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

Part 1 - Meso-saline Wastewaters: The *Spirulina* Model

Report to the Water Research Commission
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Report to the Water Research Commission on studies undertaken in the Project K5/495 'A Biotechnological Approach to the Removal of Organics from Saline Effluents'

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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

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.

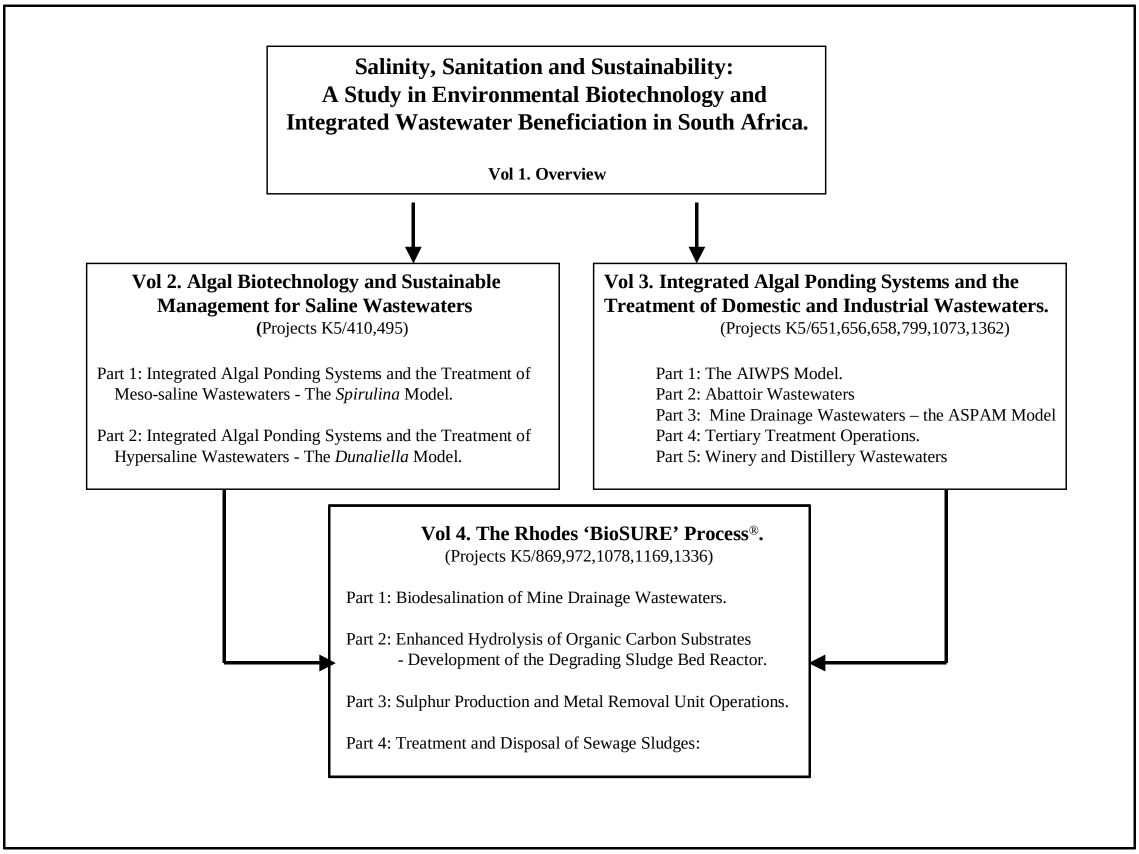

Figure P1. Research projects undertaken as components of the Water Research Commission study 'Salinity, Sanitation and Sustainability'.

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.

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CONTENTS

EXECUTIVE SUMMARY

1 SALINITY AND SUSTAINABILITY

Salinisation has long been identified as one of the single most serious threats of pollution facing the sustainability of the public water system in South Africa. Despite considerable attention devoted to the problem over many years the salinity situation has continued to deteriorate nationally. The National State of the Environment Report (DEAT, 1999), and the White Paper on Integrated Pollution and Waste Management (DEAT, 2000), have both highlighted the ongoing and, in places, the accelerating degradation of the National water resource. The White Paper has identified salinity and sanitation issues as six of the seven priority sources of pollution in the country.

While problems of saline pollution and salinisation remain widespread, and pervasive threats to sustainability of both the bio-physical and the human environments exist in most of the Southern African region, the potential technological options by which this set of problems may be addressed remain relatively limited. Of these, three main strategies have been generally pursued including elimination at source, disposal by dilution, and segregation to prevent the contamination of the rest of the water resource. Without ignoring the multi-dimensional nature of the problem, the segregation option has been the specific focus of this study of an environmental biotechnology-based approach to sustainable management for saline wastewaters.

2 PROJECT BACKGROUND

The WRC study 'Salinity Sanitation and Sustainability' was conceptualised in the late 1980s against a background of an increasing interest in the operationalisation of sustainability, around the time of the World Commission on Environment and Development (WCED, 1987), and leading to the United Nations Conference on Environment and Development in June 1992 (the Rio Earth Summit). The study of saline algal biotechnology had commenced at Rhodes University in the mid-1980s, and in 1990 Dr Oliver Hart, Research Manager at the Water Research Commission (WRC), undertook an evaluation of the research outputs of the Environmental Biotechnology Group (EBG) related to the integration of saline wastewater treatment and the production of high-value algal bio-products. It was proposed that a feasibility study be undertaken to provide a structured assessment of the wider potential in linkage of algal biotechnologies and saline wastewater treatment.

The WRC project K5/410, 'A biotechnological approach to the removal of organics from saline effluents', commenced in 1991 as a one year study, to investigate the application of Integrated Algal Ponding Systems (IAPS) in the treatment, utilisation and ultimate disposal of saline wastewaters, and to report specifically on the potential for the development of biotechnology applications associated with these systems. Against the background of this apparent potential, it was also identified as an important objective of the study to investigate how far the concept could be pushed as both a 'core technology' in sustainable wastewater treatment, and as a source of innovation within broader sustainability and 'integrated waste resource management' objectives. The project brief also called for proposals on the merits of a longer-term study into environmental biotechnology-based approaches to the salinity problem.

3 PROJECT K5/495 - 'A BIOTECHNOLOGICAL APPROACH TO THE REMOVAL OF ORGANICS FROM SALINE EFFLUENTS'

The WRC project K5/410 was undertaken primarily as a desk-study, although some issues were confirmed by initial experimental observation. Following a detailed literature review, and evaluation of algal production potential in saline wastewaters in South Africa, it was proposed that the study be taken up in an active experimental research programme. The current project K5/495 (with the same title) commenced as a five-year investigation in 1992, and it was decided that the findings and results of the initial feasibility study become incorporated in the report on project K5/495.

The broad objective of this research programme, in the first instance, was to undertake a feasibility evaluation of an integrated algal biotechnology contribution to the salinity problem. The basic question to be asked was whether the halophilic algae could be used to treat industrially generated brines, and whether production of value-added bio-products, during the course of this process, could place improved disposal practices for these wastes on a sound economic footing and thus provide a market-driven basis for their long-term sustainable management. Conversion of wastes into resources, especially in the development context where wastes might be among the few resources available, could contribute directly to sustainable development. Saline algal biotechnology appeared to offer a means for capturing nutrient values in organic wastes in the form of high-value bio-products, and thus contribute to environmental, economic and social objectives.

The enquiry was premised by a number of assumptions which served to focus the investigation:

- ❑ Of the brine disposal options that have been suggested in the literature the least attractive is the dumping of saline brines in sinks, or any variation thereof, where salt will accumulate indefinitely, creating possibly insoluble problems for the future;
- ❑ In principle, where segregation is practised, the solution of choice would be linear whereby salts are concentrated and ultimately recovered from brines, and disposed of in a way that effectively removes them finally from the environment;
- ❑ While high cost, capital and energy intensive membrane separation and evaporation technology does exist to accomplish this task, both rapidly and effectively, the disposal of reject brines remains a problem. It is likely that a 'low technology' ponding and solar evaporation process would be found to be cost effective and appropriate to the scale and time-frame of the problem. This could provide the only option for keeping saline wastewaters out of the fresh water system in the developing world context;
- ❑ The simplest application of this process would be some form of linear cascade of ponds with a rising gradient of salinity resulting in the production of a saturated brine solution, which may pass ultimately via crystallisation to final disposal;

- ❑ The loss of water associated with evaporative disposal is discouraged by DWAF. Therefore, where appropriate, saline wastewaters should be segregated and pass directly to pond impoundments, rather than via dilution and then to treatment as dilute saline and brackish wastewater streams;
- ❑ It is unlikely that a universally applicable algal culture system will be found to be appropriate for all brine streams. While some form of pooling of different brine streams is to be anticipated, separate systems will probably be required for the various forms of saline effluent.

3.1 Aims

The broad objective of Project K5/495 was to evaluate the potential of algal biotechnological systems to deal with the removal of organics and heavy metals from saline wastewaters on a scale appropriate to large-volume wastewater impoundments. The specific research aims of the project were identified at the outset as follows:

1. The development of a saline High Rate Algal Pond (HRAP) process for removal of organics from saline wastewaters, based on halophilic algae;

2. To optimise the operation of the solar evaporation Waste Stabilisation Ponding (WSP) process by the manipulation of micro-algal production;

3. The demonstration of a utility value for saline wastewaters with the recovery of value in the form of micro-algal bio-products;

4. The removal of heavy metals from saline wastewaters;

5. The application of membrane separation techniques employed for the harvesting of micro-algal biomass and recovery of bio-products.

3.2 The Tannery Waste Stabilisation Pond: A Model System.

Since the 1950s WSP have been used in a wide range of applications for the treatment of domestic and industrial wastewaters, including the treatment of tannery wastewaters (Rowswell *et al.* 1984; Pearson 1996). The WSP is one of the few cost-effective options for the treatment and final disposal of hyper-saline wastes (> 40 $g.L^{-1}$ total dissolved inorganic solids - TDIS), where dilution of the salt load into the public water system cannot be entertained (Rose *et al.* 1996). Despite the strategic significance of the technology in this application, few studies are reported in the literature which deal, in any detail, with the tannery WSP, or their application in hyper-saline wastewater treatment.

The tannery WSP was chosen as a model system for the study, as it provides, in some sense, a worst-case scenario of what may be encountered in saline wastewater impoundments. The WSP is well-established technology for the treatment and disposal of organic sewage wastewaters, and tannery effluents contain a range of components including high organic loads, heavy metals, and high levels of sulphates, ammonia, nitrates and protein nitrogen.

The results and findings of the study have been reported in the following sections:

3.2.1 The Microbial Ecology of a Tannery Waste Stabilisation Ponding System

The project commenced as a three-year study of a WSP treating tannery wastewaters. The investigation of the microbial ecology of the system was undertaken and these results shaped the remainder of the research programme. Principal factors influencing the operation of the treatment process were identified, and an attempt was made to describe how these affect the performance of the tannery WSP.

A pronounced salinity gradient establishes across these WSP, and it became apparent from pond ecology studies that the initial and final ponds of the evaporation cascade presented two clearly defined and differentiated ecosystems based on their salt concentration. The study therefore segmented at an early stage into investigations of the meso-saline compartments (< 40 $g.L^{-1}$ TDIS) where *Spirulina* sp. dominated, and the hyper-saline compartments where *Dunaliella* spp. blooms occurred from time to time. The results of the project have thus been reported in two parts. The *Spirulina* studies are detailed in this report, and the *Dunaliella* studies appear in Part 2 of the WRC report 'Integrated Algal Ponding Systems and the Treatment of Saline Wastewaters. Part 2: Hyper-saline Wastewaters - The *Dunaliella* Model' (Appendix 1).

3.2.2 The Growth and Performance of *Spirulina* in the Operation of a Tannery Waste Stabilisation Ponding System

The above studies showed growth yields in the WSP, of apparently near mono-species blooms of *Spirulina*, comparable to values reported for some commercial production systems. These blooms were, however, unpredictable and varied widely over time. Odour nuisance is a major problem in these systems and its effective control was observed to be associated with these blooms, and also an improved treatment of the wastewaters.

Studies in the microbial ecology of the system showed the primary role of ammonia concentration in the overall regulation of *Spirulina* growth, among a number of other factors. It was proposed that an effective control of these limiting factors could enable a predictable management of *Spirulina* growth in this wastewater medium, and the development of a *Spirulina*-High Rate Algal Pond (S-HRAP), as a free-standing unit operation for the treatment of tannery wastewaters. The production of *Spirulina* biomass would be a value-added by-product of such a process.

3.2.3 A *Spirulina*-HRAP Unit Operation for the Treatment of Tannery Wastewaters

The potential of the S-HRAP development was investigated in a range of laboratory photobioreactor studies. These findings led to the construction of a pilot plant at the WSP site at Mossop-Western Leathers Co. tannery in Wellington, South Africa. Following pilot studies, and the demonstration of the performance of this unit, a 2 500 m^2 full-scale S-HRAP was designed and constructed on-site by the Company for the treatment of saline tannery wastewaters (Figure 1).

3.2.4 Integration of the *Spirulina* HRAP and Waste Stabilisation Pond Operations in Tannery Effluent Treatment

The development of the S-HRAP, as an integrated unit operation in the WSP system, provided the opportunity for follow-up studies on a number of issues which had emerged earlier in the research programme. This included the potential advantages of recirculation of alkalised pond water to improve both organic loading and hydraulic retention in the unit. In addition to improved performance in the S-HRAP from recirculation, the operation of the WSP could benefit from the integration of the S-HRAP unit with reduced organic loading to the ponds, possibly a reduced pond surface area requirement, improved evaporation performance and the production of a low organic content brine, more suitable for crystallisation than that currently produced.

Figure 1. The 2 500 m^2 high rate oxidation pond at Mossop-Western Leathers Co., in Wellington, RSA.

The location of the S-HRAP at the head of the WSP system would also provide an algal overlay to the downstream ponds in the cascade. This capping effect on the anaerobic ponds could achieve a particularly effective odour control, which had been observed in the latter pond, in the pond ecology studies.

Investigation of these features was undertaken in studies which the Primary Facultative Pond (PFP) was retrofitted, and the performance of the system was evaluated together with the S-HRAP (Figure 2). It was shown that effluent treatment performance for the PFP/S-HRAP unit operations produced an effluent of a quality suitable for recycle to certain tannery operations. The linkage of these units to the WSP enabled the overlay of anaerobic ponds with algal-laden waters, the effective control of odour nuisance, and the production of an improved quality in the final effluent.

High rates of sulphate reduction were observed in the reconfigured PFP, and resulted in the near complete precipitation in this unit of heavy metal contaminants present in the tannery effluent. This resulted in the production of a *Spirulina* biomass with metal levels acceptable for use in animal feed applications. The observations of an enhanced hydrolysis of complex organic particulate solids in the sulphate reducing compartment of the PFP (a unit notoriously prone to solids build-up and requiring frequent desludging), resulted in follow-up studies on the linkage of sulphate-saline wastewater treatment and organic waste disposal. These studies using organic waste as a carbon source for biological sulphate reduction resulted in the development of the Rhodes BioSURE Process® for acid mine drainage (AMD) wastewater treatment. These studies are described in subsequent WRC project reports (Appendix 1).

3.2.5 The Harvesting and Recovery of Effluent-grown *Spirulina* Biomass

Based on the above results, studies were undertaken on the recovery of *Spirulina* biomass as a value-added by-product of the wastewater treatment operation. The harvesting and drying of *Spirulina* biomass from the Wellington WSP and the new S-HRAP unit were investigated. Nutritional analysis of harvested dried biomass was undertaken.

3.2.6 *Spirulina* Biomass Toxicology and Feed Evaluation Studies

The development of an effective value-adding function for the saline wastewater impoundment would require demonstration of the acceptability of the dried *Spirulina* biomass as a feed-grade product, both in terms of its toxicological characteristics and usefulness as a nutritional resource. Toxicological studies were undertaken including heavy metal removal through the reconfigured PFP. Feeding studies were undertaken in chickens, rainbow trout and the South African abalone, *Haliotis midae*, and indicated the production of an acceptable animal and aquaculture feed product. The dried *Spirulina* biomass was used in the development by the Rhodes University Ichthyology Department, of the novel ‘Abfeed’ for the aquaculture production of abalone.

4 CONCLUSIONS

Anecdotal observations of massive micro-algal blooms occurring on the Wellington WSP, from time to time, were confirmed in a three-year study, which represents a first detailed report of the microbial ecology of these WSP systems. Factors influencing micro-algal growth and production in the WSP were described, and it was shown that the meso-saline and hyper-saline conditions established in the separate parts of the system provided potential for the cultivation of *Spirulina* and *Dunaliella*, as near mono-species cultures.

The potential development of IAPS for the linkage of wastewater treatment and the capture of nutrient values in algal biomass production, particularly of high-value products, has been constrained by the ability to establish such mono-species growth in outdoor conditions. This objective was pursued in these studies.

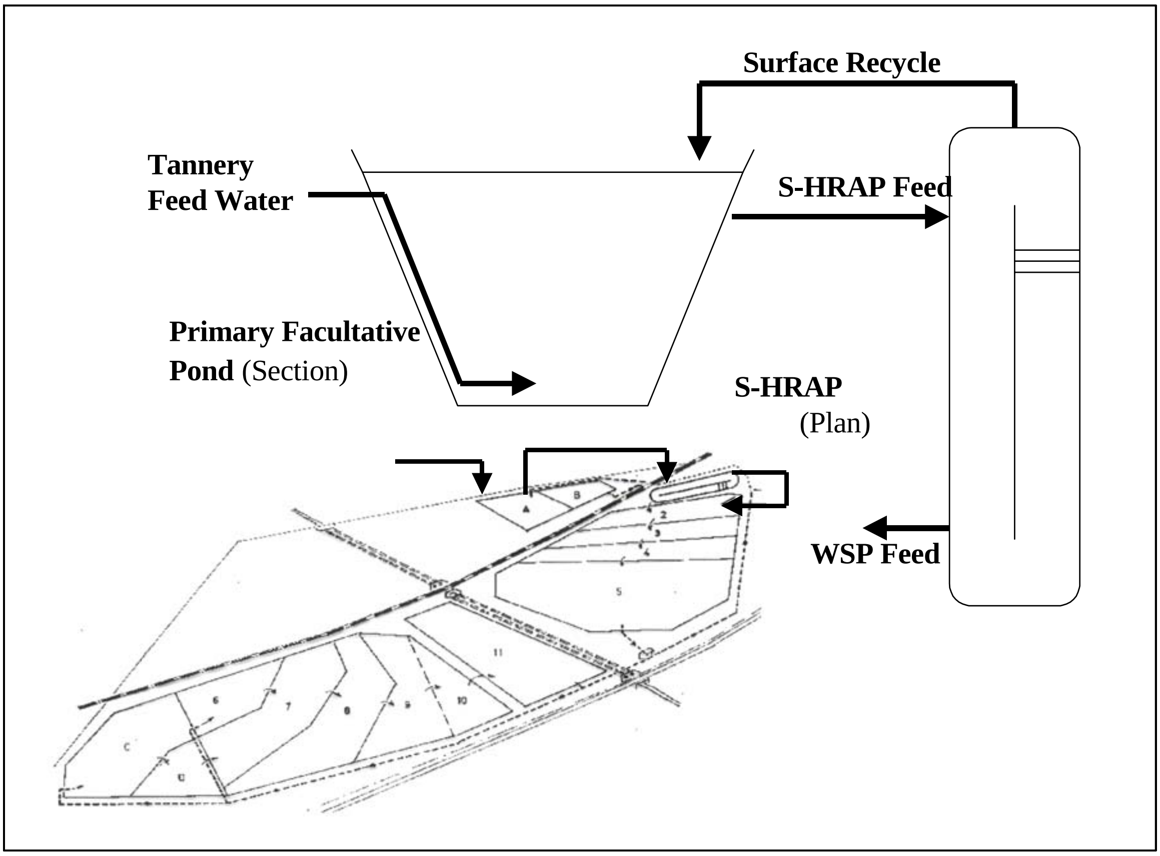

Figure 2. Configuration of the Primary Facultative Pond and *Spirulina*-HRAP (S-HRAP) constructed at the Mossop-Western Leathers Co. tannery effluent WSP site in Wellington. for the aquaculture production of abalone.

