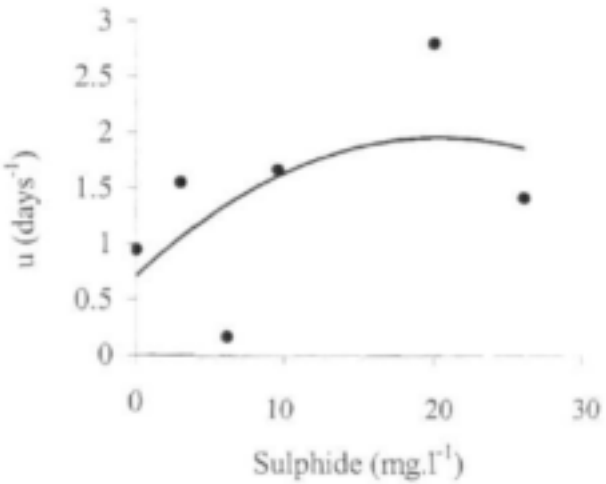


Figure 5.2. Specific growth rate (μ) of *Spirulina* sp. cultures grown in Zarrouk's medium supplemented with sulphide-rich organic medium. Trend shown by best-fit line.

A plot of specific growth rate (μ), as determined according to Middelbeek (1992), versus sulphide concentration, shows a trend of increase in μ with increasing sulphide levels (Fig. 5.2) This result appears to indicates that the organic-rich medium gives the algal cells a competitive advantage over those grown in defined medium, even in the presence of sulphide.

Following the above results, the growth of *Spirulina* sp. was measured in defined medium supplemented with sulphide in order to ascertain the effect of sulphide on algal growth without the interference of organic compounds. The sulphide concentration in the organic-rich medium was relatively low as the experiment was carried out using overflow liquor from a sulphate reducing digester which was in the initial phase of commissioning. It was thus decided to increase sulphide in the defined medium experiment to determine the level at which sulphide begins to have an effect on the growth rate.

The growth rate of *Spirulina* grown in batch culture in Zarrouk's medium supplemented with Na_2S remained relatively constant at μ=0.34-0.4 $days^{-1}$ until a sulphide concentration of 25 $mg.L^{-1}$ had been reached, after which growth declined (Figure 5.3).

It was observed that the sulphide levels in the batch cultures declined over a 24 hour period. A continuous experiment was thus undertaken to monitor the growth of *Spirulina* sp. cultures over a period of 4 days in an environment with a constant level of sulphide. The results are reported in Figure 5.4 A to F.

At daily adjusted sulphide levels between 95 $mg.L^{-1}$ and 190 $mg.L^{-1}$ the growth of the experimental and control cultures was comparable. At sulphide concentrations above

190 mg.L^{-1}, there was a marked decline in the chlorophyll *a* content of the experimental cultures, compared to the controls, with the cultures immediately entering decline phase. The rate of decline increased with increasing sulphide levels and did not appear to be affected by the dilution rate.

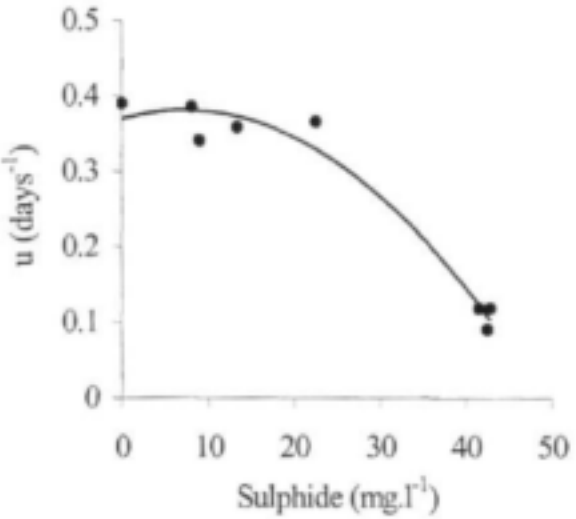


Figure 5.3. Specific growth rate (μ) of *Spirulina* sp. cultures grown in Zarrouk's medium supplemented with sulphide. Trend shown by best-fit line.

An important factor to note is the oxidation of sulphide which occurred between addition of the sulphide and sampling of the experiment for sulphide analysis, a period of approximately 10 minutes. During this period, the level of sulphide dropped considerably, but more so the higher the levels of sulphide added. The sulphide level also declined during the day, with the residual level after 24 hours varying according to the initial sulphide concentration. As may be anticipated, the highest residual level after a 24 hour period was recorded in the culture with the highest initial sulphide concentration. These experiments were conducted at pH 9-10. At these pH values very little of the sulphide would be in the gaseous form. Thus the majority of sulphide loss would have been due to the operation of oxidation mechanisms.

However, the chlorophyll *a* levels of the experimental cultures also declined as the residual sulphide concentration increased after 24 hours. This was also more noticeable at initial sulphide concentrations higher than 190 mg.L^{-1}. This indicates the presence of a biological component and possibly the operation of a sulphide detoxification mechanism. However, this possibility requires further investigation and is the subject of follow-up studies.

A plot of the specific growth rate (μ) against sulphide concentration shows the control cultures to have higher specific growth rates compared to the experimental cultures, irrespective of the sulphide concentration (Figure 5.5). When the sulphide concentration reached 190 mg.L^{-1}, the growth rate of the experimental cultures began

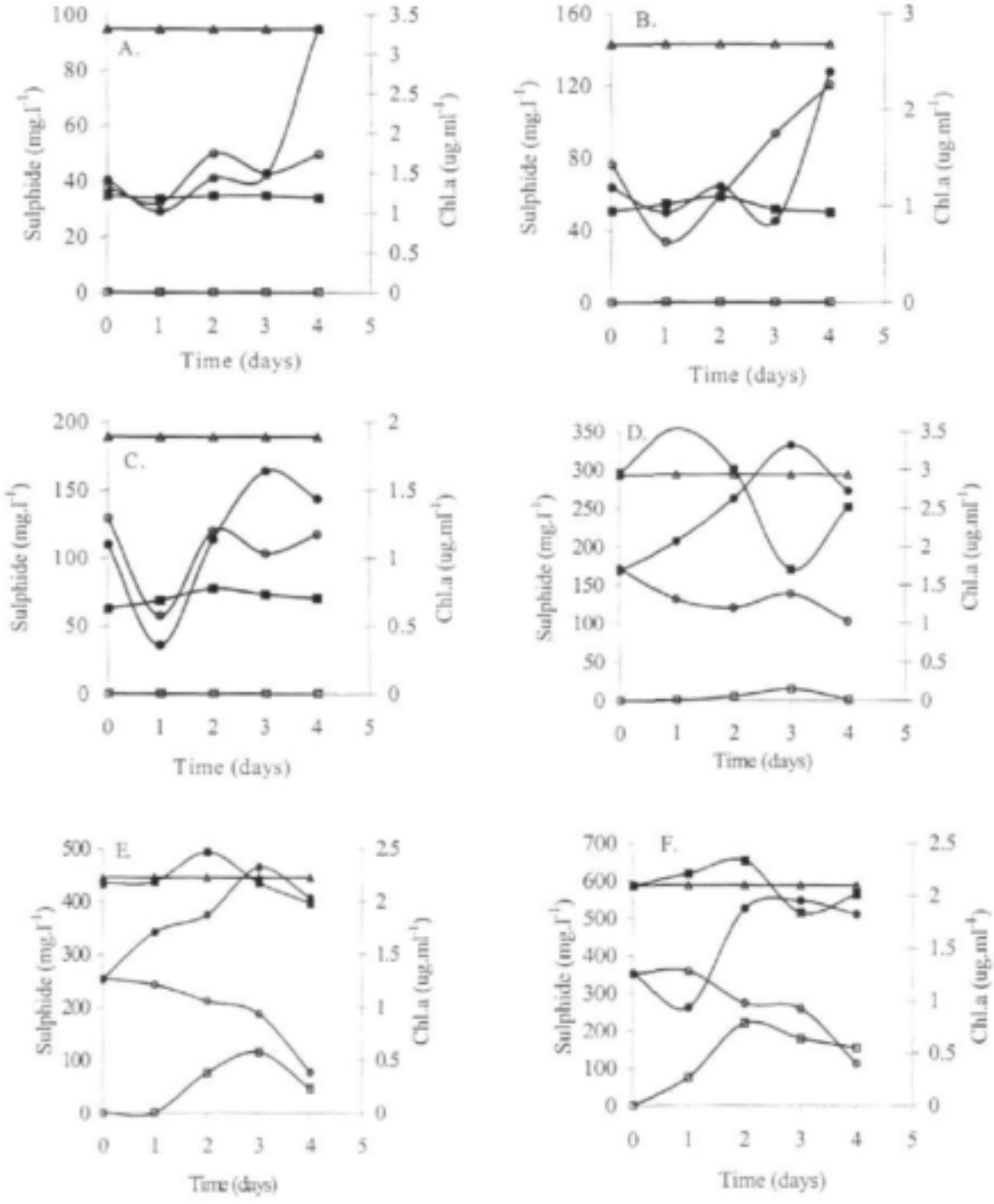


Figure 5.4. Chlorophyll *a* profile of *Spirulina* sp. grown in defined medium supplemented daily with A) 95 mg.L^{-1}, B) 145 mg.L^{-1}, C) 190 mg.L^{-1}, D) 295 mg.L^{-1}, E) 445 mg.L^{-1} and F) 590 mg.L^{-1} sulphide. Chlorophyll *a* of experimental cultures (○), chlorophyll *a* of control cultures (•), actual sulphide added (▵), residual sulphide concentration 10 minutes after feeding (▪), residual sulphide concentration 24 hours after feeding (▫).

to decline, reaching zero at a sulphide concentration of 290 mg.L^{-1}.

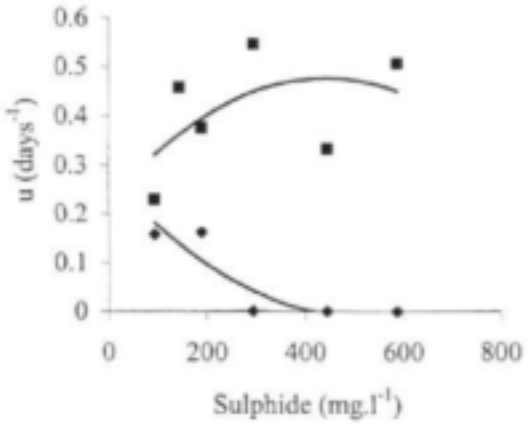


Figure 5.5. Specific growth rate (μ) of *Spirulina* sp. cultures fed defined medium supplemented with sulphide daily (♦) and control cultures fed defined medium only (▪). Trend shown by best-fit line.

From a practical point of view, it is necessary to determine whether *Spirulina* sp. can be acclimated to increasing levels of sulphide. An experiment was set up in which a culture of *Spirulina* sp. was fed Zarrouk's medium supplemented with sulphide. The level of sulphide was increased over a period of days. The cultures were operated as a batch-fed continuous system, with a set volume of medium and cells being removed every 24 hours and replaced with fresh medium supplemented with sulphide. The feed rate of the cultures was also varied to select for a fast-growing strain able to tolerate high sulphide levels.

In cultures fed at a rate of 20% v/v.day^{-1}, the sulphide concentration in the medium increased to 450 mg.L^{-1} (Figure 5.6A). Little difference between the chlorophyll *a* levels of the control and experimental cultures was observed.

At a feed rate of 30% v/v.day^{-1}, the chlorophyll *a* level of the control was slightly higher than that of the experimental culture (Figure 5.6B). However, in the cultures fed at a rate of 40%v/v.day^{-1}, the experimental cultures were able to out-compete the control cultures (Figure 5.6C).

It must be noted that the chlorophyll *a* levels of both the experimental and control cultures remained constant up to day 15, after which they increased. This would indicate that until day 15 washout conditions applied to non-adapted cells, leading to the selection of a population of adapted cells with faster growth rates, which became predominant from day 15 onwards. In Figure 5.6 C it is also evident that a population of cells which were both tolerant to sulphide and fast growing was selected for at a feed rate of 40% v/v.day^{-1}, as the experimental cultures were able to out-compete the control cultures, in terms of chlorophyll *a* levels.

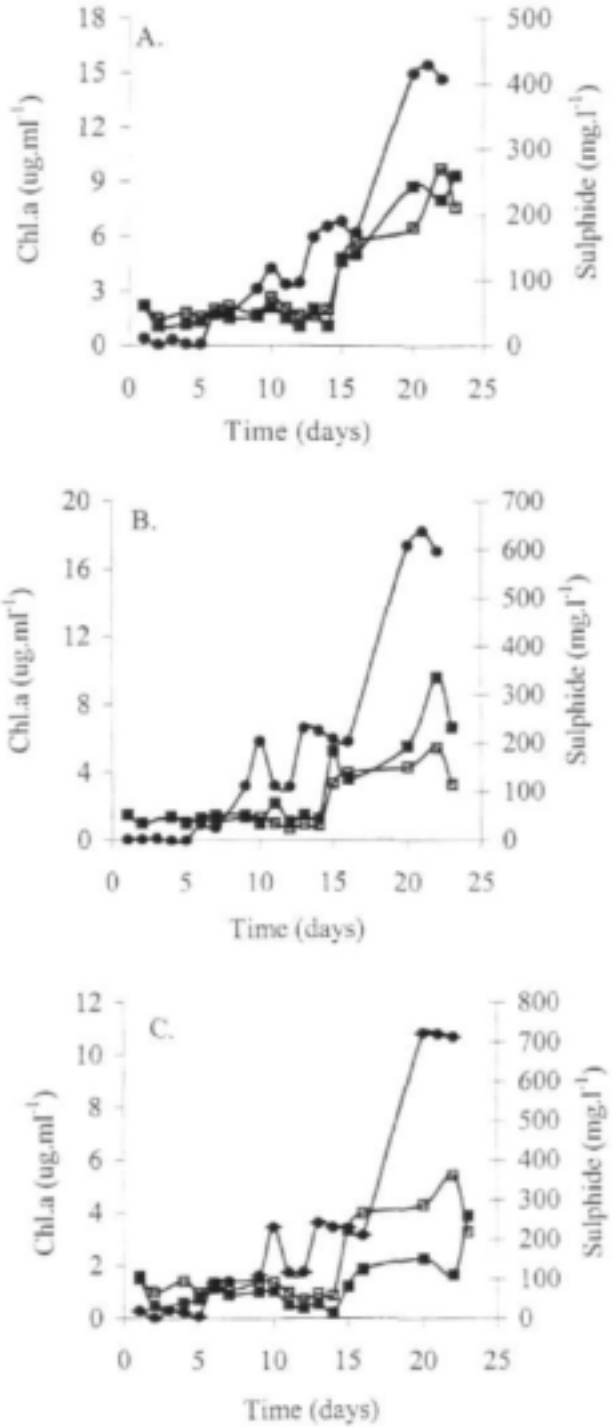


Figure 5.6. Chlorophyll *a* profile of *Spirulina* sp. cultures fed defined medium supplemented with sulphide daily at a rate of A) 20% v/v.day^{-1}, B) 30% v/v.day^{-1} and C) 40% v/v.day^{-1} (▫) compared to the control cultures fed unsupplemented medium (▪). The sulphide concentrations (•) were increased to acclimatise the micro-algae to the sulphide.

Aspects of the above experiment were scaled-up in a 50L experimental raceway to ascertain the effect of adding organic-rich sulphide medium to a micro-algal culture over a long time period. The daily addition of sulphide-rich digester overflow liquor led to a decline in the chlorophyll *a* content of the experimental culture compared to the control (Figure 5.7). However, after initial decline the chlorophyll *a* levels were relatively constant irrespective of the increase in sulphide to 150 $mg.L^{-1}$, which has been shown to be toxic to cyanobacteria. The micro-algae were also not able to completely oxidise the sulphide, with a steady accumulation in the background levels occurring.

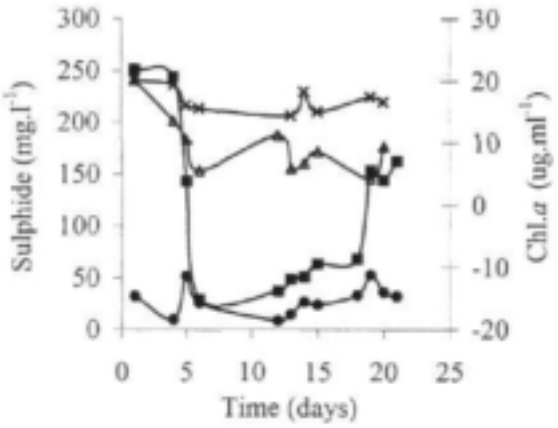


Figure 5.7. Chlorophyll *a* profile of *Spirulina* sp. fed organic-rich medium daily (▲) as compared to the control culture fed defined medium daily (×). The residual sulphide concentration 30 minutes after feeding (▪) and 24 hours after feeding (•) are shown.

The decline in chlorophyll *a* levels of *Spirulina* sp. exposed to sulphide seen in these experiments, indicates that the photosynthetic process was vulnerable to sulphide. High sulphide concentrations have previously been shown to have an adverse effect on micro-algal growth and can limit ecosystem species diversity (Pinheiro *et al.*,1987; Mara and Pearson, 1986). Sulphide is a known inhibitor of the metabolic pathway components of numerous organisms (Castenholtz, 1976). It acts as an inhibitor of catalases, peroxidases, succinate dehydrogenase, carbonic anhydrase, cytochrome oxidase and other enzymes, and also tends to combine with the iron of hemes (Evans, 1967), thus inhibiting the electron transport chain (Cohen *et al.*, 1986). Cohen *et al.* (1975) found that sulphide levels of around 9.6 $mg.L^{-1}$ inhibited Photosystem 1-driven photo assimilation reactions.

The reduction in pigment content due to the presence of sulphide leads to considerable conservation of energy that may be directed toward the biosynthesis of other cell constituents (Wyman and Fay, 1987) or towards overcoming toxicity.

Water soluble H_2S, which is in a pH-dependent equilibrium with sulphide, is considered to be the toxic component. The undissociated form of H_2S is able to penetrate passively into the cell, following diffusion laws, across both the cytoplasmic and chloroplast membranes (Howsley and Pearson, 1979). It is responsible for

sulphide toxicity to both Photosystem I (PS-I) and Photosystem II (PS-II). The ionized species of HS^- and S^{2-}, on the other hand, require active transport to penetrate the cell, as has been indicated by Howsley and Pearson (1979). This would explain why Almasi and Pescod (1996) found sulphide toxicity to algal cells to be greater at high temperatures and low pH. At the pH utilised in these experiments (pH 8.5-10), H_2S would only constitute between 0.19% and 2% of the dissolved sulphide concentration, according to the equation of Oleskiewicz *et al.* (1989). Inhibition of the cultures fed Na_2S supplemented Zarrouk's on a daily basis, as indicated by a decline in chlorophyll *a*, occurred at H_2S levels of 3.8 $mg.L^{-1}$ and above. However, in cultures which were acclimated to sulphide these levels increased to 14 $mg.L^{-1}$ H_2S, and in the continuous culture fed digester overflow, 5 $mg.L^{-1}$ H_2S. Gyure *et al.* (1987) found that the photosynthesis of an algal population in the zone of H_2S production in an acid strip mine lake was severely inhibited by H_2S concentrations of 0.19 $mg.L^{-1}$. *Anacystis nidulans,* on the other hand, exhibited complete inhibition at H_2S levels of 1.9 $mg.L^{-1}$, while Abeliovich (1980) found that 0.48 $mg.L^{-1}$ hydrogen sulphide led to a decrease in photoassimilation of CO_2 by pond algae. However, similar levels of total sulphide (between 1.15 $mg.L^{-1}$ and 3.20 $mg.L^{-1}$) were found to be inhibitory to photosynthesis in three cyanobacterial isolates at pH 8 (Howsley and Pearson,1979). Thus the level and form of sulphide which is toxic appears to vary between micro-algal species.

Cyanobacteria are oxygenic phototrophs which exhibit four different types of adaptions to sulphide based on the differential toxicity of sulphide to PS-II and PS-I, and the ability to carry out anoxygenic photosynthesis (Cohen *et al.*, 1986). In anoxygenic photosynthesis sulphide replaces water as the ultimate electron donor (Howsley and Pearson, 1979), with the production of sulphur. This involves PS I activity only and thus no oxygen is evolved (Cohen *et al.*, 1975). Although the ability to carry out anoxygenic photosynthesis has been demonstrated in a range of cyanobacteria, very little information is available for *Spirulina*. However, *Spirulina labyrinthioformis* is known to exhibit anoxygenic photosynthesis *in situ* at a sulphide concentration of 1mM (Castenholtz, 1976). Sulphur was absent from the medium in these experiments, suggesting that anoxygenic photosynthesis was not part of the mechanism involved in coping with the sulphide stress.

Cyanobacteria carrying out anoxygenic photosynthesis require a period of adaption, during which no photosynthetic activity occurs, before sulphide can be utilised (Oren and Padan, 1978). Thus it would be advantageous for them to be able to carry out oxygenic photosynthesis in the presence of sulphides. This would not only give them a competitive advantage, but the additional oxygen produced would allow for the oxidative removal of sulphide from the environment (Howsley and Pearson, 1979). A species of *Spirulina* growing in a hot spring has been found to evolve oxygen at sulphide concentrations in the range of 0.9-1.9 $mg.L^{-1}$ (Castenholtz, 1976).

5.4.2 *Dunaliella*

Figure 5.8 show plots of chlorophyll *a* as a function of time for *D. salina* cultures fed sulphide supplemented BAAM media on a daily basis.

At sulphide concentrations below 95 $mg.L^{-1}$, the experimental cultures were able to

grow, although chlorophyll *a* levels were much lower than that of the control cultures. At a sulphide concentration of 145 mg.L^{-1}, the culture stopped growing and remained in stationary phase. As the sulphide concentration increased to 190 mg.L^{-1}, the cells entered decline phase after a period of 1 day. This was in contrast to the *Spirulina* sp. cultures where the chlorophyll *a* levels of the experimental culture were the same as that of the control up to a sulphide concentration of 190 mg.L^{-1}. Thus it would appear that the *Spirulina* sp. used in this study was more tolerant to sulphide than *D. salina*. A plot of the specific growth rate (μ) against sulphide concentration shows the control cultures to have had much higher growth rates than the experimental cultures (Figure 5.9).

Higher levels of sulphide would most likely not occur in ponds in the latter stages of WSP treatment due to oxidation effects, thus negating the need to assay the effect of much higher sulphide levels on *D. salina* growth than those examined.

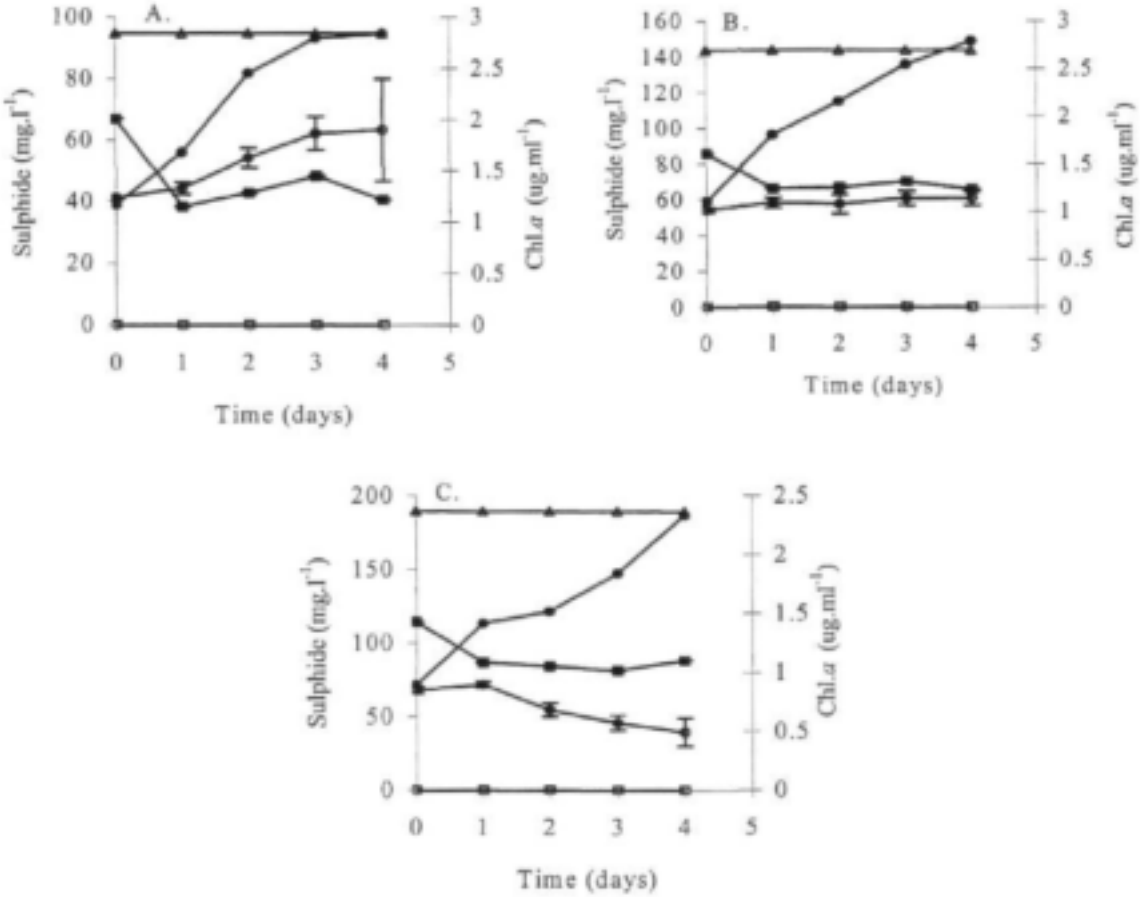


Figure 5.8. Chlorophyll *a* profile of *D. salina* grown in BAAM medium supplemented daily with A) 95 mg.L^{-1}, B) 145 mg.L^{-1} and C) 190 mg.L^{-1} sulphide. Chlorophyll *a* of experimental cultures (○), chlorophyll *a* of control cultures (•), actual sulphide added (▵), residual sulphide concentration 10 minutes after feeding (▪), residual sulphide concentration 24 hours after feeding (▫).

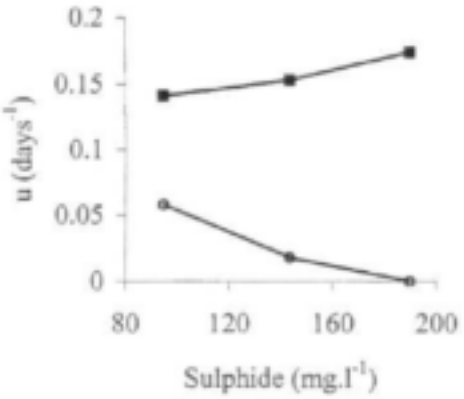


Figure 5.9. Specific growth rate (μ) of cultures of *D. salina* fed BAAM medium supplemented with different concentrations of sulphide (○) as compared to control cultures (▪) fed BAAM medium without sulphide.

5.5 CONCLUSIONS

These results show that the tannery WSP strains of *Spirulina* sp. used in this study were able to adapt to surprisingly high levels of sulphide in high pH environments. This is important if the concentration of sulphide that would be produced in facultative ponds treating AMD is considered. Rose *et al.* (1996) reported effluent sulphide levels of 1065 mg.L^{-1} entering a ponding system treating tannery effluent and sulphide levels of up to 1800 mg.L^{-1} in the outflow of the anaerobic digester treating the same effluent. *Spirulina* sp. also appears to play a role in reducing sulphide levels in its surrounding environment. The mechanism involved is under further investigation but probably involves photosynthetic oxygen production and subsequent oxidation reactions.

D. salina was found to be less resistant to sulphide than *Spirulina* sp., which may primarily be due to the lack of a cell wall, thus making it easier for the hydrogen sulphide to pass into the cell.

With the manipulation of sulphide levels in the algal ponds, possibly by the control of the residence time, it seems that algal systems might be utilised for the secondary treatment of sulphide-rich digester overflow in meso-saline, but probably to a lesser degree in hyper-saline, systems. These studies, together with previous observations of tannery ponding systems, indicated a feasibility for a HRAP-based control of the AFP, and in polishing the final water leaving the ASPAM process.

6 REMOVAL OF HEAVY METALS IN THE ASPAM SYSTEM

6.1 INTRODUCTION

The precipitation of metals in the bioreactor studies reported above, probably mainly as metal sulphide complexes, took place *in situ*. This is not ideal as the recovery of heavy metals from the metal-sulphide sludge is difficult, and metal sludges would also build-up in the reactor, thus necessitating periodic drainage and desludging. The metals may also be toxic to microbial processes occurring within the reactor. An ideal situation would thus be for at least the major part of the influent metals to be removed from solution prior to the AMD entering the anaerobic digester.

The integration of sulphidogenic and micro-algal components in the ASPAM system presents a number of mechanisms by which metals may be removed from the influent mine drainage stream. These include the formation of insoluble metal complexes (using the sulphide-rich digester overflow), precipitation by neutralisation (using recirculation from HRAP 1), and biosorption (using algal biomass and extrapolymeric substances, EPS, generated in both HRAP).

The proposed integration of algal ponds with a biological sulphate reducing reactor system to effect the removal of metals from AMD streams, is dependent on a number of factors which require further investigation in order to provide a provisional indication of feasibility. The study reported here attempted to provide clarity on some of the steps involved.

6.2 OBJECTIVES

The following objectives were identified for this component of the study:

1. To evaluate the potential for metal precipitation using the sulphide-rich effluent liquor from the sulphate reducing digesters fed a range of complex organic carbon substrates;

2. To investigate the role of the HRAP micro-algal environment in the removal of heavy metals from solution, by biosorption or by precipitation;

3. To investigate metal toxicity thresholds of the micro-algal biomass in the HRAP components of the ASPAM system.

6.3 MATERIALS AND METHODS

6.3.1 Algal cultures for metal binding studies

D. salina (CCAP 19/30) and *Spirulina* sp. (tannery WSP isolate), were maintained in BAAM and Zarrouk's medium respectively.

6.3.2 Algal cultures for polysaccharides production

Spirulina sp. was grown at 38°C under low light conditions in Zarrouk's medium. For the stress studies a mixed culture of *Dunaliella*, isolated from solar evaporation ponds in Botswana, was grown in BAAM medium at 38°C under low light conditions (< 50 μ moles.m^{-2}.sec^{-1}). The culture media were assayed for the presence of organic carbon. These conditions are referred to as stress conditions throughout the text.

6.3.3 Metal binding studies - algal biomass

Biomass from a culture of *D. salina* was harvested by centrifugation at 4420 g x10 minutes. *Spirulina* sp. biomass was harvested by filtration through a nylon gauze with a 100 μm pore size. Cultures were washed in PIPES buffer so as to remove any metal ions which may have been attached to the algal cell, and re-harvested. The respective micro-algal biomasses were then suspended in conical flasks in aqueous solutions of Cu^{2+}, Pb^{2+}, $Cr_2O_7^{2-}$ and Se^{2-} as metal salts ($CuSO_4$. $5H_2O$, $Pb(CH_3COO)_2.3H_2O$, $K_2Cr_2O_7$ and SeO_2). The conical flasks were sealed with cotton wool bungs and incubated overnight in an orbital shaker at 80 rpm at 22°C. A sample was removed and filtered through a 0.45 μm MSI nylon membrane filter. The filter was acid digested and assayed for metals. The micro-algae were then harvested and re-suspended in fresh metal solution to assess the effect of metal concentration on removal. The micro-algae were filtered and the filter assayed for metals. Experiments were performed in triplicate. The time period for metal removal was assessed by sampling at 15 minute intervals for the first 60 minutes and thereafter every 30 minutes.

The removal of zinc from a zinc refinery wastewater was also tested by the same procedure. The average concentrations of metals in the zinc effluent were as follows (mg.L^{-1}): Al, 1.46; Cd, 0.717; Co, 0.4; Cr, 2.24; Cu, 0.103; Fe, 1.8; K, 12.77; Mg, 335.4; Mn, 123.8; Nd, 166.7; Ni, 4.11; Pb, 4.987; Zn, 4.03; Ag, 0.08 and Se, 34.34.

6.3.4 Metal binding studies - extracellular organic matter

The mixed culture of *Dunaliella* was harvested from the medium by centrifugation at 5000 rpm x 20 minutes. The supernatant was retained and analysed for Total Organic Carbon (TOC). *Spirulina* sp. was harvested from the medium by filtration through a nylon gauze with a pore size of 100 μm. The supernatant was analysed for TOC. A suitable aliquot of $CuSO_4.7H_2O$ and $FeSO_4.7H_2O$ was added to a 24 mL volume sample of the supernatant. The pH of the supernatant was artificially lowered to pH 7, 5 and 3 with HCl prior to addition of the metal solutions. After a 24 hour period, the samples were centrifuged and the supernatant analysed for metals and TOC.

6.3.5 Toxicity studies

The toxicity of the metals to *D. salina* and *Spirulina* sp. was assessed by adding an aliquot of the metal stock solution to the micro-algal culture and monitoring the chlorophyll *a* content of the cells over a period of 15 days.

6.3.6 Sulphide precipitation studies

Sulphide-rich liquor from the digester treating tannery effluent was mixed with AMD, the composition of which is shown in Table 3.1, and a synthetic AMD of the following composition ($g.L^{-1}$): Na_2SO_4, 0.356; K_2SO_4, 0.049; $FeSO_4.7H_2O$, 1.045; $CaSO_4.2H_2O$, 1.61; $(NH_4)_2SO_4$, 0.198. The AMD was also mixed with Na_2S solutions of different concentrations. Sulphide-rich liquor from the digester fed algal biomass as carbon source was mixed with metal solutions of known concentration and pH standardised at pH 7.5. After a period of 10 hours, the samples were filtered through a nylon membrane filter and the supernatant analysed for metals.

6.3.7 Analysis

Chlorophyll was extracted into acetone and quantified according to Lichtenhaler (1987). Dry weight determinations were performed by filtering aliquots of the micro-algal culture through a pre-weighed GF/A filter and drying overnight in an oven at 30°C. Micro-algal biomass samples were prepared for metals analysis by digestion in 200 µl 25% HNO_3. Samples were made up to a volume of 4 mL. Metals were analysed on a Varian Atomic Absorption Spectrophotometer. Sulphides were measured by the Methylene Blue method according to Standard Methods (APHA, 1989). TOC was analysed on a Dohrmann 180 Total Organic Carbon Analyser.

6.4 RESULTS AND DISCUSSION

6.4.1 Metal removal by sulphide precipitation

The sulphide-rich liquors from sulphate reducing digesters fed tannery effluent and algal biomass, were assessed for their metal removal capacity. Comparisons were made with a synthetic sulphide solution.

6.4.1.1 Tannery-fed digester liquors

The sulphide-rich digester overflow was mixed with a synthetic mine water solution and the amount of metal removed determined. It can be seen that levels of removal far in excess of that which would be anticipated for stoichiometric sulphide precipitation were obtained (Figure 6.1).

Similar results were obtained when effluent from Grootvlei Mine, was mixed with sulphide-rich liquor from the outflow of the tank digester. The percentage iron removal from the effluent and the binding stoichiometry are shown in Table 6.1.

Table 6.1 Dissolved sulphide concentration of liquor from tannery fed digester and iron concentrations in the AMD before and after addition of the sulphide-rich digester liquors.

% Digester liquor added (v/v)	Initial Fe (mmoles)	Initial sulphide (mmoles)	Final Fe (mmoles)	Fe removed (mmoles)	% removal	Ratio initial sulphide:Fe
75502510	0.012	0.43	0.008	0.012	1.0e+10	34.84
	0.028	0.28		0.028		10.14
	0.058	0.10		0.058		1.71
	0.096	0.05		0.087		0.59

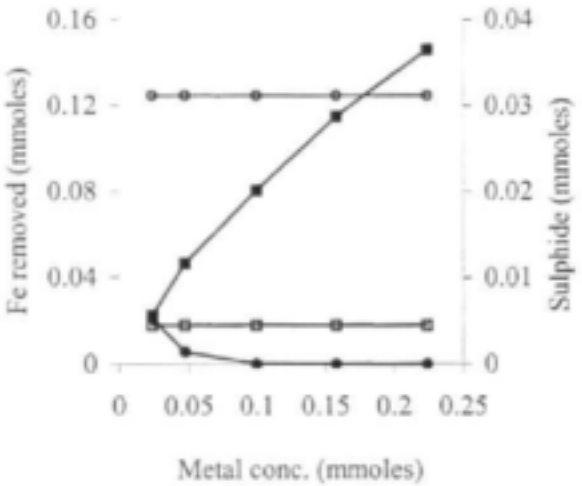


Figure 6.1. Iron removal from a synthetic mine water solution with the addition of sulphide-rich liquor from a sulphate reducing reactor treating tannery effluent. Dissolved sulphide before addition of iron (▫), dissolved sulphide after addition of iron (•), anticipated stoichiometric removal (▫), total removal obtained in the experiment (▪).

The pH of the final solution, after addition of the digester overflow, was buffered to pH 6 by the bicarbonates present in the digester overflow liquor.

In the presence of hydrogen sulphide, insoluble ferric minerals are reduced to the ferrous state. These then react immediately with further hydrogen sulphide to form a ferrous sulphide precipitate (Widdel, 1988). This occurs via the following reactions:

$$2Fe^{3+} + H_2S \rightarrow S^0 + 2Fe^{2+} + 2H^+ \quad (2)$$

$$2Fe^{2+} + H_2S \rightarrow FeS + 2H^+ \quad (3)$$

A metallic precipitate is formed when the product of the concentration of free metal ions and sulphide ions exceeds the solubility product (Ksp), which for iron is 4.2×10^{-17} (Moeller *et al.*, 1989).

In Table 6.1 it can be seen that in most cases the sulphide levels exceeded those needed for stoichiometric iron sulphide precipitation. This would account for the high levels of iron removal obtained. When sulphide levels did not exceed those needed for stoichiometric metal removal, such as in the 10%v/v solution, complete removal was

not obtained. However, removal exceeded levels anticipated from stoichiometric metal sulphide precipitation by a factor of 1.6 ie. 92% removal obtained compared to the 59% removal expected.

6.4.1.2 Algal biomass-fed digester liquors

The sulphide-rich overflow from a digester fed algal biomass was mixed with synthetic solutions of zinc, copper and iron. Synthetic metal solutions were used instead of AMD as the distance over which the AMD would have to be transported to the bioreactors was great, and would have resulted in the oxidation of the iron in the AMD, and its subsequent precipitation from solution.

The percentage removals obtained are shown in Figure 6.2. Iron removal was the highest, with between 96 and 100% removal being obtained for all levels of iron (Figure 6.2A). The percentage metal removed also increased with increasing metal levels. The opposite trend was found with copper (Figure 6.2B) and zinc (Figure 6.2C), although copper removal was much more efficient than that of zinc.

The actual amount of metal removed was also much higher for iron than for copper and zinc (Figure 6.3).

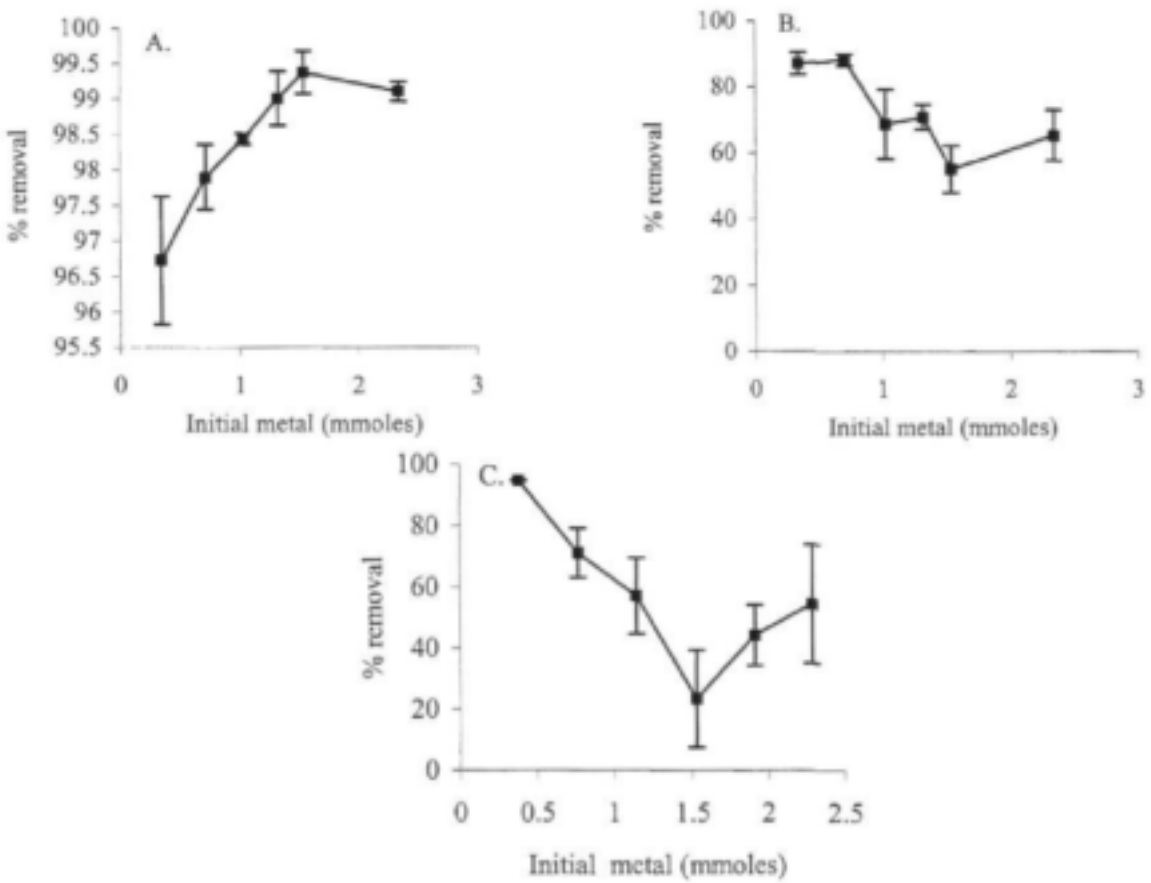
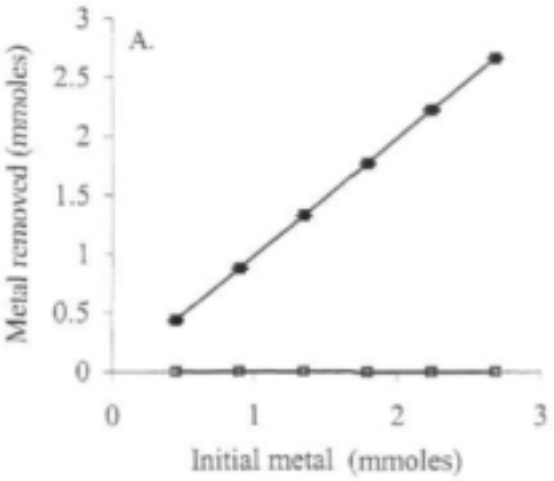
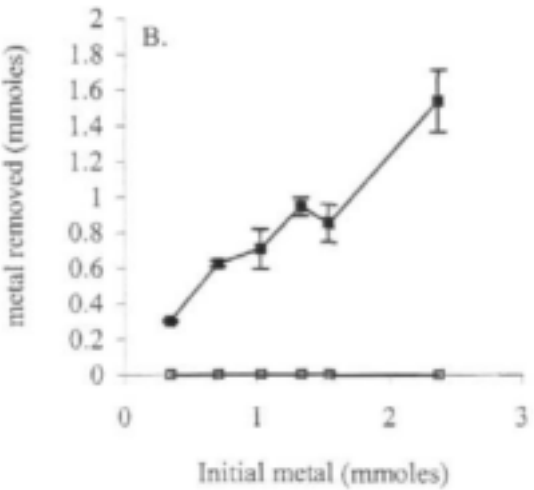


Figure 6.2. Percentage removal of A) iron, B) copper and C) zinc from solution by the addition of sulphide-rich liquor from a sulphate reducing reactor fed algal biomass.





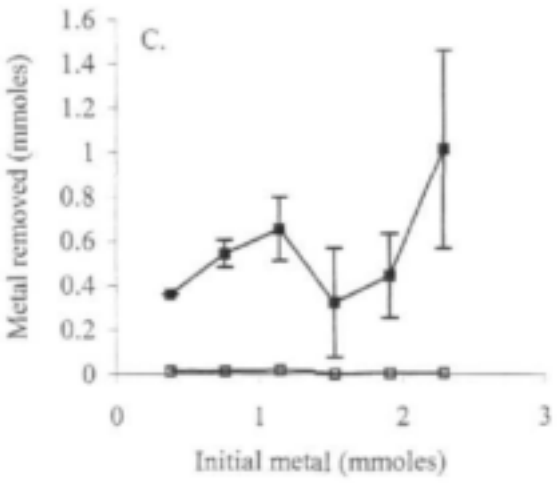


Figure 6.3. Removal of A) iron, B) copper and C) zinc from solution by the addition of sulphide-rich liquor from a sulphate reducing reactor fed algal biomass compared to controls (open square).

In all three cases, the amount of metal removed increased with increasing initial metal levels. The levels of metal removal obtained were also far in excess of those expected purely from stoichiometric sulphide precipitation, with achieved removals substantially higher than anticipated values, depending on the initial metal levels.

The above results indicate that factors other than metal sulphide formation alone plays some role in metal removal using an organics/sulphide precipitant. Two mechanisms have been identified as being responsible for heavy metal removal under anaerobic conditions. One is the interaction of the metal with hydrogen sulphide/sulphide system and the other the interaction with the carbon dioxide/carbonate system (Rivera, 1983). For every 1 mol of sulphate reduced biologically, 2 moles of HCO_3^- are produced, which combine with protons. This occurs via the following reactions:

$$SO_4^{2-} + 2CH_2O \rightarrow H^+ + HS^- + 2HCO_3^- \quad (4)$$
(Machemer and Wildeman, 1992)

$$HCO_3^- + H^+ \rightarrow H_2CO_3 \quad (5)$$
(Singh, 1992)

6.4.1.3 Synthetic sulphide solution

The addition of a pure solution of sodium sulphide to the mine water yielded similar results to that obtained with the liquor from the sulphidic tannery effluent digester, with approximately 1.5 times more metal removed than expected (Figure 6.4). This suggested that alkalinity generated during sulphate reduction is not responsible for the excess removal. Machemer and Wildeman (1992) also found that pH was not as important as sulphide precipitation in removing metals from solution. It is thought that this is due to the reducing conditions of the system and high metal solubilities (Stumm and Morgan, 1981).

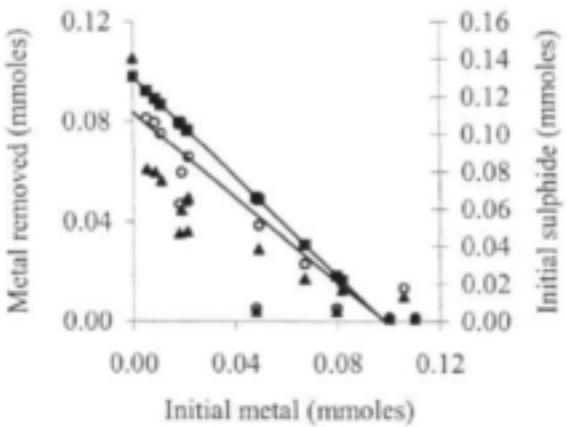


Figure 6.4. Iron removal from AMD by addition of a synthetic sulphide solution. Metal removal obtained in the experiment (▪), anticipated stoichiometric metal removal (○), initial sulphide levels before addition of AMD(▲).

Another factor playing a role may be the co-complexation of metal ions with particulate organic matter remaining in the digester effluent. Rivera (1983) had proposed a role for biosorption and the digester effluents used in these studies contained either tannery waste particulates or partly digested micro-algal cell components.

6.4.2 Metal removal by algal biomass

A number of metals other than iron are present in AMD. Some of these, such as dichromate, do not readily form sulphide complexes. Oxyanions such as selenium also do not form metal sulphide complexes, and thus an alternative method of removal is required.

D. salina and *Spirulina* sp. in BAAM and Zarrouk's medium respectively, were spiked with $Cr_2O_7^{2-}$ and Se^{2-} ions, as well as Cu^{2+} and Pb^{2+} ions, as an example of two metals which do readily form metal sulphides. The amount of metal or oxyanion associated with the algal biomass was determined. The algal biomass was repeatedly desorbed and exposed to the metal or oxyanion solution to determine the re-use potential of the algal biomass. The total percentage removal, after a single exposure of the algal biomass to the metal or oxyanion solution, is shown in Table 6.2. In terms of percentage metal removal, *D. salina* was more efficient at the lower Cu^{2+} levels than *Spirulina* sp., with 91.3% removal as compared to 63.8% removal by *Spirulina* sp. (Table 6.2). However, at the higher Cu^{2+} levels similar removal efficiencies were obtained by both species of micro-algae.

It can be seen in Figures 6.5 and 6.6, that *Spirulina* spp. removed more Cu^{2+} per µg chlorophyll *a* than did *D. salina*.

Table 6.2. Percentage metal removal from solution by algal biomass after being exposed once to the metal or oxyanion solution. The actual amount of metal (mmoles) removed is shown in brackets.

Metal	Initial levels. (mmoles)	% removal	
		Dunaliella	*Spirulina*
Cu^{2+}	0.6	91.3 (0.5)	63.8 (0.4)
Cu^{2+}	6.0	30.5 (1.8)	28.3(1.7)
Pb^{2+}	0.03	38.0 (0.01)	35.0(0.01)
Pb^{2+}	0.3	10.0 (0.03)	0(0)
$Cr_2O_7^{2-}$	0.6	4.5 (0.3)	4.8(0.03)
$Cr_2O_7^{2-}$	6.0	39.3 (2.4)	56.6(3.4)
Se^{2-}	0.09	2.7 (0.01)	1.8(0.01)
Se^{2-}	0.9	30.0(0.3)	7.8(0.07)

Thus it would appear that the removal of copper is concentration dependant, with more metal being removed at a higher external metal ion level. Literature states that at low metal levels the mass of metal ions accumulated is directly proportional to the amount of the metal ion in solution (Ting *et al.*, 1989; Ting *et al.*, 1991). This is further supported by an increase in the amount of Cu^{2+} removed by *Spirulina* sp. after a second exposure to a 6 mmole Cu^{2+} solution (Figure 6.5). However, no significant increase in the amount of Cu^{2+} associated with the algal biomass was observed in *D.*

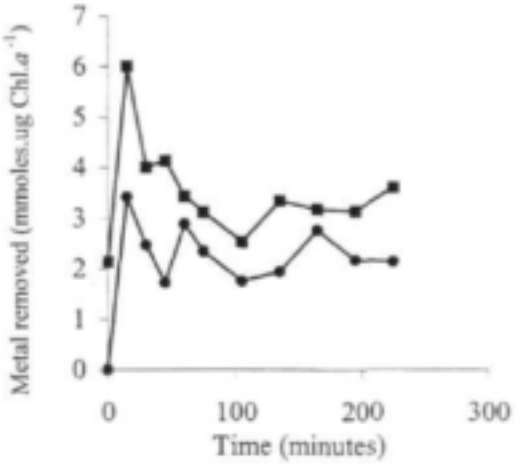


Figure 6.5. Removal of copper from solution by *Spirulina* sp. during consecutive exposure to solutions containing 6 mmoles Cu^{2+}. 1st exposure (•), 2nd exposure (▪).

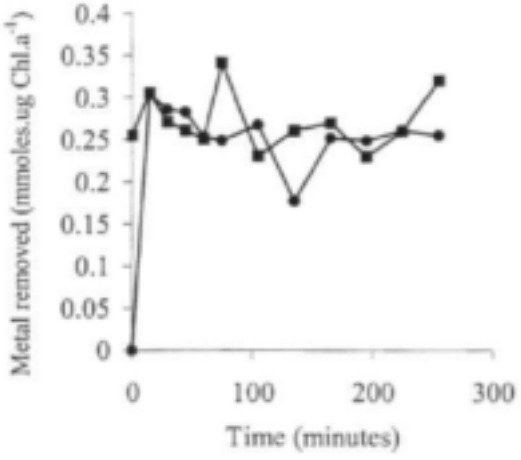


Figure 6.6. Removal of copper from solution by *D. salina* during consecutive exposure to solutions containing 6 mmoles Cu^{2+}. 1st exposure (•), 2nd exposure (▪).

salina after a second exposure to a 6 mmole Cu^{2+} solution (Figure 6.6).

The opposite result was obtained with lead, with the amount of Pb^{2+} ions associated with *D. salina* biomass increasing after the second exposure (Figure 6.7). However, when exposing *Spirulina* sp. for the second time, the amount of biomass-associated Pb^{2+} remained about the same (Figure 6.8). There was also little difference between the amount of Pb^{2+} removed at high and low levels by *Spirulina*, thus indicating that the Pb^{2+} removal process, be it precipitation or binding to the cell wall, has a threshold limit. In relation to binding this can be explained in terms of a fixed cell biomass offering a finite number of binding sites, which upon reaching saturation, do not allow for any further binding.

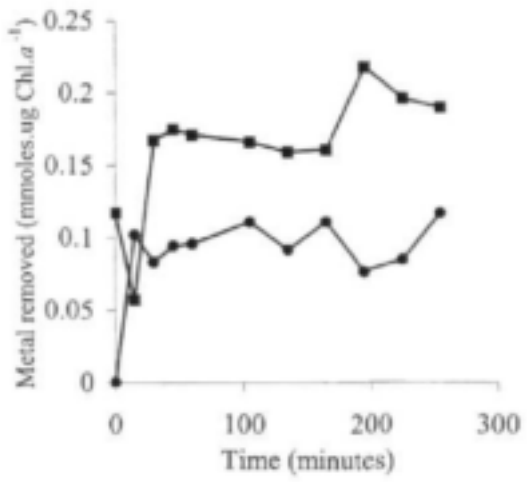


Figure 6.7. Removal of lead from solution by *D. salina* during consecutive exposure to solutions containing 0.3 mmoles Pb^{2+}. 1st exposure (•), 2nd exposure (▪).

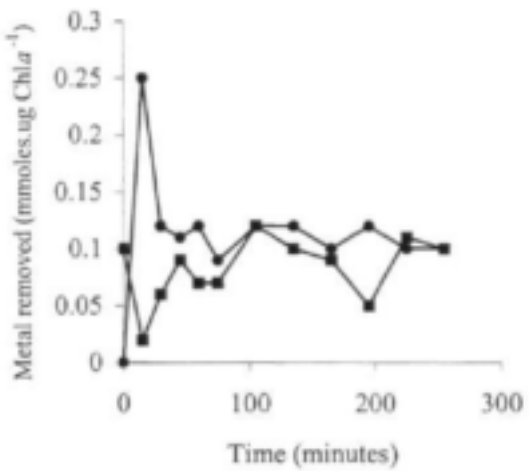


Figure 6.8. Removal of lead from solution by *Spirulina* sp. during consecutive exposure to solutions containing 0.3 mmoles Pb^{2+}. 1st exposure (•), 2nd exposure (▪).

On the other hand, more Pb^{2+} was removed when the biomass was exposed to the 0.3 mmole lead solution than the 0.03 mmole solution (Table 6.2).

Chromium removal appears to be concentration dependant, with more metal being removed from the 6.0 mmole solution than the 0.6 mmole solution (Table 6.2). However, in the case of *D. salina*, there is little difference between the amount of biomass-associated $Cr2O_7^{2-}$ after the first and second exposure (Figure 6.9) to a 6 mmole $Cr_2O_7^{2-}$ solution. In *Spirulina* sp. however, the levels of biomass associated $Cr_2O_7^{2-}$ do tend to increase after the second exposure (Figure 6.10).

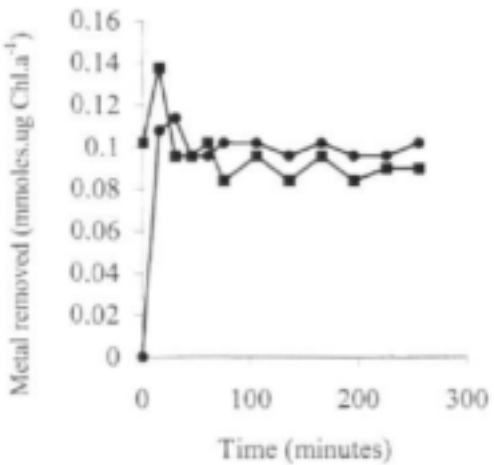


Figure 6.9. Removal of dichromate from solution by *D. salina* during consecutive exposure to solutions containing 6.0 mmoles $Cr_2O_7^{2-}$. 1st exposure (•), 2nd exposure (▪).