The S-HRAP concept was developed through laboratory, pilot plant studies to its full-scale application as a novel and patented method for the treatment of tannery wastewaters. At the same time harvesting of *Spirulina* biomass, and its toxicological assessment were undertaken, and it was shown that animal and aquaculture feed-grade micro-algal products could be recovered from the system.

The development of the hyper-saline D-HRAP application was also undertaken, involving the growth and recovery of *Dunaliella* spp. These studies are detailed in Part 2 of this report 'Integrated Algal Ponding Systems and the Treatment of Saline Wastewaters. Part 2: Hyper-saline Wastewaters - The *Dunaliella* Model' (Appendix 1).

The study achieved the principal objectives of the project and it was shown that:

- ❑ the HRAP may be developed as a useful unit operation in the treatment of saline wastewaters, and may be used for the reduction of both organic load and the removal of heavy metal contaminants;
- ❑ reduction of organic load in saline wastewater improves evaporation rates and would enable a reduced pond surface area requirement;

- ❑ removal of contaminating organic and heavy metal compounds enables the safe impoundment of saline wastewaters and eliminates the environmental threat where these become occupied as wildlife refuge sites;
- ❑ the S-HRAP could be developed as a free-standing tannery wastewater treatment operation. The S-HRAP provides an IAPS configuration which may be used for the improved operation of WSP-based tannery wastewater treatment, and also to reduce environmental impacts such as odour nuisance;
- ❑ a utility could be demonstrated for saline wastewater impoundments with the growth, and recovery of value, in the form of *Spirulina* biomass;
- ❑ the production of an algal bio-product, and the demonstration of a value-adding function for a saline effluent, indicated a possible model system for larger-scale application in sustainable management strategies for saline wastewaters;
- ❑ the enhanced hydrolysis of complex organic compounds observed in the sulphate reducing compartments of the tannery WSP indicated potential linkages for the saline and sanitation wastewater systems. The IAPS would not only provide a ‘core technology’ wastewater treatment in both systems, but disposal of sanitation system wastes in saline wastewaters could provide a basis for the capture of the substantial nutrient values in these wastes, in the form of high-value algal bio-products.

5. RECOMMENDATIONS

A number of recommendations followed from the research programme:

1. The segregation of saline wastewaters should remain an acceptable option as an alternative to their disposal by dilution in the fresh water system;

2. Active research should be undertaken on problems associated with the engineering and management of saline waste disposal facilities, such as impoundments, and on the technologies required to effect the appropriate handling of these wastes;

3. Development of value-adding operations whereby the long-term management of these wastes is placed on a sustainable economic footing, such as algal biotechnology-based production, should be pursued to commercial-scale;

4. Enhanced hydrolysis of complex organic carbon substrates in the sulphate reducing compartments of the sulphate-saline WSP system should be subjected to follow-up investigation, especially where sanitation wastes might provide an electron donor source in the biological treatment of high-sulphate wastewaters such as acid mine drainage (AMD);

5. Potential linkage of the saline and sanitation wastewater systems should be actively pursued. ‘Integrated waste resource management’ strategies should become the subject of more intensive research and innovation, and further research and development should be encouraged in this area as a basis for promoting sustainability and sustainable development objectives in wastewater management.

6. RESEARCH PRODUCTS

The WRC studies reported here led to a number of follow-up studies based on the above recommendations. These are the subject of separate project reports which are listed in Appendices 1 and 4.

Student training in IAPS treatment of saline wastewaters has included 1 Post-Doctoral Fellow, 6 PhD and 6 MSc students. Publication of the results of these studies is ongoing but currently includes 7 patents, 13 papers in refereed journals and 6 articles of general scientific interest. Publication in conference proceedings includes 3 plenary and keynote lectures, 16 international and 29 local conference presentations. The student training and publication outputs are reported in Appendix 2.

A number of industrial technology transfer exercises have been undertaken, involving the products of these studies, and are noted in Appendix 3. In addition, research spin-off developments which have resulted in associated follow-up research projects have been noted in Appendix 4 of this report.

7. FOLLOW-UP ACTIONS

In addition to the specific objectives for this project, it had also been required at the outset that the wider implications of a biotechnological approach to the saline wastewater problem should be borne in mind. In this regard a number of potential developments arose during the course of the project, and these became the subjects of follow-up WRC projects.

In implementing IAPS technology, the trade-marked Advanced Integrated Wastewater Ponding Systems (AIWPS), developed over many years by Professor William Oswald, University of California, Berkley, (Oswald, 1988a&b;1991;1995), was identified as providing many of the engineering-design aspects required to effectively manipulate algal-based wastewater treatment and biomass production operations. While this system had been principally developed in the domestic wastewater treatment application, it was considered the best model to effect the technology transfer necessary to place algal biotechnology applications development on a sound engineering basis in South Africa. A technology transfer exercise was undertaken in which a technical-scale AIWPS plant was constructed, as a component of an IAPS demonstration and research plant, at the Rhodes University Environmental Biotechnology Experimental Field Station in Grahamstown. This was based on designs by Prof Oswald and Dr Bailey Green, who also provided valuable inputs into the Wellington developments. This initiative was undertaken in a follow-up WRC project and the results are detailed in the report 'Integrated Algal Ponding Systems and the Treatment of Domestic and Industrial Wastewaters. Part1: The AIWPS Model' (Appendix 1).

A number of industrial applications of the IAPS technology were also investigated as follow-up WRC projects including abattoir and winery and distillery wastewaters (Appendix 1). The studies on hyper-saline wastewaters were followed up in a joint WRC/EBG project with Sasol Ltd., and resulted in the development of a process for β-carotene production from *Dunaliella*, scaled-up to industrial-scale operation by Sasol

Ltd. in Uppington. The development of the *Dunaliella*-HRAP (D-HRAP) for treating hyper-saline wastewaters was evaluated in the treatment of soda ash brines at Botswana Ash Co., Sua Pan, Botswana. These studies are detailed in Part 2 of this report 'Integrated Algal Ponding Systems and the Treatment of Saline Wastewaters. Part 2: Hyper-saline Wastewaters - The *Dunaliella* Model' (Appendix 1).

The potential for the linkage of the saline and sanitation wastewater systems, which had emerged from the tannery IAPS investigation, was followed up in a number of WRC studies. The construction of the IAPS demonstration and research plant treating domestic wastewater in Grahamstown, and the IAPS treating saline tannery wastewater in Wellington, had established the basis of the system as a 'core technology' linking the treatment of these wastes. The observations of enhanced hydrolysis of complex and particulate organic carbon had demonstrated a mechanism whereby the nutrient values in these wastes might be recovered in the form of value-added algal bioproducts. The use of sewage sludges (and other waste organic carbon substrates) as reagent feedstocks for the biological treatment of sulphate-saline wastewaters, such as AMD, was investigated and resulted in the development of the Rhodes BioSURE Process®. The production of treated water from AMD, as a product of the saline and sanitation linkage, is an important target given the large volumes anticipated following mine closures, and the very long time periods over which the problem is likely to affect the South African environment.

Besides environmentally sustainable advantages in a cost-effective co-disposal of these waste systems, the use of wastes as resources, an the basis of value-adding beneficiation operations, has potential to provide inputs to the wider sustainable development project. This is particularly the case in the developing world context where salinisation and desertification have been identified as primary agents constraining human development.

The objective of establishing such linkages in saline and sanitation wastewater treatment based on studies in environmental biotechnology was pursued by the EBG in a number of WRC projects and became the subject of 13 separate reports in the series 'Salinity, Sanitation and Sustainability: A Study in Environmental Biotechnology and Integrated Wastewater Beneficiation in South Africa' (Appendix 1).

ACKNOWLEDGEMENTS

The authors wish to express their thanks and appreciation to the WRC for finance and support throughout the project. In addition 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. Given the number of people involved it is difficult to acknowledge everyone individually. Nevertheless, specific inputs affecting the overall study need acknowledgement here:

- ❑ Prof William Oswald without whose pioneering work in the field over many years, and kind and generous support in this study, 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;

- ❑ Jim Moyes, Rob Newson, Bob Smith and Tony Mossop of Mossop-Western Leathers whose foresight and support for the project was quite remarkable in this somewhat conservative industry;

- ❑ 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;

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

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  Mossop-Western Leathers Co.
  SASOL Ltd.
  Botswana Ash Pty Ltd

- Academic colleagues who provided generous and valuable contributions to the programme:
  Prof John Duncan, Moira Pogrund, Prof Johan Grobbelaar (University of Orange Free State), Prof Keith Cowan (now at the University of Upsala), Dr Mark Aken (now at Anglo Coal Co.).

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Figure A 4.7. 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.

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LIST OF ABBREVIATIONS

ABP	Algal Biomass Potential
AIWPS	Advanced Integrated Wastewater Ponding Systems
ALBAZOD	Algal bacterial zoogloeal detritus
AMD	Acid mine drainage
CCAP	Culture Collection for Algae and Protozoa
CFU	Colony forming unit
Chl a	Chlorophyll a
COD	Chemical oxygen demand
DEAT	Department of Environment Affairs and Tourism
DO	Dissolved oxygen
DPM	Decay per minute
DWA	Department of Water Affairs (up to 1989)
DWAF	Department of Water affairs and Forestry (after 1989)
EBG	Environmental Biotechnology Group
EPA	Environmental Protection Agency
EPS	Extracellular polymeric substances
ESKOM	Electricity Supply Commission
FCR	Feed conversion ratio
GBq	Giga Bequerell
ha	Hectare
HRAP	High Rate Algal Ponds
S-HRAP	*Spirulina*-High Rate Algal Pond
HROP	High Rate Oxidation Pond
HRT	Hydraulic retention time
IAPS	Integrated Algal Ponding Systems
LIRI	Leather Industries Research Institute
NEMA	National Environmental Management Act
OECD	Organisation for Economic Cooperation and Development
PFP	Primary Facultative Pond
RDP	Reconstruction and Development Programme
RO	Reverse osmosis
SADC	Southern African Development Community
SGR	Specific growth rate
SMME	Small micro and medium enterprise
SCP	Single cell protein
SRB	Sulphate reducing bacteria
STR	Stirred tank reactor
TDS	Total dissolved solids
TDIS	Total dissolved inorganic solids
TKN	Total Kjeldal nitrogen
UN	United Nations
VFA	Volatile fatty acid
WISA	Water Institute of Southern Africa
WRC	Water Research Commission
WSP	Waste Stabilisation Pond

NOTATION

A wide range of terms have 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 SALINITY, SUSTAINABILITY AND ALGAL BIOTECHNOLOGY

1.1 BACKGROUND

Salinisation has been identified as one of the single most serious pollution threats facing the public water system in South Africa (Commission of Enquiry into Water Matters, 1970; DWA,1986; Stander,1987; DWAF, 1990; Herold & Bailey, 1996, DEAT, 1999, 2000, Urban-Econ, 2000). The implications of a widespread and creeping salinisation had been addressed in the late 1960s by the Commission of Enquiry into Water Matters (1970), and its recommendation, of a broad-based co-operative research and remedial strategy to deal with this and other water-related strategic problems, was one of the motivating reason for the establishment of the Water Research Commission (WRC) in 1971 (Section 2 of the Water Research Act No. 34 of 1971). Despite considerable attention devoted to the problem over many years, the salinity situation has continued to deteriorate nationally. The National State of the Environment Report (DEAT, 1999), and the White Paper on Integrated Pollution and Waste Management (DEAT, 2000), have both again highlighted the ongoing and, in places, the accelerating degradation of the National water resource. The White Paper identified salinity and sanitation issues as six of the seven priority sources of pollution facing the country and those requiring most urgent attention (DEAT, 2000).

The problem is of course not new, and long-range prospects of reversing ongoing salinisation are bleak. It has been a constant threat to organised human activity since the collapse of mankind's first major irrigation project along the Tigris and Euphrates rivers, and with it the Sumerian civilization around 2000 BC (Toynbee, 1972; Sinnigen and Robinson, 1981). These lands, which then supported an estimated population of 15-25 million people, remain desolate and unproductive to the present time, and with a current population of less than 10 million people (de Villiers, 1999).

Following the explosive development of agriculture in latter part of the 20th Century around 17% of the world's agricultural land is now irrigated, with some countries relying almost exclusively on irrigated agriculture (Egypt 100%, and Pakistan 70%). However, salinisation still remains one of the principal constraints. Salinity now impacts most irrigation systems at some level, including South Africa (Herold and Bailey, 1996), with water logging and salinisation affecting 80-110 million ha fertile soil worldwide, and spreading at a rate of about 1 million ha.year^{-1} (Ramsar, 1996). In Pakistan two million ha irrigation land has been decommissioned due to salinity, and in the USA 30% of all irrigated land now has yield reductions due to salinity (de Villiers, 1999).
However, dependence on irrigation is increasing, and a UN study on Global Change and Sustainable Development (UN, 1997) has projected that rising population and increasing demand for animal protein is expected to require a doubling of current global food production over the next 30 years. The bulk of this will need to come from irrigated land, and an associated increased water consumption of 50-100% will be required to achieve this yield (UN, 1997).

Without irrigation, the present area under cultivation in the world would meet the minimum food needs of less than half the current human population (Kijne, 1999).

In much of sub-Sahara Africa the food requirement is now near or beyond the existing capacity of rain-fed agriculture. To balance the food deficit, production must rise by an estimated 3.3% annually until 2025 (Lake and Souare, 1997). Irrigated land has generated more than half the increase in global food production from the mid-1960s to the mid-1980s, and the FAO predicts that irrigation will continue to expand at 0.8% annually in the Developing World. The rate of expansion in Africa is expected to be higher because of land availability and the low starting point. Currently the 4% of irrigable land which is under irrigation in the SADC region produces about 30% of all agricultural production (Shela, 1996). However, even if it were possible to triple Africa's irrigated areas, the available water resource would come under severe stress. Of the 65 countries worldwide which will be facing water shortages by 2050, it is anticipated 32 will be in Africa (OECD, 1998).

While the threats to agriculture of irrigation salinisation are worldwide trends affecting food production and population issues, this is not the only source of the problem. South Africa faces substantially more immediate contributions to salinity from both natural sources, and those relating to rapid urbanisation and a range of other human activities.

Geological formations in South Africa, such as the Karroo shales, are well impregnated with salts and, when periodically inundated with flood waters, are leached. With an elevated water table, they may give rise to highly saline base flows (Schultz,1988). This is especially apparent in the arid western region of the country (Walmsley *et al*., 1999), but also affects the gold fields and coal mines in the Gauteng, Mpumulanga and Northern Kwa Zulu-Natal regions. Evaporative losses and flow reduction in rivers due to upstream diversion, or frequent drought conditions, also serve to raise salt levels.

Salinity is, however, also the inevitable result of most human productive activity - urban, industrial as well as agricultural. It is the inevitable result of water usage, especially where recycle plays an important role in the overall water economy, as is the case in the Vaal River system, and other regions of Southern Africa. It is the inevitable consequence of increased abstractions, consumptive use and return flows (Foster,1990). The DWA 'Salts Project' (DWA,1989), which surveyed sources of saline effluent in the Witwatersrand area, identified mine dumps and diffuse urban runoff as principal sources of pollution, with industrial sources contributing only part of the overall problem. Scott (1995) has shown that gold mine closure in the East Rand Basin would be followed by the decanting of substantial additional sources of salinity, which will ultimately enter the Vaal River system.

The costs of salinity to urban, industrial and downstream agricultural users have been estimated by Heynike (1981;1987) and du Plessis (1984) and have been the subject of subsequent revision by Steffen, Robertson and Kirsten (1989), DWAF (1990) and Urban-Econ (2000).

1.2 REMEDIAL INTERVENTIONS

While problems of saline pollution and salinisation remain widespread and pervasive threats to sustainability of both the biophysical and the human environments, worldwide and in Southern Africa, the potential options by which this set of problems may be addressed remains relatively constrained. Of these, three principal strategies have generally been pursued including elimination, disposal by dilution, and segregation to prevent the contamination of the rest of the water resource.

1.2.1 Elimination

Eradication of saline pollution at point source by application of general standards, has proved difficult to enforce, where at all possible, and has generally foundered on the perennial question - ‘what to do with the waste brines produced ?’ Attention has thus focussed on methods to achieve sustainability by the complete elimination of pollution sources through upstream changes in process design and production methods. While this provides one of the principal strategies for achieving long-range environmental sustainability objectives (Jansen, 1997), it can at best only offer a partial solution in the Southern African context. Geological sources of salinity, decanting mines and degrading ash dumps, and a wide range of urban and industrial activities may be anticipated to continue to have an impact on salinity, particularly in the water-scarce inland conurbations in Southern Africa.

While strenuous efforts should continue to be directed at reducing point source contributions to saline pollution, it is evident that other options also need to be considered.

1.2.2 Dilution

A programme was undertaken in 1977 to develop a hydro-salinity model to simulate the movement of water and salt through the Vaal River Pretoria/Witwatersrand/Vereeniging complex, and to evaluate the use of fresh water from the Vaal Dam to reduce salinity at the Barrage off-take (Herold, 1980; Stewart, Sviridov & Oliver,1985). Follow-up application reports on the blending option have also appeared (DWAF,1990), and on the basis of these findings Herold & Triebel (1990) identified the blending scheme as being the most cost-effective and ameliorative measure. Since this time, water in the Vaal Barrage has been quite successfully maintained within an upper limit of 300 mS/m (John Geldenhuis, pers. comm., 2001).

While providing an effective management of the salinity problem, Herold & Triebel (1990), however, also warned that too ambitious a blending target cannot be maintained indefinitely without incurring a severe penalty in terms of system yield, given anticipated growth in water demand for the PWV area over the following decades. Stander (1987) has argued that the dilution and blending option should be seen as a short term, or temporary, solution for handling the problem of saline flows. The process is inherently wasteful of fresh water and depends on adequate supplies which are not assured. Application of control measures aimed, in the long term, at the source of saline pollution should be favoured (Stander, 1987).

After some years of the implementation of dilution strategies, Foster (1990) had noted that by the end of the 1980s the position remained one of continuously increasing salinity, with water quality management strategies and pollution control measures, including dilution, tending to conceal this underlying trend.

1.2.3 Segregation

The principal alternatives to the salinity dilution option are segregation in one form or another. The practicality of these options has been investigated in the DWA "Salts" project (DWA ,1989b), which indicated that a significant portion of the total salt load leaving the municipalities in their effluents can be isolated in a relatively small fraction of the total volume. Jones *et al.* (1989) estimated that while mine dewatering and mine-dump seepage produces 25% of the annual salt load reaching the Vaal Barrage (variously estimated at around 400 000t/year), this is discharged in only 5% of the associated effluent volume.

The viability of the segregation option, including desalination, depends ultimately on the provision of adequate methods for brine disposal. Buckley *et al*., (1987) had commented that the lack of provision of adequate methods for brine disposal had rendered the authorities responsible for pollution control powerless to enforce the desalination of effluents, despite available legislation. Whereas disposal sites for virtually all solid, toxic and other wastes can be found or engineered, sites for the effective disposal of brines have generally not been well investigated. In the main, saline concentrates are currently either stored in one form or another, mainly in ponds and ash dams, or in some cases fill valuable volume in toxic landfill sites.