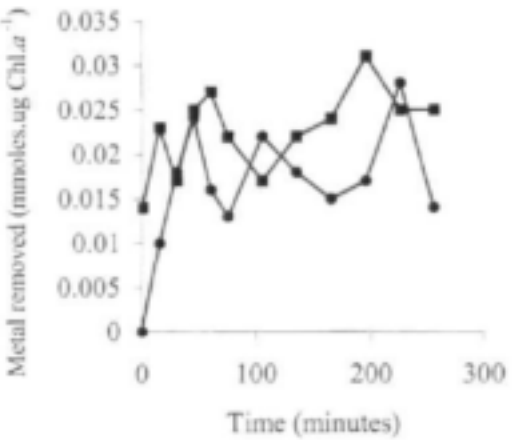


Figure 6.10. Removal of dichromate from solution by *Spirulina* sp. during consecutive exposure to solutions containing 6.0 mmoles $Cr_2O_7^{2-}$. 1st exposure (•), 2nd exposure (▪).

Cr (VI) ions were obtained by diluting $K_2Cr_2O_7$ such that the dichromate (Cr_2O_7) would carry an overall charge of -2. This would not allow for binding with the anionic groups on the algal cell surface. Thus it can be speculated that the majority of uptake can be attributed to an active transport across the cell membrane, related to the metabolic activities of the cells, or due to metal precipitation. Saraiva and Frazier (1975) were unable to demonstrate any active mode of radio labelled chromium absorption, with only 1.4% of the total radioactivity being detected in the algal cells. However, chromate (VI) can easily cross cell membranes as the phosphate-sulphate carrier in the cell membrane also transports chromate anions (Gauglhofer, 1990).

D. salina was found to remove more selenium than *Spirulina* sp. (Table.6.2). There was not much increase in the amount of biomass-associated selenium between the first and second exposure to a 0.9 mmole Se^{2-} solution (Figure 6.11). Selenium is also in the anionic form and thus will not bind to the cell wall. However, from Table 6.2, it can be see that more selenium was removed from the 0.9 mmole Se^{2-} solution than from the 0.09 mmole solution. Gerhardt and Oswald (1990b) reported that no direct algal uptake of selenium occurs.

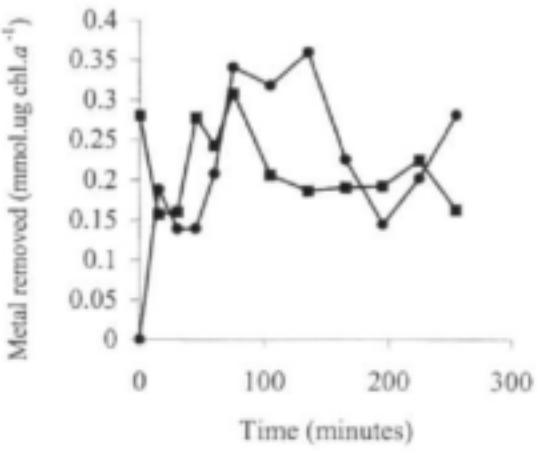


Figure 6.11. Removal of selenium from solution by *D. salina* during consecutive exposure to solutions containing 0.9 mmoles Se^{2-}. 1st exposure (•), 2nd exposure (▪).

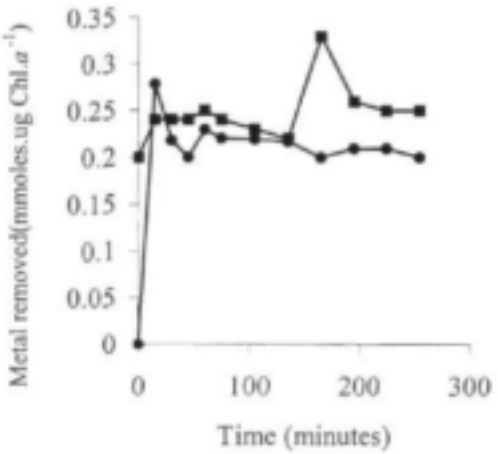


Figure 6.12. Removal of selenium by *Spirulina* spp. during consecutive exposure to solutions containing 0.9 mmole Se^{2-}. 1st exposure (•), 2nd exposure (▪).

T

The results for the *D.salina* and *Spirulina* sp. biomass studies on selenium were broadly comparable.

6.4.3 Metal toxicity effects

The toxicity of copper and its effect on photosynthesis was assessed by growing both *D. salina* and *Spirulina* sp. in medium containing 0.6 mmoles and 6 mmoles Cu^{2+}. As can be seen in Figures 6.13 and 6.14, *Spirulina* sp. is more tolerant to 6 mmoles Cu^{2+} than *D. salina*, with cultures able to sustain growth. However, in all cases the chlorophyll *a* levels were much lower than that of the control cultures.

Examination of the *D. salina* cells under the microscope after exposure to 6 mmole copper showed that the cells had lysed (Figure 6.15). This in turn may explain why no increase in biomass-associated Cu^{2+} was noted after a second exposure of *D. salina* biomass to a 6.0 mmole Cu^{2+} solution. Copper is know to have toxic effects on algae and $CuSO_4$ is used as an algicide due to its ability to disturb cell functions (De Haan *et al.*, 1981). This toxicity is mainly due to the excessive binding of copper to the cells, resulting in their increased permeability and a resultant release of potassium. Copper has also been shown to be more toxic intracellularly than other heavy metals (Davies, 1983) as it binds to metabolically active sites (Overnell, 1975; Davies, 1983), and interferes with pigment biosynthesis as well as inhibiting the photosynthetic electron transport chain (Baron *et al.*, 1995).

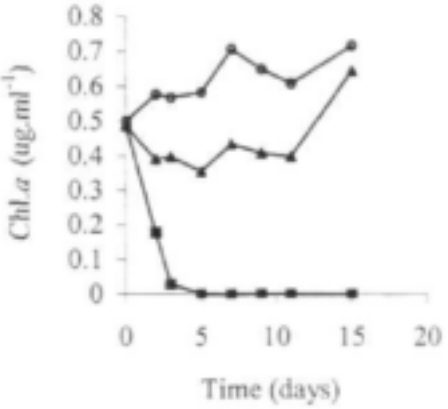


Figure 6.13. Growth of *D.salina* in the presence of 6.0 mmoles (▪) and 0.6 mmoles (▲) Cu^{2+} in relation to a control (○) which did not contain Cu.

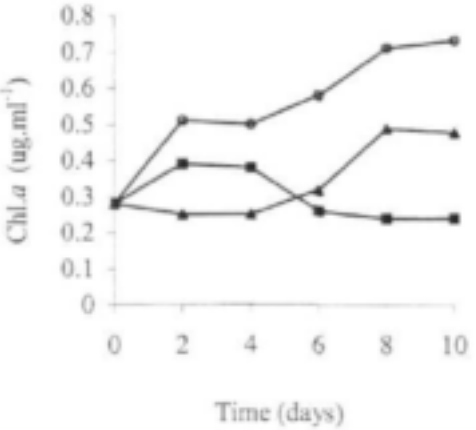


Figure 6.14. Growth of *Spirulina* sp. in the presence of 6 mmoles (▪) and 0.6 mmoles (▲) Cu^{2+} in relation to a control (○) which did not contain Cu.

Lead did not appear to have an effect on the growth of *D. salina* (Figure 6.15) or *Spirulina* sp. (Figure 6.16), with there being little difference between the chlorophyll *a* content of the experimental and control cultures.

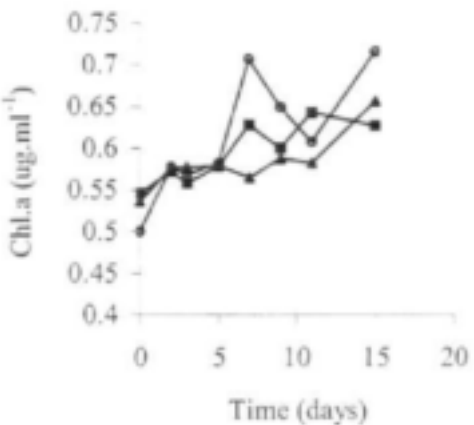


Figure 6.15. Growth of *D. salina* in the presence of 0.3 mmoles (▪) and 0.03 mmoles (▲) Pb^{2+} in comparison to a control (○) grown in the absence of Pb^{2+}.

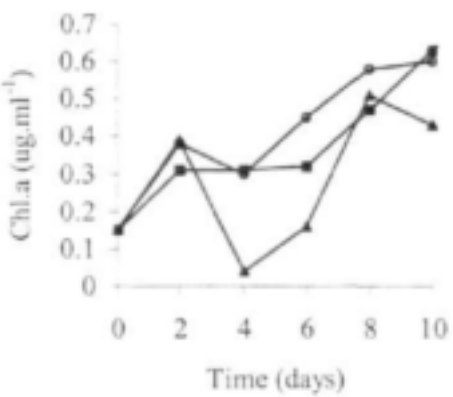


Figure 6.16. Growth of *Spirulina* sp. in the presence of 0.3 mmoles (▪) and 0.03 mmoles (▲) Pb^{2+} in comparison to a control (○) grown in the absence of Pb^{2+}

As stated by Pace *et al.* (1977) the effect of lead is more pronounced towards the end of the logarithmic phase of growth. Davies (1983) also showed that the chlorophyll content of populations of phytoplankton was the same in the presence of lead as in its absence, as did Overnell (1975) for *Dunaliella tertiolecta*. However, Hollibaugh *et al.* (1980) found that lead caused a decrease in chlorophyll production at a concentration of 1 µM. This is true for *Spirulina* sp., with a marked difference

between the chlorophyll *a* content of the control and experimental cultures. However, it is interesting to note that the cultures exposed to 0.3 mmole Pb^{2+} had higher chlorophyll *a* levels than those exposed to 0.03 mmole Pb^{2+} (Figure 6.16). Lead in the acetate form has previously been found to be stimulatory to cultures of *Chlamydomonas* (Hutchinson, 1973).

Chromium also appears to be very toxic to both *D. salina* (Figure 6.17) and *Spirulina* sp. (Figure 6.18), with the chlorophyll *a* levels of the experimental cultures being much lower than that of the control cultures. However, in comparison to *Spirulina* sp., *D. salina* appears to show more tolerance to the lower concentration of dichromate.

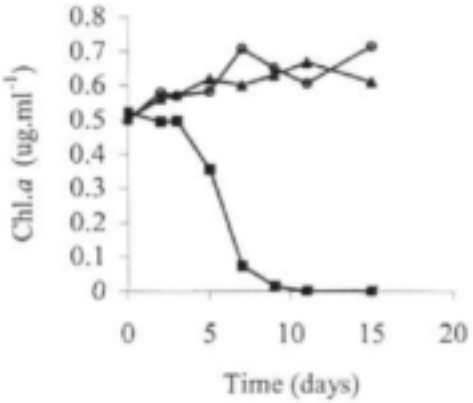


Figure 6.17. Growth of *D. salina* in the presence of 6 mmoles (▪) and 0.6 mmoles (▲) $Cr_2O_7^{2-}$ in comparison to a control (○) grown in the absence of $Cr_2O_7^{2-}$.

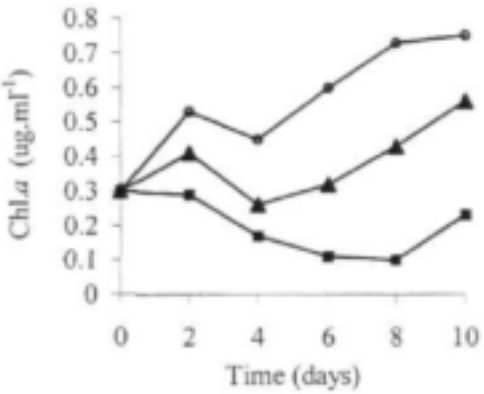


Figure 6.18. Growth of *Spirulina* sp. in the presence of 6 mmoles (▪) and 0.6 mmoles (▲) $Cr_2O_7^{2-}$ in comparison to a control (○) grown in the absence of $Cr_2O_7^{2-}$

Selenium also appears to have a slightly toxic effect on both *D. salina* (Figure 6.19) and *Spirulina* sp. (Figure 6.20), although more apparent in *D. salina*.

Gerhardt and Oswald (1990a,b) reported the growth of *Dunaliella* in HRAP in the presence of 30 $\mu g.L^{-1}$ selenium, while Gerhardt *et al.* (1994) reported that selenium levels of 330 $\mu g.L^{-1}$ did not inhibit algal growth. However, the levels used in this study were much higher. Shrift (1961) found that selenomethionine interfered with cell division of the alga *Chlorella* leading to the formation of giant cells. A natural selenium cycle has also been well documented with uptake of selenium by microorganisms and plants, and subsequent transformation into various inorganic and organic compounds (Besser *et al.*, 1989).

6.4.4 Metal removal by binding to extracellular organic matter

Considering the toxicity demonstrated by the metals such as copper and chromium to the growth of the algae, the use of a continuous direct contact system, in which the AMD is fed into a HRAP, may not be feasible, especially if these metals occur in high concentrations. It is against this background that the metal binding potential of

the extracellular organic matter produced by the micro-algae was investigated. This would allow separation of the growth of the micro-algae from metal removal, as purely the organic carbon-rich medium would be in contact with the metal-rich effluent.

The organic carbon-rich media used in these metal removal studies was produced by *D. salina* and *Spirulina* spp. under conditions of high temperature and low light. Light microscope studies of the algal cells revealed the presence of gelatinous-like matter surrounding cells, and in some cases holding the cells together. Staining with Ruthenium Red, a cationic dye, showed this matter to be acidic in nature and thus amenable for metal binding (micrographs not shown).

Work done by Giordano *et al.* (1994) showed the presence of a cationic periplasmic coat surrounding cells of *D. salina* grown under conditions of low CO_2 or in the presence of NH_4^+. Huntsman (1972) also found that 27% of the organic carbon excreted by senescent cells of *Dunaliella tertiolecta* was cationic in composition. However, very little work has been done to characterise the organic component or evaluate its potential for metal removal. A water soluble extracellular polysaccharide produced by *Spirulina platensis*, calcium spirulan (Ca-SP) has also been shown to be sulphated and thus may be useful for metal removal.

Figure 6.21. shows the amount of copper removed from solution by addition of extracellular organic carbon produced by *D. salina* grown under stress conditions. Under the conditions used in this experiment, metal removal by binding to the organic matter decreases with increasing pH. The amount of copper removed by chemical precipitation in turn increases with increasing pH.

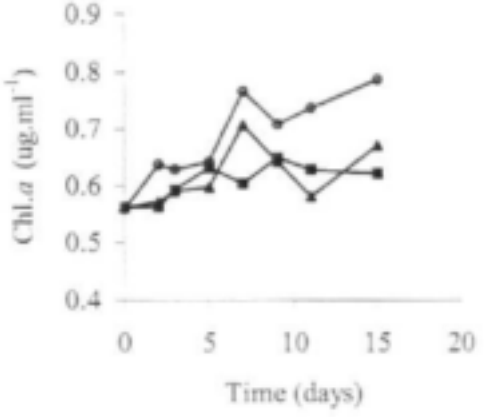


Figure 6.19. Growth of *D. salina* in the presence of 0.9 mmoles (▪) and 0.09 mmoles (▲) Se^{2-} as compared to a control (○) grown in the absence of Se^{2-}.

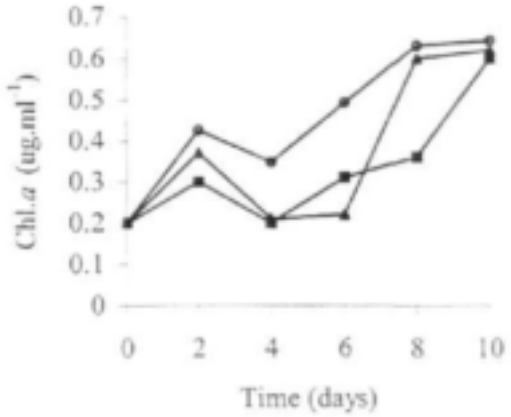


Figure 6.20. Growth of *Spirulina* sp. in the presence of 0.9 mmoles (▪) and 0.09 mmole (▲) Se^{2-} as compared to a control (○) grown in the absence of Se^{2-}.

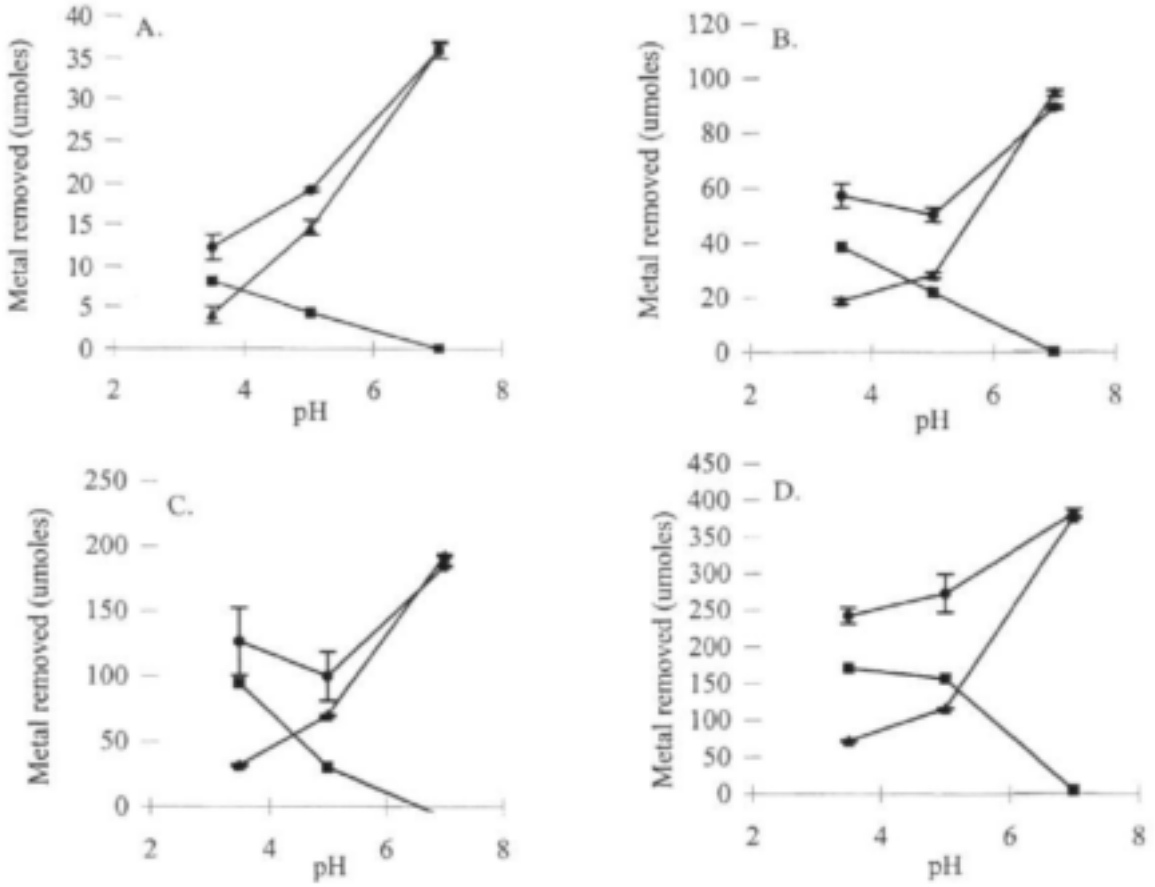


Figure 6.21. Removal of copper from solutions containing A) 40 µmoles B) 100 µmoles, C) 200 µmoles and D) 500 µmoles Cu^{2+} at a pH of 3.5, 5 and 7. Removal obtained in the experiment (•), removal due to precipitation (▲), removal due to binding to organic matter produced by *D. salina* (■).

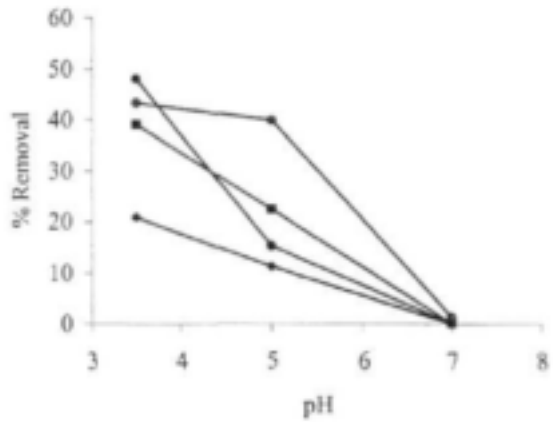


Figure 6.22. Percentage Cu^{2+} removal from solutions containing 40 µmoles (♦), 100 µmoles (■), 200 µmoles (•) and 395 µmoles (○) Cu^{2+} by binding to extracellular organic matter produced by *D. salina* under stress conditions.

The percentage Cu^{2+} removal obtained ranged between 20 and 50%, with higher removal efficiency obtained at the higher initial metal concentrations (Figure 6.22). There is also a linear relationship between pH and percentage removal, especially at the lower copper concentrations. The relationship between total Fe^{2+} removal, removal due to chemical precipitation and removal due to binding to extracellular organic matter is shown in Figure 6.23. As was the case with copper, chemical precipitation increases as the pH increases.

The percentage removal of iron by binding to extracellular organic matter produced by *D. salina*, on the other hand, does not show a linear relationship, with the highest percentage removal being obtained at pH 5. There is also an inverse relationship between percentage removal and initial metal concentration, with the highest removal obtained at the lowest concentration Fe^{2+} solutions (Figure 6.24).

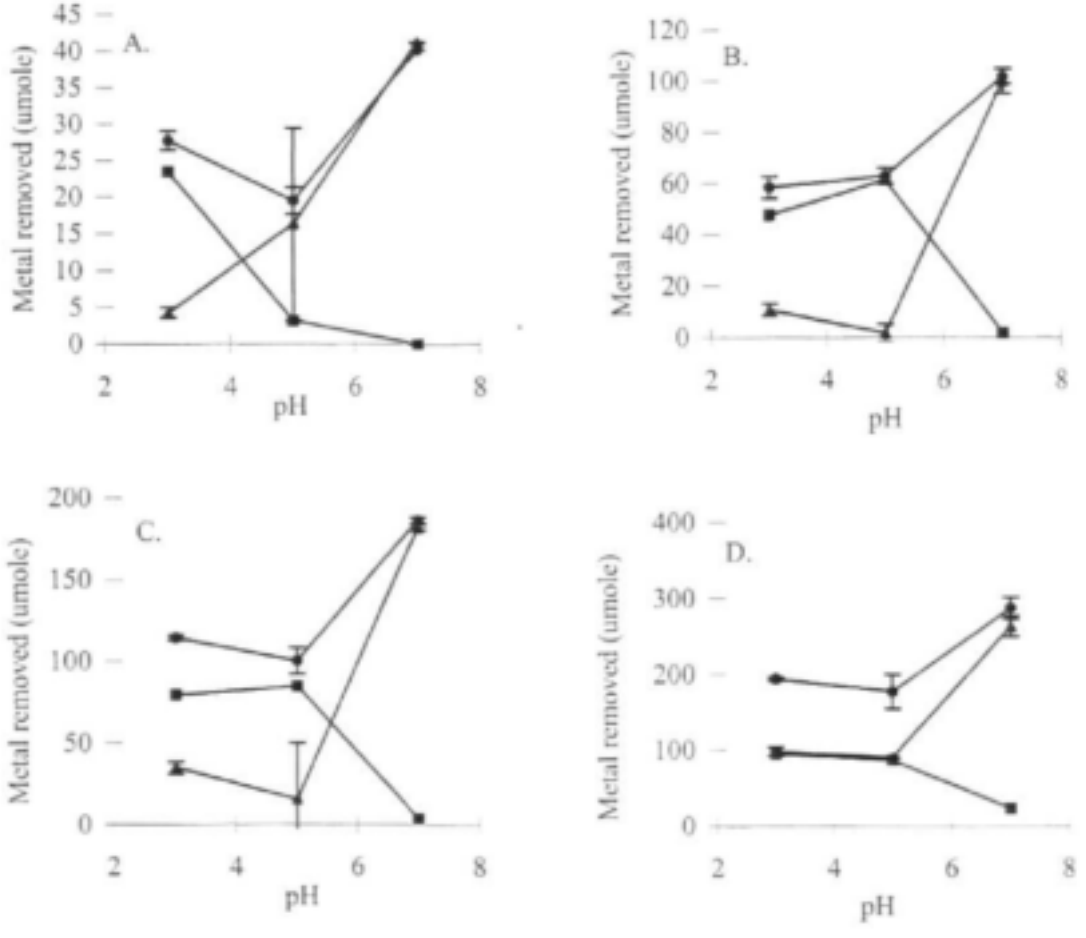


Figure 6.23. Removal of ferrous iron from solutions containing A) 45 µmoles, B) 112 µmoles, C) 225 µmoles and D) 450 µmoles Fe^{2+} at a pH of 3.5, 5 and 7. Removal obtained in the experiment (•), removal due to precipitation (▲), removal due to binding to organic matter produced by *D. salina* (■).

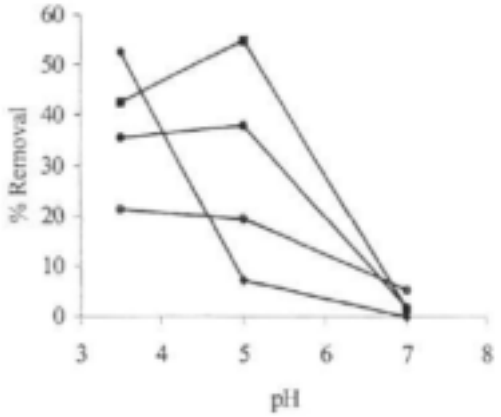


Figure 6.24. Percentage Fe^{2+} removal from solutions containing 45 µmoles (○), 112 µmoles (▪), 225 µmoles (•) and 450 µmoles (♦) Fe^{2+} by binding to extracellular organic matter produced by *D. salina* under stress conditions.

The removal of copper by binding to organic matter produced by *Spirulina* sp. shows similar results to that obtained with *D. salina* organic matter. The percentage removal of copper ranges from 0 to 50% with the lowest removals at the highest pH values (Figure 6.25). The highest removal by binding to organic matter was also shown to occur in the metal solution with the highest concentration.

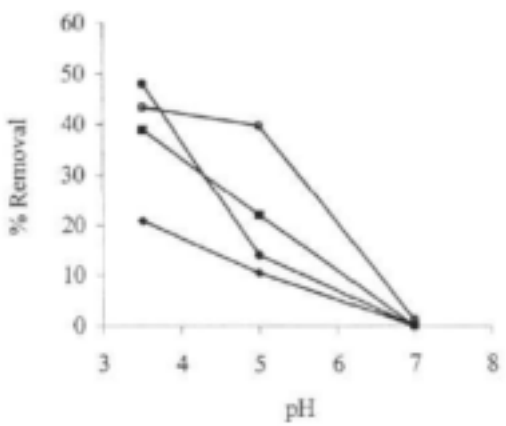


Figure 6.25. Percentage Cu^{2+} removal from solutions containing 40 µmoles (♦), 100 µmoles (▪), 200 µmoles (•) and 395 µmoles (○) Cu^{2+} by binding to extracellular organic matter produced by *Spirulina* sp. under stress conditions.

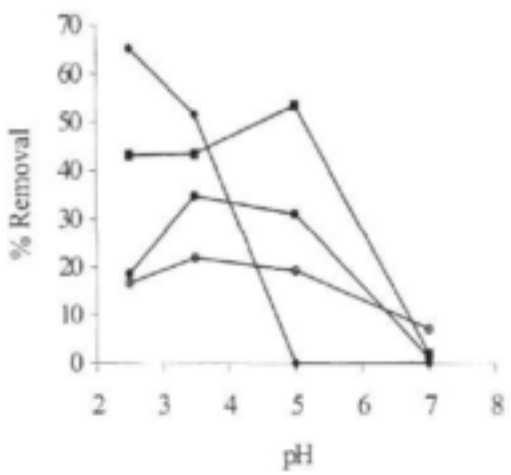


Figure 6.26. Percentage Fe^{2+} removal from solutions containing 45 µmoles (♦), 112 µmoles (▪), 225 µmoles (•) and 450 µmoles (○) Fe^{2+} by binding to extracellular organic matter produced by *Spirulina* sp. under stress conditions.

Iron removal by binding to organic carbon produced by *Spirulina* sp. did not show similar results (Figure 6.26).

At the lowest Fe^{2+} levels, most removal was shown at a pH of 2.5. At pH 5 or above, no removal by binding was shown. At the higher Fe concentrations the highest removal was obtained between pH 3 and 5, with no removal by binding occurring at pH 7 (Figure 6.26).

The relationship between total copper and iron removed and removal due to binding and precipitation are shown in Figures 6.27 and 6.28 respectively.

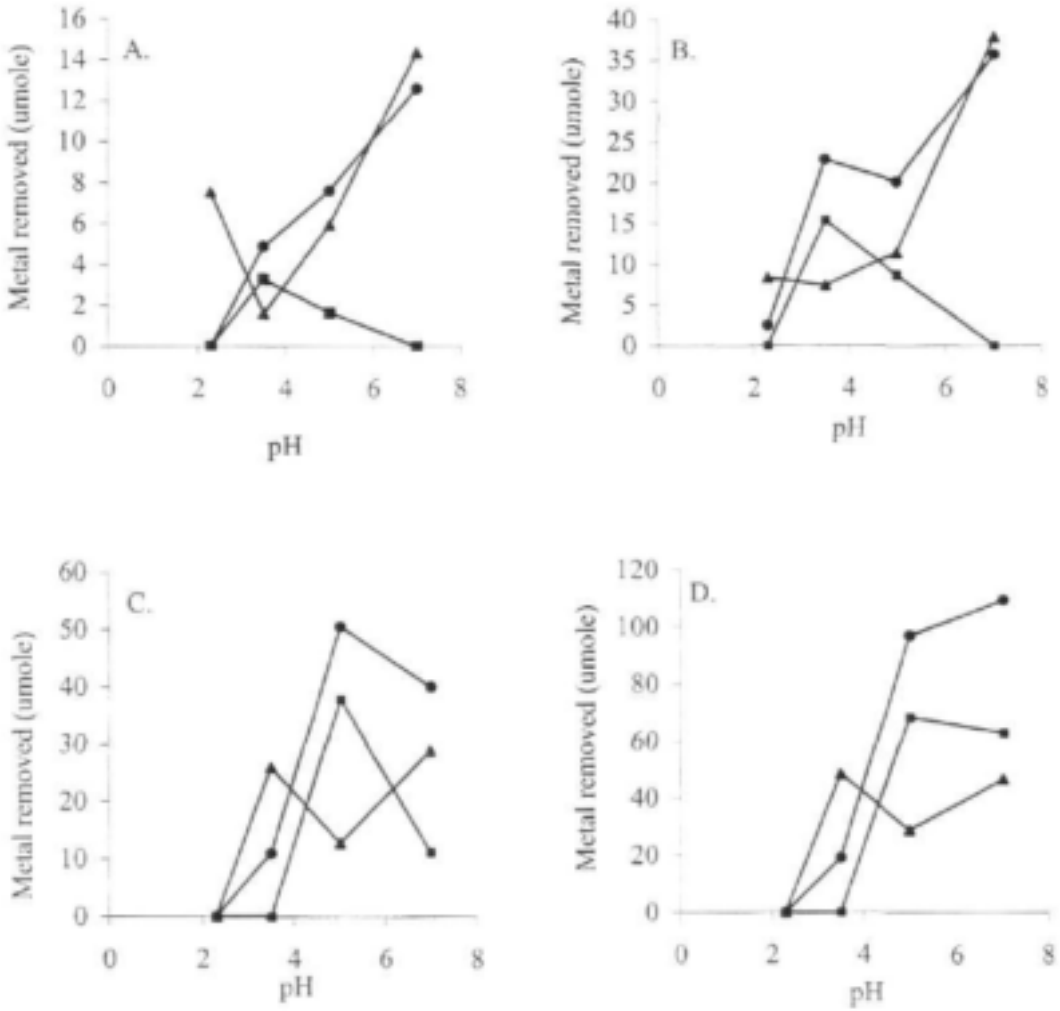


Figure 6.27. Removal of copper from solutions containing A) 16 µmoles, B) 40 µmoles, C) 80 µmoles and D) 160 µmoles Cu^{2+} at a pH of 2.5, 3.5, and 7. Removal obtained in the experiment (•), removal due to precipitation (•), removal due to binding to organic matter produced by *Spirulina* sp. (▪).

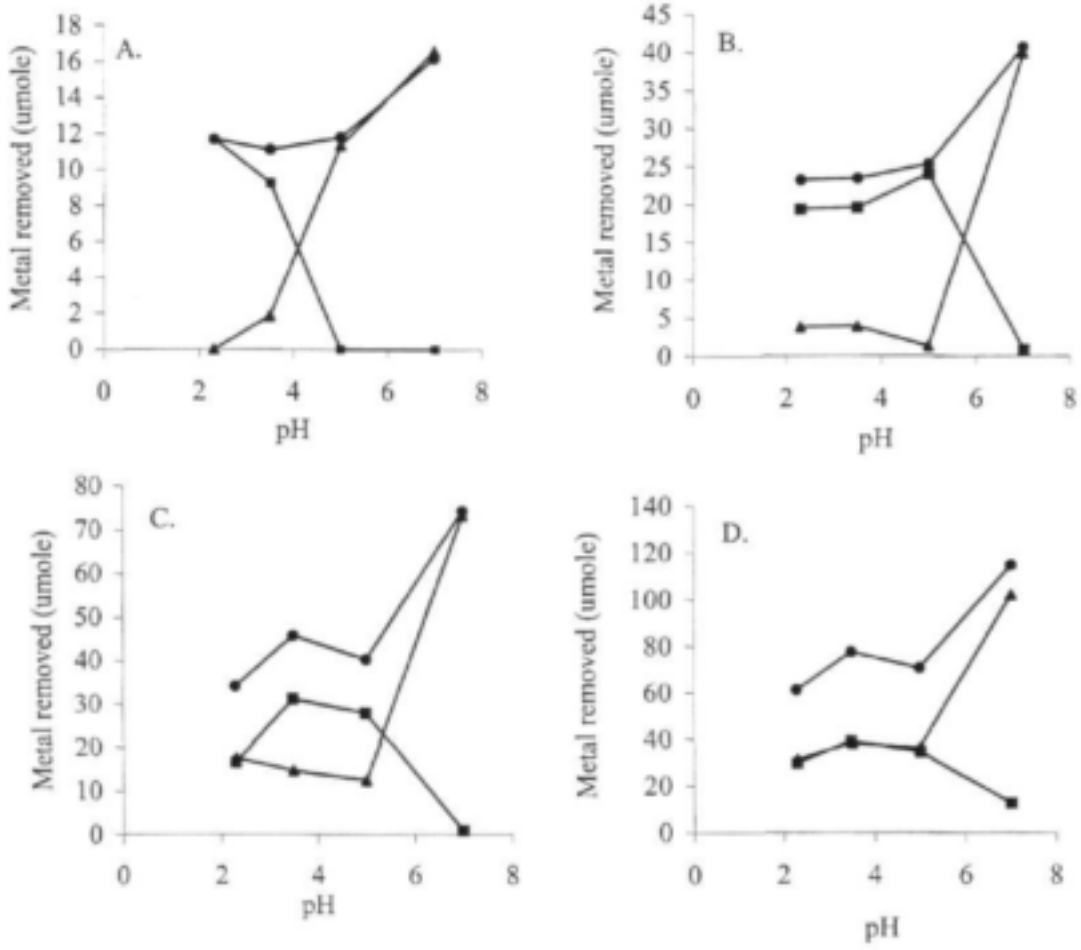


Figure 6.28 Removal of iron from solutions containing A) 18 µmoles, B) 45 µmoles, C) 90 µmoles and d) 180 µmoles Fe^{2+} at pH 3.5, 5 and 7. Removal obtained in the experiment (•), removal due to precipitation (▲), removal due to binding to organic matter produced by *Spirulina* sp. (■).

The binding of metals to organics has also been found to be pH dependent by other researchers (Farrah and Pickering, 1978). An increase in pH was found to increase the uptake of Cu, Pb, Zn and Cd by humic acid (Beveridge and Pickering, 1980) and cellulose (Farrah and Pickering, 1978).

The pH of the media will affect the speciation of the metal ion in solution. This can be seen in Figure 6.29. For example, in seawater at pH 8.5, Cu occurs as $Cu(OH)_2$, whereas in acidic lake waters, it may occur as the divalent cation. As already noted conditions in high rate oxidation ponds will lead to the formation of hydroxide and carbonate ions, which together with the extracellular organic matter have the potential for metal removal by precipitation. The solubility of certain metal oxides and hydroxides is shown in Figure 6.30.

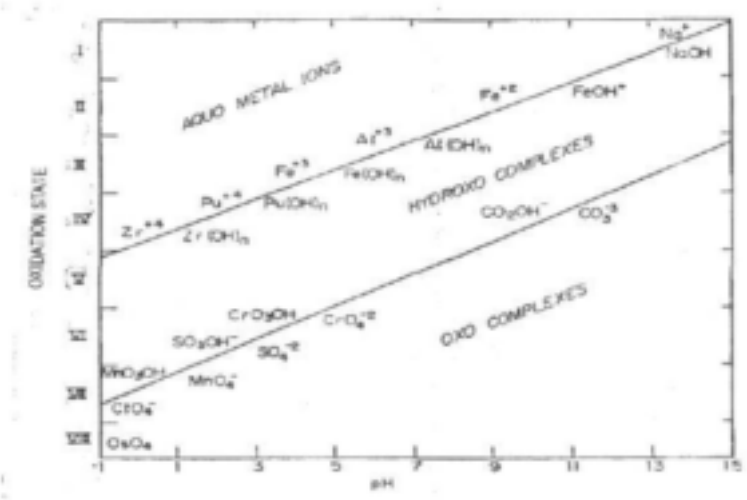


Figure 6.29. Predominant pH range for the occurrence of aquo, hydroxo, hydroxo-oxo and oxo complexes for various oxidation states (From Stumm and Morgan, 1981).

The limiting stability relations for iron is shown in Figure 6.30. This shows that at a pH below 3.5, Fe exists either as Fe^{2+} or Fe^{0}. Whereas at pH 5 and above, $FeCO_3$ and $Fe(OH)_3$ tend to form thus accounting for the higher removal attributed to binding to organic matter obtained at low pH values. Hydroxide ions have also been found to often have a stronger affinity for Fe^{3+} than organic or inorganic bases (Stumm and Morgan, 1981).

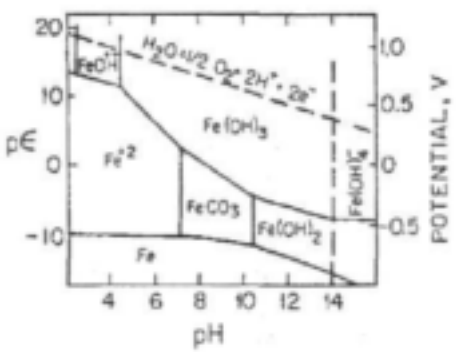


Figure 6.30. pe-pH diagram for iron (From Stumm and Morgan, 1981).

The level of organic carbon in solution was measured before and after addition of the metal solution. As expected it was found that the metal removal and organic carbon removal correlated. The higher the organic carbon removal, the higher the metal removal. Examples of this are shown in Figures 6.31 and 6.32. Irrespective of the metal concentration in solution, complete removal of the organic carbon from solution was not obtained. This suggests that not all of the organic carbon produced was acidic in nature and thus does not bind cations.

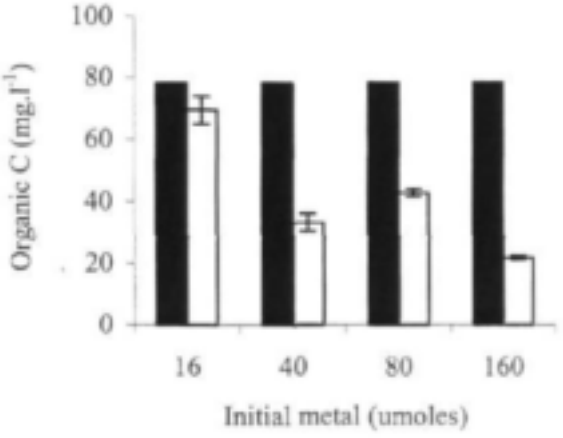


Figure 6.31. Levels of organic carbon in solution at pH 5 before (■) and after (□) addition of varying amounts of Cu^{2+} ions. The organic carbon was produced by *Spirulina* sp. grown under stress conditions.

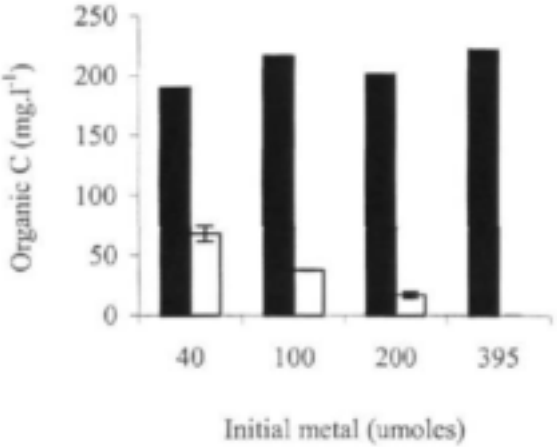


Figure 6.32. Levels of organic carbon in solution at pH 5 before (■) and after (□) addition varying amounts of Cu^{2+} ions. The organic carbon was produced by *D. salina* grown under stress conditions.

6.5 CONCLUSIONS

This study has demonstrated the operation of a number of metal removal mechanisms functioning in micro-algal systems, each of which could be integrated into a system for the remediation of metal-rich wastewaters. The use of sulphide-rich liquors from sulphate reducing anaerobic digesters to precipitate heavy metals from solution showed positive results, with metal removal being obtained in excess of that anticipated from stoichiometric sulphide precipitation. This was especially true when sulphide-rich liquor from a sulphate reducing digester fed micro-algal biomass was utilised, with substantially more removal being achieved than anticipated.

The removal of metals by micro-algal biomass, although not excellent, is quite capable of dealing with small amounts of metal ions, such as those in the overflow from the facultative pond. Metal removal was less effective when anions such as dichromate, and oxyanions such as selenium were used. However, unlike copper and dichromate, which proved to be highly toxic to both species of micro-algae, selenium and lead did not have much effect on the growth of *D. salina* and *Spirulina* sp., as shown by an increase in their chlorophyll *a* levels. The toxicity of the metals to algal growth demonstrated here could be a problem, especially in the operation of a continuous system, and biomass removal from HRAP 2 would be important.

Metal removal by binding to extracellular organic carbon produced by algae grown under stress conditions in HRAP 1 was shown to be very successful, especially at acidic pH values. Mechanisms for the removal of heavy metals from acidic effluents have not previously been reported, thus making this an important mechanism especially in cases where the pH of acidic effluents cannot be elevated by biological means.

Clearly a number of metal removal mechanisms are available in the integrated

ponding approach to AMD treatment, and these may be incorporated into the ASPAM system in a number of ways:

- In a simplified ponding system, or where large volumes of wastewater need to be treated, metal precipitation could be allowed to take place directly in the anaerobic digester;

- Alternatively, the sulphide-rich anaerobic digester liquors could be recycled and blended with the incoming AMD in a separate unit operation, to initiate neutralisation and metal precipitation outside of the anaerobic digester. A disposable metal sludge would be produced in this operation;

- Residual metals, following sulphide precipitation, would then pass to HRAP 1, with the metal removal mechanism in this system resulting from both binding to micro-algal cells as well as EPS produced by the micro-algal biomass. The micro-algal biomass and complexed EPS may then be removed by settling or, together with the bound metals, may be fed to the anaerobic digester, serving as a further carbon source for biological sulphate reduction. The dissociation of the metal from the micro-algal biomass and polymeric substances would take place in the anaerobic reactor, with the subsequent formation of metal sulphides *in situ*. The HRAP 2 would then serve as a final polishing step for the overflow from the facultative pond;

- The stress conditions in HRAP 1, receiving partly neutralised acidic waters from the metal precipitation step, may be manipulated to maximise EPS production by micro-algal biomass produced either in HRAP 1 or fed back from HRAP 2. The results reported here show that improved production of EPS would contribute to both the precipitation operation and adsorption functions in HRAP 1;

- The carbonate-rich alkaline water, produced as a result of the pH elevation in HRAP 1, could be used to neutralise the incoming AMD, also thereby contributing to heavy metal removal prior to its discharge into an anaerobic digester.

7 NEUTRALISATION OF ACID MINE DRAINAGE IN THE ASPAM SYSTEM

7.1 INTRODUCTION

Both the cyanobacteria and eucaryotic micro-algae possess mechanisms for actively acquiring inorganic carbon from the external medium, which leads to the elevation of the pH of the surrounding environment (Borowitzka, 1982). This mechanism elevates the CO_2 concentration around the active site of the primary photosynthetic carboxylating enzyme, ribulose biphosphate (Rubisco) (Badger and Price, 1992), to levels higher than can be obtained by simple diffusion, thus enabling micro-algae to utilise both CO_2 and HCO_3^- as carbon substrates, and to grow at very low CO_2 concentrations (Ramazanov *et al.*, 1996). In the case of cyanobacteria, in alkaline conditions it is HCO_3^- transport that largely serves to support the supply of CO_2 for photosynthesis (Miller *et al.*, 1990; Reinhold *et al.*, 1991) The position with eucaryotic micro-algae is more complicated as the active uptake of CO_2 and HCO_3^- may occur at both the plasma membrane and chloroplast envelope (Badger and Price, 1992). The increase in the pH of the medium seems to be due to the rapid conversion of extracellular HCO_3^- to CO_2 and OH^- either by carbonic anhydrase (CA), or a carbonic anhydrase like moiety (Shiraiwa *et al.*, 1993). When the CO_2 is rapidly removed, the OH^- ion is left in solution where it combines with H^+ ions, thereby consuming acidity.

Thus micro-algal photosynthesis may be used as a source of alkalinity (acid consumption) in addition to that generated by SRB, and can play an important role in the neutralisation of acid mine drainage in the lower pH range. The micro-algae are able to handle effluent feeds at pH values both lower and higher than the normal SRB range of pH 6 - 9 (Widdel, 1988).

The micro-algae used in moderating AMD pH must have the ability to survive in the effluent, thereby making it a viable and continuous process. Thus it was of importance to consider not only the alkalising function of the micro-algae, but also their growth in the effluent concerned, and its effect on their productivity. (The alkalisation term refers to the ability of the algae to elevate the pH of their surrounding environment by the conversion of HCO_3^- in solution to OH^- and CO_2, and does not involve the production of net alkalinity).

In the ASPAM system (Figure 1.2) the micro-algal component in HRAP 1 would provide a preliminary neutralisation step prior to the SRB digester, with deficiencies in growth rate made up by recycling biomass directly from HRAP 2. The integrated system is importantly dependent on the practical realisation of these expectations, and these studies were undertaken to test the feasibility of the assumptions.

7.2 OBJECTIVES

The following objectives were identified for this component of the study:

1. To evaluate the capacity of a range of micro-algal species to elevate the pH in

acidic industrial effluents;

2. To evaluate micro-algal growth potential in these acidic industrial effluents.

7.3 MATERIALS AND METHODS

7.3.1 Micro-algal cultures

An *Anacystis* sp. was isolated from acid mine drainage effluent from an abandoned coal mine. Axenic stock cultures of *Anacystis* and *D. salina* (CCAP 19/30) were grown in 1% w/v NaCl BAAM and BAAM respectively. A stock culture of *Spirulina* sp. (tannery IAPS isolate), was grown in Zarrouk's medium. The cultures were grown at 27°C and maintained under a 16hr light 8hr dark cycle, illuminated by cold white fluorescent light.

7.3.2 Experimental

Cultures of *D. salina* and *Anacystis* in the logarithmic phase of growth were harvested by centrifugation, while cultures of *Spirulina* sp. were harvested by filtration through a nylon mesh with a pore size of 100 µm and resuspended in the media of interest. The pH of the medium was adjusted using either NaOH or HCl and the pH measured at time intervals. The effect of the external carbonic anhydrase inhibitor acetozolamide was assayed by resuspending a culture of *Spirulina* sp. in AMD containing 100 µM acetozolamide. The pH was measured at time intervals.

Cells were grown in different carbon supplemented regimes. The term 'low CO_2-grown cells' refers to growth in medium rich in bicarbonate. The term 'high CO_2-grown cells' refers to growth in medium deficient in inorganic carbon other than CO_2.

7.3.3 Analysis

The pH was measured on a Cyberscan 1500 pH meter. Inorganic carbon was analysed on a Dohrmann 180 Total Organic Carbon Analyser. Chlorophyll was extracted into acetone and quantified according to the method of Lichtenhaler (1987).

7.4 RESULTS AND DISCUSSION

7.4.1 *Spirulina* sp.

Initial experiments were carried out to determine whether *Spirulina* sp. could elevate the pH of raw AMD effluent. Low CO_2-grown *Spirulina* sp. cells were suspended in acid mine drainage effluent from Grootvlei Mine, with an initial pH of 3.1. and illuminated. Analysis of the effluent indicated the absence of inorganic carbon (Ci). No biological alkalisation response was observed, irrespective of whether or not the pH was artificially elevated to 3.5 or 4.5 with NaOH (Figure 7.1). Similar results were obtained for high CO_2-grown *Spirulina* sp. cells (results not shown).

However, with the addition of a small amount of bicarbonate an elevation in pH was

observed (Figure 7.2). A control in which bicarbonate was added to the acid mine drainage effluent without algae was performed in order to assess the effect of purely bicarbonate addition on the pH of the effluent. As can be seen in Figure 7.3, an increase in pH with the addition of a bicarbonate solution was observed.

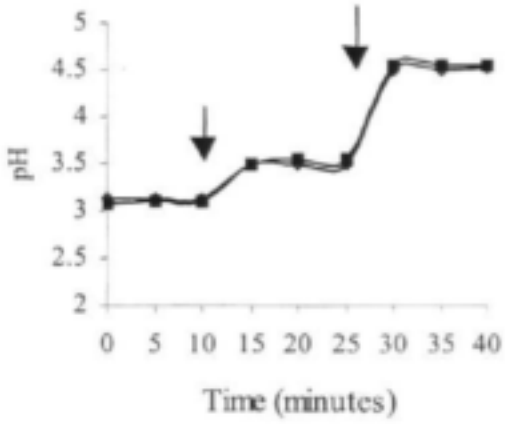


Figure 7.1. A pH profile of *Spirulina* sp. in AMD effluent under illumination (▪) and in the dark (◊). The arrows indicate the points at which the pH was artificially elevated by the addition of NaOH.

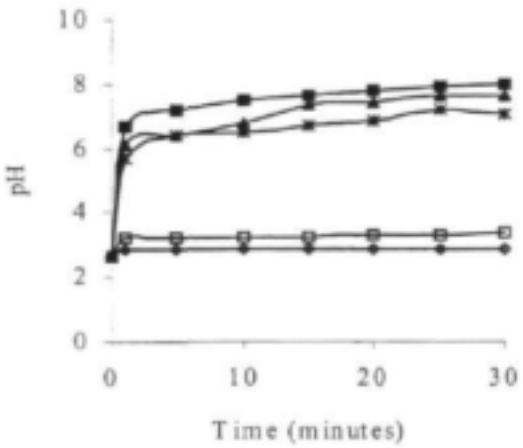


Figure 7.2. A pH profile of *Spirulina* sp. in AMD effluent after addition of 500 µmoles (▪), 250 µmoles (▲), 150 µmoles (×), 100 µmoles (▫) and 50 µmoles (◊) $NaHCO_3$ to the effluent.

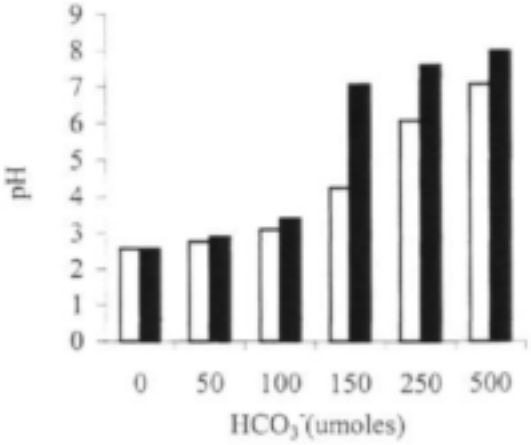


Figure 7.3. Elevation of pH of AMD effluent by the addition of increasing concentrations of $NaHCO_3$ in the presence (■) and absence (□) of *Spirulina* sp.

However, the pH elevation observed in the presence of the algae was greater than can be attributed purely to the presence of bicarbonate ions.

From this it can be seen that a minimum initial bicarbonate level is required for the algae-mediated elevation of pH to occur, with this value ranging between 100-150 µmoles HCO_3^-, where pH might be elevate by up to 3 pH units or more.

The supply of CO_2 to phytoplankton cells is limited by molecular diffusion through the unstirred layer surrounding cells in aqueous media and the rates of uncatalysed dehydration of bicarbonate (Riebesell *et al.*, 1993). The carbonic species together with the OH^- and H^+ ions of the water exist in a state of dynamic equilibrium described by the following reactions:

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \quad (6)$$

$$H_2CO_3 \rightleftharpoons H^+ + HCO_3^- \quad (7)$$

$$HCO_3^- \rightleftharpoons H^+ + CO_3^{2-} \quad (8)$$

$$H_2O \rightleftharpoons H + OH^- \quad (9)$$

As can be seen in Figure 7.4, pH is an indicator of the relative concentrations in the water of these species which affect the availability of carbon for micro-algal photosynthesis (Azov, 1982).

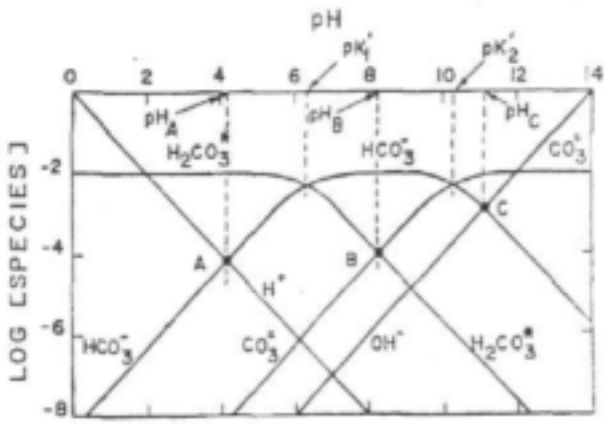


Figure 7.4. pH distribution of carbonic species (From Stumm and Morgan, 1981).

At pH1, virtually all DIC is in the form of CO_2 or H_2CO_3, whereas HCO_3^- is completely absent (Geib *et al.*, 1996). In the case of the AMD effluent used in this study, no DIC is present and thus the cyanobacteria are dependent on atmospheric CO_2 to supply their Ci needs. CO_2 exchange between a water body and a gas phase (air) depends on the difference in partial pressure of CO_2 between the water and air and mixing conditions in the water body. In waters with a high level of eutrophication there are large diurnal fluctuations in pH due to photosynthesis and respiration by algae and other plants. Thus a driving force is established for CO_2 absorption from the air during the daylight. Under low Ci conditions, active transport of CO_2 across the cell membrane occurs, and with no need for the activity of an external CA. No elevation of the pH occurs.

Spirulina sp. are cyanobacteria whose habitat is characterised by high alkalinity and pH. In order for *Spirulina* sp. to be used in a biological treatment system for the elevation of pH, it must be able to grow in the effluent. To assess this we suspended

Spirulina sp.cells in AMD effluent at different pH values and monitored the change in pH of the effluent (Figure 7.5) and its growth (Figure 7.6) over a period of days. It was decided to use pH values of 3, 4 and 5, as pH 4 is the cross-over point for decreasing H^+ concentration and increasing HCO_3^- concentration (Figure 7.4), while pH 3 and 5 lie on either side of this point.

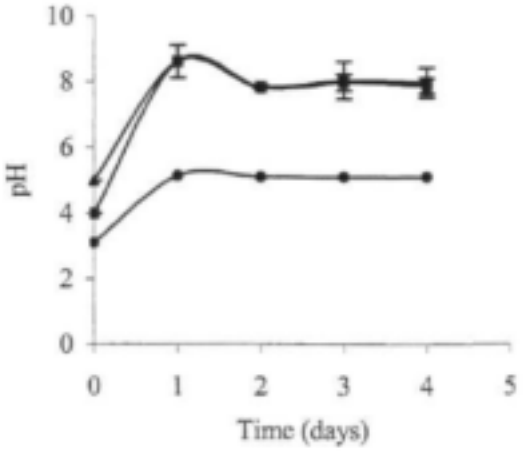


Figure 7.5. Elevation of pH of AMD by *Spirulina* sp. from an initial pH of 3 (•), 4 (■) and 5(▲).