Numerous methods have been suggested at various times for handling segregated brine concentrates including co-disposal on solid waste disposal sites, mine tailing dams and in ash quenching; irrigation directly onto land; vapour compression, evaporation and crystallization; transport to the coast and discharge to sea; deep well injection and dumping in disused gold mines; incorporation into products such as fluting and liner board; salt water sinks or lakes that can be used for recreational purposes; solar evaporation ponding producing solid salt or soda ash products, using saline impoundments for the co-disposal of organic solid wastes; saline algal aquaculture in ponds with the production of high value products such as fine bio-chemicals, biomass or fish (DWA, 1986; Stander, 1987; Fijen, 1988; Moolman, 1990; Rose *et al.* 1992,1996 &1998).

1.2.4 Alternative Technologies

The need to undertake a thorough evaluation of technical alternatives to the prevailing dilution option was recognised at a WRC workshop where South Africa's future salinity research requirements were prioritised (du Plessis,1990). Alternative use for saline water and salinity control were identified as among those research needs with the highest priority, and second only to the existing programmes set up to assess future salinisation trends. The final NATSURV report also identified similar research priorities in this field (WRC,1989). Moolman (1990), who had pioneered work in this area warned that while novel ideas for handling the salinity problem should be prioritised these should also be treated with caution since, while theoretically sound, they may be fraught with numerous unforseen practical problems.

While it seems clear that no one solution is likely to provide a cure-all for every requirement or eventuality of segregated saline waste disposal, the pro-active engagement with the practical issues noted by Moolman is required if appropriate technological alternatives are to be available, and in place, if and when circumstances so require. It may be argued that while the initial research investment may be exploratory or developmental in the first instance, under the pressure of natural selection among alternative technologies, options may mature into viable solutions for at least some components of the overall problem. Jansen (1997) has noted that to meet the timetables set by the sustainability agenda, the breakthroughs in technology required will have to be initiated now to guarantee their availability in the next 2 to 4 decades.

1.2.5 Key Questions

The above considerations raise important questions regarding the need for pro-active development of alternative technological options for dealing with individual saline waste streams:

- ❏ Will the positive cost/benefits of the dilution options change adversely in the future resulting in increasing pressure on the fresh water resource, and with increasing salinisation damage and disbenefits to downstream users?
- ❏ Will segregation of saline concentrates at source become an increasingly attractive option for the user as compliance with the general standard is enforced?
- ❏ If so, will segregation to ponds and salt water sinks remain an option of choice for individual users or collective disposal operations, especially in the context of developing world options?
- ❏ And determined by the above, do biological systems offer potential technological advantages for dealing with some of the problems and disadvantages associated with the cost effective impoundment of saline wastewaters such as evaporative losses of water, possible groundwater contamination and threats to wildlife?
- ❏ Given the range of existing physico-chemical technologies for dealing with saline wastes, can a programme of biotechnological innovation in this area make a satisfactory contribution to the development of cost-effective alternative processes for the improved handling, treatment and the possible beneficiation of these wastes?
- ❏ Does beneficiation, as operations that add value by transforming materials

from a raw-material status into finished products, provide an incentive for the long-term sustainable management of saline water impoundments?

- If so, what existing biotechnologies may be appropriate, and where is innovation required to demonstrate the viability of engineerable full-scale processes?

The overall sense of a deteriorating salinity problem, possibly somewhat concealed, and probably under only partial reprieve by dilution operations, seems to warrant a pro-active investment in the development of alternative technological options for dealing with the problem. It was against this background that the current study was undertaken to evaluate biotechnological approaches to the sustainable management of saline wastewaters.

1.3 BRINE DISPOSAL - PROBLEM OR RESOURCE?

The principle that should govern any solution to the salinity problem is that it must not create more environmental problems than it solves. Verstraete and Huysman (1988), in identifying research needs in Environmental Biotechnology, point out that many conventional treatment techniques in current use provide an often perfect illustration of Murphy's Law - they merely transform one problem into another. To be specific, the solution to the problem must not cost more than the cost of the problem.

The gravity, central relevance and long-term implications of the decisions to dilute or to segregate, have been noted by Stander (1987). In concluding a detailed assessment of the salinity issue, he warned that opting for easy, costly solutions for present day problems could result in an enormous debt burden for future generations, for whom such solutions would be either exorbitant or prohibitive. "This would be an awesome burden of guilt for the administrator, the researcher and the industrialist"(Stander, 1987).

While ‘clean technology’ and prevention strategies offer the ideal goal, it is evident that numerous instances do occur where a dilution or segregation strategy may provide the only option to prevent serious, and possibly permanent, degradation of the national water resource, and the associated biophysical environment. This is especially obvious in the developing world where technological options available to deal with the salinity problem may be quite limited.

The incentive to manage the saline waste is clearly an integral part of the salinity problem itself, and costs associated with its disposal are a major limitation in the development of long-term strategies. Since the principle of cost, in its widest sense, will dictate the most appropriate solutions to the problem, those technological options which offer the potential of value recovery in some form or other, would warrant particular attention. Recovery of value could imply a wide range of options, but where the direct recovery of a by-product is implied, the generation of credits of this type, against waste disposal, has been a well-established means of improving the cost effectiveness of industrial processes.

The principle of value recovery is not new to waste management, and while exercising caution with regard to oversimplification, wastes have, nevertheless, been regarded as something of a golden opportunity for biotechnology (Hacking, 1986). The boundary between wastes and by-products is arbitrary - where utility increases a waste becomes a by-product. The value of wastes is determined by their opportunity costs, and this increasingly acquires a higher negative value with rising disposal charges, and penalties imposed by environmental legislation. As environmental awareness increases, rising waste treatment costs become more acceptable to society and industry. This eventually tips the balance in favour of integrating waste treatment and some form of economic activity.

While no general rules apply, it seems that in particular cases of water pollution the identification of opportunity value may be the only way to ensure long-term environmental sustainability. Investment in incentive-driven research, and investigation of value-adding opportunities to drive effectively managed segregation strategies, has been identified elsewhere. An important aspect of the Salton Sea Restoration Project in California has been to include the development of economic benefits and the enhancement of recreational uses as principal drivers in the reclamation of healthy fish and wildlife resources, and their habitats (USBR, 2000). The Australian National Dry Land Salinity Programme has set up a special study project on Options for Productive Use of Salinity - OPUS, which is tasked to investigate opportunities and incentives available for adding value to saline wastes (NDSP, 1999). Initial findings in this study have identified numerous opportunities in saline agronomy, forage halophytes, horticulture, innovative industries and aquaculture, including production of algal bio-products.

Value recovery, then, plays a potentially pivotal role in driving ‘integrated waste resource management’ objectives, and it is in this sense that the concept of value recovery, as a principle for placing the sustainability of salinity remediation solutions on a solid economic foundation, has provided a basic objective of the WRC study reported here. It has been argued that since amelioration of cost is at the root of the problem, what is required in defining the research direction is a change in mindset, and that the potential of brine wastes be re-evaluated as a resource rather than a problem. In this regard it is important that the objectives of sustainability be clearly stated.

1.4 SUSTAINABILITY AND INTEGRATED WASTE RESOURCE MANAGEMENT

Concepts of sustainabiliy have been at the core of deep concerns emanating from the environmental movement over the past half century or more. However, it is only relatively recently that the close and complex association between sustainability in the environment, and human development objectives, have been noted. The World Conference on Environment and Development (WCED, 1987), in focussing on the issues of inter-generational equity, defined sustainable development as development “that meets the needs of the present without compromising the ability of future generations to meet their own needs”. The global debate on sustainability gained formal currency through the adoption, by most nations, of the Agenda 21 protocol at the United Nations Conference on Environment and Development, the Earth Summit, in Rio de Janeiro, in June 1992.

The discourse surrounding sustainable development is both complex and controversial. While sustainability has emerged as a principal feature of social and political life in the 21st Century, it is nevertheless defined quite simply by two apparently conflicting goals, now nearly universally pursued around the globe - the improvement of environmental quality on the one hand, while simultaneously achieving large and sustained increases in economic activity and human development on the other. Although some have seen the evolution of sustainable development as a flag of convenience under which many ships sail (Adams, 1993), the term ‘sustainability’, as it relates to the resilience of biophysical ecosystems, may nevertheless be clearly distinguished from the different, but closely related political, economic and social contexts of ‘sustainable development’.

Jansen (1997) has noted that the broad goal of achieving sustainability over the long-term requires the fundamental renewal of the whole means by which human needs are fulfilled. Given the revolutionary nature of the technological transformation that is required to achieve the necessary fusion of economic and environmental objectives, it is necessary that technologies meet two principal criteria. First, they must transform human activities into resource-light consumption patterns, and second, in terms of non-renewable resource depletion, new technology is required to enable societies to live strictly off nature’s income, rather than consuming its capital. Technological transformation for environmental sustainability is thus a process that reduces environmental damage, per unit of added value, fast enough to outpace production increases (Speth, 1990).

Some environmental economists have argued that depletions in ‘natural capital’ may be replaced by future developments in ‘human capital’ (Whelan, 1989), and specifically in the form of technological innovation. Since it is not possible to leave future generations with the same quantities of exhaustible resources that are available to current generations, sustainable development requires technical progress in the use, extraction and development of renewable or reproducible resources which fulfill the same functions as exhaustible resources (Bowers, 1997).

This has led to the development of a new environmental economics where growth is taken as part of the solution, and no longer part of the problem (Fritsch *et al.*, 1993). Environmental concern emerges as a force propelling economic growth. Shifting consumer demand spurs innovation, trimming down resource usage lowers production costs, and environmental technology opens up new markets. The environmental predicament is redefined as a problem of efficient resource allocation. Natural resources are considered grossly undervalued and therefore wastefully allocated, while human resources along with technology are under-utilised. Sachs (1999) has noted that the business objective of achieving “eco-efficiency” has emerged as a strategy of considerable innovative power.

Nevertheless, the matter is no foregone conclusion and profound technological development is required for any real progress to be made towards the development of a sustainable future. It is in this regard that a ‘closed system’ approach has been proposed as a mechanism by which the goals of sustainability may be achieved. Here human activities and the associated technological environment need to be detached as far as possible from the biophysical environment related to natural systems. The ‘closed system’ calls for a technological thrust that will enable the transformation of industrial activity, and other anthropogenic impacts, from materials-intensive high-throughput processes to resource-light systems that use fuel and raw materials efficiently, rely on inputs with low environmental costs, generate little or no waste, recycle residuals, and release only benign effluents (Heaton *et al.*, 1991). Above all, the condition of sustainability can only be met where renewable resources replace the use of finite exhaustible reserves.

However, it is apparent that fully functional and appropriately engineered ‘closed systems’ may still lie some way in the future. Jansen (1997) has examined the requirements for operationalising sustainable development in Europe and notes that short-term instruments, such as policy and system management approaches, including demand management strategies, are to be found typically in 5-year planning programmes. The development of integrated technologies, and environment-directed products, are located rather in 20 year environmental policy plans, and these longer-term approaches require that exploratory innovation in sustainable technology development becomes embedded in the overall R&D process. In identifying the critical role of the ‘technology push’ approach, Jansen further notes that to meet the timetables set by the sustainability agenda, the breakthroughs in technology required will have to be initiated now to guarantee their availability in the next 2 to 4 decades.

Suzuki (2000) has examined factors constraining the development of ‘closed systems’ and notes that in existing industries, achieving ‘zero emissions’ seems more difficult, since eco-restructuring needs to start from the currently operating system, and this has already been fully optimised within the open market economy. The development of key technologies for the establishment of completely closed material cycles will depend, to some extent, on the development of new industry clusters established specifically around this concept. These developments are largely dependent on pro-active approaches and are among the prime long-term opportunities of environmental biotechnology.

It is in regard to the need for immediate responses to environmental problems that ‘integrated waste resource management’ has developed as an intermediate strategy with the principal focus on optimising the conversion of waste into resources within the current production paradigm. Recovery, recycle, reuse and waste beneficiation, especially of water, form the principal targets. This presents a route whereby a myriad of small incremental steps may lead in time to the implementation of grand plan ‘closed systems’ which do successfully effect a change in the means by which human needs are fulfilled. This study investigated algal biotechnology as a potential source of innovation to achieve the ‘integrated waste resource management’ of saline wastewaters.

1.5 ALGAL BIOTECHNOLOGY

Algal biotechnology developed rapidly in the 1970s and 1980s, and the potential of aquatic photosynthetic production to deal with long-term resource replacement and sustained, environmentally sound economic growth has been frequently identified (Hall,1986; Oswald, 1995). Photosynthesis is the most abundant energy-storing process on earth (Ben-Amotz and Avron,1989), and the use of micro-algae (including cyanobacteria) for the industrial production of biomass, and speciality chemical products, has long been recognised, and has been the subject of extensive review (Grobbelaar, 1980; Shelef and Soeder,1980; Richmond,1986; Barclay and McIntosh,1986; Borowitzka & Borowitzka,1988; Lembi,1988; Stadler *et al.*, 1988; Cresswell *et al.*, 1989).

Despite the enormous genetic potential of the micro-algae, and the observation that they can produce nearly all the biological compounds currently obtained from conventional land crops, very few large-scale outdoor algal production systems have been successfully established (Richmond,1996). Dubinsky (1986) has made the point that the occasionally observed algal water bloom has been the ‘philosopher's stone’ of applied algology, offering optimistic potential for pure culture which is difficult to reproduce under controlled conditions. Oswald (1988a) has noted that "There are no sustained axenic cultures out of doors in the real world."

With the possible exception of *Chlorella* and *Scenedesmus* cultivation as fresh water systems, the only really successful product-oriented outdoor algal biotechnology systems have been those where the exotic growth requirements of the algae in culture act as an environmental selection factor, ensuring the exclusion of competitors, and the maintenance of a mono-species system. The two notable cases are the halophilic alga *Dunaliella salina*, which grows in hyper-saline brine solutions (> 40 $g.L^{-1}$ total dissolved inorganic solids - TDIS) producing β-carotene and glycerol, and *Spirulina* spp. which grows best in meso-saline alkaline environments (< 40 $g.L^{-1}$ TDIS). *Spirulina* biomass has a high market value as a health food, a high protein feed additive in specialist aquaculture rations, and in the recovery of vitamins, fatty acids and the pigments phycocyanin and phycoerythrin (Richmond,1996). The control of contamination and predation in algal cultures, in the absence of extreme culture conditions, has been identified as one of the major limitations to be overcome in the future development of algal biotechnology (De Pau and Persoone,1988; Ben-Amotz and Avron,1989).

The selective action of salinity on aquatic populations is a well described ecological principle. Goldman and Horne (1983) have noted in saline lakes that as salinity increases there is a decrease in the diversity of organisms present, with an increase in productivity based on a few abundant species. In reviewing the current status and future prospects of algal biotechnology, Richmond (1986) has identified saline systems as offering the most immediate potential to progress towards economically feasible processes. He has identified significant economic advantages in cultivating the halophilic algae as salt-tolerant crops, utilizing land

and brackish water that is unsuitable for the production of conventional agricultural land crops.

While food production in the form of micro-algal biomass still suffers from the problems of low product values, which are common to other single cell protein (SCP) projects (Hall,1986), the biosynthesis of high-cost speciality products is likely to provide the 'research pull' on which future algal biotechnology will develop. In this regard, recent developments in micro-algal genetic manipulation could offer future possibility for the production of a range of economically significant metabolites, currently obtained from terrestrial plants, or from heterotrophic organisms cultured in expensive bioreactors (Richmond 1986; Craig *et al.*, 1988).

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.

Despite the substantial promise of sustainable technologies offered by these developments in algal biotechnology, little progress has been recorded in either the development or the implementation of integrated wastewater treatment and algal bio-product value recovery systems.

1.5.1 Waste Stabilisation Ponds and Wastewater Treatment

Waste Stabilisation Ponds have been constructed in a wide range of applications for the treatment of domestic and industrial wastewaters (Pearson 1996), including tannery effluents (Figure 1.1), and operate in most climatic zones of the world (De Pau and Salamoni 1991).

Figure 1.1 The Waste Stabilisation Pond evaporative disposal cascade treating tannery wastewaters at the Mossop-Western Leathers Co. Wellington.

The rapid development of the technology since the 1950s has been reviewed in detail by Mara & Marecos Do Monte (1987), and Mara *et al.* (1996). Early studies of these systems identified the role of micro-algae in their successful operation (Gotaas and Oswald, 1954), and the High Rate Algal Pond (HRAP) concept developed from attempts to optimise and intensify the function of the micro-algal components (Grobbelaar *et al*., 1988). The detailed theory of the HRAP has been reviewed by Shelef *et al.* (1980); Abeliovich (1986); Oswald (1988 a&b) and De Pau & Salamoni (1991), but mainly as applied to the treatment of domestic wastewaters. The algal raceway has become the standard bioreactor design for algal biotechnology developments (Richmond, 1996).

In the HRAP (Figure 1.2), an association is established between high populations of algae and heterotrophic microorganisms, with photosynthetic oxygen production providing for the degradation of organics, and the algae, in turn, utilizing the inorganic nutrients and CO_2 produced. While the advantages of the process were at first thought to be limited to this mutual microbial association, it was subsequently realised that the algal component also contributes significantly to the degradation of low molecular weight compounds, being responsible for up to 50% of the heterotrophic uptake of organics from the system (Abeliovich,1980 & 1986).

Figure 1.2 The High Rate Algal Pond showing paddle wheel and raceway. This system treating domestic wastewaters was constructed at the Grahamstown Disposal Works.

A polished effluent, suitable for discharge to the public water system is produced, following the removal, by settlement, of the algal-bacterial-zoogloeal detritus (ALBAZOD) biomass. The albazod from domestic wastewater treatment, however, has little value more than as a cheap protein supplement, and the cost of its separation from the waste stream has been a negative factor (Soeder,1986). Overall yields 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).

The precise control of algal species in these systems treating non-saline wastewaters is not possible, with wide fluctuations in type following shock loading, seasonal changes and variations in effluent content. Abeliovich (1986) has noted a trend in species dominance, dependant on organic load, with highly polluted water containing *Euglena* and *Chlamydomonas*, and in decreasing order of organic load *Scenedesmus*, *Chlorella* and *Micractinium* species. The standing algal crop can reach 1.0-1.5 g.L^{-1}dry wt. in the HRAP (Abeliovich,1980; Pearson *et al.*, 1987).

Despite the above, 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 micro-algal biotechnologies to be exploited as a multi-purpose system. Shelef (1987) has noted that Integrated Algal Ponding Systems (IAPS) epitomises the principles of water and nutrient recycling and closes the cycle of waste to primary biomass more rapidly than any other outdoor process; converting organic wastes into an algal biomass rich in protein, while stripping out nutrients. Furthermore all this is accomplished without mechanical aeration but capitalising only on solar energy and, following algal harvesting, producing a high quality effluent not surpassed in quality by other biological or physico-chemical wastewater treatment processes.

Notwithstanding these developments, and despite a concerted phase of research development in algal biotechnology, and the demonstration of the IAPS as among the most efficient wastewater treatment operations available, these systems had not found widespread application.

2 THE RESEARCH PROGRAMME

2.1 BACKGROUND

The study of saline algal biotechnology commenced at Rhodes University in the mid-1980s with an investigation of factors regulating stress physiology in the extreme halophile *Dunaliella salina* by Peter Rose, Mark Aken and Keith Cowan. An evaluation of the organism's growth in saline wastewaters contaminated with organic compounds, such as tannery effluents, and the associated removal of these compounds, led to an investigation of micro-algal growth in saline evaporation WSP systems. This followed observations, made by Roger Rowswell of the Leather Industries Research Institute (LIRI), of extensive blooms of micro-algal growth occurring, at times, in tannery WSP. Follow-up investigations of these phenomena by Peter Rose and Roger Rowswell at The Western Tanning Company, Wellington (now Mossop-Western Leathers) showed at times near mono-species blooms of a *Spirulina* sp., and various species of *Dunaliella* (Rose *et al*., 1996).

Studies on the removal of organics and heavy metals associated with micro-algal growth, and a process for the production of β-carotene by *D. salina* in these systems, had commenced in the Department of Biochemistry and Microbiology.

In 1990 Dr Oliver Hart, Research Manager at the WRC, undertook an evaluation of the preliminary research outputs related to the above studies and available at that time. It was proposed that a feasibility study undertake a structured assessment of the practical potential of linking algal biotechnologies and saline wastewater treatment.

The Rhodes University EBG was commissioned in 1991, by the WRC, to undertake Project K5/410, 'A biotechnological approach to the removal of organics from saline effluents', as a one year study, to investigate the application of IAPS in the treatment, utilisation and ultimate disposal of saline wastewaters, and to report specifically on the potential for the development of algal biotechnology applications associated with these systems. Against the background of this apparent potential, it was also identified as an important objective of the study to investigate how far the concept could be pushed as both a 'core technology', and as a source of innovation within broader sustainability and 'integrated waste resource management' strategies. The project brief also called for proposals on the merits of a longer-term study into biotechnology-based approaches to the salinity problem.

The preliminary inquiry undertaken as part of Project K5/410 included both experimental and desktop study components, and the following was noted:

- ❑ Algal biotechnology had made rapid advances, particularly in the 1970s and 1980s, and a basis for sustainability had been demonstrated with the identification of the technical potential for saline wastewater treatments available, at least theoretically, in the application of IAPS;

- ❑ Potential for the development of a sustainable approach to the problem was identified in terms of technical sustainability, as technology-that-lasts (low-construction costs, low-operator skills needs, reliability, flexibility and upgradeability); resource sustainability (potential to segregate saline wastes, closed system operation; recovery of nutrients and resources, 'integrated waste resource management'); economic sustainability (value-adding opportunities in the form of potentially high-value bio-products); social sustainability (service provision, job creation);

- ❑ While engineering design of algal ponding systems in wastewater treatment applications 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' approaches. Few commercially successful examples were available, and little had been reported on saline wastewater applications;

- ❑ IAPS 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 applications potential, specifically in the context of saline wastewaters and sanitation. 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 wastes and sanitation appeared to extend beyond the common use of a core IAPS technology for the treatment of a variety of different waste types. The potential use of saline wastewater in the reticulation of sewage, sewage sludge disposal in low-grade saline wastewaters, and also its use as a carbon source in the biological treatment of high sulphate-saline wastewaters, such as acid mine drainage (AMD) and tannery wastewaters, emerged from the study;

- ❑ In addition to salinity, IAPS systems 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;

- ❑ The treatment of saline wastewaters in evaporation ponding cascades provided a salinity gradient for the production of a succession of micro-algal crops, depending on their salinity requirements;

- ❑ The WSP used for treatment of tannery effluents and disposal of high chloride and sulphate salinities provided a useful model for investigating the linkage of algal biotechnology and wastewater treatment in the evaporation ponding cascade;

- ❑ Saline wastewater impoundments offer important opportunities for restoring wetland environments and providing habitats especially for threatened waterfowl species;

- The removal of organic and heavy metal contaminants from such saline wastewater impoundments is necessary to prevent their concentration within the system, afterwards entering ecosystems via the food chain and causing wild life deaths and deformations. IAPS offers a potential pretreatment for saline wastewaters prior to impoundment.

2.2 PROJECT K5/495 - 'A BIOTECHNOLOGICAL APPROACH TO THE REMOVAL OF ORGANICS FROM SALINE EFFLUENTS'

Based on the findings reported for Project K5/410, the current project K5/495 (with the same title) commenced as a five-year investigation in 1992.

The broad objective of this research programme, in the first instance, was to undertake a feasibility evaluation of an integrated algal biotechnology solution to the salinity problem. The basic question to be asked was whether halophilic algae could be used to treat industrially generated brines, and generating products of value which, in turn, could provide an economic basis for environmentally sound and sustainable disposal practices for these wastes.

A number of assumptions served to focus the investigation:

- Of the brine disposal options that have been suggested in the literature, and detailed above, the least attractive is the dumping of saline brines in sinks, or any variation thereof, where salt will accumulate indefinitely, creating possibly insoluble problems for the future;
- In principle, where segregation is practised, the solution of choice should be linear whereby salts are concentrated and ultimately recovered from brines and disposed of in a way that effectively removes them finally from the environment;
- While high-cost, capital- and energy-intensive membrane separation and evaporation technology does exist to accomplish this task, both rapidly and effectively, it is more likely that a 'low technology' ponding and solar evaporation process would be found to be more cost effective and appropriate to the scale and time-frame of the problem;
- The simplest application of this process would be some form of linear cascade of ponds with a rising gradient of salinity resulting in the production of a saturated brine solution, which may pass via crystallisation to final disposal;
- The loss of water associated with evaporative disposal is discouraged by DWAF and therefore segregated and concentrated saline wastewaters, possibly including desalination reject brines, should pass to pond impoundments rather than be disposed of as dilute salinities and brackish water streams;
- It was unlikely that a universally applicable algal culture system would be found to be appropriate for all brine streams. While some form of pooling of different brine streams could be anticipated, separate systems would probably be required for the various forms of saline effluent.

2.2.1 Aims

The broad objective of Project K5/495 was to evaluate the potential of algal biotechnology systems to deal with the removal of organics and heavy metals from saline wastewaters on a scale appropriate to large water impoundments. The specific research aims of the project were identified at the outset as follows:

1. The development of a saline HRAP process for removal of organics from saline wastewaters, based on halophilic algae;
2. To optimise the operation of the solar evaporation Waste Stabilisation Ponding (WSP) process by the manipulation of micro-algal production;
3. The demonstration of a utility value for saline wastewaters with the recovery of value in the form of micro-algal bioproducts;
4. The removal of heavy metals from saline wastewaters;
5. The application of membrane separation techniques employed for the harvesting of micro-algal biomass and recovery of bioproducts.

2.2.2 Expected Benefits from the Study

Based on the above, it was anticipated that the study would:

- ❏ Provide water resource managers and waste treatment operators with an expanded range of technological options on which to base decisions relating to the disposal of saline wastewaters;
- ❏ Provide a better understanding of the potential contribution to the salinity treatment field offered by biological processes;
- ❏ Determine which biotechnological options warrant more detailed study, particularly practical problems which remain unresolved, and to determine where additional research effort should be invested in further bioprocess development;
- ❏ Provide a demonstration of the viability of selected biotechnological processes for treating and handling saline wastewaters and/or the functionality of individual unit operations in a critical path research approach.

2.2.3 The Tannery WSP: A Model System.

The effective linkage of micro-algal production and saline water treatment requires appropriate reactor design. Oswald (1995) has noted that ponds provide the most cost effective reactors for liquid waste management and the efficient capture of solar energy, enabling the reclamation of water, nutrients, and energy from organic wastes. Earthwork reactors, per unit volume, cost at least an order of magnitude less than alternative steel-reinforced concrete structures.

In addition to a wide range of applications the WSP has been used for the treatment of tannery wastewaters (Rowswell *et al.* 1984; Pearson 1996). The Rhodes University Leather Industries Research Institute (LIRI) recommended their use by the Leather Industry in South Africa and a number have been constructed as zero discharge terminal evaporation pond cascades (Shuttleworth 1978; Rowswell *et al.* 1984). The WSP is one of the few cost-effective options available for the treatment and final disposal of hyper-saline wastes where dilution of the salt load into the public water system cannot be entertained (Rose *et al.* 1996). Despite the strategic significance of the technology in this application few studies are reported in the literature which deal, in any detail, with the tannery WSP or their application in hyper-saline wastewater treatment.

The reasons for the choice of the tannery WSP as a model system for this study included:

- ❑ The use of the WSP is well-established technology for the treatment and disposal of high chemical oxygen demand (COD) loads in wastewaters;
- ❑ In addition to COD, tannery effluents contain a range of components including heavy metals and high levels of sulphates, ammonia, nitrates and protein nitrogen, providing, in some sense, a worst-case scenario of what may be encountered in saline impoundments;
- ❑ A wide ranging gradient of salinity concentrations occurs across the evaporation cascade, providing for the evaluation of sequential dominance of a range of different micro-algal species dominating meso-saline and hyper-saline environments;
- ❑ WSP are designed to treat large volume flows of wastewater which provides a useful model for evaluating their application as appropriate reactors for the treatment of other large volume saline wastewater flows such as AMD;
- ❑ The low-cost and quasi-passive nature of WSP makes for sustainability where treatment requirements are anticipated to stretch over long periods of time, again as is predicted for AMD in the gold fields and coal mines in the Gauteng and Mpumalanga regions.

Malfunction in WSP do occur, particularly as they age (Lawty *et al*., 1995), and the study provided an opportunity to investigate a role for algal biotechnology innovation in dealing with aspects of this including:

- ❑ Reduced organic loading potential due to accumulation of organics and dissolved solids;
- ❑ Severe odour problems;
- ❑ The unpredictability of algal blooms;
- ❑ The toxic effects of effluent on algal production rates and the quality of the harvested algal biomass.

The WSP operated by the Mossop-Western Leathers Co. Wellington, South Africa, was used as the system on which this study was based.

2.2.4 Meso-saline and Hyper-saline Wastewaters

Based on the research objectives outlined above, and the selection of the tannery WSP as a model system for the investigation of algal production linked to saline wastewater disposal it became apparent from pond ecology studies that the initial and final ponds of the evaporation cascade presented two clearly defined ecosystems. The study therefore segmented at an early stage into investigations of the meso-saline compartments where *Spirulina* sp. dominated, and the hyper-saline compartments where *Dunaliella* spp. predominated. The results of the project have thus been reported in two parts. The *Spirulina* studies are reported in this volume, and the *Dunaliella* studies appear in Part 2 of this WRC report 'Integrated Algal Ponding Systems and the Treatment of Saline Wastewaters. Part 2: Hyper-saline Wastewaters - The *Dunaliella* Model'.

Following initial studies on the ecology of tannery effluent WSP the factors influencing growth and performance of *Spirulina* in tannery effluent was investigated with insights acquired on the management of inhibitory factors. This also provided an indication of the biomass yield potential which might be anticipated from the system. At the same time studies commenced on the utility of *Spirulina* biomass harvested from tannery effluent ponds. Not only was it important to determine the feed quality of the biomass, but also the potential toxicity for use as animal feed at an early stage in the beneficiation exercise.

Broadly positive outcomes to these initial studies led to the development of the *Spirulina*-HRAP (S-HRAP) as a free-standing unit operation in tannery effluent treatment. Process development followed from laboratory-based flask and photo-bioreactor studies to pilot and full-scale implementation of the S-HRAP. Based on these findings it is possible to address certain of the problems which plague the tannery effluent WSP application, including sulphide/ammonia odour problems, reduced evaporation rates, organic and salinity accumulation. Finally the S-HRAP was included in an integrated operation of the WSP.

This study has been reported in the following sections:

1. The Microbial ecology of a tannery WSP system;
2. The growth and performance of *Spirulina* in the operation of a tannery WSP system;
3. A S-HRAP unit operation for the treatment of tannery wastewaters;
4. Integration of the S-HRAP and WSP operations in tannery effluent treatment;
5. The harvesting and recovery of effluent-grown *Spirulina* biomass;
6. *Spirulina* biomass toxicology and feed evaluation studies.

Aspects of these investigations were the subject of PhD theses by Kevin Dunn (1998) and Genevieve Boshoff (1999), and an MSc thesis by Brenton Maart (1993).

3 THE MICROBIAL ECOLOGY OF A TANNERY WASTE STABILISATION PONDING SYSTEM

3.1 INTRODUCTION

Since the 1950s WSP have been used in a wide range of applications for the treatment of domestic and industrial wastewaters (Pearson 1996). Despite their early use in the treatment of tannery effluents, both in South Africa and in other parts of the world, few studies are reported in the literature which deal with this application in any detail (Shuttleworth 1978; Rowswell *et al.* 1984; Rose *et al.* 1996).

Despite early contributions to rational modelling of WSPs based on chemical reaction engineering principles (Marais & Shaw, 1961; Oron & Shelef, 1980), both Wood (1987) and Middlebrooks (1987) have identified problems and shortcomings associated with predictive models in use. Pescod (1996) has drawn attention to the complex interaction of factors operating in the WSP, and Middlebrooks (1987) has noted the critical need for fundamental studies on the complex physical and biological factors operating in these systems in order to derive the simplifying assumptions required for more reliable mathematical modelling. The biology of WSP treating tannery wastewaters has not been the subject of detailed report.

This project commenced as a three-year study of a WSP treating tannery wastewaters. The investigation of the microbial ecology of the system was undertaken and the results provided the basis on which the remainder of the biotechnology research and development undertaking was founded. Principal factors influencing the operation of the treatment process were identified and an attempt was made to describe how these affect the performance of the tannery WSP.

3.2 METHODS

Two sampling procedures were followed over the course of the three-year study and were influenced by the evaluation of sampling methodologies in WPS reported by Pearson *et al*. (1987). A subjective grab sampling protocol was used to draw samples at various points across the WSP cascade from Ponds A to 11 (Figure 3.1). In this procedure an attempt was made to gain a sample as representative as possible of the conditions prevailing in the ponds at the time of sampling. An objective sampling protocol was followed for the vertical sampling of the pond water column. Here samples were drawn at various depths from fixed points on a grid drawn on the pond site plan, regardless of upwelling, micro-algal rafting and other variable circumstances prevailing on the pond's surface at the time of sampling. The various samples drawn from a particular depth in a pond were pooled before analysis. Analytical procedures followed A.P.H.A. Standard Methods (1989). Measurements of photosynthetic productivity were also made at the grid points noted above using the [^{14}C]-sodium bicarbonate CO_2 fixation method described in A.P.H.A. Standard Methods and modified by Oren (1992). Sample measurement was undertaken in a Beckman LS3150T scintillation counter. Dissolved oxygen (DO) was determined using a YSI Model 57 DO meter. Salinity was measured using an Atago salinity refractometer, and photosynthetically available radiation (PAR) using a Skye

Instruments 210 light sensor and SDL 2580 intelligent base unit. Light attenuation was measured using the Secchi Disk method and calculations, following Ramus (1985). Bacterial identification was based on descriptions in Bergey's Manual of Determinative Bacteriology (Stanley *et al.*, 1989). Eucaryotic algae and cyanobacteria (referred to collectively as the micro-algae or algae in this text) were identified using type strains from the Culture Collection for Algae and Protozoa (CCAP). Chlorophyll a (Chl a) was measured according to the method of Lichtenthaler (1987). Photosynthetic bacterial pigments were extracted into 100% acetone and absorbance measured in a Shimadzu spectrophotometer. Biogas was collected through a tube attached to an inverted cone located above the floor of Pond A and gas was analysed in a Chrompak gas chromatograph.

3.3 RESULTS AND DISCUSSION

3.3.1 System Operation

The WSP system operated by the Mossop-Western Leathers Co. in Wellington, RSA, and commissioned in 1964, was a 13.6 ha cascade of 15 ponds with a total capacity of 197 000m^3 (Figure 3.1). Daily production averaged 1500 hides processed to wet blue leather with an effluent load to the ponds of about 460 m^3.day^{-1}. The raw effluent was segregated in the tannery into alkaline lime-sulphide and acidic tanning liquor streams, and passed through pre-treatment including sulphide oxidation, blending and aeration, followed by flocculant-assisted settling of a portion of the solids fraction.

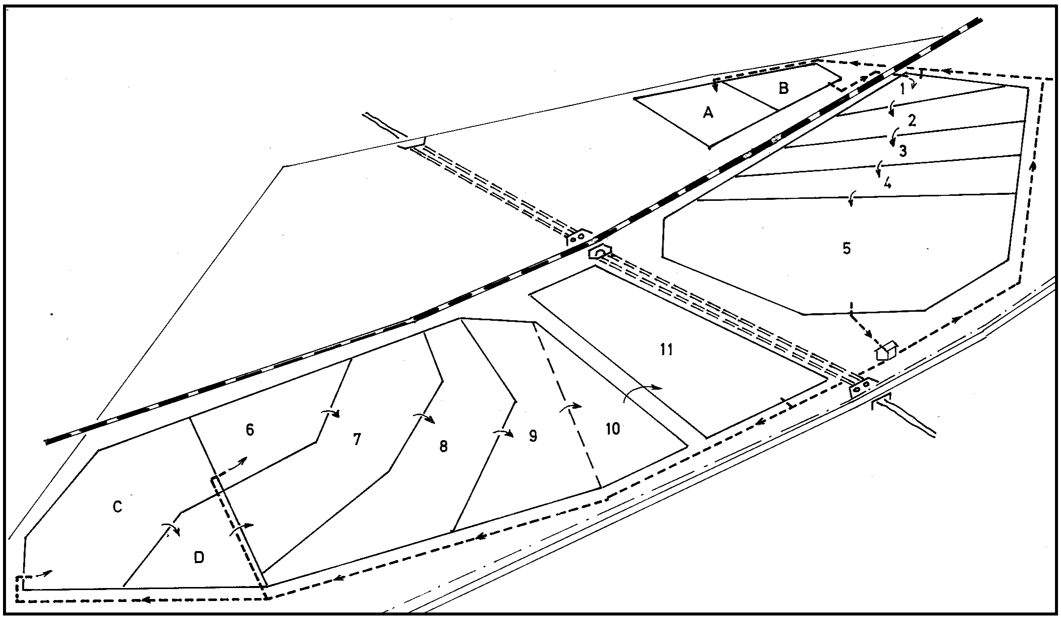


Figure 3.1 Plan diagram of the Waste Stabilisation Pond tannery wastewater treatment operation at Mossop-Western Leathers Co. tannery in Wellington showing the effluent treatment flow path from Pond A through Pond 5, and from the collection sump to Pond 11 via Ponds C to 10.

Effluent entered the WSP at Pond A which operated initially as an Anaerobic Pond, flowed through Ponds B to 5 (which were also functionally anaerobic) where it was pumped from a collection sump to Pond C and then flowed to Pond 10. Pond 11 served as a sink for final concentrates and stormwater runoff from the site and Ponds C to 11 operated as Facultative Ponds. Pond A was subsequently converted to operate as a Primary Facultative Pond (PFP) with the installation of mechanical surface aerators. This development is described in Chapter 6. The cascade functioned as a zero-discharge process in an area where the hot, dry summers of a Mediterranean climate enable the operation of a positive evaporation balance in the ponds (annual evaporation > 1.5 $m.year^{-1}$). While the study documented here relates to the investigation of the Wellington plant only, the broad trends which are described were also noted in separate observations of a tannery WSP operated by East Cape Tanning Co. in Uitenhage, RSA; at Okapuko in Namibia and in a number of tannery ponds observed at various times elsewhere in Africa.

Carre *et al.* (1983) estimate an environmental impact for tannery wastes of 1600 population $equivalents.ton^{-1}$ hides processed. However, the direct load to the WSP system, reported in Tables 3.1 and 3.2 and Figure 3.2, is somewhat less than that value, given solids removal in the pretreatment steps described. Surface loading to the system at 10.7 $gCOD.m^{-2}.day^{-1}$ is comparable to sewage WSP (Gomes De Sousa, 1987; Bucksteeg, 1987) but volumetric loads of 7.4g COD. $m^{-3}.day^{-1}$ are quite low. Despite the 85% COD reduction which is evident across the ponding system, it had become overloaded over the 36 years of its operation and, during North Westerly winds, has been the source of serious odour offense to the local community which is situated some 3 km downwind from the site. Wellington has become affectionately known as ‘Smellington’ in local circles.