The cells suspended in AMD at pH of 3 were unable to grow and rapidly entered decline phase. However, they were able to elevate the pH of the effluent to around pH 5. Those placed into AMD at pH 4 and 5 managed to overcome inhibitory effects, were able to grow actively and showed characteristic logarithmic phase and decline phase of growth measured as chlorophyll a (Figure 7.6) They were also able to elevate the pH of the effluent to pH 8 and above. The initial increase in pH occurred in a matter of seconds and appears to be related to the biomass concentration, expressed here as µg chl.*a*.mL^{-1} of culture, with higher chlorophyll *a* levels corresponding to larger pH changes (Figure 7.7).

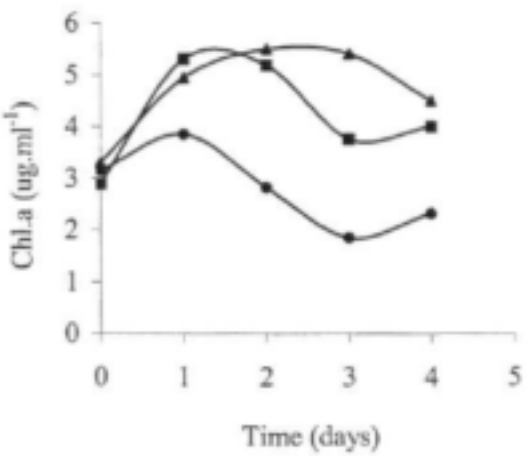


Figure 7.6. Chlorophyll *a* profile of *Spirulina* sp. grown in AMD with an initial pH of 3 (•), 4 (■) and 5 (▲).

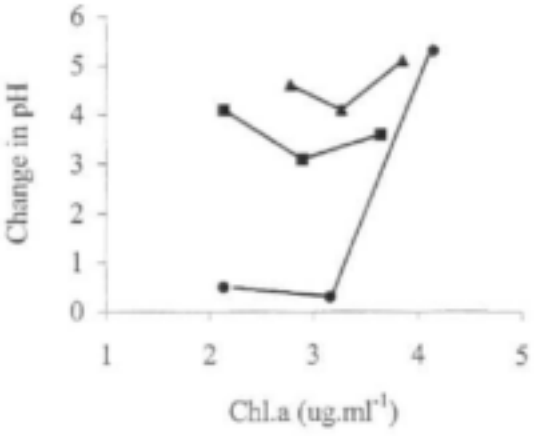


Figure 7.7. Change in pH of AMD from an initial pH of 3 (•), 4 (▪) and 5 (▲) in relation to the chlorophyll *a* concentration of cultures of *Spirulina* sp.

Assays were carried out to measure the effect of the CA inhibitor acetozolamide (AZ) on the elevation of the pH of AMD. The *in vivo* effects of CA inhibitors depend on their lipid solubility (Maren, 1984). AZ is not very lipid soluble, and is unable to penetrate the plasma membrane of intact cells, and thus only inhibits the external carbonic anhydrase. As can be seen in Figure 7.8, cultures in the presence of AZ were able to elevate the pH, but to a lesser extent than those in the absence of AZ. This may be due to the AZ levels not being high enough to mediate complete inhibition of CA, or it may indicate that not all of the pH elevation can be attributed to an external CA.

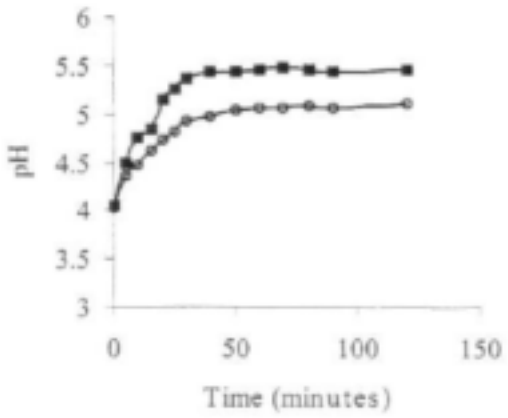


Figure 7.8. Elevation of pH of AMD effluent by *Spirulina* sp. in the presence (▫) and absence (▪) of acetozolamide.

So far it has been shown that *Spirulina* sp. has the ability to elevate the pH of acid mine drainage effluents. However, the potential use of this biological alkalisation function for the treatment of other acidic effluents also needed to be determined. The ability of *Spirulina* sp. to elevate the pH of an effluent from a zinc refinery was thus evaluated. No biological alkalisation function was observed at pH 2.5. The addition to the effluent of $NaHCO_3$, and macro-nutrients such as KNO_3, H_3PO_4 and K_2SO_4 which are required for micro-algal growth, also did not lead to an elevation in pH (Figure 7.9). However, when the effluent was neutralised, the algae were able to increase the pH to values above 10 (Figure 7.10).

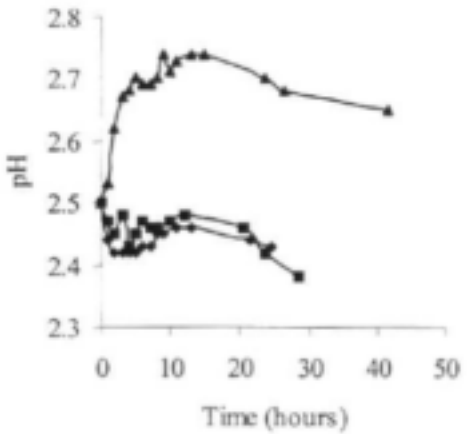


Figure 7.9. Elevation of pH of zinc refinery effluent supplemented with HCO_3 (▪), macro-nutrients (▲) and with no supplementation (♦) by *Spirulina* sp.

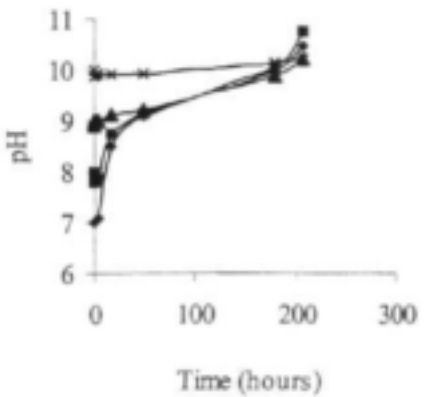


Figure 7.10. Elevation of pH of zinc refinery effluent from an initial pH of 7 (♦), 8 (▪), 9 (▲) and 10 (×) by *Spirulina* sp.

7.4.2 *Dunaliella*

It was noted in laboratory cultures that the alga *D. salina* was able to elevate the pH of its surrounding medium from 8.5 to above 10. The question arose as to the lowest initial pH at which the biological alkalisation function would operate. Cultures of *D. salina* were placed in BAAM medium, the pH of which had been artificially reduced to 4, 5 and 6. As can be seen in Figure 7.11, at an initial pH of 4 the micro-algae are able to elevate the pH only one pH point to 5. However, when the initial pH was 5 or 6, the micro-algae was able to further increase it to almost 9.

Dunaliella spp. grow in an extremely wide range of environments. They are strict photoautotrophs and require inorganic carbon for survival. The pH of the surrounding medium affects many processes associated with algal growth and metabolism, including the availability of CO_2 for photosynthesis and the availability and uptake of ions (Borowitzka and Borowitzka, 1988). Most *Dunaliella* have been shown to tolerate a wide range of pH values. *D. salina* has been shown to tolerate a pH in the range of 5.5 to 10.0 (Baas-Becking, 1930). However, the growth of *Dunaliella* in acid mine drainage effluent has not been reported.

As can be seen in Figure 7.12, the chlorophyll *a* levels of *D. salina* exposed to AMD, supplemented with NaCl, at pH 3.5, 4 and 5, declined over a period of days. Unlike the *Spirulina* sp. which were able to survive and grow, *D. salina* immediately entered decline phase. Although effective elevation of pH was achieved within the first day of the experiment, the pH levels had declined to their initial levels after 3 days (Figure 7.13).

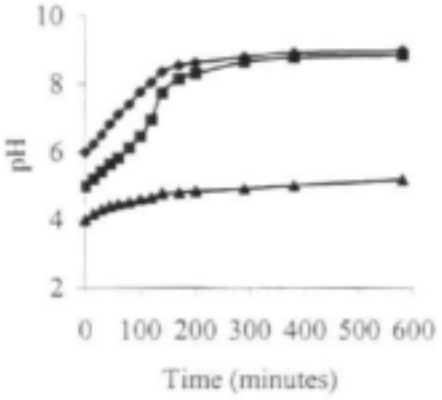


Figure 7.11. Elevation of pH of BAAM medium from an initial pH of 4 (▲), 5 (■) and 6 (◆) by *D. salina*.

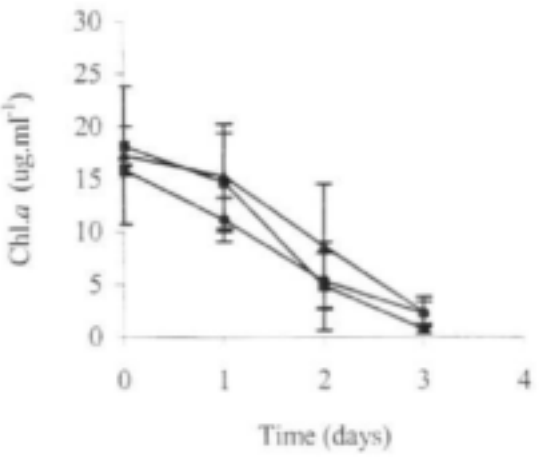


Figure 7.12. Chlorophyll *a* profile of *D. salina* grown in AMD with an initial pH of 3.5 (•), 4 (■) and 5 (▲).

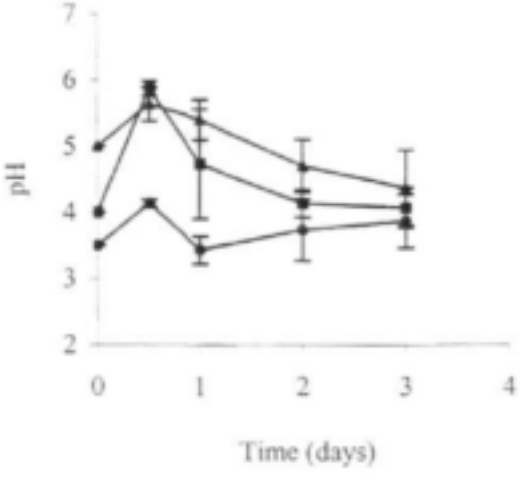


Figure 7.13. Elevation of pH of AMD from an initial pH of 3.5 (•), 4 (■) and 5 (▲) by *D. salina*.

The initial increase in pH also seems to be related to the initial chlorophyll *a* concentration of the micro-algal population (Figure 7.14).

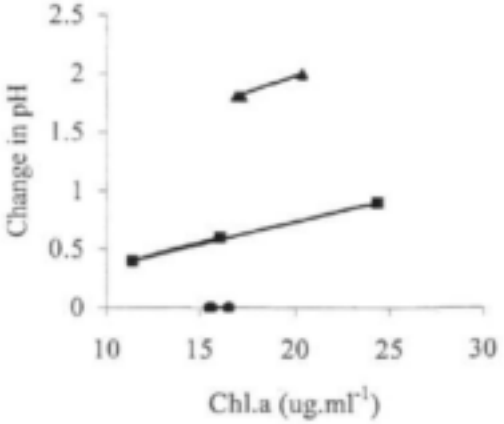


Figure 7.14. Change in pH of AMD from an initial pH of 3.4 (•), 4 (■) and 5 (▲) in relation to the initial chlorophyll *a* concentration of a culture of *D. salina*.

The *D. salina* culture was also assessed for its ability to elevate the pH of a zinc refinery effluent. As was the case with *Spirulina* sp., even when $NaHCO_3$ and macro-nutrients such as NH_4Cl, KNO_3 and H_2PO_4 which are required for micro-algal growth, were added to the effluent, the micro-alga was unable to elevate the pH of the effluent successfully (Figure 7.15).

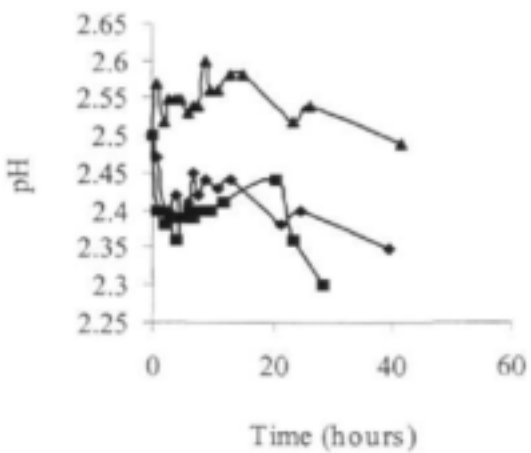


Figure 7.15. Elevation of pH of zinc refinery effluent by *D. salina* after supplementation with HCO_3^- (■), macro-nutrients (▲) and no supplementation (◆).

7.4.3 *Anacystis*

Neither *Spirulina* sp. nor *D. salina* are endemic to acidic metal-rich wastewaters. It was thus necessary to determine if an acidophilic micro-alga isolated from this environment would demonstrate a different biological alkalisation function.

The ability of the AMD strain of *Anacystis* to elevate the pH of 1% NaCl BAAM

medium was monitored. As can be seen in Figure 7.16, when the initial pH of the medium is below 4, no biological elevation of pH is observed. However, at an initial pH of 4 and above, biological pH elevation occurs.

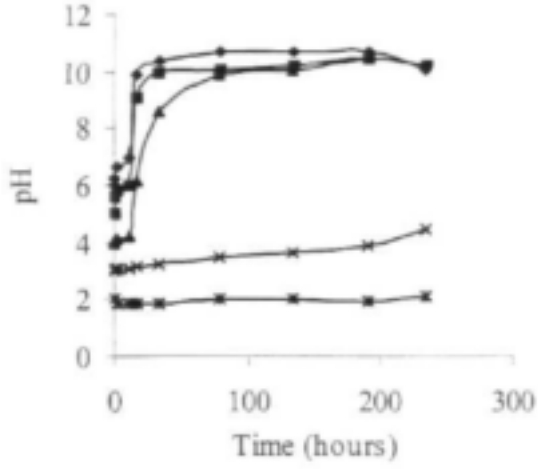


Figure 7.16. Elevation of pH of BAAM from an initial pH of 2 (*), 3 (×), 4 (▲), 5 (■) and 7 (♦) by a species of *Anacystis*.

At pH 3 and 4 BAAM has a Ci content of approximately 54 and 72 mg.L^{-1} C respectively (Figure 7.17). At the high salinity of BAAM medium (8 % NaCl), the activity of the carbonic species is reduced and the pKa moved to the left of the pH scale, i.e. more HCO_3 present at low pH values in saline waters than freshwaters. Thus one would expect more HCO_3^- present in the BAAM compared to the 1% NaCl BAAM at the same pH.

However, *D. salina* was only able to elevate the pH of BAAM when it had an initial pH of 5 or above as compared to *Anacystis*, which was able to elevate it from pH 4. This would tend to indicate that *Anacystis* was better adapted to utilising HCO_3^- from the surrounding medium and was also more tolerant of low pH values. *D. salina* is known to have a pH optimum of 8 or above, whereas the *Anacystis* species isolated from the mine water was obviously more adapted to acidic conditions. This can be seen by its ability to grow in acid mine drainage effluent (Figure 7.18).

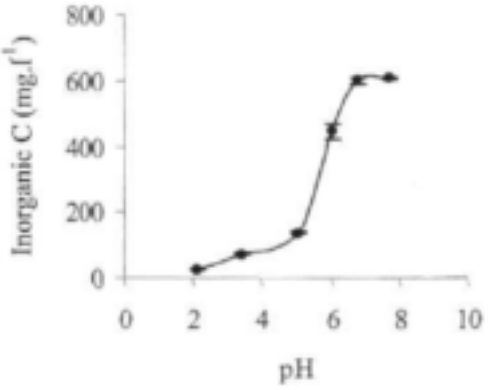


Figure 7.17. Inorganic carbon content of BAAM medium at different pH values.

However, *Anacystis* was also not successful in elevating the pH of a zinc refinery effluent (Figure 7.19) irrespective of the addition of HCO_3^- or macro-nutrients necessary for micro-algal growth. This would suggest either severe metal inhibition or that HCO_3^- utilisation is not a serious factor in the process.

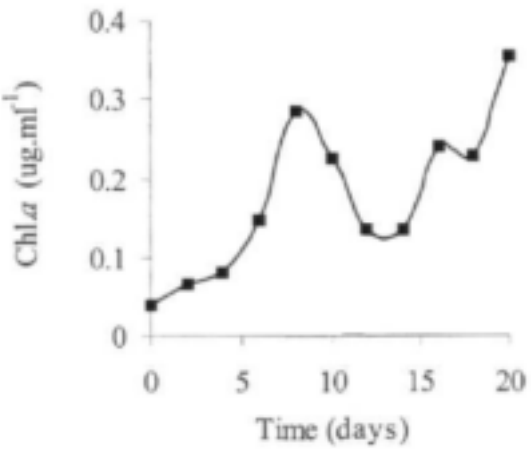


Figure 7.18. Chlorophyll *a* profile of *Anacystis* spp. in AMD effluent.

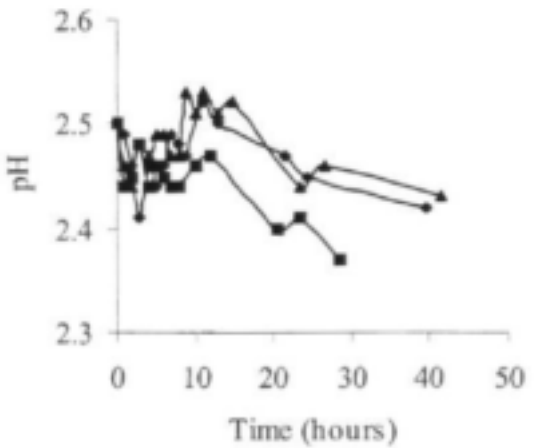


Figure 7.19. Elevation of pH of zinc refinery effluent by *Anacystis* spp. after supplementation with HCO_3^- (▲), macro-nutrients (♦), and no supplementation (■).

7.5 CONCLUSION

Spirulina sp. was found to be able to elevate the pH of AMD effluent and at the same time sustain active cell growth. However, this required an initial concentration of bicarbonate ions to be present in solution. This is important in terms of practical application, as AMD is usually bicarbonate depleted, and it would need to be supplemented by chemical addition. Two options thus exist for the integration of this alkalising function into the process.

1. The bicarbonate-rich overflow from the facultative pond (bicarbonate is generated in the sulphate reduction process), together with the metal-rich AMD, can be fed via HRAP 2 into HRAP 1, thus making the bicarbonate ions available in pH elevation functions. Addition of actively growing biomass from HRAP 2 would be important in sustaining micro-algal biomass in the more stressful conditions of HRAP 1.

2. The alkaline-rich overflow from the HRAP can be blended with the metal-rich acid mine water thus neutralising it as well as contributing to controlled metal removal.

D. salina on the other hand was not able to carry out this function and thus does not appear to be a candidate for the remediation of acidic effluents. However, the micro-alga *Anacystis* was able to elevate the pH of BAAM medium and is able to grow in acid mine drainage effluents, thus making it a candidate for AMD remediation.

The combination of metal ion toxicity and acidity needs to be addressed, and in this regard removal of the major part of the heavy metal load in the precipitation unit operation, before entering HRAP 1, would play an important part in overcoming possible toxic effects.

8 CONCLUSIONS AND RECOMMENDATIONS

The WRC Project K5/656, reported here, developed around the concept of linkage between saline wastewater treatment and the co-disposal of organic wastes using IAPS technology (Rose, 2002). Applications of algal ponding systems had been extended in a number of novel respects in the course of the preceding Project K5/495 (Rose *et al.*, 2002a), and in particular the treatment of high sulphate-salinity tannery wastewaters had been demonstrated in both technical- and full-scale installations. IAPS application studies had also been undertaken for domestic wastewaters in Project K5/651 (Rose *et al.*, 2002b), and for abattoir wastewaters in Project K5/658 (Rose *et al.*, 2002c). Efficient rates of sulphate reduction observed in the tannery system, the mobilisation of complex organic carbon compounds as electron donor sources, and the effective removal of heavy metals indicated the potential apparent in the AFP design to handle various aspects of the complex issues related to AMD treatment. Given National focus on the mine drainage problem associated with Grootvlei Mine in 1996, and the long-term problems to be addressed in the drainage of the East Rand Basin, and other gold and coal mining areas, it was decided to focus the project on this problem.

Although little had been previously reported on the treatment of AMD in active IAPS-type systems, the tannery effluent study and substantial comment on extensive and passive AMD treatment systems in the literature, was used to construct the proposed ASPAM model (Figure 1.2). While considerable experience could be called on to support the practical feasibility of aspects of the individual unit operations making up the process, certain important gaps were identified in knowledge available to warrant proceeding to pilot-scale evaluation of the integrated AMD treatment application. A critical path research methodology was used to identify knowledge gaps and those areas requiring experimental investigation prior to scale-up evaluation studies which would follow at a subsequent stage.

The objective of the project was thus to undertake studies required to demonstrate the conceptual feasibility of the ASPAM model. Issues identified in the initial study included an evaluation of electron donor sources, the UD as suitable reactor vessels for sulphate reduction activity, the precipitation of heavy metals and the neutralisation of the acid stream using the sulphidic and alkaline products of the integrated ponding system.

It is evident that the micro-algal component of the system plays a crucial role in the performance of a number of the unit operations constituting the process.

8.1 ELECTRON DONOR SOURCES AND THE POND AS BIOREACTOR

While a wide number of organic carbon sources have been investigated and reported in the literature as potential electron donors for sulphate reduction in extensive and passive AMD treatment systems, these have not previously been considered in the context of the UD associated with the AFP. This unit in the AIWPS design, developed and refined by Oswald over a period of many years (Oswald, 1988 a&b), provides the combination of optimised anaerobic and aerobic biological processes. It has

apparently not been previously investigated as a sulphide generation reactor.

Algal biomass, tannery wastes and sewage sludge solids were investigated in separate studies utilising the UD bioreactor configuration. Each organic carbon source was demonstrated to provide effective electron donor sources for sulphate reduction in a series of laboratory and pilot-scale studies. While the algal biomass study did not investigate COD:SO_4 reduction ratios below 8:1, both tannery and sewage sludge showed optimal reduction ratios around 2:1. Substantial production of sulphide was demonstrated in each case. In addition, preliminary observations made during the tannery IAPS study (Project K5/495), of an accelerated hydrolysis of complex organic carbon substrates within the biosulphidogenic environment, were confirmed in this investigation. The unsuspected manner in which carbon intermediates become available to sulphate reduction activity in these systems has become the subject of a subsequent follow-up investigation as the Rhodes BioSURE Process® for AMD treatment in WRC Projects K5/869 and 972 (Appendix 1).

Sewage sludges and tannery wastes both provide useful opportunities for co-disposal together with AMD treatment. Where long-term treatment will be required, as is likely to be the case in the Witwatersrand gold mines, and low-cost is, as always, a key consideration in the treatment of waste streams, the integration of sewage and AMD management appears to offer substantial advantages in sustainability.

Micro-algal biomass was shown to provide an electron donor source for sulphate reduction competitive with both sewage sludges and tannery wastewaters. While this application was not evaluated at pilot-scale in this study, a number of reports on feeding algal biomass to fresh water methanogenic UD-type digesters has been documented (Gerhardt *et al.*, 1994). While micro-algal production in the ASPAM ponding system provides an independence from external electron donor sources, the practicality of this option will depend on the volume flow to be treated and the pond surface areas available for pond construction. Clearly, remotely operated systems treating relatively small AMD volumes could conceivably be managed utilising only the micro-algal biomass production of the system.

In both the remotely-located applications, and those treating large volume flows, the pond provides an effective choice of bioreactor. Oswald (1995) has noted that ponds are at least an order of magnitude cheaper to construct than conventional concrete reactors, and considerably more so than highly engineered steel reactors. In long-term AMD treatment applications the lower operational costs associated with pond management vs constructed bioreactor controls would assume major significance over time.

8.2 HEAVY METAL REMOVAL

A number of factors can be brought to bear in optimising the removal of contaminating heavy metals in the ASPAM system. Although only a selected group of metals was investigated, the studies reported here showed that metal sulphide complexation, on cycling sulphidic AFP contents to influent AMD, was not the only mechanism involved in precipitate formation. It was evident that metal carbonate

and/or hydroxide formation might also be involved, as well as the adsorption of metal complexes to particulate organic carbon remaining in the AFP stream.

In addition to the adsorption properties of the micro-algal biomass itself, or its partly degraded particulate residues in the AFP stream, the production of EPS by these organisms may play an important role in adsorbing and removing metals, prior to the neutralisation reactions and the formation of alkaline metal sludges. Studies undertaken here showed that EPS binding was most efficient at acidic pH values, and also that the production of EPS may be maximised under conditions of physiological stress pertaining in the HRAP 1 unit. While growth in this unit may be slow, provision may be made to feed biomass forward from HRAP 2 where cell mass may be produced at optimised growth rates.

The HRAP 2 unit also provides a final polishing step where remaining metal contamination may be reduced to very low residuals within the range of the required discharge standards.

8.3 NEUTRALISATION OF THE ACID STREAM

The effective neutralisation of the acid stream before discharge and reaching the anaerobic compartment of the AFP is a critical requirement. Although the SRB have quite a wide pH range compared to MPB, this does not generally fall much within the acid range. However, where acidotolerant or acidophilic SRB have been identified, their growth rate has been generally slow (Johnson, pers.comm. 2000).

While the results reported here indicate that micro-algal growth in HRAP 1 may be relied on to provide an impressive elevation of the pH of the influent stream, this only occurs reliably above pH 4, the crossover point where HCO_3^- species exceed the H^+ concentration (Stumm and Morgan, 1981). However, AMD is also generally low in carbonate, and the system is thus dependent on the sulphate reduction reaction in the AFP for net addition of carbonate to the system. It seems therefore that a preliminary alkalisation step is required, and coincides with the addition of AFP effluent to the influent AMD in the metal precipitation step.

8.4 CONCLUSIONS

The results of these studies enabled a number of conclusions to be drawn about the performance of the proposed ASPAM unit operations:

- The anaerobic pit in the AFP may be used as an UD reactor, suitable for the biosulphidogenic digestion of a range of complex organic carbon sources, as electron donors, for the production of sulphide, alkalinity and particulate organic carbon fractions;

- Certain micro-algal species have been shown to be fairly resistant to sulphide toxicity, and algal-enriched oxygenated water from the HRAP 2 may be passed to the surface of the AFP to control odour and reoxidise residual sulphide levels in the outflow;

- In addition to providing actively growing micro-algal biomass for supplementing HRAP 1, the HRAP 2 unit would provide final polishing and residual metal removal from the treated AMD stream;

- AFP contents may be cycled directly to the metal precipitation step where a number of mechanisms would be involved in the precipitation of heavy metals contaminating the influent AMD stream. The provision of carbonate to subsequent algal acid consumption reactions would be an important component of the recycle operation in carbon deficient AMD streams;

- Following the metal precipitation operation the HRAP 1 unit would complete the neutralisation of the stream prior to its passing to the AFP. Production of EPS as a result of physiological stress in this unit would provide a metal binding function under the more acidic conditions prevailing, and together with alkalisation (acid consumption) in HRAP 1, these liquors would contribute to metal removal in the precipitation unit.

The studies reported here had indicated a preliminary feasibility for the ASPAM model in certain important respects, as an IAPS configuration to be applied in AMD treatment.

8.5 RECOMMENDATIONS

It is apparent that the functionality of an IAPS approach in the treatment of AMD would depend on a wide range of factors. While certain additional factors have been clarified here, at both laboratory and pilot-scale, these results relate to the evaluation of independent free-standing unit operations. However, the results of the study indicate that sufficient information may now be at hand to warrant proceeding to the next stage investigation. This should allow the integration of the various component unit operations of the system into the fully integrated ASPAM process, and with process piloting to determine the mass balances and flow regulation required between the various units.

Given the anticipated growth in the AMD problem nationally, with pending closure of increasing numbers of both coal and gold mines, the long-term nature of the problem, and the relatively high cost of existing treatment technologies, it is recommended that the WRC consider proceeding to scale-up evaluation of the ASPAM system. Particular areas of application to be investigated should include treatment of large volume AMD flows, and also quasi-passive applications where the generation of algal biomass would provide some independence from external carbon sources.