Table 3.1 Dissolved Oxygen, colour and odour distribution across the Waste Stabilisation Ponding system.

	Effluent	Pond A	Pond 5	Pond 6	Pond 11
Dissolved Oxygen ($mg.L^{-1}$)	<0.01	<0.01	<0.01	3.98	4.71
Chlorophyll a ($mg.L^{-1}$)	0	0	0.24(0.12)	3.73(0.8)	3.3(0.4)
Colour	brown	brown	red	green	green
Odour	+++	+++	+++	+	0

(+++=seriously offensive; +=noticeable not unpleasant; 0=none)

3.3.2 Microbial Ecology

A number of clearly established gradients formed across the ponding cascade, and Figure 3.2 reports the results of the sampling programme in which the effluent treatment performance in the WSP was measured in composite grab samples drawn from ponds over the course of the study period. The observed gradients determined the principal features of the microbial ecology particular to the tannery WSP, and in turn the performance of the biological treatment processes operating within the system. Well-defined, and rather dramatic, colour changes occurred across the pond

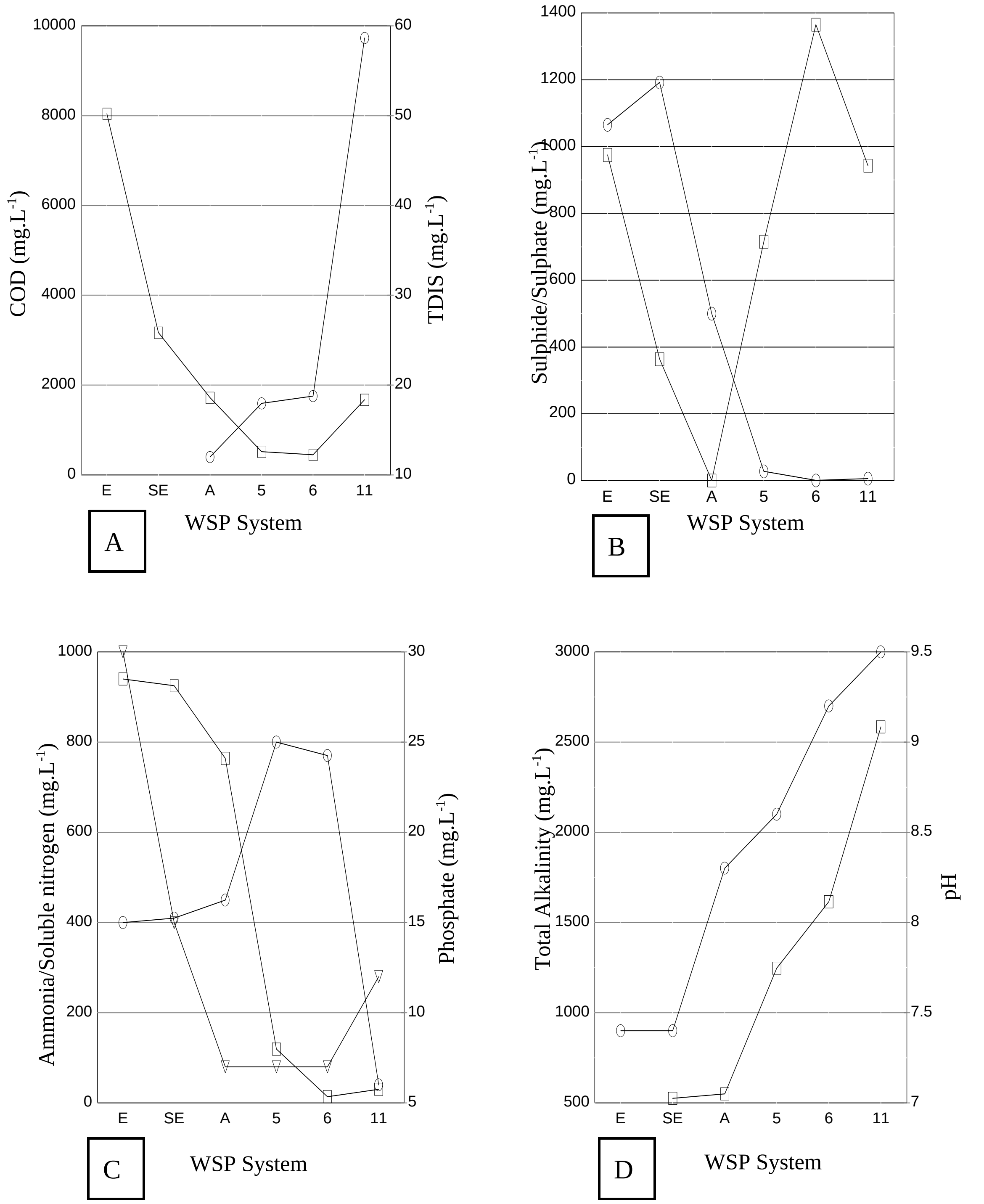


Figure 3.2 Effluent treatment performance across the Waste Stabilisation Ponding system (E=raw effluent; SE=settled effluent; Ponds A,5,6,11). A: Chemical Oxygen Demand (COD)=open square; Total Dissolved Inorganic Solids (TDIS)=open circle. B: Sulphate as SO_4 =open square; Sulphide as Na_2S=open circle. C: Ammonia as NH_3=open square; Total Soluble Nitrogen as TKN=open circle; Phosphate as PO_4=open triangle. D: Total Alkalinity as $CaCO_3$=open square; pH=open circle.

cascade which also correlate quite closely with rising salinity, DO and pH levels (Table 3.1 and 3.2). The brown/grey colour of the effluent and Pond A contents changed to a bright pink/dark purple in the anaerobic Ponds 1 to 5. These ponds were dominated by large populations of purple sulphur bacteria, various *Chromatium spp.*, and purple non-sulphur bacteria including *Rhodobacterium spp.* Green sulphur bacteria of *Chlorobium spp.* occurred in smaller numbers with the occasional observation of *Dunaliella salina* and *Dunaliella viridis*, depending on season. Bacterial photosynthesis and lithotrophic metabolism resulted in the oxidation of sulphide at the pond surface which manifested as a film of white elemental sulphur which, at times, blew into thick windrows against the pond levees. Sulphide oxidation resulted in the pronounced elevation of sulphate levels through Pond 5.

In Pond 6 the colour changed abruptly to dark green due to the blooming, at times, of near monocultures of the cyanobacterium *Spirulina* sp. Table 3.1 reports the increasing levels of Chl a across the system. Although the particular strain which dominated the system has not been finally classified, it is morphologically distinct and has been provisionally identified as an alkalophilic strain of *Spirulina platensis*. Massive blooms of this organism occurred and large rafts of biomass drifted on the surface, and accumulated on the sides of the levees. As the salinity rose through Ponds 9 and 10, numbers of *Spirulina* decreased and small mixed blooms of the halophilic chlorophytes *Dunaliella salina* and *Dunaliella viridis* occurred. Despite the water colour change to dark green, *Rhodobacterium sp.* remained the dominant component of the bacterial population in the latter ponds and their cell numbers ($6.4x10^4$) did not change significantly. However, the Rhodobacterial population was decolourised, with a reduction in photosynthetic bacterial pigment concentration observed. This response to growth in the dark water column was shown, in a series of flask studies, to be related to a shift from photosynthetic to heterotrophic metabolism influenced by the *Spirulina*-induced elevation of DO levels.

Table 3.2 Analysis of effluent feed and ponded effluent at various points across the Wellington Waste Stabilisation Ponding system.

	Effluent	Pond A	Pond 5	Pond 6	Pond 11
Total alkalinity as $CaCO_3$ ($mg.L^{-1}$)	525	-	1246	1615	2585
Dissolved Oxygen ($mg.L^{-1}$)	< 0.005	< 0.005	< 0.005	3.98	4.71
Chemical 0xygen Demand ($mg.L^{-1}$)	3173	1722	522	450	1677
Sulphide as Na_2S ($mg.L^{-1}$)	1192	500	28	<1	6
Sulphate as SO_4 ($mg.L^{-1}$)	364	<1	715	1365	943
Ammonia as NH_3 ($mg.L^{-1}$)	925	764	119	14	30
Phosphate as PO_4 ($mg.L^{-1}$)	15	7	7	7	12
pH	7.40	8.30	8.60	9.20	9.50

The results of the flask study are reported in Figure 3.3, which shows rhodobacterial pigment production in Pond 6 effluent in the light (control), and compares levels of pigment produced in the absence of oxygen (nitrogen headspace) with reduced pigment production in the presence of both oxygen and *Spirulina* photosynthesis.

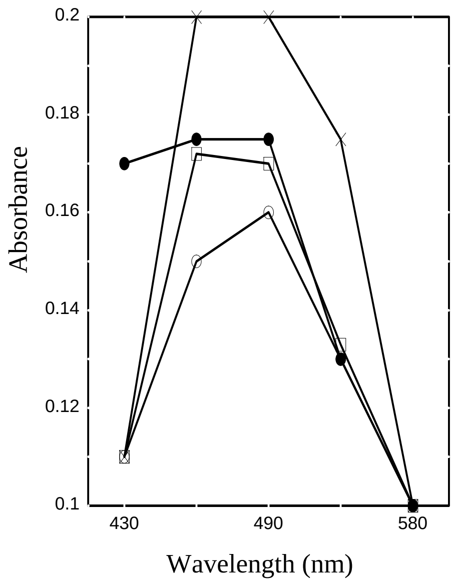


Figure 3.3 Rhodobacterial pigment production in pond effluent following growth in aerobic and anaerobic environments. Cross=nitrogen headspace; open circle=oxygen headspace; closed circle=growth in the presence of photosynthesising *Spirulina;* open square=control.

Photosynthetic oxygen production, and the elevation of DO and alkalinity levels commencing in Pond 6, resulted in sharply reduced sulphide and ammonia levels and also correlated with a marked reduction in odour release. De Pau and Salamoni (1991) have reported a chemical trapping mechanism in ponding systems, whereby odour-causing sulphides and mercaptans are detained in oxygenated and high pH surface waters, to be followed in due course by their biological oxidation.

The results of the sampling programme across the ponds indicated the operation of mature sulphur and nitrogen biogeochemical cycles, as principal determinants of the biological processes in the system, and explained, in part, the remarkable blooms of *Spirulina* in the latter ponds. The trends, summarised in Figure 3.2, show sulphate reduction in the anaerobic compartments followed by nearly complete oxidation of sulphide in the facultative ponds. Ammonia removal from the system may be linked to an increase in total alkalinity and pH, and rising nitrate levels, which indicates the operation of both chemical stripping and biological consumption mechanisms (Pano & Middlebrooks, 1982). Abeliovich (1980) and Konig *et al*. (1987) report ammonia toxic thresholds for *Spirulina* of around100mg.L^{-1}, and this threshold is reached somewhere between ponds 5 and 6. While ammonia concentration may play the primary role in regulating *Spirulina* blooms, the reduction in both COD and sulphide levels, and a rising salinity gradient across the pond cascade, contributed to a point, reached around pond 6, where conditions favoured the prolific growth of the mesohaline, alkalophilic strain of *Spirulina*, which occurred in this system.

3.3.3 Water Column in Anaerobic Ponds

In addition to the subjective grab sample study across the ponding system, the results of which are reported above, a programme of water column sampling, at fixed stations on a pre-determined grid in each pond was also carried out over the 3 years of the study. These results are summarised in Tables 3.3 and 3.4.

Table 3.3 Analysis of anaerobic pond water column strata. Samples were drawn at three levels through anaerobic ponds at each of the seasons over the three year study period and averaged results reported. Standard deviation in brackets.

	Surface	0.75m	1.5m	Average
pH	8.63	8.64	8.66	8.64
Dissolved Oxygen (mg.L^{-1})	<0.01	<0.01	<0.01	<0.01
Total Dissolved Solids (g.L^{-1})	13.6(2.19)	13.6(2.19)	37.6(30.66)	21.6(11.7)
Chemical Oxygen Demand (mg.L^{-1})	760(43)	841(74)	1074(241)	892 (163)
Sulphide as Na_2S (mg.L^{-1})	20(10)	25(15)	30(18)	25(5)
Sulphate as SO_4 (mg.L^{-1})	759(212)	719(183)	667(152)	715(46)
Ammonia as NH_4 (mg.L^{-1})	54(26)	61(30)	65(27)	60(5.6)
Nitrate as NO_3 (mg.L^{-1})	44(24)	41(24)	35(20)	40(4.6)
Phosphate as PO_4 (mg.L^{-1})	27(5)	32(3)	35(3)	31(4)
Chlorophyll a (mg.L^{-1})	0.319(0.157)	0.171(0.07)	0.147(0.021)	0.212( )
Light PAR (μmoles.m^{-2}.sec^{-1})	571(216)	0	0	-
Temperature (°C)	22.1(7.9)	19.8(5.1)	19(4.8)	19.9( )

Following the retrofitting of Pond A with mechanical surface aerators, an aerobic surface layer was established, with an oxypause at 0.5m depth, and providing two clearly defined compartments. It operated effectively thereafter as a PFP. Effluent inflow to the base of the pond (4m depth) was subjected to active anaerobic digestion. The loading rate of around 1500 kg.ha^{-1}.day^{-1} was more than twice the recommended rate for anaerobic ponds, and the approximately 45% reduction in COD effected in this unit was somewhat less than the 60-70% reduction reported by Oswald *et al.* (1994) for fermentation pits incorporated into an Advanced PFP treating domestic sewage. It was, however, consistent with reduced rates for the anaerobic digestion of tannery wastes reported by Jackson-Moss (1990) and Lalitha *et al.* (1994). Methane content of biogas sampling ranged around 20% with virtually complete conversion of sulphate to sulphides in the anaerobic compartment of pond A, by a stable population of sulphate reducing bacteria (Figure 3.2 B).

Conditions similar to those observed above prevailed throughout ponds 1 to 5 which, despite a well-developed salinity gradient at lower levels, functioned effectively as mixed anaerobic ponds (Table 3.3). A slight thermal gradient was evident during the summer months, and light extinction, and hence also levels of chl a decreased rapidly down the water column. Sulphide/sulphate, ammonia and COD levels showed a modest increase with depth, thereby indicating that, while some oxygen is available at the surface, DO is close to zero, and more stringent anaerobiosis prevails at the lower levels of these ponds. An average 85% COD reduction is achieved through this stage of the system, which is consistent for anaerobic pond operation as reported by Pescod (1996).

3.3.4 Water Column in Facultative Ponds

Unlike the anaerobic ponds, the water in Ponds 6-11 was well-stratified throughout most of the year. An overlay of warmer oxygenated water covered an anaerobic bottom zone, implying true facultative function (Table 3.4). The sulphate/sulphide, ammonia and COD gradients present through the water column indicate the operation of two distinct compartments. Table 3.4 data suggests these are defined by the availability of light, and hence by photosynthetically produced oxygen. However, seasonal change in the temperature gradient also correlated closely with chl a and DO profiles in the water column over the same period. Oswald (1988b) has noted a near 90% conversion of light to heat energy by micro-algae. High micro-algal growth at the surface in summer is associated with light extinction at lower levels, and hence also reduced chl a concentrations.

Table 3.4 Analysis of facultative pond water column strata. Samples were drawn at three levels through facultative ponds at each of the seasons over the three year study period and averaged results reported. Standard deviation in brackets.

	Surface	0.75m	1.5m	Average
pH	9.19(0.19)	9.18(0.19)	9.2(0.18)	9.19(0.19)
Dissolved Oxygen ($mg.L^{-1}$)	16.7(1.27)	0.28(0.16)	<0.01	5.6(0.76)
Total Dissolved Solids ($g.L^{-1}$)	50.5(16.9)	51.1(17.2)	54.7(18.6)	52.1(17.5)
Chemical Oxygen Demand ($mg.L^{-1}$)	557(58)	623(70)	730(135)	636(87.6)
Sulphide as Na_2S ($mg.L^{-1}$)	9(5)	14(5)	76(9)	33(6.3)
Sulphate as SO_4 ($mg.L^{-1}$)	1066(547)	923(402)	832(348)	940(432)
Ammonia as NH_4 ($mg.L^{-1}$)	22(1)	25(2)	30(4)	26(2)
Nitrate as NO_3 ($mg.L^{-1}$)	15(4.6)	14(2.3)	19(14)	16(6.9)
Phosphate as PO_4 ($mg.L^{-1}$)	25(2)	26(2)	29(1)	27(2)
Chlorophyll a ($mg.L^{-1}$)	3.95(0.27)	2.63(0.32)	1.63(0.007)	2.74(0.2)
Light PAR ($\mu moles.m^{-2}.sec^{-1}$)	571.6(216.2)	0	0	-
Temperature (^oC)	22.2(5.8)	18.9(5.1)	18.6(3.5)	19.9(4.8)

These waters were well-stratified as indicated by the temperature profile. Lower growth at the surface in winter was associated with increased penetration of light and higher chl a recorded at lower pond levels. The temperature profile indicated less stratification in ponds during winter. The chl a and DO gradients were sustained even in winter, and no serious mixing of these ponds was observed during the 3 year study. The oxygen-rich cap on the Facultative Ponds accounts, in part, for the efficient control of odour nuisance in this part of the system. Pronounced salinity and temperature gradients also occurred in the Facultative Ponds.

3.3.5 Photosynthetic Productivity of Pond System

Photosynthetic productivity measurements through the water column are reported in Figure 3.4 and relate almost entirely to *Spirulina* activity. Taken together these results indicate that at lower levels growth is light-limited during summer, while temperature effects probably account for the surface limitations noted during winter.

The photosynthetic productivity of the ponding system was estimated on the basis of carbon fixation values recorded in ponds over the various seasons. While mid-summer fixation rates approached 15 $gC.m^{-2}.day^{-1}$, which is in the upper range reported for fully optimised sewage HRAP (Oswald, 1995), winter values fell to about 4 $gC.m^{-2}.day^{-1}$, providing an annual average production of around 7.5 $gC.m^{-2}.day^{-1}$ across the 4ha of the Facultative Ponds. Figure 5 shows higher carbon fixation at the pond surface in summer and at lower levels in winter. Richmond (1988) has reported experimental yields for *Spirulina* of up to 30 $gC.m^{-2}.day^{-1}$, while the average values recorded here compare favourably with the 8-12 $gC.m^{-2}.day^{-1}$ reported for outdoor culture basins reported by Fox (1988).

It is thus apparent that total *Spirulina* biomass generated through the facultative component of the system could reach a level of about 110 ton.year^{-1}. This translates into an estimated oxygen production in the system of around 165 tons O_2.year^{-1}, or a solar energy conversion equivalent to mechanical oxygenation of 165 000 kW hrs (based on values provided by Oswald, 1988b). Given the elevation of mass transfer barriers due to the salinity of the system, actual expenditure of mechanical energy to achieve this level of oxygen actually transferred would be anticipated to be substantially higher than this figure.

3.4 CONCLUSIONS

The observation of substantial and sustained blooms of *Spirulina* in the tannery WSP, and the relatively high productivity values which have been reported, was one of the most noteworthy outcomes of the study. Subsequent observations of other tannery WSP systems indicate that the occurrence of *Spirulina* at a specific point along the chemical, physical and biological gradients which establish across these systems, is a particular and characteristic feature of the tanning WSP environment. The authors have not found this phenomenon previously documented or explained. Blooms of *Dunaliella* spp. occurred primarily in the terminal hyper-saline ponds in the WSP system.

The growth of *Spirulina* clearly determined the aerobic status of the latter ponds and the stratification and aerobic capping was observed to provide particularly successful and stable odour control in the facultative ponds. The potential to manipulate algal growth to effect odour control in the WSP system was also an important outcome of the study, especially given the level of conflict between the tannery and the local community over the odour nuisance issue. The substantial oxygen production potential demonstrated for the system has raised the prospect of dealing with system malfunction, and also the possible enhancement of the tannery effluent treatment process itself, through the engineering of an algal biotechnology process approach to the treatment of these effluents. In this regard the objective would be to manipulate the ponding process to allow the establishment of *Spirulina* growth in the first ponds of a WSP, thus ensuring an effective control across the whole system.

The investigation and development of a micro-algal management system for the operation of tannery WSP was identified as a primary goal for the project, and these investigations are described in the subsequent chapters of the report. The WSP study showed potential for the cultivation of two micro-algal species occurring as the dominant forms at different points along the salinity gradient which establishes across the pond cascade - *Spirulina* in the medium salinity, alkaline ponds in the middle of the cascade, and *D. salina* in the terminal hyper-saline ponds.

Spirulina biomass production is one of the few algal biotechnologies which has been successfully commercialised on any scale, and the dried product, depending on grade, commands a sustained demand on world markets (Richmond, 1996). The investigation and further development of the potential for *Spirulina* biomass production in the tannery WSP was investigated and is also reported in subsequent chapters. The investigation of a role for *Dunaliella* spp. in hyper-saline wastewater treatment is detailed in part 2 of this project report series (Appendix 1).

4 THE GROWTH AND PERFORMANCE OF *SPIRULINA* IN THE OPERATION OF A TANNERY WASTE STABILISATION PONDING SYSTEM

4.1 INTRODUCTION

The occurrence of near-monospecies blooms of the cyanobacterium *Spirulina* in tannery WSP, and their role in shifting ponds from anaerobic to facultative and aerobic operation, was observed in the pond ecology studies reported above. A number of factors characteristic of the system were identified, and might explain the constraints regulating the distribution of the particular species observed in the system. Clarity in this regard would be important in any attempt to develop a micro-algal management approach based on algal biotechnology developments.

The objective of the study reported in this section was to characterise the principal factors regulating and controlling *Spirulina* growth in tannery wastewaters. The tannery WSP also provided an opportunity to study mechanisms controlling general micro-algal performance in saline and hyper-saline environments. Factors investigated include the nutrient status of the ponds, which influences *Spirulina* growth, the possible presence of toxic or growth inhibitory factors, and the effect of the various gradients which exist across the ponding system. The role of organic nutrition in the growth and performance of *Spirulina* in these systems was also investigated.

4.2 METHODS

Analytical procedures used are described in section 3.2. Analysis of pond contents are outlined in Tables 3.1 and 3.2, and Figure 4.1 shows the characteristic distribution pattern of *Spirulina* across the system. Chl a levels reported relate almost entirely to the presence of this organism. Zarouk's medium (Zarouk, 1966) was used for the cultivation of *Spirulina*.

In the study of organic nutrition, colony forming units (CFU) were determined for each flask at the commencement of each experiment, to reduce or eliminate the possible role of bacterial mineralisation of the carbon source, and hence the release of labeled CO_2. [^{14}C]-glycine (3.81 GBq. mmol^{-1}) and D-[^{14}C]-glucose (10.9 GBq.mmol^{-1}) (Amersham), were added to the washed cultures containing either a concentration range of unlabeled glycine and glucose (Merck) respectively. One set of cultures contained no added glucose and glycine. Flasks were incubated at 25 ^{o}C in a constant environment room. Control cultures for each treatment were exposed to continuous illumination at 158 mol.m^{-2}.s^{-1}, while the other set was kept in total darkness. At time intervals of 5 minutes, 3 hours and 6 hours, 1 mL of culture medium was removed, filter washed with sterile defined medium (x3), and the cells transferred to aqueous scintillant (Packard). Culture supernatant was likewise transferred in 100 mL aliquots to aqueous scintillant and counted in a Beckman LS3150T scintillation counter and results adjusted to reflect disintegration per minute (dpm) values. The results reflect a triplicate mean. Zarrouk's inorganic mineral medium was used as the defined medium control (Richmond, 1986).

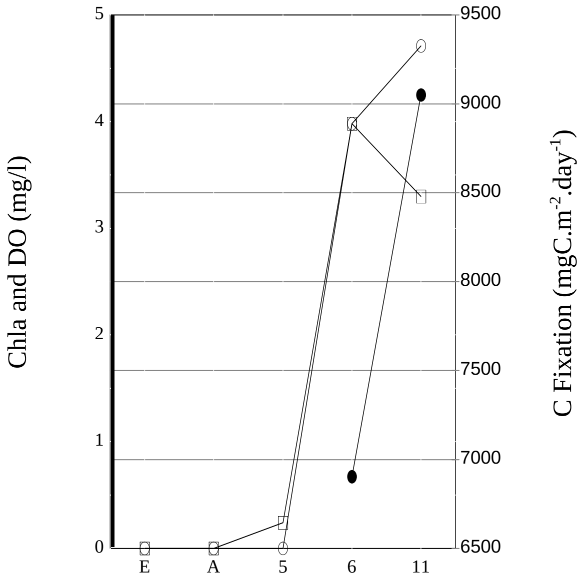


Figure 4.1 Distribution of micro-algal biomass across the Waste Stabilisation Pond cascade(A-11) as indicated by chlorophyll a and dissolved oxygen levels, and carbon fixation rates (open square=chlorophyll a; open circle=dissolved oxygen; closed circle=carbon fixation rate).

4.3 RESULTS AND DISCUSSION

4.3.1 Nutrient Status

The nutrient status of the tannery effluent feed to the WSP, and the capacity of the system to sustain *Spirulina* growth through the phases of pond treatment, was evaluated on the basis of the Algal Biomass Potential (ABP) method described by Oswald (1988a). An analysis of the major elements present in the effluent during the various stages of the ponding cascade is reported in Table 4.1 and is compared to Zarrouk's defined inorganic medium. The ABP calculation for the effluent assumes a carbon:nitrogen:oxygen ratio for the cyanobacterial biomass of 50:8:1 (Oswald, 1988a). The derived ABP values for the effluent through the various stages of the pond cascade are reported in Table 4.2.

Richmond (1986) has noted that *Spirulina* growth may remain uninhibited in the presence of high levels of sodium as long as the potassium:sodium ratio remains below 5. This does not appear to be a constraining factor in the effluent medium examined. Neither calcium, which precipitates in the system, nor magnesium exceeded toxic levels reported by Mitchell & Richmond (1988). While carbon values appear to be limiting in the initial effluent an increase occurs through the system, most probably due to photosynthetic activity, and reaches about half of full requirement by Pond 6. Although nitrogen is clearly not limiting in the effluent itself, as may be anticipated in this type of waste, levels are reduced as ammonia is removed and could possibly become a factor limiting growth later in the system.

Table 4.1 Composition of tannery wastewater Waste Stabilisation Pond effluents and defined inorganic Zarrouk's medium.

(mg/L)	Defined Medium	Tannery Effluent	Pond 5	Pond 7	Pond 11
Calcium	13.3	226	-	-	-
Carbon	2800	957	1246	1615	2585
Chloride	527	4048	7157	11651	24184
Iron	0.4	10.7	6.2	0.65	6.14
Magnesium	7.0	260	171	191	190
Nitrogen500	717	125	63	54	
Phosphorus	62	19	28	23	24
Potassium	125	127	118	276	259
Sodium	3800	3090	2890	6790	8690
Sulphur	151	195	167	209	316

The phosphate requirement is fulfilled throughout the ponds. The calculated ABP values provide an indication that nutritional factors alone do not explain the restricted distribution of *Spirulina* growth to the latter ponds in the WSP system.

Table 4.2 The Algal Biomass and Oxygen Release Potential (ABP) calculated for defined medium, tannery wastewater and Waste Stabilisation Pond effluents.

ABP	Defined Medium	Tannery Effluent	Anaerob.Pond(5)	Fac. Pond(7)	Terminal Pond (11)
Carbon	5600	1914	2492	3230	5170
Nitrogen6250	8962		1562	787	675
Phosphorus	6250	1900	2400	2300	240

4.3.2 Toxicity

The possibility that factors toxic to *Spirulina* growth may limit the organism's occurrence earlier in the pond cascade was evaluated in flask culture studies of a tannery effluent dilution series inoculated with the WSP *Spirulina* isolate. While the results of this study (illustrated in Figure 4.2) confirm previous observations of the complete inhibition of *Spirulina* growth in undiluted effluent, the effect is concentration related with the 50% and 20% dilutions of pre-treated effluent showing some growth, but only following a lag period of 4 to 5 days.

Growth could not be sustained at all in untreated raw effluent. However, below the 20% dilution of pretreated effluent a growth stimulation effect is observed with higher cell yields produced in the effluent dilution compared to the Zarouk's defined medium controls. In this case no lag phase was observed.

Analysis of the pre-treated tannery effluent is reported in Table 3.2 and shows the presence of high levels of ammonia, sulphate and sulphides, and COD. Of these, ammonia levels are substantially higher than the 100 $mg.L^{-1}$ toxic threshold for *Spirulina* reported by Abeliovich (1983) and Konig *et al*. (1987), and indicate the operation of at least one inhibitory factor in the effluent. Growth enhancement observed in the 10% and 5% effluent dilutions, where the ammonia concentration fell below the reported toxic threshold, tends to confirm this observation. In the WSP cascade the appearance of *Spirulina* in the latter ponds is also associated with the observed reduction in ammonia concentration below the 100 $mg.L^{-1}$ toxic threshold level.

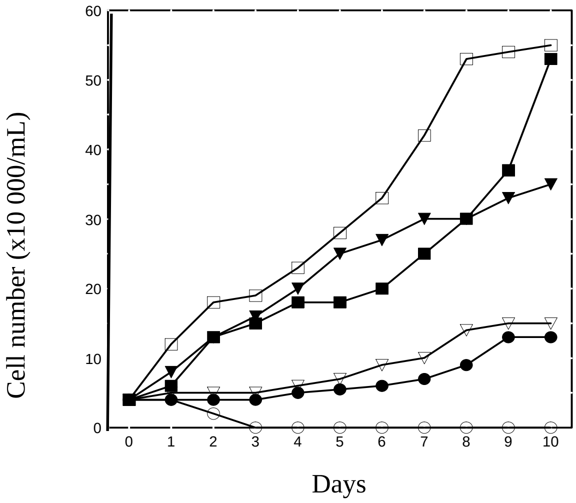


Figure 4.2 Growth of *Spirulina* in a dilution range of tannery effluent and in Zarouk's defined mineral medium (closed square=control in defined medium; open square=5% effluent; closed triangle=10% effluent; open triangle= 20% effluent; closed circle=50% effluent; open circle=100% effluent).

4.3.3 Pond Gradients

It was noted above that a number of well defined physical, chemical and nutrient gradients establish across the tannery WSP cascade, and that these could influence the observed patterns of *Spirulina* growth. The interaction of a number of factors, possibly impacting synergistically on the growth rate of *Spirulina*, were evaluated in a series of grid matrix experiments using the 5% effluent dilution as the control medium. These included the interaction of salinity and pH, which rise together through the system, and the concentrations of bicarbonate and phosphate, and also bicarbonate and ammonia, which change with respect to one another over the course of the ponding process. The results are reported in Figures 4.3-4.5.

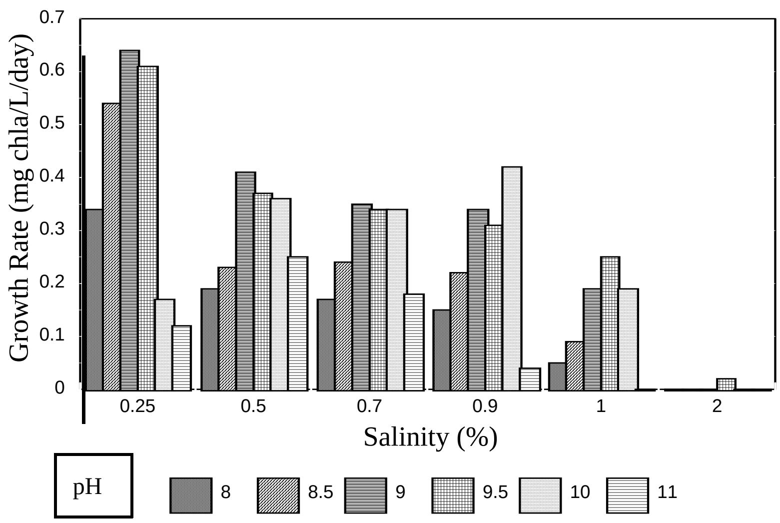


Figure 4. 3 A matrix study of *Spirulina* growth performance in tannery effluent comparing the effects of increasing pH and salinity values (pattern bars=pH).

Figure 4.3 shows that while optimum growth occurs at lower salinity levels and at pH values between 9.0 and 9.5, growth is, nevertheless, sustained at salinities between 1-2% and at pH 11. While these values agree with maxima reported by Richmond (1986), the results indicate that neither pH nor salinity factors limit *Spirulina* growth in the tannery effluent medium.

The results reported in Figure 4.4 show a bicarbonate stimulation of growth in the effluent medium of up to 5-fold, demonstrating a quite severe carbon limitation, which had been indicated by the APB calculation. A number of reports have shown growth enhancement of *Spirulina* with the addition of bicarbonate to a range of wastewaters (Chaudari *et al*., 1980; Materassi *et al*., 1984; Mitchell & Richmond, 1988). The addition of phosphate had little impact on growth in the effluent control cultures, confirming indications provided in the ABP calculation. While phosphate addition over a range of concentrations, together with bicarbonate in the grid matrix study, produced no enhancement in the *Spirulina* growth rate, higher levels of phosphate addition did appear to be inhibitory at the elevated growth rates achieved. Maximum growth response was observed at an addition of 0.2M bicarbonate.

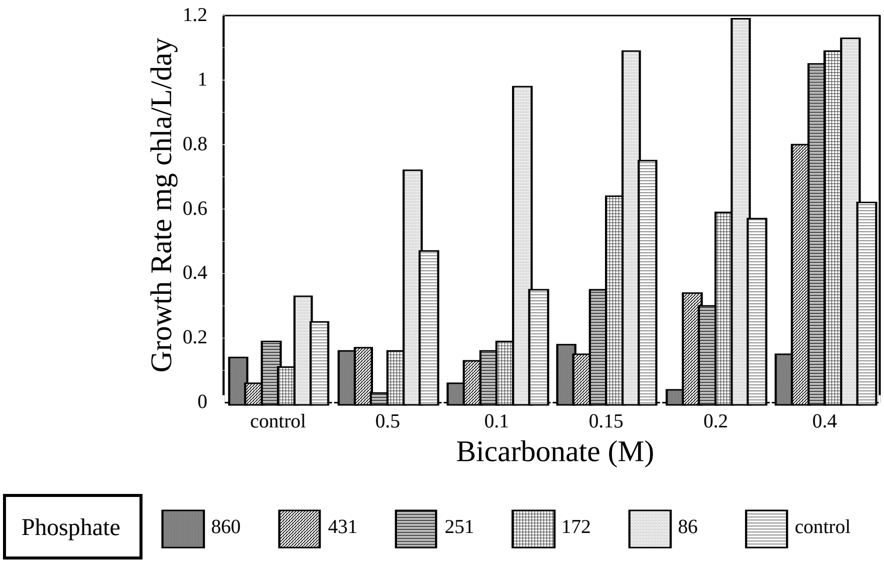


Figure 4.4 A matrix study of *Spirulina* growth performance in tannery effluent comparing increasing concentrations of bicarbonate and phosphate (pattern bars=phosphate mg.L^{-1}).

The growth rates of *Spirulina* in the bicarbonate/ammonia grid matrix study reported in Figure 4.5 again demonstrated the growth stimulatory effects of bicarbonate in the tannery effluent medium. Ammonia is clearly used as a nitrogen source by *Spirulina* with the best growth rates in the series achieved with the addition of 20 mg.L^{-1} ammonia and 0.2 M bicarbonate. In these studies ammonia toxicity was found to completely inhibit growth between 40-60 mg.L^{-1} without bicarbonate addition, and between 60-80 mg.L^{-1} ammonia at the bicarbonate-induced elevated growth rates.

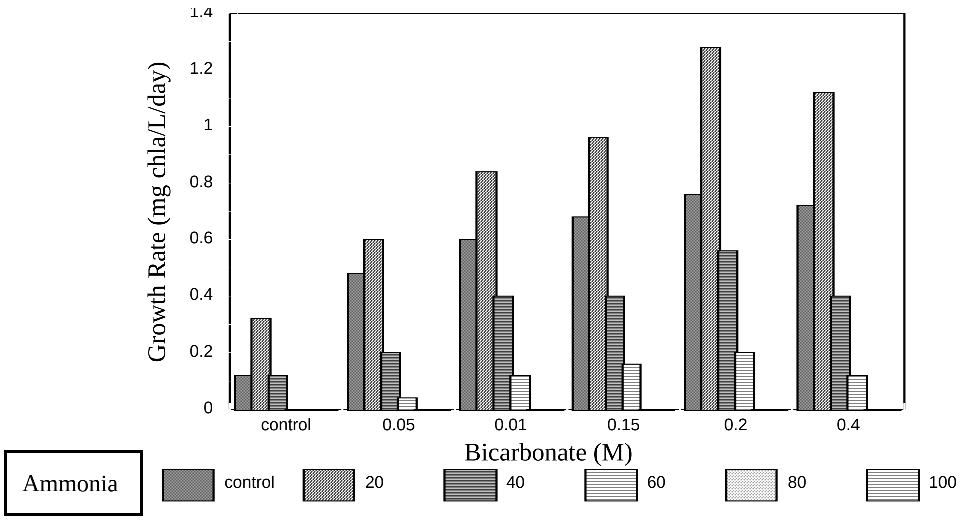


Figure 4.5 A matrix study of *Spirulina* growth in tannery effluent comparing increasing concentrations of bicarbonate and ammonia (pattern bars=ammonia mg.L^{-1}).

While Richmond (1988) has reported the use of ammonia by *Spirulina* as a nitrogen source, which appears to be confirmed in this study, the toxicity threshold is somewhat lower in this medium than the 100 mg.L^{-1} value reported for sewage and pure culture media (Abeliovich & Azov, 1976; Konig *et al*., 1987; Soares *et al*., 1995). The pH-induced shift of ammonia to the ionised form, a process which takes place across the pond cascade as alkalinity rises across the system, would serve to reduce the ammonia toxicity effect (Chevalier & de la Noue, 1985). The effect of carbonate addition to the tannery effluent medium serves to confirm this point.

4.3.4 Organic Nutrition

Radio-labeled nutrient uptake studies were undertaken to determine a possible role for organic nutrition in the enhancement of *Spirulina* growth observed in the lower dilutions of tannery effluent compared to defined mineral media. Figures 4.6 - 4.9 report the incubation of washed cultures of the WSP *Spirulina* isolate, in Zarrouk's defined medium, together with [^{14}C]-glucose and [^{14}C]-glycine, in both the presence and absence of light.

The association of the labeled compounds with the biomass fraction, in the light incubation studies, was observed to occur very shortly after this addition to the culture medium, and then to increase over the remaining incubation period. The addition of increasing concentrations of the unlabeled substance, in each case, resulted in reduced uptake of the [^{14}C] label indicating a competitive quenching effect on label association with the *Spirulina* biomass. This, together with the continued and increasing label-biomass association noted above, indicates that more than surface binding appeared to be involved in the process observed. The results suggest that the uptake and internalisation of these organic compounds by the organism occurs during its growth in tannery effluent.