8.6 RESEARCH PRODUCTS

The studies reported here led to a number of research spin-off developments in follow-up studies based on the above recommendations. These are the subject of separate project reports which are listed in Appendix 1 and 3.

Student training in IAPS treatment of saline wastewaters has included 3 PhD and 2 MSc students. The results of these studies have been instrumental in leading to the publication of 1 patent, 9 papers in international journals and 6 articles of general scientific interest. Publication in conference proceedings includes 5 plenary and key note papers, 17 international and 29 local conference presentations. The student training and publication outputs are reported in Appendix 2.

8.7 FOLLOW-UP ACTIONS

The observation, in the tannery pond studies (K5/495), of enhanced hydrolysis of complex organic carbon compounds in the sulphidogenic environment, and the preliminary investigation, in this study, of their use as electron donor substrates in sulphate reduction activity, had indicated a useful linkage of waste organic carbon and sulphate-saline wastewater treatment. A more rigorous study of the mechanisms involved in the enhanced hydrolysis process was undertaken in follow-up WRC Projects K/5 869 and K/5 972 (Appendix 1). These studies have led to a number of follow-up actions which are noted here, and are the subject of subsequent WRC reports in the 'Salinity, Sanitation and Sustainability' series (Appendices 1 and 3):

- The follow-up investigation on the use of sewage sludge as a carbon source in AMD treatment resulted in the development of the Rhodes BioSURE Process® and was scaled up from laboratory studies to pilot-scale evaluation at the Grootvlei Mine, Springs (Report 9 and 10);
- The effective solubilisation of sewage sludge observed in the above study led to the evaluation of the BioSURE Process® in sewage sludge disposal. This process has been the subject of technical-scale evaluation in a joint WRC/EBG project with Erwat Co., at the Ancor Works in Springs, South Africa (Report 12);
- Evaluation of alternative carbon sources has led to an investigation of maize lignocellulose wastes in collaboration with Eskom. In this sustainable development programme the BioSURE Process® provides the initial operation in an AMD beneficiation process whereby treated water is used in downstream agro-industrial job creation. Providing economic sustainability for communities following mine closure provides a basis for long-term environmentally sustainable AMD management;
- The studies in sulphidogenic enhanced hydrolysis were applied to the development of AMD treatment in passive systems in a Department of Arts Culture Science and Technology Innovation Fund programme in collaboration with Pulles Howard and de Lange;
- The sulphur recovery unit operation to effect a biodesalination of the AMD was developed in follow-up WRC Projects K5/1078 and K5/1336 (Report 11);
- Collaborative studies on the enzymology of sewage sludge solubilisation have

been undertaken in the Department of Biochemistry Microbiology and Biotechnology at Rhodes University with Professor Chris Whiteley and Dr Bret Pletschke. These studies have led to a separate WRC programme in environmental enzymology;

- Collaborative studies on the modelling of the BioSURE Process®, and the aqueous chemistry of the metal precipitation operations, have been undertaken with the Departments of Chemical and Civil Engineering at the University of Cape Town, involving Professors Geoff Hansford and Dick Loewenthal and Dr Alison Lewis.

9 REFERENCES

Abeliovich, A. 1980. Factors limiting algal growth in high rate oxidation ponds. In: Shelef, G. and Soeder, C.J. (eds.) Algal Biomass Production and Use. Elsevier/North Holland, Amsterdam.

Abeliovich, A.1986. Algae in wastewater oxidation ponds. In: A. Richmond (ed.), Handbook of Micro-algal Mass Culture, CRC Press, Boca Raton.

Almasi, A. and Pescod, B. 1996. Wastewater treatment mechanisms in anoxic stabilisation ponds. Wat. Sci. Technol., 33: 125-132.

Anderson, K.L., Tayne, T.A. and Ward, D.M. 1987. Formation and fate of fermentation products in hot springs cyanobacterial mats. Appl. Envir. Microbiol., 53: 2343-2352.

Andrews, G. 1989. An examination of the kinetics of coal pyrite decomposition. In: Scheiner, G., Doyle, F.M. and Katawa, S. (eds.), Biotechnology in Minerals and Metals Processing. Society of Mining Engineers, Littleton.

APHA, 1989. Standard Methods for the Examination of Water and Wastewater. 15th Edition. American Public Health Association, Washington D.C.

Azov, Y. 1982. Effect of pH on inorganic carbon uptake in algal cultures. Appl. Environ. Micro., 43: 1300-1306.

Baas-Becking, L.G.M. 1930. Observations on *Dunaliella viridis* Teodoresco. Stanford University.

Badger, M.R. and Price, G.D. 1992. The CO_2 concentrating mechanism in cyanobacteria and microalgae. Physiologia Plantarum, 84: 606-615.

Banister, S.S. and Pretorius, W.A. 1998. Optimization of sludge acidogenic fermentation for biological nutrient removal. Water SA, 24: 35-41.

Barnes, L.J., Janssen, F.J., Sherren, J., Versteegh, J.H., Kock, R.O. and Scheeren, P.J.H. 1991. A new process for the microbial removal of sulphate and heavy metals from contaminated waters extracted by a geohydrological control system. Trans. Ichem., 69: 184-186.

Baron, M., Arellano, J.B. and Gorge, J.L. 1995. Copper and photosystem II: A controversial relationship. Physiologia Plantarum, 94: 174-180.

Barton L.L. 1995. Sulphate Reducing Bacteria. Plenum Press, New York.

Barton L.L. and Tomei F.A. 1995. Characteristics and activities of sulphate-reducing bacteria. In: Barton LL (ed) Sulphate Reducing Bacteria. Plenum Press, New York.

Béchard, G., Rajan, S. and Gopuld, W.D. 1993. Characterization of a microbial process for the treatment of acidic drainage. In: Torma, A.E., Apel, M.L. and Brierly, C.L. (eds.)

Biohydrometallurgical Technologies. The Minerals, Metals and Materials Society.

Ben-Amotz, A. and Avron, M. 1983. On factors which determine massive β-carotene accumulation in the halotolerant algae *Dunaliella bardawil*. Plant Physiol., 72: 592-597.

Bender, J., Lee, R.F. and Phillips, P. 1995. Uptake and transformation of metals and metalloids by microbial mats and their use in bioremediation. J. Indust. Microbiol., 14: 113-118.

Besser, J.M., Huckins, J.N., Little, E.A. and La Point, T.W. 1989. Distribution and bioaccumulation of selenium in aquatic microcosms. Environmental Pollution, 62: 1-12.

Beverdige, A. and Pickering, W.F. 1980. Influence of humate-solute interactions on aqueous heavy metal ion levels. Water Air and Soil Pollution, 14: 171-185.

Bhattacharya, S.K., Uberoi, V. and Dronamraju, M.M. 1996. Interaction between acetate-fed sulfate reducers and methanogens. Wat. Res., 30: 2239-2246.

Borowitzka, M.A. 1982. Mechanisms in algal calcification. Progress in Phycological Research, 1: 137-177.

Borowtizka, M.A. and Borowwitzka, L.J. 1988. Dunaliella. In: Borowtizka, M.A. and Borowitzka, L.J. (eds.) Micro-algal Biotechnology. Cambridge University Press, Cambridge.
Boshoff, G. (1998) Development of integrated biological processing for the biodesalination of sulphate- and metal-rich wastewaters.

Boshoff, G., Duncan, J. and Rose, P.D. 1996. An algal-bacterial intergrated ponding system for the treatment of mine drainage waters. J. Appl. Phycology 8 (4-5): 442.

Brady D., Stoll, A.D., Starke, L. and Duncan, J.R. 1994. Chemical and enzymatic extraction of heavy metal binding polymers from isolated cell walls of *Saccharomyces cerevisiae*. Biotech. Bioeng., 44: 297-302.

Braun, M., and Stolp, H. 1985. Degradation of methanol by a sulphate-reducing bacterium. Arch. Microbiol. 58: 786-793.

Brierley, J.A. and Brierley, C.L. 1983. Biological accumulation of some heavy metals - Biotechnogical applications. In: Westbroek, P. and de Jong, E.W. (eds.), Biomineralization and Biological Metal Accumulation. D. Riedel Publishing Company.

Buisman, C.J.N., Post, R., Ijspeert, P., Geraats, G. and Lettinga, G. 1989. Biotechnological process for sulphide removal with sulphur reclamation. Acta Biotechnol. 9: 255-267.

Burgess, S.G. and Wood, L.B. 1961. Pilot plant studies in production of sulphur from sulphate enriched sewage sludge. J. Sci. Food Agric., 12: 326-341.

Butlin, K.R., Selwyn, S.C and Wakerlery, D.S. 1956. Sulphide production from sulphate-

enriched sewage sludges. J. Appl. Bacteriol., 19: 3-15.

Carre, M.C., Vulliermet, A. and Vulliermet, B. 1983. Environment and Tannery. Centre Technique du Cuir, Lyon.

Castenholtz, R.W. 1976. The effect of sulfide on the bluegreen algae of hot springs. 1. New Zealand and Iceland. J. Phycol., 12: 54-68.

Chen, C.I., Mueller, R.F. and Griebe, T. 1994. Kinetic analysis of microbial sulfate reduction by *Desulfovibrio desulfuricans* in an anaerobic upflow porous media filter bioreactor. Biotech. Bioeng., 43: 267-274.

Chian, E.S.K. and DeWalle, F.B. 1983. Removal of heavy metals from a fatty acid wastewater with a complete mixed anaerobic filter. Proceedings 38th Industrial Waste Conference, Purdue University, West Lafayette, IN.

Choi, E. and Rim, J.M. 1991. Competition and inhibition of sulfate reducers and methane producers in anaerobic treatment. Wat. Sci. Tech., 23: 1259-1264.

Cohen, Y. 1984. Oxygenic photosynthesis, anoxygenic photosynthesis and sulfate reduction in cyanobacterial mats. In: Klug, M.J. and Reddy, C.A. (eds.), Current Perspectives in Microbial Ecology. American Society for Microbiology, Washington DC.

Cohen, Y., Padan, E. and Shilo, M. 1975. Facultative anoxygenic photosynthesis in the cyanobacterium *Oscillatoria limnetica*. J. Bact., 123: 855-861.

Cohen, Y., Jorgensen, B.B., Revsbech, N.P. and Poplawski, R. 1986. Adaption to hydrogen sulphide of oxygenic and anoxygenic photosynthesis among cyanobacteria. Appl. Envir. Microbiol., 51: 398-407.

Colleran, E. 1997. Uses of bacteria in bioremediation. In: Sheehan, D. (ed.), Bioremediation Protocols. Humana Press, Totowa, New Jersey.

Conradie, P.J.A. and Grutz, P.W.E. 1973. The treatment of acid mine waste in a mixture with sewage sludge in an anaerobic digester. Report to the Chamber of Mines (File No. w6/534/3). National Institute for Water Research, Pretoria.

Corbett, C.J. 2001. The Rhodes BioSURE Process in the Treatment of Acid Mine Drainage Wastewaters. MSc Thesis, Rhodes University, Grahamstown.

Davies, A.G. 1983. The effects of heavy metals upon natural marine phytoplankton populations. Progress in Phycological Research, 2: 113-145.

Davis, J.S. 1993. Biological management for problem solving and biological concepts for a new generation of solar saltworks. Seventh Symposium on Salt, 1: 611-616.

DEAT (Department of Environment Affairs and Tourism). 1999. The National State of the Environment Report. Department of Environment Affairs and Tourism, Pretoria.

DEAT (Department of Environment Affairs and Tourism). 2000. White paper on integrated pollution and waste management. Department of Environment Affairs and Tourism, Pretoria.

De Baere, L.A., Devocht, M., Van Assche, P. and Verstraete, W. 1984. Influence of high NaCl and NH_4Cl salt levels on methanogenic associations. Water Res., 18: 543-548.

De Haan, H., De Boer, T. and Hoguela, H. 1981. Metal binding capacity in relation to hydrology and algal periodicity in Tjeukemeer, The Netherlands. Archiv Fuer Hydrobiologie, 92: 11-23.

De Pauw, N. and Salomoni, C. 1991. The use of microalgae in wastewater treatment: achievements and constraints. In: P. Madoni (ed.), Biological Approach to Sewage Treatment Processes: Current Status and Perspectives. Perugia.

DeWalle, F.B., Chian, E.S.K. and Brush, J. 1979. Heavy metal removal with completely mixed anaerobic filter. J. Water Poll. Control Fed. 51:22-36.

Dunn, K.M. 1998. The biotechnology of high rate algal ponding systems in the treatment of saline tannery wastewaters. PhD. Thesis, Rhodes University, Grahamstown.

Du Preez, L.A., Odendaal, J.P., Maree, J.P. and Ponsonby, M. 1992. Biological removal of sulphate from industrial effluent using producer gas as energy source. Environ. Technol., 13: 875-882.

Elefsiniotis, P. and Oldham, W.K. 1994. Anaerobic acidogenesis of primary sludge: the role of solids retention time. Biotech. Bioeng., 44: 7-13.

Evans, C.L. 1967. The toxicity of hydrogen sulphide and other sulphides. Q. J. Exp. Physiol., 52: 231-248.

Fallon, R.D. and Brock, T.D. 1979. Decomposition of blue-green algal (cyanobacterial) blooms in Lake Mendota, Wisconsin. Appl. Environ. Micro., 37: 820-830.

Farrah, H. and Pickering, W.F. 1978. The effect of pH and ligands on the sorption of heavy metal ions by cellulose. Aust. J. Chem., 31: 1501-1509.

Filip, D.S., Peters, T., Adams, V.D. and Middlebrooks E.J. 1979. Residual heavy metal removal by an algae-intermittent sand filtration system Wat. Res. 13: 305-313.

Fox, R.D. 1983. Nutrient preparation and low cost basin construction for village production of Spirulina. In: Proceedings of the 4th International Meeting of SAA, Villeneuve 'd Ascq, France . 15-17 September.

Gadd, G.M. and White, C. 1993. Microbial treatment of metal pollution - a working biotechnology? TIBTECH 11: 353-359

Gale, N.L. and Wixson, B.G. 1979. Control of heavy metals in lead industry effluents by algae and other aquatic vegetation. In: Conf. Management and Control of Heavy Metals in the Environment, London.

Garlick, S., Oren, A. and Padan, E. 1977. Occurrence of facultative anoxygenic photosynthesis among filamentous and unicellular cyanobacteria. J. Bact,. 129: 623-629.

Gauglhofer, J. 1990. Chromium in tannery effluents and its impact on the environment. Proceedings of the 42nd Convention of the SLTC, Drakensberg, South Africa.

Gazea, B., Adam, K. and Kontopoulos, A. 1996. A review of passive systems for the treatment of acid mine drainage. Minerals Engineering, 9: 23-42.

Geib, K., Golldack, D. and Gimmler, H. 1996. Is there a requirement for an external carbonic anhydrase in the extremely acid-resistant green algal *Dunaliella acidophila*? Eur. J. Phycol., 31: 273-284.

Genschow, E., Hegemann, W. and Aschke, K. 1996. Biological sulfate removal from tannery wastewater in a two-stage anaerobic treatment. Wat. Res., 30: 2072-2078.

Gerhardt, M.B. and Oswald, W.J. 1990a. Reduction of selenate from agricultural drainage water using anaerobic bacteria grown on algal substrate. Proceedings of the Summer Natl.Meeting., Am. Inst. Chem. Eng.

Gerhardt , M.B. and Oswald, W.J. 1990b. Final Report: Microalgal-bacterial treatment for selenium removal from San Joaquin Valley drainage waters. SEEHRL Report no. 90-1. San Joaquin Valley Drainage Program, U.S.Bureau Reclamation, Sacramento, California. Sanitary Engineering Environmental Health Research Laboratory, University of California, Berkeley.

Gerhardt, M.B., Green, B., Newman, R.D., Lundquist, T.J., Tresan, R.B. and Oswald, W.J. 1994. Removal of selenium using a novel algal-bacterial process. Research Journal Water Pollution Control Federation, 63: 799-805.

Giordano, M., Davis J.S., and Bowes, G. 1994. Organic carbon release by *Dunaliella salina* (Chlorophyta) under different growth conditions of CO_2, nitrogen and salinity. J. Phycol., 30: 249-257.

Gotaas, H.B. and Oswald, W.J. 1954. Studies of algae in sewage oxidation ponds. Sanitary Engineering Research Laboratory Report, University of California, Berkeley, USA.

Greene, B. and Bedell, G.W. 1990. Algal Gelsor immobilized algae for metal recovery. In: Alatsuka, I. (ed.), Introduction to Applied Phycology. Academic Publishing, The Hague.

Grobicki, A. and Stuckey, D.C. 1992. Hydrodynamic characteristics of the anaerobic baffled reactor. Wat. Res. 23(3): 371-378.

Gunnison, D. and Alexander, M. 1975. Basis for the susceptibility of several algae to microbial decomposition. Can. J. Microbiol., 21: 619-628.

Gyure, R.A., Konopka, A., Brooks, A. and Doemel, W. 1987. Algal and bacterial activities in acidic (pH3) strip mine lakes. Appl. Envir. Microbiol., 53: 2069-2076.

Hammack, R.W. and Edenborn, H.M. 1992. The removal of nickel from mine water using bacterial sulfate reduction. Appl. Microbiol. Biotechnol., 37: 674-678.

Hammack, R.W., Edenborn, H.M. and Dvorak, D.H. 1994. Treatment of water from an open-pit copper mine using biogenic sulfide and limestone: a feasibility study. Wat. Res., 28: 2321-2329.

Hart, M.J. and Madgwick, J.C. 1987. Utilisation of algae as a sole nutrient for micro-organisms biodegrading manganese dioxide. Bull. Proc. Australas. Inst. Min. Metall., 292: 61-63.

Herrera, L.J., Hernández, P. Ruiz, and Gantenbein, S. 1991. *Desulfovibrio desulfuricans* growth kinetics. Environ. Toxicol. Water Qual. 6:225-238.

Holan, Z.R., Volesky, B. and Prasetyo I. 1993. Biosorption of cadmium by the biomass of marine algae. Biotech. Bioeng., 41: 819-825.

Hollibaugh, J.T., Seibert, D.L. and Thomas, W.H. 1980. A comparison of the acute toxicities of ten heavy metals to phytoplankton from Saanich Inlet, B.C., Canada. Estuarine and Coastal Marine Science, 10: 93-105.

Howsley, R. and Pearson, H.W. 1979. pH dependent sulphide toxicity to oxygenic photosynthesis in cyanobacteria. FEMS Microbiology Letters, 6: 287-292.

Hudson, B.J.F. and Karis, I.G. 1974. The lipids of the alga Spirulina. Journal of Science, Food and Agriculture, 25: 759-763.

Huntsman, S.A. 1972. Organic excretion by *Dunaliella tertiolecta*. J. Phycol., 8: 59-63.

Hutchinson, T.C. 1973. Comparative studies of the toxicity of heavy metals to phytoplankton and their synergistic interactions. Water Pollut. Res. Can., 8: 68-90.

Isa, Z., Grusenmeyer, S. and Verstraete, W. 1986. Sulfate reduction relative to methane production in high-rate anaerobic digestion: Technical aspects. Appl. Environ. Micro., 51: 572-579.

Jennett, J.C., Hassett, J.M. and Smith, J.E. 1979. Control of heavy metals in the environment using algae. Heavy metals in the environment. Intl. Conf. London.

Johnson, D.B. 1995. Acidophilic microbial communities: Candidates for bioremediation of acidic mine effluents. International Biodeterioration & Biodegradation, 35: 41-45.

Jorgensen, B.B., Cohen, Y. and Revsbech, N.P. 1986. Transition from anoxygenic to oxygenic photosynthesis in a *Microcoleus chthonoplastes* cyanobacterial mat. Appl. Envir. Microbiol., 51: 408-417.

Juby, G.J.G. 1992. Membrane desalination of service water from gold mines. J. S. Afr. Inst. Min. Metall., 92: 65-69.

Juby, G.J.G. and Pulles, W. 1990. Evaluation of Electrodialysis Reversal for the desalination of brackish mine service water. WRC Report No. 179/90. Water Research Commission. Pretoria.

Juby, G.J.G., Schutte, C.F. and van Leeuwen, J. 1996. Desalination of calcium sulphate scaling mine water: Design and operation of the SPARRO process. Water SA., 22: 161-172.

Kalin, M., Cairns, J. and McCready, R. 1991. Ecological engineering methods for acid mine drainage treatment of coal wastes. Resources, Conservation and Recycling, 5: 265-275.

Kim, B.M. 1984. Membrane-based solvent extraction for selective removal and recovery of metals. J. Mem. Sc., 21: 5-19.

Koster, I.W., Rinzema, A., De Vegt, A. and Lettinga, G. 1986. Sulfide inhibition of the methanogenic activity of granular sludge at various pH levels. Wat. Res., 20: 1561-1567.

Kroen, W.K. and Rayburn, W.R. 1984. Influence of growth status and nutrients on extracellular polysaccharide synthesis by the soil alga *Chlamydomonas mexicana* (Chlorophyceae). J. Phycol., 20: 253-257.

Kroiss, H. and Wabnegg, F.P. 1983. Sulfide toxicity with anaerobic wastewater treatment. Proceedings of the European Symposium on Advanced Anaerobic Waste Treatment (AWWT). The Hague.

Kuenen, J.G. and Robertsen, L.A. 1992. The use of natural bacterial populations for the treatment of sulphur containing wastewater. Biodegradation, 3:239-254.

Kuyucak, N. and Volesky, B. 1989. The mechanism of cobalt biosorption. Biotech. Bioeng., 33: 823-831.

Kuyucak, N. and St-German, P. 1994. In-situ treatment of acid mine drainage by sulfate reducing bacteria in open pits. 1. Scale-up experiences. Proceedings of the International Land Reclamation and Mine Drainage Conference and the Third International Conference on the Abatement of Acidic Mine Drainage, Pittsburgh, April 24-29.

Laubscher, R.K. 1992. The culture of *Dunaliella salina* and the production of β-carotene in tannery effluents. MSc Thesis, Rhodes University, Grahamstown.

Lens, P.N., De Poorter, M.P., Cronenberg, C.C. & Verstraete, W.H. 1995. Sulphate reducing and methane producing bacteria in aerobic wastewater treatment systems. *Wat. Res., 29*: 871-880.

Li, Y.Y., Lam, S. and Fanf, H.H.P. 1996. Interactions between methanogenic, sulfate-reducing and syntrophic acetogenic bacteria in the anaerobic degradation of benzoate. Wat. Res., 30: 1555-1562.

Lichtenhaler, H.K. 1987. Chlorophylls and carotenoids:pigments of photosynthetic biomembranes. Methods in Enzymology, 148: 350-371.

Maart, B. 1993. The biotechnology of effluent-grown *Spirulina* and application in aquaculture nutrition. MSc Thesis. Rhodes University, Grahamstown.

Machemer, S.D. and Wildeman, T.R. 1992. Adsorption compared with sulfide precipitation as metal removal processes from acid mine drainage in a constructed wetland. J. Contaminant Hydrology, 9: 115-131.

Mann, H. and Fyfe, W.S. 1988. Biogeochemical cycling of the elements in some freshwater algae form gold and uranium mine districts. Biorecovery 1: 3-26.

Mara, D.D. and Pearson, H. 1986. Artificial freshwater environment: waste stabilisation ponds. In: Rehm, H.J. and Red, G. (eds.), Biotechnology-A Comprehensive Treatise 8, Weinheim, Verlagsgesellschaft. .

Mara, D.D. and Marecos Do Monte, M. 1987. Waste stabilization ponds. Wat. Sci. Tech., 19: 1-41.

Mara, D.D., Pearson, H.W. and Silva, S.A. 1996. Waste stabilisation ponds: technology and applications. Wat. Sci. Technol., 33: 1-262.

Maree, J.P. and Hill, E. 1989. Biological removal of sulphate from industrial effluent and concominant production of sulphur. Wat. Sci. Technol., 21: 265-267.

Maree, J.P., Gerber, A. and Hill, E. 1987. An integrated process for biological treatment of sulfate-containing industrial effluent. Journal Water Pollution Control Federation, 59: 1069-1074.

Maren, T.H. 1984. The general physiology of reactions catalyzed by carbonic anhydrase and their inhibitions by sulfonamides. Ann. N.Y. Acad. Sci., 429: 568-575.

Middelbeek, E.J. 1992. Growth in batch culture. In: Cartledge, T.G. (ed.) In vitro cultivation of micro-organisms. Butterworth-Heinemann Ltd.

Miller, A.G., Espie, G.S. and Canvin, D.T. 1990. Physiological aspects of CO_2 and HCO_3^- transport by cyanobacteria: a review. Can. J. Bot., 68: 1291-1302.

Mizuno, O., Li, Y.Y. and Noike, T. 1994. Effects of sulfate concentrations and sludge retention time on the interaction between methane production and sulfate reduction for butyrate. Wat. Sci. Tech., 30: 45-54.

Moeller, T., Bailar, J.C., Kleinberg, J., Guss, C.O., Castellian, M.E. and Metz, C. 1989. Chemistry with inorganic qualitative analysis. 3rd Edition. Harcourt Brace Jovanovich Publishers.

Molepane, N.P. 1999. Sulphate reduction utilising hydrolysis of complex carbon sources. MSc Thesis, Rhodes University, Grahamstown.

Neytzell-De Wilde, F.G. 1992. Reassesment of the strategy with respect to industrial effluent discharge with special reference to advanced technology treatment methods. Phase 1: Industrial effluent discharge problem areas. WRC Report No. 407/1/92. Water Research Commission, Pretoria.

Nyns, E.J., Naveau, H.P., Chome, R. and Bertrand, Y. 1979. Digesters - a world-wide review. In: Proceedings of the first International symposium on anaerobic digestion, University College, Cardiff, Wales. D.A. Stafford, B.I. Wheatley and D.E. Hughes (eds.), Applied Science Publishers Ltd., London.

Odom, J.M. and Singleton, R. 1993. The Sulphate Reducing Bacteria: Contemporary Perspectives. Springer-Verlag, New York.

Okabe, S., Nielsen, P.H., Jones, W.L. and Characklis, W.G. 1995. Sulfide product inhibition of *Desulfovibrio desulfuricans* in batch and continuous cultures. Wat. Res., 29: 571-578.

Oleszkiewicz, J.A. and Hilton, B.L. 1986. Anaerobic treatment of high sulphate wastes. Canadian Journal of Civil Engineering 13: 423-428.

Oleszkiewicz, J.A., Marstaller, T. and McCartney, D.M. 1989. Effects of pH on sulfide toxicity to anaerobic processes. Environmental Technology Letters, 10: 815-822.

Oren, A. and Padan, E. 1978. Induction of anaerobic, photoautotrophic growth in the cyanobacterium *Oscillatoria limnetica*. J. Bact., 133: 558-563.

Oren, A. and Shilo, M. 1979. Anaerobic heterotrophic dark metabolism in the cyanobacterium *Oscillatoria limnetica* : sulfur respiration and lactate fermentation. Arch. Microbiol., 122: 77-84.

Oswald, W.J. 1988a. Micro-algae and waste-water treatment. In: M.A. Borowitzka and L.J. Borowitzka (eds.), Micro-algal Biotechnology. Cambridge University Press, Cambridge.

Oswald W.J. 1988b. Large-scale algal systems (engineering aspects). In: M.A. Borowitzka and L.J. Borowitzka (eds.), Micro-algal Biotechnology, Cambridge University Press, Cambridge.

Oswald, W.J. 1988c. The role of microalgae in liquid waste treatment and reclamation. In: Lembi, C.A. and Waaland, J.R. (eds.) Algae and Human Affairs. Cambridge University Press, Cambridge.

Oswald, W.J. 1990. Advanced integrated wastewater pond systems. Proceedings of the 1990 ASCE Convention EE Div/ASCE. San Francisco, California, November 5-8.

Oswald, W.J. 1995. Ponds in the Twenty-first Century. Wat. Sci. Tech. 31(12):1-8.
Oswald, W.J., Golueke, C.G., Cooper, R.C., Gee, H.K. and Bronson, J.C. 1963. Water reclamation, algal production and methane fermentation in waste ponds. Int. J. Air Wat. Poll., 7: 627.

Oswald, W.J., Gotaas, H.B., Golueke, C.G. and Kellen, W.R. 1957. Algae in waste-water treatment. Research Forum - algae in waste treatment. 29(4):437-457.

Overnell, J. 1975. The effect of heavy metals on photosynthesis and loss of cell potassium in two species of marine algae, *Dunaliella tertiolecta* and *Phaeodactylum tricornutum*. Marine Biol., 29: 99-103.