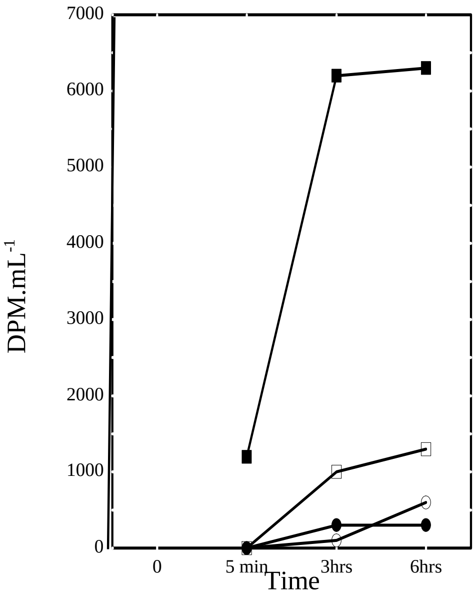


Figure 4.6 Uptake of D-[^{14}C]-glucose by *Spirulina* incubated in the light in defined medium, without the addition of unlabeled glucose and also together with the addition of increasing concentrations of unlabeled glucose (closed square=unlabeled glucose not added; open square=addition 1mg.L^{-1} unlabeled glucose; closed circle=addition 10 mg.L^{-1} unlabeled glucose; open circle=addition 50 mg.L^{-1} unlabelled glucose).

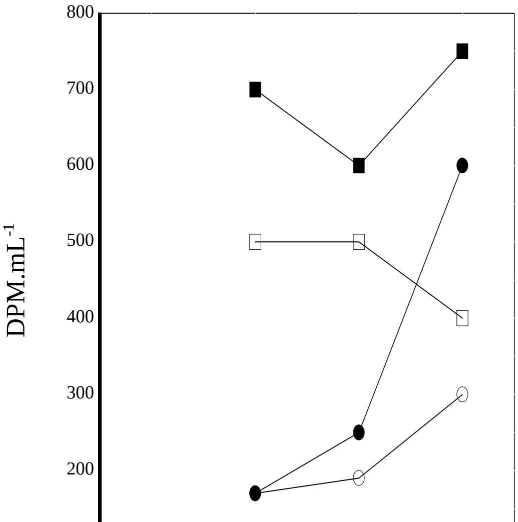


Figure 4.7 Uptake of D-[^{14}C]-glucose by *Spirulina* incubated in the dark in defined medium, without the addition of unlabelled glucose and also together with the addition of increasing concentrations of unlabeled glucose (closed square=unlabeled glucose not added; open square=addition 1mg.L^{-1} unlabeled glucose; closed circle=addition 10 mg.L^{-1} unlabeled glucose; open circle=addition 50 mg.L^{-1} unlabeled glucose).

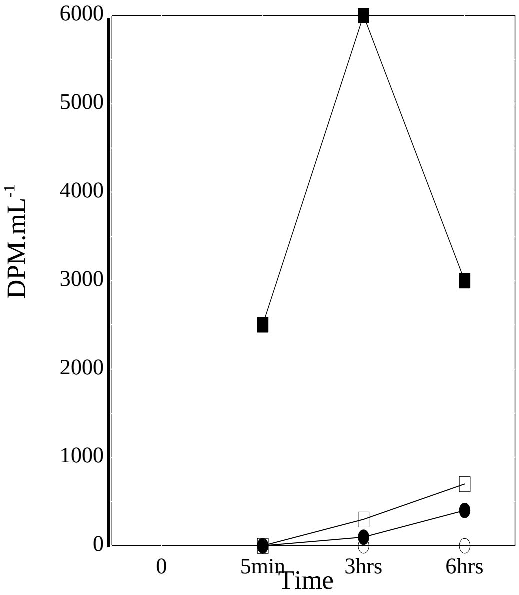


Figure 4.8 Uptake of [^{14}C]-glycine by *Spirulina* incubated in the light in defined medium, without the addition of unlabeled glycine and also together with the addition of increasing concentrations of unlabeled glycine (closed square=unlabeled glycine not added; open square=addition 0.5mg/l unlabeled glycine; closed circle=addition 1 mg.L^{-1} unlabeled glycine; open circle=addition 10 mg.L^{-1} unlabeled glycine).

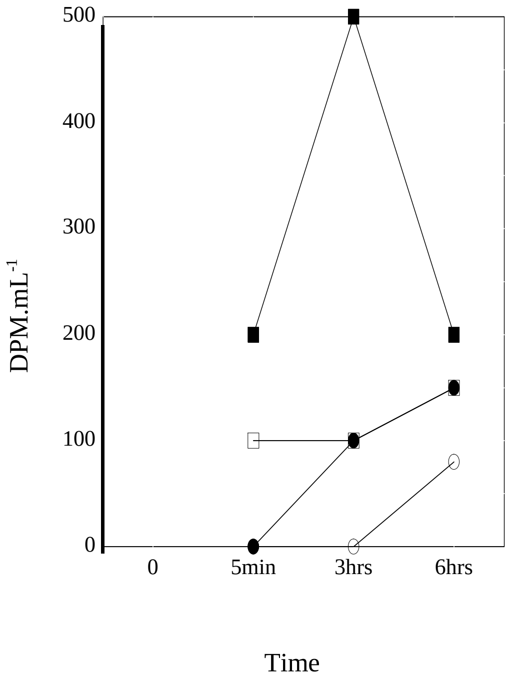


Figure 4.9 Uptake of [^{14}C]-glycine by *Spirulina* incubated in the dark in defined medium, without the addition of unlabeled glycine and also together with the addition of increasing concentrations of unlabeled glycine (closed square=unlabeled glycine not added; open square=addition 0.5mg/l unlabeled glycine; closed circle=addition 1 mg.L^{-1} unlabeled glycine; open circle=addition 10 mg.L^{-1} unlabeled glycine).

While the above trends were also observed in the dark incubation studies, indicating probable uptake of glycine, the results for glucose uptake in the dark are less convincing. Uptake studies for glucose and glycine were also undertaken in the tannery effluent medium. As may be anticipated uptake rates were low in this organic rich medium, and a competitive quenching of label uptake most probably accounts for the absence of uptake observed (results not shown).

4.4 CONCLUSIONS

1. Nutritional limitation does not appear to play a substantial role in the failure of micro-algae to establish themselves in the early ponds of the tannery WSP cascade. On the contrary, in the case of *Spirulina*, growth is substantially enhanced in appropriate dilutions of tannery effluent compared to defined culture medium.

2. The results of the toxicity experiments show that the presence of high levels of ammonia and sulphide in the tannery effluent act as inhibitors of *Spirulina* growth, and are directly involved in suppressing micro-algal performance in the early stages of the system. Ammonia toxicity thresholds are somewhat lower than reported values for domestic wastewater and pure culture systems.

3. Uptake of organic compounds present in the effluent may play some role in the growth stimulatory effect observed. This would accord with previous reports of organic nutrition in the cyanobacteria (Ciferri, 1983; Labarre *et al*., 1987; Anderson & Mackintosh, 1991).

4. It is apparent from the above results that while the nutrient status in the effluent feed to the system is apparently adequate to sustain *Spirulina* growth, and that ammonia/pH and sulphide/pH toxicity effects act, at least in part, to limit *Spirulina* growth, the impact of these factors is reduced, and is finally surmounted by the organism at a point within the gradients which establish across the pond cascade. In the WSP studied this occurs around pond 6 where ammonia levels fall below a 40-60 $mg.L^{-1}$ toxic threshold which seems to apply in this system. At this point possible sulphide toxicity has already been relieved due to both elevation in pH, and a net reduction in total sulphide content of the pond effluent.

5. The addition of bicarbonate extends the range of ammonia concentrations at which *Spirulina* will continue to grow. This result indicates that the strategy of recirculating water from terminal ponds to stimulate algal growth in primary WSP could depend, in part at least, on the alkalinity content which accumulates in the latter ponds due to photosynthetic activity. A number of mechanisms may be involved. Possible carbon limitation of *Spirulina* growth in the tannery effluent would be alleviated together with the enhanced consumption of ammonia at the elevated growth rate. Ammonia stripping would accelerate as pH rose and sulphide toxicity would decline as the equilibrium shifted to the ionised species.

6. The results of the flask studies indicate the possibility that *Spirulina* could be successfully cultivated in an HRAP paddle raceway-type application fed with pretreated tannery effluent at an appropriate loading rate, as has been reported for defined media (Fox, 1983) and cattle wastes (Mitchell & Richmond, 1988). The predictable generation of consistent biomass yields in this way, and loaded to the surface of the anaerobic ponds to effect an aerobic micro-algal capping of odour generating anaerobic compartments, would represent a useful application of algal biotechnology in upgrading the operation and performance of the tannery WSP. This would have particular significance given the severe odour problems associated with the tannery application of WSP systems. Oswald (1991) has described retention of the benefits of an algal biomass recirculation strategy in the Advanced Integrated Wastewater Ponding System (AIWPS) application by using a sub-surface draw-off from the anaerobic pond. This establishes what is in effect a two-compartment system with sustained micro-algal growth in an aerobic upper layer above an anaerobic lower zone.

   The HRAP as a retrofit, or design add-on to the tannery WSP, could overcome problems encountered with recirculation procedures such as the large blending ratios required to achieve adequate odour control, and the uneven and unpredictable production of micro-algal biomass in the latter ponds of a WSP cascade. This applies especially where oxygenation and dilution are the principal mechanisms involved in generating the anoxic or facultative, rather than anaerobic, conditions desired.

7. A surprising finding was the enhanced growth rates and biomass yields observed for *Spirulina* grown in the tannery effluent medium compared to defined inorganic media. Production of *Spirulina* biomass, as a specialist feed ration, or as a health supplement, presents one of the few algal biotechnologies to have been successfully commercialised (Richmond, 1996). Costs of defined medium formulation represent one of the major charges to production in *Spirulina* systems and the prospect of a value-adding utility for tannery effluent as a growth medium for the commercial production of this organism highlights the objective of value recovery from wastewater treatment which has been noted by numerous authors (Oswald, 1995; Pearson, 1996). The scale-up evaluation and development of a *Spirulina*-based approach to tannery effluent treatment, with both micro-algal capping of tannery WSP and the recovery of *Spirulina* biomass, have been the subject of follow-up studies which are reported in the following section.

5. A *SPIRULINA* HRAP UNIT OPERATION FOR THE TREATMENT OF TANNERY WASTEWATERS

5.1 INTRODUCTION

Both pond ecology and laboratory flask studies of *Spirulina* growth in tannery wastewaters had yielded preliminary indications that tannery effluent could, under certain conditions, provide a suitable medium for *Spirulina* growth equivalent, if not superior, to defined inorganic growth media. These studies also seemed to strengthen early indications that the tannery WSP system could provide the basis for an algal biotechnology innovation enabling both value-adding functions in the form of micro-algal biomass recovery, and improved and sustainable management of saline wastewaters.

The HRAP raceway which has evolved as the standard bioreactor of algal biotechnology systems (Richmond, 1996), seemed to offer an obvious mechanism for the practical development of these possibilities, both as a free-standing unit operation in tannery effluent treatment, but also integrated into the operation of WSP dedicated to the treatment of these wastewaters.

The studies reported in this chapter relate to the development of a *Spirulina* HRAP (S-HRAP) process as an independent unit operation in the treatment of tannery wastewaters. The scale-up evaluation of the process was undertaken from laboratory photobioreactor investigations, through pilot plant studies, to the design and construction of a full-scale S-HRAP producing both *Spirulina* biomass and treated tannery wastewaters.

5.2 METHODS

Tannery effluent used in both the photobioreactor and pilot HRAP studies was sourced from the Mossop-Western Leathers Tannery in Wellington, South Africa. A New Brunswick Instruments Bioflow III microprocessor-controlled fermenter was converted to operate as a photobioreactor and illuminated with cool white light. Control cultures were grown in Zarrouk's defined mineral medium (Richmond, 1986). The S-HRAP pilot plant and full-scale installations were designed and constructed as described, and analytical procedures followed methods already described.

5.3 RESULTS AND DISCUSSION

5.3.1 Photobioreactor Studies

The linkage of tannery effluent treatment with *Spirulina* growth and biomass production was examined in a series of photobioreactor studies. Conditions were optimised to achieve logarithmic growth of the WSP *Spirulina* isolate, first in Zarrouk's defined inorganic growth medium. The reactor was then operated over a period of several weeks in fed batch mode with tannery effluent fed at the equivalent of 5% of reactor volume.day^{-1}. This effluent dilution had been shown, as previously noted, to sustain good growth in flask studies. Figure 5.1 illustrates the effluent treatment performance achieved with this feed regime. An organic load reduction of 98% was observed at an overall COD removal rate of 19.5%.day^{-1}. While the high levels of ammonia present in the effluent were reduced by nearly 98% over the first few days, levels increased again gradually as protein degradation occurred.

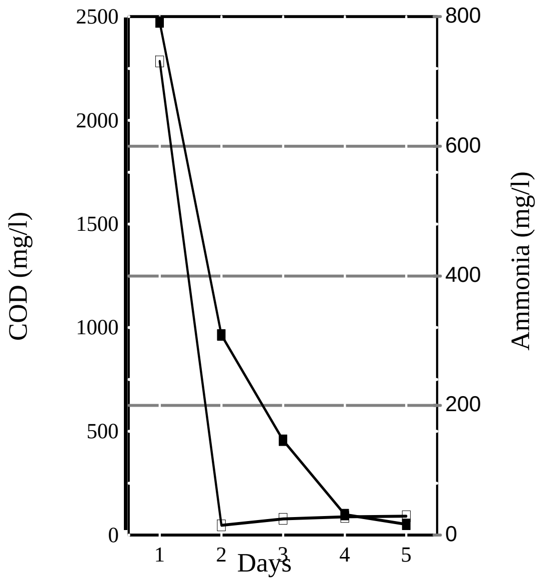


Figure 5. 1 COD and ammonia removal in a *Spirulina* photobioreactor showing a typical 5-day batch fed sequence with a tannery effluent loading rate equivalent to 5% reactor volume.day^{-1}. (Closed square=COD; open square=ammonia).

A continuous feed regime was then used to determine optimum effluent loading rates to the *Spirulina* culture, and in this study the photobioreactor was fed at daily loading rates of 3%, 5% and 8% of reactor volume. Steady state was achieved for each loading regime through at least 3 replacements of reactor volume, with the exception of the 8% feed rate where culture collapse intervened before this time. Table 5.1 reports analysis of the displaced reactor volumes and compares treatment effected under the different loading regimes. While sulphides were oxidised completely (results not shown), and a 90% reduction of COD and ammonia was achieved at the 3% feed, effluent load reduction was less efficient at the higher loading rates. Where the ammonia level exceeded 80 mg.L^{-1} the *Spirulina* culture started to show a decline in both chl a and cell numbers, a process which culminated in culture collapse and cell death. The 5% loading produced the highest sustainable feed rate to the system, and also highest DO and biomass productivity.

The ability of the *Spirulina* culture to sustain the possible accumulation of inhibitory factors through long-term continuous feeding of tannery effluent at a particular loading rate was evaluated in terms of the oxygen regeneration time. This was measured by operating the steady state continuous culture in batch mode over a period of hours, and following the treatment of a slug load of effluent fed to the reactor equivalent to the daily loading rate. Dissolved oxygen recovery to the previous resting steady state value was used to determine the appropriate feed interval for each concentration of effluent fed to the photobioreactor. It was found that the removal of the major part of the organic load coincided with the point where photosynthetic oxygen production started to exceed the total oxygen demand of the system. At the 5% loading regime oxygen regeneration times averaged 21 hours while at the 8% loading rate the equivalent regeneration required 40 hours. This procedure indicated the gradual accumulation of a treatment deficit at the higher daily loading regime and explained the eventual culture collapse which was observed under these conditions. Figure5.2 shows the photobioreactor DO profile responding to a batch feed mode at the 5% loading regime.

Table 5.1 Tannery effluent treatment performance in a *Spirulina* photobioreactor operated in continuous culture mode and fed at different loading rates expressed as a percentage of reactor volume. Analysis reflects photobioreactor status after at least three reactor volume displacements at each loading rate. Standard deviation in brackets.

	Effluent	3%	5%	8%
Chemical Oxygen Demand ($mg.L^{-1}$)	2474(1810)	213(102)	347(50)	563(85)
Ammonia as NH_3 ($mg.L^{-1}$)	731(98)	41(18)	61(18)	135(38)
Nitrate as NO_3 ($mg.L^{-1}$)	569(106)	15(9.6)	32(13.3)	34(27)
Phosphate as PO_4 ($mg.L^{-1}$)	19(12.5)	<1	<1	<1
Salinity ($g.L^{-1}$)	10(1.6)	30	30	30
Dissolved Oxygen ($mg.L^{-1}$)	0.01	11.93(1.84)	12.69(1.25)	11.8(1.2)
pH	8.2	10	10	10
Biomass ($mg.L^{-1}$)	-	173(42)	173(43)	167(11)

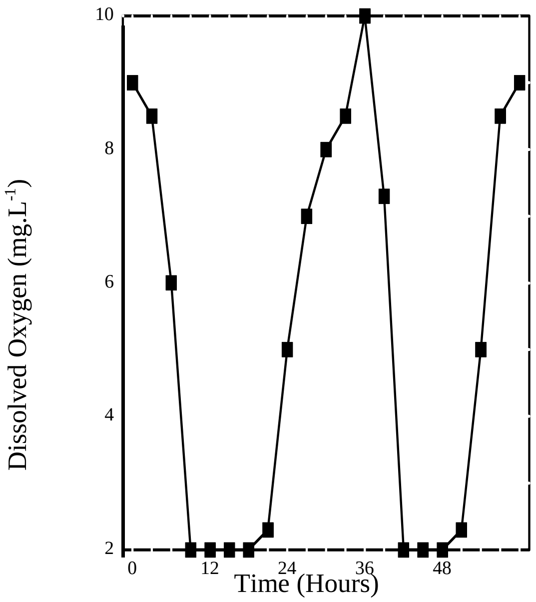


Figure 5.2 Dissolved oxygen profile in the *Spirulina* photobioreactor fed at the 5% continuous loading regime then operated in batch-fed mode to demonstrate the oxygen regeneration time.

5.3.2 S-HRAP Pilot Plant Study.

Based on the photobioreactor studies reported above, a scale-up evaluation of the *Spirulina* treatment process was undertaken in two 80 m^2 pilot S-HRAP paddle wheel-driven raceway ponds, constructed outdoors on-site at the Mossop-Western Leathers Co. Tannery in Wellington (Figure 5.3). Effluent feed to these ponds followed partial treatment through the PFP in the WSP (for plan diagram of this system see Figure 3.1). This sequence followed the flow-path described for the AIWPS described by Oswald (1991). The pilot HRAP units were seeded with *Spirulina* biomass sourced from the tannery's WSP system.

The pilot HRAP units were operated in a batch-fed mode, in the first instance, to establish operating conditions, and Figure 5.4 records a typical batch treatment sequence. The organic load was reduced by 85% at an overall rate of 7%.day^{-1} and ammonia by 93% at an overall rate of 8%.day^{-1}. Biomass increased by nearly 5%.day^{-1} in this mode of operation.

Figure 5.3 The S-HRAP pilot plant constructed at Mossop-Western Leathers Co., Wellington, showing one of the 80 m^2 High Rate Algal Ponds.

Cultures performed poorly at operating depths greater than 15cm and a linear flow velocity of 18 cm.sec^{-1} proved to be optimal for the S-HRAP.

Following the initial optimisation of the pilot S-HRAP they were operated in continuous feed mode at daily loading regimes of 3%, 5%, 8% and 10% tannery effluent. The loading rates were determined on the basis of treatment performance indications derived in the photobioreactor study. Tables 5.2 and 5.3 illustrate effluent load reduction and treatment performance achieved in the continuously fed pilot S-HRAP operated for at least 4 weeks at each loading regime, both for summer and winter conditions. Once again the 5% loading proved to be the highest level commensurate with year round sustainable *Spirulina* growth. Ammonia increased to 120 and nearly 200 mg.L^{-1} in the 8% and 10% continuous load regimes, leading ultimately to system failure. It is evident from the daily percentage removal values for COD and ammonia achieved in this study that successful continuous operation required a daily removal for these components as close to the optimum daily effluent loading rate as possible. As shown in the bioreactor study, deviation from this optimum results in an overall accumulation of effluent components, and leads ultimately to failure of the continuously operated culture.