Pace, F., Ferrara, R. and Del Carratore, G. 1977. Effects of sub-lethal doses of copper sulphate and lead nitrate on growth and pigment composition of *Dunaliella salina* Teod. Bulletin of Environmental Contamination and Toxicology, 17: 679-685.

Parkin, G.F., Lynch, D.L., Kuo, W.C., van Kueren, E.L. and Bhataacharya, S.K. 1990. Interaction between sulfate reducers and methanogens fed acetate and propionate. J. Water Pollut. Control Fed., 62: 780.

Pescod M.B. 1996. The role and limitations of anaerobic pond systems. Wat. Sci. Tech. 33: 11-22.

Peters, R.W. and Ku, Y. 1985. Batch precipitation studies for heavy metal removal by sulphide precipitation. AiChe Symposium Series, 81: 9-27.

Phillips, T. 1994. Stress manipulation in *Dunaliella salina* and dual - stage β-carotene production. PhD Thesis, Rhodes University, Grahamstown.

Pinheiro, H.M., Reis, M.T. and Novais, J.M. 1987. A study of the performance of a high rate photosynthetic pond system. Wat. Sci. Tech., 19: 237-241.

Pipes. W.O. 1961. Sludge digestion by sulphate reducing bacteria. Proc. 15th Ind. Waste Conf., Purdue University, Lafayette, Indiana.

Pipyn, P and Verstraete, W. 1979. Waste classification for the digestibility in anaerobic systems. In: D.A. Stafford, B.I. Wheatley and D.E. Hughes (eds.), Proceedings of the first International symposium on anaerobic digestion, University College, Cardiff, Wales. , Applied Science Publishers Ltd., London.

Postgate, J.R. 1984. The sulphate-reducing bacteria. Cambridge University Press, Cambridge.

Pronk, J.T. and Johnson, D.B. 1992. Oxidation and reduction of iron by acidophilic bacteria. Geomicrobiology Journal 10:153-171.

Pulles, W., Howie, D., Otto, D and Easton J. 1995. A manual on mine water treatment and management practices in South Africa. WRC Report No. TT 80/96. Water Research Commission, Pretoria.

Quillet, M. 1975. Rechereche sur les substances glucidiques elabores pae les spirulines. Annales de la Nutrition et de l'Alimentation, 29: 553-561.

Ramazanov, Z., Rawat, M., Mason, C.B. and Moroney, J.V. 1996. Ultrastructural and biochemical adaption of algal cells to limiting CO_2 concentrations. Sci. Mar., 60: 141-148.

Reinhold, L., Kosloff, R. and Kaplan, A. 1991. A model for inorganic carbon fluxes and photosynthesis in cyanobacterial carboxysomes. Can. J. Bot., 69: 984-988.

Reis, M.A.M., Almeida, J.S., Lemos, P.C. and Carrondo, M.J.P. 1992. Effect of hydrogen sulphide on growth of sulfate reducing bacteria. Biotech. Bioeng., 40: 593-600.

Richmond, A.E. 1986. Microalgaculture. CRC Critical Reviews in Biotechnology, 4: 369-438.

Riebesell, U., Wolf-Gladrow, D.A. and Smetacek, V. 1993. Carbon dioxide limitation of marine phytoplankton growth rates. Nature, 361: 249-251.

Rivera, A.L. 1983. Heavy metal removal in a packed bed, anaerobic upflow (anflow) bioreactor. Journal WPCF, 55: 1450-1456.

Robb, G.A. 1994. Environmental consequences of coal mine closure. The Geographical Journal 106: 33-40.

Robinson, J.D.F. and Robb, G.A. 1995. Methods for the control and treatment of acid mine drainage. Coal International July 1995: 152-156.

Rose, P.D. 1992. Treatment of saline effluent. RSA Patent 91/5069.

Rose, P.D. 2002. Salinity, Sanitation and Sustainability: A Study in Environmental Biotechnology and Integrated Wastewater Beneficiation in South Africa. Volume 1. Overview. WRC Report No TT 187/02. Water Research Commission, Pretoria.

Rose, P.D. and Cowan,A..K. 1992. A process for the production of useful products from saline media. RSA Patent 91/5070; Australian Patent 19345/92; USA Patent 906536; EPO Patent 92306077.4; Israel Patent102357.

Rose, P.D., Maart, B.A., Dunn, K.M., Rowswell, R.A. and Britz, P. 1996. High rate algal oxidation ponding for the treatment of tannery effluents. Wat. Sci. Tech., 33: 219-227.

Rose, P.D., Boshoff, G.A., van Hille, R.P., Wallace, L.C.M., Dunn, K.M. and Duncan, J.R. 1998. An integrated algal sulphate reducing high rate ponding process for the treatment of acid mine drainage wastewaters. Biodegradation 9:247-257.

Rose, P.D., Dunn, K.M., Maart, B.A. and Shipin, O. 2002a. Salinity, Sanitation and Sustainability: A Study in Environmental Biotechnology and Integrated Wastewater Beneficiation in South Africa. Volume 2. Integrated Algal Ponding Systems and the Treatment of Saline Wastewaters. Part1: Meso-saline Wastewaters - The *Spirulina* Model. WRC Report No TT 188/02. Water Research Commission, Pretoria.

Rose, P.D., Hart, O.O., Shipin, O. and Ellis, P.J. 2002b. Salinity, Sanitation and Sustainability: A Study in Environmental Biotechnology and Integrated Wastewater Beneficiation in South Africa. Volume 3. Integrated Algal Ponding Systems and the Treatment of Domestic and Industrial Wastewaters. Part1: The AIWPS Model. WRC Report No TT 190/02. Water Research Commission, Pretoria.

Rose, P.D., Hart, O.O., Shipin, O. and Müller, J.R. 2002c. Salinity, Sanitation and Sustainability: A Study in Environmental Biotechnology and Integrated Wastewater Beneficiation in South Africa. Volume 3. Integrated Algal Ponding Systems and the Treatment of Domestic and Industrial Wastewaters. Part 2: Abattoir Wastewaters. WRC Report No TT 191/02. Water Research Commission, Pretoria.

Rose, P.D., Corbett, C.J., Whittington-Jones, K. and Hart, O.O. 2002d. Salinity, Sanitation and Sustainability: A Study in Environmental Biotechnology and Integrated Wastewater Beneficiation in South Africa. Volume 4. The Rhodes BioSURE Process®. Part 1: Biodesalination of Mine Drainage Wastewaters.WRC Report No TT 195/02. Water Research Commission, Pretoria.

Rowswell R.A., Cooper D.A. and Shuttleworth S.G. 1984. Evaporation ponds: A solution for tannery effluent disposal. J. Soc. Leather Technol. Chem. 69: 123-129.

Saha, S. 1993. Treatment of aqueous effluent for fluoride removal. Wat. Res., 27: 1347-1350.

Saraiva, M.C. and Fraizier, A. 1975. Contamination par le ^{51}Cr et le ^{109}Cd de cultures de l'algue Dunaliella bioculata. Marine Biology, 29: 343-350.

Scheeren, P.J.H., Kock, R.O. & Buisman, C.J.N. 1993. Geohydrological contaminant system and microbial water treatment plan for metal contaminated groundwater at Budelco. International Symposium World Zinc.

Schrift, A. 1973. Metabolism of selenium by plants and microorganisms. In: Klayman, D.L. and Gunther, W.H.H. (eds.), Organic Selenium Compounds:Their Chemistry and Biology. John Wiley and Sons, New York.

Scott, R. 1995. Flooding of Central and East Rand Gold Mines: An investigation into controls over the inflow rate, water quality and the predicted impacts of flooded mines. WRC Report No. 486/1/95. Water Research Commission, Pretoria.

Shelef G., Azov Y., Moraine, R. and Oron, G. 1980. Algal mass production as an integral part of wastewater reclamation system. In: Algae Biomass Production and Use, G. Shelef and C.J. Soeder (eds.), Elsevier, Amsterdam..

Shiraiwa, Y., Goyal., A and Tolbert. N.E. (1993). Alkalisation of the medium by unicellular green algal during uptake of dissolved inorganic carbon. Plant Cell Physiol., 34: 649-657.

Shrift, A. 1961. Biochemical interrelations between selenium and sulfur in plants and microorganisms. Fed. Proc. Fed. Am. Soc. Exp. Biol., 20: 695-702.

Shuttleworth S.G. 1978. The evaluation of tannery effluent treatment - guidelines for further investigations. J. Soc. Leather Technol. Chem. 62, 87.

Silver, M. 1989. Control of acid mine drainage including coal pile and ash pond seepage. In: Hammer DA (ed), Constructed Wetlands for Wastewater Treatment, Lewis Publishers, Inc., Chelsea, MI.

Singh, K. 1992. Treating acid mine drainage with BSR. Pollution Engineering, : 66-67.

Soeder, C.J. 1986. An historical outline of applied algology, In: A. Richmond (ed.), CRC Handbook of Microalgal Mass culture. p. 25-44. CRC Press Inc., Boca Raton.

Speece, R.E. 1983. Anaerobic biotechnology for industrial wastewater treatment. Environ. Sci. Technol., 17: 416.

Stewart, W.D.P. and Pearson, H.W. 1970. Effects of aerobic and anaerobic conditions on growth and metabolism of blue-green algae. Proc. R. Soc. London Ser. B., 175: 293-311.

Stumm, W. and Morgan, J.J. 1981. Aquatic Chemistry. 2nd Edition. Wiley Interscience, New York.

Thauer, R.K. 1982 . Dissimilatory sulphate reduction with acetate as electron donor. Philos. Trans. R. Soc. London. B. Biol. Sci., 298: 467-471.

Ting, Y.P., Lawson, F. and Prince, I.G. 1989. Uptake of cadmium and zinc by the alga *Chlorella vulgaris*. Part 1. Individual ion species. Biotech. Bioeng., 34: 990-999.

Ting, Y.P., Lawson, F. and Prince, I.G. 1991. Uptake of cadmium and zinc by the alga *Chlorella vulgaris*: Part 11. Multi-ion situation. Biotech. Bioeng., 37: 445-455.

Toerien, D.F. and Maree, J.P. 1987. Reflections on anaerobic process biotechnology and its impact on water utilization in South Africa. Water SA, 13: 137-144.

Tornabene, T.G., Bourne, T.F., Raziuddin, S. and Ben-Amotz, A. 1985. Lipid and lipopolysaccharide constituents of cyanobacterium *Spirulina platensis* (Cyanophyceae, Nostocales). Marine Ecology: Progress Series, 22: 121-125.

Uberoi, V. and Bhattacharya, S.K. 1995. Interactions among sulfate reducers, acetogens, and methanogens in anaerobic propionate systems. Wat. Environ. Res., 67: 330-339.

Ueki, K., Kotaka, K., Itoh, K. and Ueki, A. 1988. Potential availability of anaerobic treatment with digester slurry of animal waste for the reclamation of acid mine water containing sulphate and heavy metals. J. Ferment. Technol., 66: 43-50.

Umita, T., Nenov, V., Omura, T., Aizawa, J. and Onuma, M. 1988. Biological ferrous-iron oxidation with fluidized bed reactor. Water Pollution Control in Asia.

Uziel, M. 1978. Solar energy fixation and conversion with algal bacterial systems. Ph.D Thesis, University of California, Berkeley.

Van Houten, R.T., Hulshoff-Pol, L.W. and Lettinga, G. 1994. Biological sulphate reduction using gas-lift reactors fed with hydrogen and carbon dioxide as energy and carbon source. Biotechnol. Bioeng. 44:586-594.

Van Zyl, H.C. 1996. Environmental systems in Amcoal. Mining Environment management, March issue (pp.18-21).

Vavilin, V.A., Rytov, S.V. and Lokshina, L.Y. 1996. A description of hydrolysis kinetics in anaerobic degradation of particulate organic matter. Bioresource Technol., 56: 229-237.

Walmsley, R.D., Walmsley, J.J. and Silberbauer, M. 1999. Fresh water systems and resources. The National State of the Environment Report. Department of the Environment and Tourism, Pretoria.

Wentzel, M.C., Mbewe, A. and Ekama, G.A. 1995. Batch test for measurement of readily biodegradable COD and active organism concentrations in municipal waste waters. Water SA, 21: 117-124.

Whang, J.S., Young, D. and Pressman, M. 1982. Soluble sulphide precipitation for heavy metals removed from wastewaters. Environ. Prog., 1: 110-113.

Whittington-Jones, K. 2000. Sulphide enhanced hydrolysis of primary sewage sludge: Implications for the bioremediation of acid mine drainage. PhD Thesis, Rhodes University, Grahamstown.

Whittington-Jones, K.J., Corbett, C.J. and Rose, P.D. 2002e. Salinity, Sanitation and Sustainability: A Study in Environmental Biotechnology and Integrated Wastewater Beneficiation in South Africa. Volume 3. Integrated Algal Ponding Systems and the Treatment of Domestic and Industrial Wastewaters. Part 5: Winery and Distillery Wastewaters. WRC Report No TT 196/02. Water Research Commission, Pretoria.

Widdel, F. 1988. Microbiology and ecology of sulfate and sulfur reducing bacteria. In: Zehnder A (ed.), Biology of anaerobic microorganisms. Wiley Interscience, New York.

Widdel, F. and Hansen, T.A. 1992. The dissimilatory sulphate-and sulphur-reducing bacteria. In: Ballows, A., Trüper, H.G., Dworkin, M., Harder, W. and Schleifer, K-H. (eds.), The Prokaryotes, Vol I (p 583-624). Springer-Verlag, Berlin.

Wieder, R.K. 1993. Ion input/output budgets for five wetlands constructed for acid coal mine drainage treatment. Water, Air, and Soil Pollution, 71: 231-270.

Wilde, E. and Benemann, J.R. 1993. Bioremoval of heavy metals by the use of microalgae. Biotech. Adv., 11: 781-812.

Wildeman T., Brodie G.A. and Gusek J.F. 1991. Draft Hand Book for Constructed Wetlands Receiving Acid Mine Drainage. US Environmental Protection Agency, Ohio.

Wyman, M. and Fay, P. 1987. Acclimation to natural light climate. In: Fay, P. and van Baalen, C. (eds.), The Cyanobacteria. Elsevier, Amsterdam.

Younger, P.L., Curtis, T.P., Jarvis, A. and Pennell, R. 1997. Effective passive treatment of aluminium-rich acidic colliery spoil drainage using a compost wetland at Quaking Houses, County Durham. J. Chartered Inst. Water Environ. Mgt., 11: 200-208.

Zarrouk, C. 1966. Contribution a l' Etude d' une Cyanophyceae. Influence de Divers Facteurs Physiques et Chimiques sur la Croissance et la Photosynthese de Spirulina maxima. Thesis, University of Paris, France.

Zhang, T.C. and Noike, T. 1994. Influence of retention time on reactor performance and bacterial trophic populations in anaerobic digestion processes. Wat. Res., 28: 27-36.

Ziminik, P.R. 1988. Binding and removal of aluminium ions in waters by an algal biomass. Analytical Letters, 21: 1383-1396.

10 APPENDICES

APPENDIX 1

WRC STUDY 'SALINITY SANITATION AND SUSTAINABILITY' - PROJECT REPORTS

The WRC study which has been summarised here developed out of a number of closely interrelated studies, undertaken for the WRC by the Rhodes University Environmental Biotechnology Group, over a 10 year period. The detailed findings associated with this work will be published separately as individual project reports. The following lists the WRC reports which cover the various investigations dealt with in the programme. The individual WRC projects under which the various studies were undertaken are listed separately below:

Report 1

Salinity, Sanitation and Sustainability: A Study in Environmental Biotechnology and Integrated Wastewater Beneficiation in South Africa.
Volume 1. Overview.

Report 2

Salinity, Sanitation and Sustainability: A Study in Environmental Biotechnology and Integrated Wastewater Beneficiation in South Africa.
Volume 2. Integrated Algal Ponding Systems and the Treatment of Saline Wastewaters.
Part1: Meso-saline Wastewaters - The *Spirulina* Model.

(Project K5/495: A Biotechnological approach to the removal of organics from saline effluents - Part 1.)

Report 3

Salinity, Sanitation and Sustainability: A Study in Environmental Biotechnology and Integrated Wastewater Beneficiation in South Africa.
Volume 2. Integrated Algal Ponding Systems and the Treatment of Saline Organic Wastewaters.
Part 2: Hyper-saline Wastewaters - The *Dunaliella* Model.

(Project K5/495: A biotechnological approach to the removal of organics from saline effluents - Part 2.)

Report 4

Salinity, Sanitation and Sustainability: A Study in Environmental Biotechnology and Integrated Wastewater Beneficiation in South Africa.

Volume 3. Integrated Algal Ponding Systems and the Treatment of Domestic and Industrial Wastewaters. Part1: The AIWPS Model.

(Project K5/651: Appropriate low-cost sewage treatment using the integrated algal high rate oxidation ponding process.)

Report 5

Salinity, Sanitation and Sustainability: A Study in Environmental Biotechnology and Integrated Wastewater Beneficiation in South Africa.
Volume 3. Integrated Algal Ponding Systems and the Treatment of Domestic and Industrial Wastewaters.
Part 2: Abattoir Wastewaters.

(Project K5/658: Algal high rate oxidation ponding for the treatment of abattoir effluents.)

Report 6

Salinity, Sanitation and Sustainability: A Study in Environmental Biotechnology and Integrated Wastewater Beneficiation in South Africa.
Volume 3. Integrated Algal Ponding Systems and the Treatment of Domestic and Industrial Wastewaters.
Part 3: Mine Drainage Wastewaters - The ASPAM Model.

(Project K5/656: Appropriate low-cost treatment of sewage reticulated in saline water using the algal high rate oxidation ponding system.)

Report 7

Salinity, Sanitation and Sustainability: A Study in Environmental Biotechnology and Integrated Wastewater Beneficiation in South Africa.
Volume 3. Integrated Algal Ponding Systems and the Treatment of Domestic and Industrial Wastewaters.
Part 4: System Performance and Tertiary Treatment Operations.

(Project K5/799: Development and monitoring of integrated algal high rate oxidation pond technology for low-cost treatment of sewage and industrial effluents;
Project K5/1073: Extension of applications and optimisation of operational performance of algal integrated ponding systems technology in appropriate low-cost treatment of industrial and domestic wastewaters.
Project K5/1362: Development and technology transfer of IAPS applications in upgrading water quality for small wastewater and drinking water treatment systems.)

Report 8

Salinity, Sanitation and Sustainability: A Study in Environmental Biotechnology and Integrated Wastewater Beneficiation in South Africa.
Volume 3. Integrated Algal Ponding Systems and the Treatment of Domestic and Industrial Wastewaters.
Part 5: Winery and Distillery Wastewaters.

(Project K5/1073: Extension of applications and optimisation of operational performance of algal integrated ponding systems technology in appropriate low-cost treatment of industrial and domestic wastewaters.)

Report 9

Salinity, Sanitation and Sustainability: A Study in Environmental Biotechnology and Integrated Wastewater Beneficiation in South Africa.
Volume 4. The Rhodes BioSURE Process®.
Part 1: Biodesalination of Mine Drainage Wastewaters.

(Project K5/869: Biological sulphate desalination and heavy metal precipitation in industrial and mining effluents using the IAPS.)

Report 10

Salinity, Sanitation and Sustainability: A Study in Environmental Biotechnology and Integrated Wastewater Beneficiation in South Africa.
Volume 4. The Rhodes BioSURE Process®.
Part 2: Enhanced Hydrolysis of Organic Carbon Substrates - Development of the Recycling Sludge Bed Reactor.

(Project K5/972: Process development and system optimisation of the integrated algal trench reactor process for sulphate biodesalination and heavy metal precipitation in mining and industrial effluents.)

Report 11

Salinity, Sanitation and Sustainability: A Study in Environmental Biotechnology and Integrated Wastewater Beneficiation in South Africa.
Volume 4. The Rhodes BioSURE Process®.
Part 3: Sulphur Production and Metal Removal Unit Operations.

(Project K5/1078: Development and piloting of the integrated biodesalination process for sulphate and heavy metal removal from mine drainage water incorporating co-disposal of industrial and domestic effluents;
Project K5/1336: Scale-UP development of the Rhodes BioSURE Process® for sewage sludge solubilisation and disposal.)

Report 12

Salinity, Sanitation and Sustainability: A Study in Environmental Biotechnology and Integrated Wastewater Beneficiation in South Africa.
Volume 4. The Rhodes BioSURE Process®.
Part 4: Treatment and Disposal of Sewage Sludges.

(Project K5/1169: Intermediate scale-up evaluation of the Rhodes Process for hydrolysis and solubilisation of sewage sludges in a sulphate reducing bacterial system.)

PROJECTS

The following lists the WRC Projects under which the studies in this series have been undertaken, and also the relevant reports in which the detailed results have been documented:

Project K5/410

A Biotechnological approach to the removal of organics from saline effluents.

Report: 1. Salinity, Sanitation and Sustainability: A Study in Environmental Biotechnology and Integrated Wastewater Beneficiation in South Africa.
Volume 1. Overview.

Project K5/495

A Biotechnological approach to the removal of organics from saline effluents.

Report: 2. Salinity, Sanitation and Sustainability: A Study in Environmental Biotechnology and Integrated Wastewater Beneficiation in South Africa.
Volume 2. Integrated Algal Ponding Systems and the Treatment of Saline Wastewaters. Part1: Meso-saline Wastewaters - The *Spirulina* Model.

Report: 3. Salinity, Sanitation and Sustainability: A Study in Environmental Biotechnology and Integrated Wastewater Beneficiation in South Africa.
Volume 2. Integrated Algal Ponding Systems and the Treatment of Saline Organic Wastewaters. Part 2: Hyper-saline Wastewaters - The *Dunaliella* Model.

Project K5/651

Appropriate low-cost sewage treatment using the integrated algal high rate oxidation ponding process.

Report 4: Salinity, Sanitation and Sustainability: A Study in Environmental Biotechnology and Integrated Wastewater Beneficiation in South Africa.
Volume 3. Integrated Algal Ponding Systems and the Treatment of Domestic and Industrial Wastewaters. Part1: The AIWPS Model.

Project K5/656

Appropriate low-cost treatment of sewage reticulated in saline water using the algal high rate oxidation ponding system.

Report 6: Salinity, Sanitation and Sustainability: A Study in Environmental Biotechnology and Integrated Wastewater Beneficiation in South Africa.
Volume 3. Integrated Algal Ponding Systems and the Treatment of Domestic and Industrial Wastewaters. Part 3: Mine Drainage Wastewaters - The ASPAM Model.

Project K5/658

Algal high rate oxidation ponding for the treatment of abattoir effluents.

Report 5: Salinity, Sanitation and Sustainability: A Study in Environmental Biotechnology and Integrated Wastewater Beneficiation in South Africa.
Volume 3. Integrated Algal Ponding Systems and the Treatment of Domestic and Industrial Wastewaters. Part 2: Abattoir Wastewaters.

Project K5/799

Development and monitoring of integrated algal high rate oxidation pond technology for low-cost treatment of sewage and industrial effluents.

Report 7: Salinity, Sanitation and Sustainability: A Study in Environmental Biotechnology and Integrated Wastewater Beneficiation in South Africa.
Volume 3. Integrated Algal Ponding Systems and the Treatment of Domestic and Industrial Wastewaters. Part 4: System Performance and Tertiary Treatment Operations.

Project K5/869

Biological sulphate desalination and heavy metal precipitation in industrial and mining effluents using the IAPS.

Report 9: Salinity, Sanitation and Sustainability: A Study in Environmental Biotechnology and Integrated Wastewater Beneficiation in South Africa.
Volume 4. The Rhodes BioSURE Process®. Part 1: Biodesalination of Mine Drainage Wastewaters.

Project K5/972

Process development and system optimisation of the integrated algal trench reactor process for sulphate biodesalination and heavy metal precipitation in mining and industrial effluents.

Report 10: Salinity, Sanitation and Sustainability: A Study in Environmental Biotechnology and Integrated Wastewater Beneficiation in South Africa.
Volume 4. The Rhodes BioSURE Process®. Part 2: Enhanced Hydrolysis of Organic Carbon Substrates - Development of the Recycling Sludge Bed Reactor.

Project K5/1073

Extension of applications and optimisation of operational performance of algal integrated ponding systems technology in appropriate low-cost treatment of industrial and domestic wastewaters.

Report 7: Salinity, Sanitation and Sustainability: A Study in Environmental Biotechnology and Integrated Wastewater Beneficiation in South Africa.
Volume 3. Integrated Algal Ponding Systems and the Treatment of Domestic and Industrial Wastewaters. Part 4: System Performance and Tertiary Treatment Operations.

Report 8: Salinity, Sanitation and Sustainability: A Study in Environmental Biotechnology and Integrated Wastewater Beneficiation in South Africa.
Volume 3. Integrated Algal Ponding Systems and the Treatment of Domestic and Industrial Wastewaters. Part 5: Winery and Distillery Wastewaters.

Project K5/1078

Development and piloting of the integrated biodesalination process for sulphate and heavy metal removal from mine drainage water incorporating co-disposal of industrial and domestic effluents.

Report 11: Salinity, Sanitation and Sustainability: A Study in Environmental Biotechnology and Integrated Wastewater Beneficiation in South Africa.
Volume 4. The Rhodes BioSURE Process®. Part 3: Sulphur Production and Metal Removal Unit Operations.

Project K5/1169

Intermediate scale-up evaluation of the Rhodes Process for hydrolysis and solubilisation of sewage sludges in a sulphate reducing bacterial system.

Report 12: Salinity, Sanitation and Sustainability: A Study in Environmental Biotechnology and Integrated Wastewater Beneficiation in South Africa.
Volume 4. The Rhodes BioSURE Process®. Part 4: Treatment and Disposal of Sewage Sludges.

Project K5/1336

Scale-up development of the Rhodes BioSURE Process® for sewage sludge solubilisation and disposal.

Report 11: Salinity, Sanitation and Sustainability: A Study in Environmental Biotechnology and Integrated Wastewater Beneficiation in South Africa.
Volume 4. The Rhodes BioSURE Process®. Part 3: Sulphur Production and Metal Removal Unit Operations.

Project K5/1362

Development and technology transfer of IAPS applications in upgrading water quality for small wastewater and drinking water treatment systems.

Report 7: Salinity, Sanitation and Sustainability: A Study in Environmental Biotechnology and Integrated Wastewater Beneficiation in South Africa.
Volume 3. Integrated Algal Ponding Systems and the Treatment of Domestic and Industrial Wastewaters. Part 4: System Performance and Tertiary Treatment Operations.

APPENDIX 2

RESEARCH PRODUCTS

2.1 STUDENTS TRAINED

2.1.1 PhD Students

G. Boshoff (1998) Development of integrated biological processing for the biodesalination of sulphate and metal-rich wastewaters.
K. Dunn (1998) The biotechnology of high rate algal ponding systems in the treatment of saline tannery wastewaters.
K. Whittington-Jones (2000) Sulpide-enhanced hydrolysis of primary sewage sludge: implications for the bioremediation of sulphate-enriched wastewaters.

2.1.2 MSc Students

P. Molepane (2000) Sulphate reduction utilising hydrolysis of complex carbon sources.
C.J. Corbett (2001) The Rhodes BioSURE Process in the Treatment of Acid Mine Drainage Wastewaters.

2.2 PUBLICATIONS

2.2.1 Patents

1. Rose P.D., Duncan, J.R., van Hille, R.P., Boshoff, G.A. 1999. Use of ponds to treat sulphate solutions and ASPAM process. RSA 99/4585 (Final). US patent pending.

2.2.2 Papers

1. Brady, D., Letebele, B., Duncan, J.R. and Rose, P.D. 1994.Bioaccumulation of metals by *Scenedesmus, Selenastrum* and *Chlorella* algae. Water S.A. 20: 213-218.
2. Rose, P.D. and Hart, O.O. 1996. The saline water algal high rate oxidation pond- capacity building in the developing world. Abstract - Journal of Applied Phycology 8(4-6):456.
3. Boshoff, G., Duncan,J. and Rose P.D. 1996. An algal-bacterial integrated ponding system for the treatment of acid drainage waters. Abstract - Journal of Applied Phycology 8(4-6):442.
4. Rose,P.D., Boshoff, G.A., van Hille, R.P., Wallace, L.M.C., Dunn, K.M. and Duncan, J.R. 1998. An integrated algal sulphate reducing high rate ponding process for the treatment of acid mine drainage wastewaters. Biodegradation 9:247-257.
5. van Hille, R., Boshoff, G., Rose, P. and Duncan, J. 1999. A continuous process for the biological treatment of heavy metal contaminated acid mine water. Resource Conservation and Recycle, 27:157-167.
6. C.G. Whiteley, P. Heron, B.Pletschke, P.D. Rose, S. Tshivhunge, F.P. van Jaarsveld and K.Whittington-Jones. 2002. The Enzymology of Sludge Solubilisation Utilising Sulphate Reducing Systems. Properties of Proteases and Phosphatases. Enz., Microbiol. Tech. (Accepted, 2002).

7. C.G. Whiteley, P.Rose, B.Pletschke and X.Melamane. 2002. Environmental enzymology: Enzymology of accelerated sludge solubilisation: Properties of Lipases. Water Research. (Accepted, 2002).
8. C.G. Whiteley, P.Rose, and B.Pletschke. 2002. Environmental enzymology: Enzymology of accelerated sludge solubilisation: Role of ATP Sulphurylases. Enz., Microbiol. Tech. (Accepted, 2002).
9. C.Whiteley, B.Pletschke, P. Rose and N.Ngesi. 2002. Specific Sulphur Metabolites Stimulate β-Glucosidase Activity in an Anaerobic Sulphidogenic Bioreactor. Biotech. Letts. (Accepted, 2002).

2.2.3 General Articles

1. Gibbs, S. 1995. Sewage Treatment Plants: Algae offer a cheaper way to clean up wastewater. Scientific American, 273:27.
2. Rose, P.D., Maart, B.A., Dunn, K.M., Rowswell, R.A. and Brits, P. 1995. Ponding presents Potential. Leather 83-90, September 1995.
3. Claasen, J. 1997. Alge suiwer water en maak geld. Landbouweekblad,20-22, 28 Februarie,1997.
4. Rose, P.D. 1997. Algal integrated ponding in Wellington. Rhodes University Environmental Biotechnology Group Occasional Publication.
5. Rose, P.D. 1997. The algal integrated ponding system. Rhodes University Environmental Biotechnology Group Occasional Publication.
6. Corbett CJ, Whittington-Jones K, Hart OO and Rose PD. 2001. Biological Treatment of Acid Mine Drainage Wastewaters using a Sewage Sludge Carbon Source. Chemical Technology, November/December 2001.

2.2.4 Conferences

2.2.4.1 Plenary and Keynote Papers

1. Rose, PD., Boshoff, GA., van Hille, RP., Wallace, L., Dunn, KM. and Duncan, JR. 1998. An integrated algal sulphate reducing high rate ponding process for the treatment of acid mine drainage wastewaters. European Union Summer School: The Biological Sulphur Cycle - Environmental Science and Technology. Wageningen, The Netherlands, April 19-24, 1998.
2. Rose, P.D. 1999. Integrated biological treatment of metal and sulphate enriched drainage waters utilising low-cost complex organic carbon sources. European Union Conference on the Aznalcolar Mine Disaster. Seville, Spain, January, 1999.
3. Rose, P.D. 2000. Sulphidogenic hydrolysis of complex organic carbon - just sewage or a valuable resource in environmental remediation? 11th Biennial Congress of the South African Society for Microbiology. BIOY2K, Grahamstown, January 2000.
4. Rose, P.D., Clarke, A., Roman, H., Madekane, M. and Nagabushana, N. 2002. The microbial ecology of lignocellulose degradation in biosulphidogenic environments. 12th Biennial Congress of the South African Society for Microbiology. Bloemfontein, 2-5 April, 2002.
5. Rose, P.D. 2002. The biological sulphur cycle: Part of the problem or basis for sustainable bioprocess innovation? WISA Biennial Conference, Durban, 19-23 May, 2002.

2.2.4.2 International Conferences

1. Rose, P.D., Maart, B.A., Dunn, K.M., Rowswell, R.A. and Britz, P. 1995. High Rate Oxidation Ponding for the Treatment of Tannery Effluents. 3rd IAWQ International Specialist Conference on Waste Stabilisation Ponds, Brazil.
2. Boshoff, G.A., Duncan, J.R., Burton, S.G. and Rose, P.D. 1995. The removal of heavy metals from industrial effluents by sulphate reducing bacteria. Proceedings of Society for General Microbiology first Joint meeting with the American Society for Microbiology on Bioremediation, Aberdeen, Scotland, 1995.
3. Boshoff, G.A., Duncan, J.R. and Rose, P.D. 1996. Algal integrated ponding system for the treatment of mine drainage waters. Proceedings of 7th International Conference of Applied Algal Biotechnology, Knysna, April 1996.
4. Rose, P.D. and Dunn, K. 1996. The integrated Photosynthetic high rate oxidation pond for treating tannery waste waters. Proceedings of 7th International Conference of Applied Algal Biotechnology, Knysna, April 1996.
5. Boshoff, G. and Rose, P. 1998. Algal biomass as a carbon source in sulphate reducing ponding treatment of acid mine drainage water. European Union Summer School: The Biological Sulphur Cycle - Environmental Science and Technology. Wageningen, The Netherlands, April 19-24, 1998.
6. Boshoff, G. and Rose, P. 1998. The use of tannery wastewater as a carbon source for sulphate reduction and heavy metal removal. European Union Summer School: The Biological Sulphur Cycle - Environmental Science and Technology. Wageningen, The Netherlands, April 19-24, 1998.
7. Duncan, JR., van Hille, RP., Boshoff, GA., Wallace, L. and Rose, PD. 1998. Biological treatment of metal containing wastewater using an integrated approach. 4th Intl. Symposium on Envir. Biotechnol., Belfast, Ireland.
8. Duncan, JR., van Hille, RP., Boshoff, GA., Wallace, L. and Rose, PD. 1998. Biological treatment of metal containing wastewater using an integrated approach. Proc. 4th Intl. Symp. Envir. Biotechnol., Belfast, Ireland.
9. van Hille, RP., Boshoff, GA., Rose, PD. and Duncan, JR. 1998. A continuous process for the biological treatment of heavy metal contaminated acid mine water. Proc. 4th Intl. Symp. Envir. Biotechnol., Belfast, Ireland.
10. Boshoff, GA., Duncan, JR. and Rose, PD. 1998. Heavy metal sequestration by microalgal photosynthate released in high rate algal ponding treatment of acid mine drainage. 4th Intl. Symp. Envir. Biotechnol., Belfast, Ireland.
11. Boshoff, GA., Duncan, JR. and Rose, PD. 1998. Microalgal biomass: An independent carbon source for sulphate reduction in an algal ponding treatment of acid mine drainage. Proc. 4th Intl. Symp. Envir. Biotechnol., Belfast, Ireland.
12. Heron, P., Ngesi, N., Tshivunge, S., van Jaarsveld, F., Rose, P. and Whiteley, C. 2000. Environmental enzymology: enzymology of the accelerated primary sewage sludge solubilisation and bioremediation of acid mine drainage. Fifth International Symposium on Environmental Biotechnology. Kyoto, Japan, July 9-13.
13. C.G.Whiteley, P.Rose, B.Pletschke, F van Jaarsveld, P.Heron, N.Ngesi, S.Tshivhunge. 2000. Environmental Enzymology: Enzymology of Accelerated Primary Sewage Sludge Solubilisation and Bioremediation of Acid Mine Drainage. International Symposium on Environmental Biotechnology, Kyoto, Japan, July 9-13.
14. C.G.Whiteley, B.I.Pletschke, P.D Rose and A.S Tshivhunge. 2001. Environmental Enzymology: Enzymology of Accelerated Primary Sewage Sludge Solubilisation: Effect of

sulphate, sulphite and sulphide on proteases; International Water Association: Asia Waterqual; Fukuoka, Japan, 2001.
15. C.G.Whiteley, B.I.Pletschke and P.D Rose. 2001. Environmental Enzymology: Enzymology of Accelerated Primary Sewage Sludge Solubilisation: Effect of Sulphurylases; International Water Association: Anaerobic Digestion; Antwerpen, Belgium, 2001.
16. C.G.Whiteley, B.I.Pletschke and P.D Rose. 2001. Environmental Enzymology: Enzymology of Accelerated Primary Sewage Sludge Solubilisation: Effect of Proteases; International Water Association: Sludge Management; Taipei; Taiwan; 2001.
17. C.G.Whiteley, X.Melamane, B.Pletschke, P.Rose. 2002. Effect of Lipases on the Acceleration of Solubilisation of Primary Sewage Sludge. Environmental Biotechnology 2002; IWA Conference. New Zealand, 2002.

2.2.4.3 Local Conferences

1. Boshoff, G., Leukes, W., Jacobs, E., Sanderson, R. and Rose P.D. 1994. Efficiency of zinc removal by microalgae immobilised on hollow-fibre ultrafiltration membranes. Proc Eight Biennial Conference South African Society for Microbiology Grahamstown.
2. Boshoff, G., Duncan, J and Rose, P. 1994. The precipitation of heavy metals by sulphate reducing bacteria in a mixed bioreactor. Proceedings of Eight Biennial Congress of the South African Society of Microbiology. Rhodes University, Grahamstown, June 1994.
3. Boshoff, G., Leukes, W., Jacobs, E., Sanderson, R and Rose, P. 1994. Efficiency of zinc removal by microalgae, immobilised on hollow fibre ultrafiltration membranes. Proceedings of First WISA/MTD Seminar, Van Stadens, November, 1994.
4. Boshoff, G., Duncan, J and Rose, P. 1995. The utilisation of algal biomass as a carbon source for sulphate reducing bacteria. Proceedings of All-African Biotechnology Conference, Pretoria, November 1995.
5. Boshoff, G.A., Duncan, J.R. and Rose, P.D. 1996. The removal of heavy metals from industrial effluents by sulphate reducing bacteria. Proceedings of Water Institute of Southern African Biennial Conference, Port Elizabeth, May 1996.
6. Boshoff, G.A., Duncan, J.R. and Rose, P.D. 1996. The production of extracellular polysaccharides by the salt-tolerant alga under different environmental conditions. South African Society for Microbiology. June 1996.
7. Rose, P.D., Hart, O.O., Barnard, J., Shipin, O. and Boshoff, G. 1997. Algal biotechnology and water treatment. Second South African Biotechnology Conference, Biotech SA '97, Grahamstown. January 1997.
8. Boshoff, G.A., Radloff, S., Duncan, J.R. and Rose, P.D. 1997. Biological sulphate removal for the treatment of acid mine drainage - a statistical perspective. Second South African Biotechnology Conference, Biotech SA '97, Grahamstown. January 1997.
9. Molepane, N.P., Boshoff, G.A. and Rose, P.D. 1997. The culture of *Dunaliella* and the production of extracellular polysaccharide under different environmental conditions. Second South African Biotechnology Conference, Biotech SA '97, Grahamstown. January 1997.
10. van Hille, R.P., Wallace, L.C.M., Boshoff, G., Rose, P.D. and Duncan, J.R. 1997. Second South African. Biotechnology Conference, Biotech SA '97, Grahamstown. January 1997.
11. Rose, P.D., Boshoff, G.A., van Hille, R.P., Wallace, L.M.C., Dunn, K.M., Hart, O.O. and Duncan, J.R. 1998. Treatment of acid mine drainage water in an integrated sulphate reducing high rate ponding process. WISA '98, Cape Town.
12. Wallace, L.M.C., Boshoff, G.A., Duncan, J.R. and Rose, P.D. 1998. A microbial sulphate

reducing system utilised for the precipitation of heavy metals from refinery wastewaters. WISA '98, Cape Town.
13. van Hille, R. P., Boshoff, G.A., Rose, P.D. and Duncan, J.R. 1998. A continuous process for the biological treatment of heavy metal contaminated acid mine drainage. WISA '98, Cape Town

14. Boshoff, G.A., Duncan, J.R. and Rose P.D. 1998. Sulphide toxicity to microalgae. WISA '98, Cape Town
15. Dekker, L.G., Clark, S,J., Hart, O.O. and Rose, P.D. 2000. Dentirification and tertiary treatment of domestic wastewaters using stress manipulation in algal ponds. Biotech SA 2000, BIOY2K Grahamstown, January 2000.
16. Nightingale, L., van Hille, R.P., Rose, P.D. and Duncan, J.R. 2000. Algal alteration of carbonate species equilibria: bioremediation potential. Biotech SA 2000, BIOY2K Grahamstown, January 2000.
17. Wallace, L.C.M., Rose, P.D. and Duncan, J.R. 2000. Competitive metal ion removal from zinc refinery wastewater treated with sulphide-containing anaerobically digested sewage sludge. Biotech SA 2000, BIOY2K Grahamstown, January 2000.
40.
18. Whittington-Jones, K., Corbett, C.J.,. Whiteley, C., van Jaarsveld, F. and Rose, P.D. 2000. Enhanced hydrolysis of primary sewage sludge under sulphate reducing conditions. Biotech SA 2000, BIOY2K Grahamstown, January 2000.
19. Whittington-Jones, K., Corbett, C.J., Whiteley, C., van Jaarsveld, F. and Rose, P.D. 2000. Enhanced hydrolysis of primary sludge under sulphate reducing conditions. SASBMB, BIOY2K Grahamstown, January 2000.
20. Rose, P.D. 2000. The Rhodes BIOSURE process: the piloting of an active process for the treatment of acid mine drainage wastewaters. WISA Minewater Conference, BIOY2K Grahamstown, January 2000.
21. Corbett, C., Whittington-Jones, K. and Rose, P.D. 2000. Biological treatment of acid mine drainage wastewaters using a sewage sludge carbon source. WISA Biennial Conference, Sun City, 28 May - 1 June, 2000.
22. Ngesi, N., van Jaarsveld, F., Rose, P.D. and Whiteley, C.G. 2000. Envvironmental enzymology; role of cellulases and glycohydrolases in sewage sludge solubilisation. WISA Biennial Conference, Sun City, 28 May - 1 June, 2000.
23. Heron, P., Ngesi, N., Tshivunge, S., van Jaarsveld, F., Rose, P.D. and Whiteley, C.G. 2000. Biochemical context of the enzymology of accelerated primary sewage sludge solubilisation. WISA Biennial Conference, Sun City, 28 May - 1 June, 2000.
24. Heron, P., Ngesi, N., Tshivunge, S., van Jaarsveld, F., Rose, P.D. and Whiteley, C.G. 2000. Environmental enzymology: enzymology of accelerated primary sewage sludge solubilisation. WISA Biennial Conference, Sun City, 28 May - 1 June, 2000.
25. Tshivunge, S., van Jaarsveld, F., Rose, P.D. and Whiteley, C.G. 2000. Environmental enzymology: identification, isolation, kinetics, characterisation and inhibition of proteases. WISA Biennial Conference, Sun City, 28 May - 1 June, 2000.
26. Roman, H.J., Madikane, M., Nagabhushana, K.S. and Rose, P.D. 2002. Lignocellulose as a carbon source in the biological treatment of mine drainage wastewaters. WISA Biennial Conference, Durban, 19-23 May, 2002.
27. Clarke, A.M. and Rose, P.D. 2002. Microbial ecology of the degrading packed bed reactor. WISA Biennial Conference, Durban, 19-23 May, 2002.
28. Whiteley, C.G., Melamane, X., Pletschke, B. and Rose, P.D. 2002. The enzymology of

sludge solubilisation utilising sulphate reducing systems - the role of lipases. WISA Biennial Conference, Durban, 19-23 May, 2002.
29. Ackhurst, T., Watson, S., Rose, P.D., Whiteley, C.G. and Pletschke, B. 2002. Accelerated sludge solubilisation under sulphate reducing conditions: the effect of hydrolytic enzymes on sludge floc size distribution and EPS composition. WISA Biennial Conference, Durban, 19-23 May, 2002.

APPENDIX 3

RESEARCH SPIN-OFF DEVELOPMENTS

The studies described here have resulted in a number of follow-up studies undertaken by the Rhodes EBG, and also by colleagues and collaborators, and certain of these projects have developed a life of their own. Aspects of these spin-off developments are described here, the outcomes of which will be detailed in separate reports.

3.1 THE RHODES BIOSURE PROCESS®

3.1.1 Mine Drainage Wastewater Treatment

Fundamental studies were undertaken to explain the enhanced hydrolysis of organic particulate solids and sludges in the sulphate reducing environment. Application of these findings in the treatment of AMD as optimised reaction outside the IAPS environment, utilising sewage sludges as the carbon source, resulted in the development of the Recycling Sludge Bed Reactor (RSBR) and the Rhodes BioSURE Process®. The linkage of saline and sanitation wastewater treatment would provide a sustainable management for the AMD problem for the long periods of time over which the decanting mine waters are expected to flow. The process was scaled up and evaluated in a pilot plant at Grootvlei Mine near Springs (Figure A 4.6). These studies are detailed in WRC report ' The Rhodes BioSURE Process®. Part 1: Biodesalination of Mine Drainage Wastewaters'.

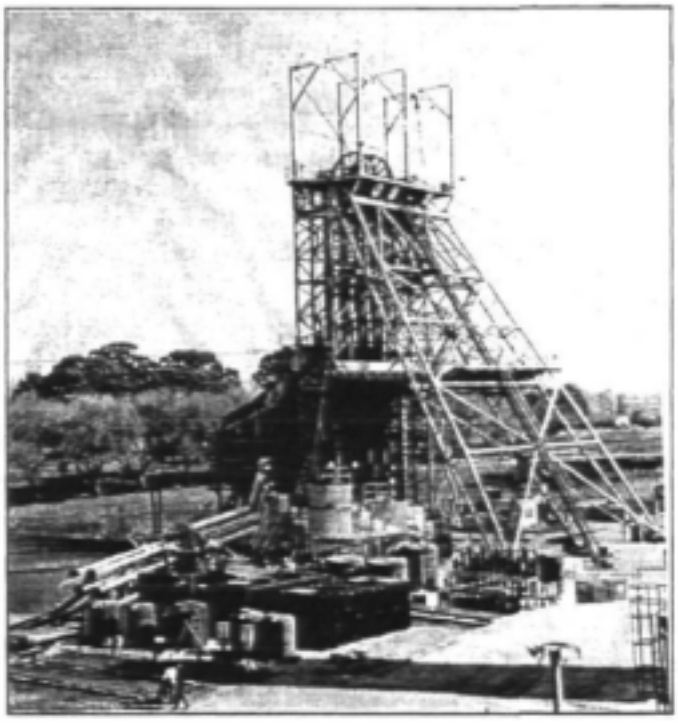


Figure A 3.1 Headgear at the No.4 shaft Grootvlei Mine with the Rhodes 'BioSURE' Process pilot plant in the foreground.

3.1.2 Treatment for Sewage Sludge Solids

The effective use of sewage sludge as a carbon source for AMD treatment in the Rhodes 'BioSURE' process led to an investigation of the process in an application where the solubilisation and disposal of sewage sludges would be the primary objective. This has been undertaken in collaboration with the East Rand Water Care Company (Erwat) in a joint WRC/Erwat/Rhodes University project, and it has been shown that in addition to solids solubilisation and the removal of heavy metals, a high levels of sludge disinfection is achieved. A technical-scale plant has been constructed at the Erwat Ancor Works for the scale-up evaluation of the process. This development has been based on the scale-up of the RSBR described above, and the 2ML reactor (Figure A 3.2) is fed with a sulphate-rich wastewater along a 2.5 km pipeline from the Grootvlei Mine. These studies will be reported in WRC report 'The Rhodes BioSURE Process®. Part 4: Treatment and Disposal of Sewage Sludges' (Report 12).

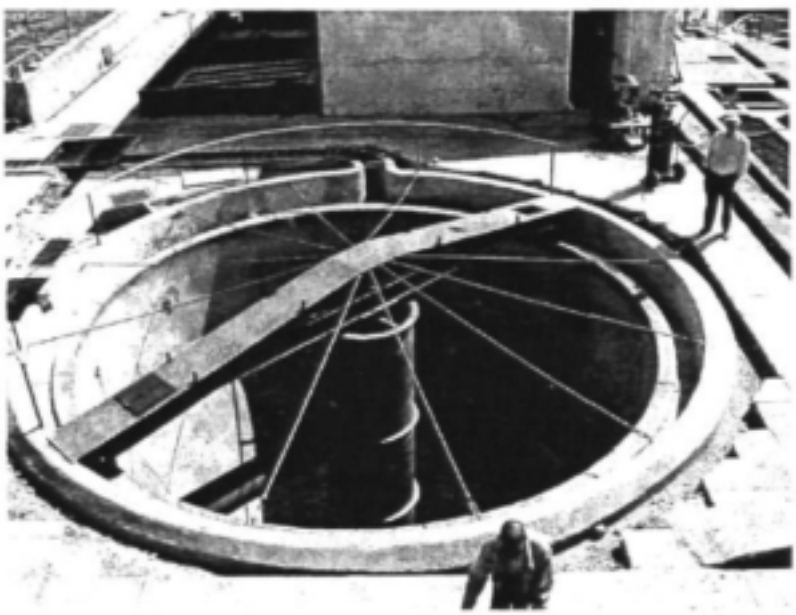


Figure A 3.2. The 2 ML scaled-up Recycling Sludge Bed Reactor in the BioSURE® sewage sludge solubilisation technical-scale plant constructed at Erwat's Ancor Works in Springs. Surface struts provide supports for a covering membrane.

3.2 INNOVATION FUND PROJECT ON PASSIVE TREATMENT SYSTEMS

An investigation of passive systems for the treatment of mine drainage wastewaters have been investigated in a Department of Arts Culture Science and Technology (DACST) Innovation Fund project led by Pulles Howard and De Lange. Current research is investigating the application of the sulphate reducing Degrading Packed Bed Reactor, based on the RSBR development in the BioSURE® process. Lignocellulosic wastes are used as the feedstock in this process.

3.3 ESKOM SUSTAINABLE DEVELOPMENT PROJECT

Eskom have undertaken the application of various aspects of the WRC study in a programme to establish a comprehensive 'integrated wastewater resource management' approach to coal mining wastewaters. In addition to water treatment, the project aims to develop aspects of the value recovery and beneficiation findings which have developed in the WRC study, including job creation and community rehabilitation initiatives in preparation for mine closure. This undertaking involves the use of the Rhodes BioSURE® Process as a pretreatment to reverse osmosis membrane desalination, effecting the removal of metals, sulphate, calcium and other scaling salts, and the return of the treated water to mining and power generation requirements. The saline reject streams would pass to *Spirulina* and *Dunaliella* solar evaporation ponding cascades where biomass and fine biochemical production would be used as a component of downstream beneficiation operations. The ESKOM project would ultimately handle a minewater stream of 20 Ml.day^{-1}, and provides an opportunity for the large-scale evaluation of the 'integrated wastewater resource management' objectives which formed one of the major motivations for the WRC study 'Salinity Sanitation and Sustainability'.

3.4 BIODESALINATION AND SULPHUR RECOVERY

A biodesalination of sulphate saline wastewaters may be achieved in a sulphide oxidation unit operation, where elemental sulphur (S^o) would be a final by-product of the treatment process. Follow-up studies on sulphide oxidation bioprocess development, both at laboratory- and pilot-scale, were undertaken in WRC and a joint WRC/DACST Innovation Fund projects. These resulted in the development of the Floating Sulphur Biofilm Reactor (Figure A3.3) and will be described in WRC Report, 'Salinity, Sanitation and Sustainability: A Study in Environmental Biotechnology and Integrated Wastewater Beneficiation in South Africa. Volume 4. The Rhodes BioSURE Process®. Part 3: Sulphur Production and Metal Removal Unit Operations' (Report 11).

Figure A 3.3 The Floating Sulphur Biofilm Reactor developed for sulphur recovery and biodesalination of reduced sulphate-saline wastewaters

3.5 METALS AND BIOHYDROMETALLURGY

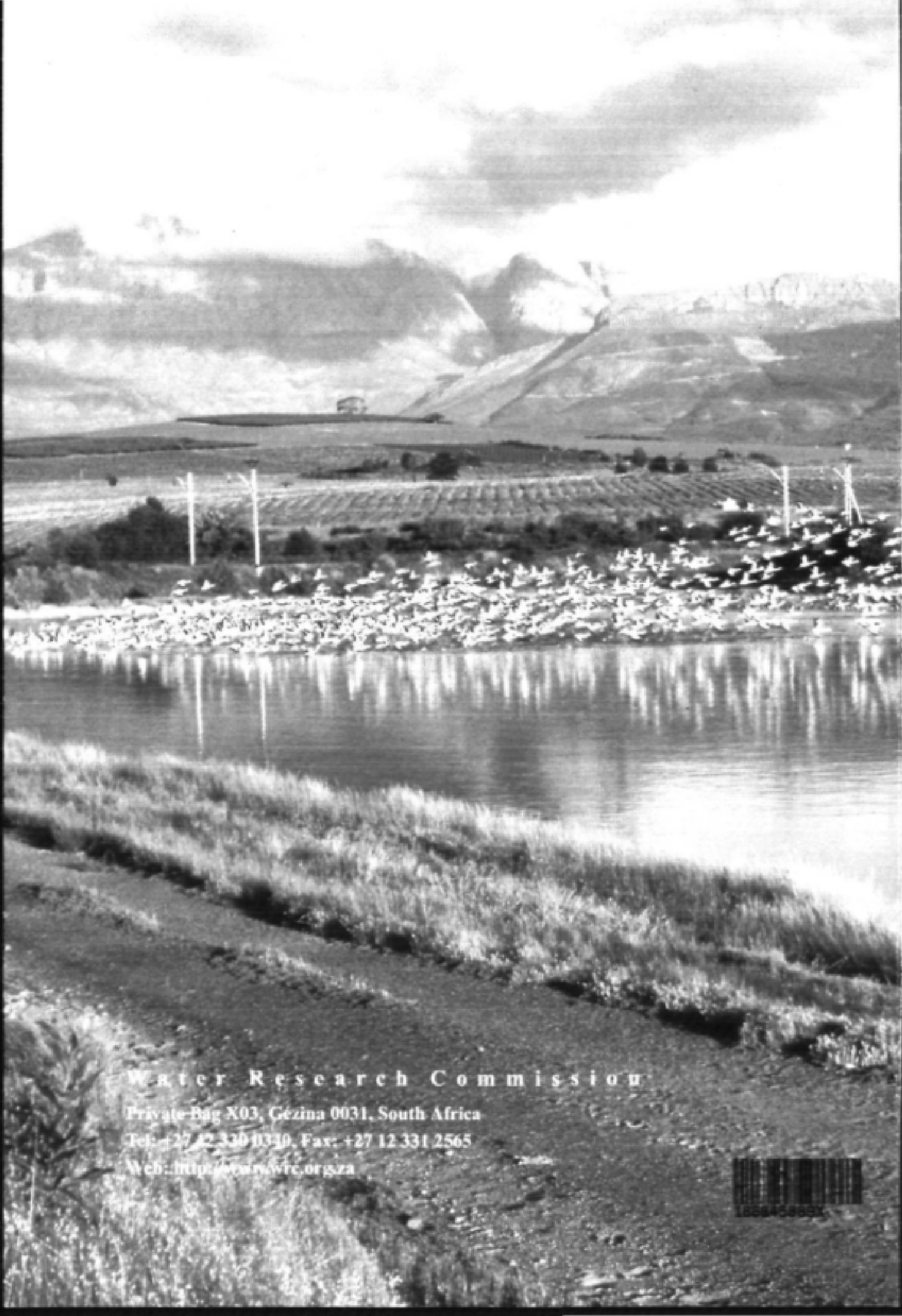
The early studies in metal removal in algal ponding systems explored the role of bioadsorption and bioaccumulation by microbial biomass. Algal ponding systems were shown to provide the basis for process development in free-standing HRAP systems such as the ASPAM development. These studies were undertaken in collaboration with Prof John Duncan and this now forms an independent WRC programme in biohydrometallurgy at Rhodes University.

3.6 UCT DEPARTMENTS OF CHEMICAL AND CIVIL ENGINEERING

Following the biotechnology studies and bioprocess developments, relating to the Rhodes BioSURE® Process reported in Chapter 7, a number of collaborative studies commenced with the UCT Departments of Chemical and Civil Engineering, to undertake the modelling of particular aspects of the system. Both computer models of the process and descriptive accounts of the aqueous chemistry of these systems have resulted, and these initiatives have developed into separate WRC projects.

3.7 THE ENZYMOLOGY OF SLUDGE HYDROLYSIS

Preliminary studies had shown a relationship between the physico-chemical conditions prevailing in the RSBR and enzymatic activity resulting in the sludge hydrolysis and solubilisation patterns observed. These studies have become the focus of a separate programme in environmental enzymology under the leadership of Chris Whiteley and Bret Pletschke.



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