Photosynthetic productivity in the HRAP study is reported in Table 5.3 for both summer and winter operating conditions. Performance was somewhat lower than the 7487 mgC.m^{-2}.day^{-1} average recorded for *Spirulina* blooms in the WSP ecology study, and at a dry mass equivalent of around 9000 mg dry matter.m^{-2}.d^{-1} this is well below the 15 000-20 000 mg dry matter.m^{-2}.d^{-1} reported by Oswald (1988a) for sewage HRAP. However, the yields established in this study do compare well with the 7300-9500 mg dry matter.m^{-2}.d^{-1} reported by Saxena *et al.* (1983), for summer conditions, which fall to 5000 mg dry matter.m^{-2}.d^{-1} in winter. Fox (1983) has recorded carbon fixation yields of 8000-12000 mgC.m^{-2}.day^{-1} for *Spirulina* grown in latrine wastes. While WSP surface water temperatures may vary by no more than 5-8^{0}C over the seasons at the Wellington site, incident light, measured as photosynthetically available radiation at noon, will vary between 500 and 1300 moles.m^{-2}.sec^{-1} from winter to summer conditions, which will substantially influence total photosynthetic production levels achieved.

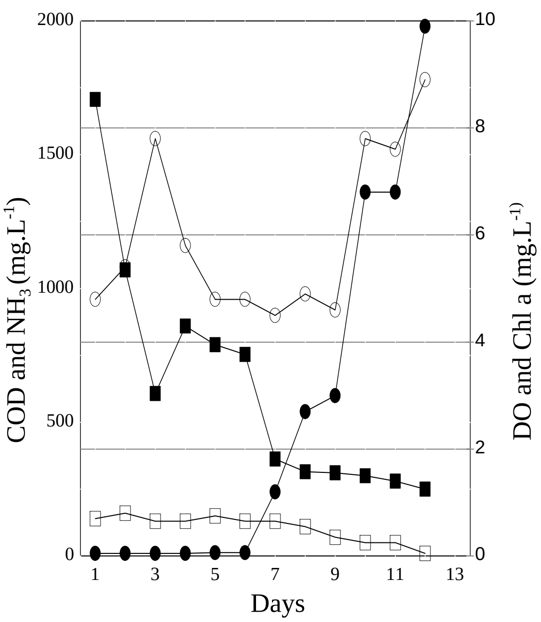


Figure 5.4 A typical start-up sequence for the 80 m^{2} S-HRAP pilot installation (closed square=chemical oxygen demand; open square=ammonia; closed circle=dissolved oxygen; open circle=chlorophyll a).

Table 5.2 Performance of the 80m^2 pilot High Rate Algal Pond installation continuously fed tannery effluent at different daily loading regimes, expressed as a percentage of reactor volume. Analysis reflects pond status at steady state after at least four weeks operation at each loading rate.Standard deviations in brackets.

	Effluent	3%	5%	8%	10%
Chemical Oxygen Demand (mg.L^{-1})	2472(1810)	330(66.2)	413(81.8)	781(70.7)	924(87)
Ammonia (mg.L^{-1})	731(98)	22.5(15.4)	58.4(22.1)	120(40.2)	199(39)
Dissolved Oxygen (mg.L^{-1})	0.01	10.5(1.65)	9.5(o.7)	8.2(0.6)	5(0.6)
pH	8.2(0.1)	8.4(0.2)	8.2(0.2)	8.4(0.1)	8.4(0.1)
Biomass (mg.L^{-1})	-	729(69)	625(93)	234(6)	173(7)

5.3.3 Recirculation

The effects of an ammonia-related regulation of *Spirulina* growth in tannery effluent had been demonstrated in both the laboratory and pilot plant studies and it was evident that hydraulic loading to the S-HRAP would be substantially determined by this factor. Abeliovich (1980) has described the manipulation of loading rates and retention times in sewage HRAP to overcome effects inhibitory to algal growth. Reduction of ammonia levels by aeration stripping has been described by Pano and Middlebrooks (1982), and Chaudari *et al.* (1980) have used bicarbonate to reduce its toxicity. Recirculation of algae-rich waters to improve pond performance was reportedly first used by Abbott in 1958, in a large WSP near Cape Town, South Africa, but Pescod (1996) reports that the mechanisms involved in the success of recirculation are not well understood and have not been well researched.

Table 5.3 Performance of 80 m^2 pilot High Rate Algal Pond installation continuously fed tannery effluent and reflecting load removal rates, total load removed and biomass productivity expressed as the average areal carbon fixation rate for summer and winter seasons .

Effluent loading rates	3%		5%		8%		10%	
Percentage load removed	total	per day	total	per day	total	per day	total	per day
Chemical Oxygen Demand(%)	87	2.6	83	4.2	68	5.5	63	6.3
Ammonia (%)	97	2.9	92	4.6	83	6.7	73	7.3
Carbon fixation(mg C.m^{-2}.day^{-1})								
Summer	10374		7194		5141		4450	
Winter	3671		2866		1998		1242	
Average	7022		5030		3569		2846	

Ammonia oxidation and removal in the Wellington WSP was shown to be associated with both rising alkalinity and DO levels through the cascade, and it was proposed that recirculation of these waters to the S-HRAP could be used to partly control the ammonia regulation effect. However, large recirculation ratios of up to 2.5:1 are generally required in WSP operations (Shelef & Kanarek,1993), and in the case of zero-discharge evaporation ponding systems, highly concentrated saline water would be returned to the front of pond cascades, and thus potentially distort evaporative performance and system function.

The following studies were undertaken to test the recirculation hypothesis and to determine whether this should be included in operating procedures for S-HRAP integrated into WSP systems.

Table 5.4 reports a laboratory flask study simulating ammonia oxidation and stripping in tannery effluent, both with and without the addition of alkalinity derived from the simulated recirculation of alkaline pond water. A 0.2:1 ratio of water addition from the terminal pond in the WSP cascade at Wellington (pH 9.9 and total alkalinity 6500mg.L^{-1}) was identified as optimal in preliminary studies. Flasks were subjected to a regime of gentle aeration over the 3-day study periods recorded.

Table 5.4 Ammonia stripping of tannery effluent by aeration simulating, in 3-day flask studies, the effect of a 0.2:1 recirculation of alkalinity from terminal ponds in a tannery WSP cascade.

	pH	Total Alkalinity (mg.L^{-1})	Ammonia (mg.L^{-1})			
			Day 0	Day 1	Day 2	Day3
Tannery effluent (without recirculation)	8.1	2900	730	581	448	420
Tannery effluent (with recirculation)	9.83	5800	584	146	50	0

Table 5.5 reports the effects on *Spirulina* growth of the 0.2:1 addition of the terminal pond water to tannery effluent, and to Zarrouk's defined medium, compared to unamended tannery effluent controls. The addition of alkalinity elevates the ammonia toxic threshold to *Spirulina* growth in this system from around 60 to above 80 mg.L^{-1}, and provides for a doubling of the photosynthetic carbon fixation rate. The differences in effluent treatment effected by the recirculation simulation exercise showed higher ammonia and organic load reductions were achieved with recirculation, and that a daily volumetric loading rate of 8%, without recirculation, is comparable to loading at 15% with the addition of recirculation.

Table 5. 5 Photosynthetic productivity of *Spirulina* measured in a photobioreactor study fed tannery effluent at different daily loading rates, and comparing unamended effluent with the addition of 0.2:1 terminal pond water to effluent and to Zarouk's defined medium. Results are reported as mgC.m^{-2}. day^{-1} and reflect the mean of three values with standard deviation in brackets.

	5%	15%	25%
Tannery Effluent(unamended)	4349(1751)	2752(2020)	379(300)
Tannery effluent(plus alkalinity)	8306(2649)	5173(4816)	519(113)
Zarouk's medium(plus alkalinity)	12690(1655)	6758(2919)	615(130)

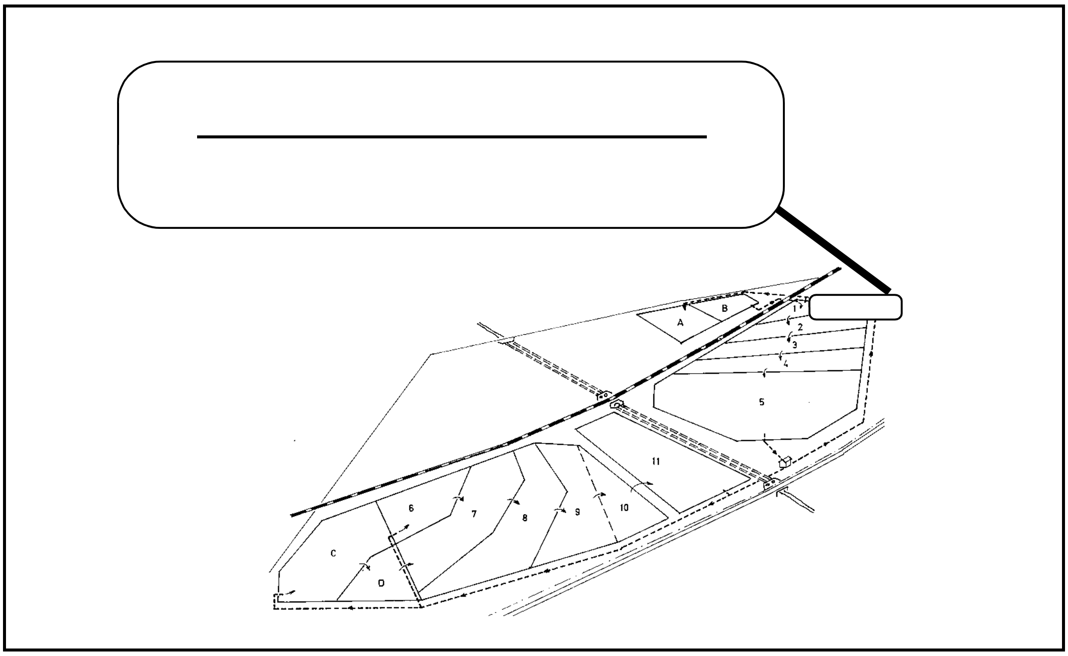
Practical considerations and pipework constraints on-site at the Wellington WSP excluded the scale-up evaluation of the alkalinity recirculation strategy in the S-HRAP pilot plant study. The above results were, however, used in the design of the full-scale S-HRAP, and the pond water recirculation procedures were investigated together with the operational evaluation of this system.

5.3.4 Full-scale High Rate Algal Pond

The decision to proceed to the design and construction of a full-scale S-HRAP was based on the findings outlined above. A 2500 m^2 paddle wheel-driven S-HRAP was designed following criteria described by Oswald (1988b) to undertake an approximate 30-fold scale-up of the pilot S-HRAP study.The unit was constructed as a retrofit adjacent to the WSP operated as a zero discharge terminal evaporation disposal system by Mossop-Western Leathers in Wellington, South Africa. The unit was designed to treat 15% of the tannery's total effluent flow of 450 $m^3.day^{-1}$ and its location is illustrated in plan drawing Figure 5.5. Pipework enabled the HRAP to receive partly treated effluent from the primary anaerobic ponds A/B in this system and thus facilitated its evaluation as a component of the WSP cascade flow path, or as a stand-alone effluent treatment unit operation based on the AIWPS design concept. An operating depth of 0.3 m and linear flow velocity of 20 $cm.sec^{-1}$ were found to be optimal. The HRAP was seeded with *Spirulina* biomass from the WSP.

5.3.4.1 Design

The successful design of a large-scale algal treatment system requires the consideration not only of the application desired but also a host of factors, many of which are uncontrollable in the natural environment (Oswald, 1988). Some factors requiring consideration in design include the specific application, media



requirements, and local climatological conditions.

Figure 5.5 The location of the full-scale S-HRAP constructed on the site of Pond 1 at the WSP treating tannery wastewaters at Mossop-Western Leathers, Wellington.

The general sequence for the scale-up of biotechnological processes followed Hacking (1986) and Trilli (1986), and was adhered to throughout this phase of the project. System design followed procedures outlined by Oswald (1988b).

For design purposes a daily production of 600 m^3 of combined pre-treated tannery effluent was assumed:

$$\text{Design Flow (Q)} = 600 \ m^3.day^{-1}$$

The ultimate Biochemical Oxygen Demand (BOD_{ULT}) of the combined pre-treated tannery effluent was 1124 $mg.L^{-1}$ (based on a COD of 2474 $mg.L^{-1}$ and BOD:COD ratio of 1:2.2). So the organic loading rate to the system (L_o) would be:

Organic Loading Rate (L_O) = 675 $Kg.day^{-1}$

The high rate ponds were designed on the basis of organic loading rate and solar energy. Assuming a conservative BOD_{ULT} in the influent waste-water of 1124 $mg.L^{-1}$ and assuming a conservative combined removal in the PFP of 60 %, the S-HRAP influent BOD_{ULT} (I_{HRP}) would be:

$$I_{HRP} = 0.4 \text{ x } 1124 \text{ mg.L}^{-1} \text{ BOD}_{ULT} = 450 \text{ mg.L}^{-1} \text{ BOD}_{ULT}$$

The operation of the HRAP would include a 20 % recirculation of Pond 11 water, in order to adjust the pH and alkalinity of the effluent from the facultative pond and to minimize the toxic effect of the ammonia (Studies on which this assumption was based are outlined in the following chapter). Therefore, the influent BOD_{ULT} (I_{HRP}) would be:

$$I_{HRP} = 360 \text{ mg.L}^{-1} \text{ BOD}_{ULT}$$

In order to oxidise the influent organic load, the concentration of algal cells (Cc) in a HRAP expressed in $mg.L^{-1}$ is normally set equal to the influent BOD. Again this rule of thumb includes a safety factor as algae produce net oxygen through photosynthesis in an amount between 1.6 and 1.9 of their cell dry weight mass. Using an empirically derived formula, the depth of light penetration (d_L) expressed in centimeters can be determined by dividing 6000 by the influent BOD:

$$d_L = 6000 / 360 \text{ mg.L}^{-1} \text{ BOD}_{ULT} = 16.66 \text{ cm}$$

The depth of light penetration is normally 2/3 of the optimal HRAP depth, so the optimal depth of the HRAP (d_{HRP}) will be:

$$d_{HRP} = 3/2 \text{ x } 16.66 = 24.99 \text{ cm}$$

Assuming an average solar insolation for Wellington of 268.36 $cal.cm^{-2}.day^{-1}$ and a photosynthetic efficiency of 3.5 %, the optimal hydraulic residence time (θ) in days can be calculated by the following equation:

$$hCc = SAF\theta$$

where h is the heat of combustion of algae in $cal.mg^{-1}$, S is the solar energy flux expressed in $cal.cm^{-2}.day^{-1}$, A is the area occupied by 1 liter in the S-HRAP expressed in cm^2, and F is the photosynthetic efficiency. Therefore,

$$\theta = (5.5 \text{ cal.mg}^{-1} \text{ x } 360 \text{ mg.L}^{-1}) / (268.36 \text{ cal.cm}^{-2}.\text{day}^{-1} \text{ x } 1000 \text{ cm}^3 / 24.99 \text{ cm x } 0.035)$$
$$= 5.26 \text{ days}$$

The overflow velocity ($v_{OVERFLOW}$) is calculated:

$$v_{OVERFLOW} = d_{HRP} / \theta = 0.2499 \text{ m} / 5.26 \text{ days} = 0.0475 \text{ m.day}^{-1}$$

Therefore, the organic loading rate for the HRAP (L_{HRP}) will be:

$$L_{HRP} = 0.0475 \text{ m.day}^{-1} \text{ x } 360 \text{ mg.L}^{-1} \text{ BOD}_{ULT} = 17.10 \text{ g BOD}_{ULT}.\text{m}^{-2}.\text{day}^{-1}$$

This organic load would by definition require 17.10 grams of oxygen.m^{-2} .day $^{-1}$. Assuming that algae produce 1.6 times their dry weight cell mass of dissolved oxygen, the necessary concentration of algae will be:

$$Cc = 17.10 \text{ g } O_2.\text{m}^{-2}.\text{day}^{-1} / 1.6 = 10.68 \text{ g.m}^{-2}.\text{day}^{-1}$$

Based on a flow to the S-HRAP (Q_{HRP}) of 720 m^3.day^{-1} (influent plus recirculation), the maximum area required for the S-HRAP (A_{HRP}) will be:

$$A_{HRP} = Q_{HRP} / V_{OVERFLOW} = 720 \text{ m}^3.\text{day}^{-1} / 0.0475 \text{ m.day}^{-1} = 15157.89 \text{ m}^2$$

Assuming a channel width of 10 meters, the channel length (CL) will be:

$$CL = A_{HRP} / 10 \text{ m} = 15157.89 \text{ m}^2 / 10 \text{ m} = 1515.78 \text{ m}$$

The calculations indicated that S-HRAPs covering an area of 1.5 Ha. would effectively treat the total effluent produced. Based on the extensive information gathered, it was decided to proceed with the design of a 2500 m^2 HRAP (Figure 5.6), capable of processing around 15 % of the daily effluent volume. Design diagrams are illustrated in Appendix 2.

5.3.4.2 Construction

The initial construction involved the preparation of the site area. Pond 1 was drained, allowed to dry out and the base was then cleared of sludge and rocks before being levelled. The site was built up to ground level and compacted to 90 % to ensure a firm base on which to build. Floating foundations of concrete were cast for the outer and central dividing walls of the S-HRAP. The outer and central dividing walls were then built using concrete blocks and light wire reinforcement.

The walls were sealed by plastering with concrete, while the joints were covered with bitumen cloth painted with a non-degradeable sealant. Clay walls were built by back filling to surround the S-HRAP walls, to provide additional support and to raise the ground level adjacent to the S-HRAP. The floor of the S-HRAP was built up to the correct level with fill, levelled and compacted. The floor was sealed with a layer of aluminium silicate which was covered with a thin layer of compacted coarse river sand.

The areas at both ends of the raceway were sealed by casting sections of concrete. These concrete sections served to cover the pipework, surround the paddle wheel and provide an area for cleaning. Circular walls were constructed at both ends of the S-HRAP, to produce the effect of forming channels which result in improved movement of the water in the S-HRAP, and reducing the formation of dead spaces. The area around the paddle wheel was built so as to form a funnel which results in turbulence and more efficient waterflow from the paddles, thus improving the mixing in the S-HRAP.

Figure 5.6 The 2 500 m^2 high rate oxidation pond at Mossop-Western Leathers Co., in Wellington, South Africa.

The paddle wheel (Figure 5.7) was constructed from marine plywood which was treated to prevent damage from UV radiation and the high salinity of the effluent water. The paddle wheel was constructed in 2 halves, to ensure that the paddles were off-set, which eliminates the 'pulsing' action and thereby reducing the size and periodicity of the waves in the S-HRAP.

The paddle wheel was driven by an electrical motor mounted alongside with speed control. The level of the water in the S-HRAP was controlled either via wooden slats which could be removed from an overflow box, or by the amount of harvesting performed (by means of a submerged pump). The tannery effluent was gravity fed into the S-HRAP via a splitter box from the PFP. Harvesting was performed using a 80μ vibrating screen to recover the *Spirulina* sp. biomass which was then sun-dried.

Figure 5.7 Paddle wheel of the High Rate Algal Pond at Mossop-Western Leathers Co., in Wellington.

5.3.4.3 Costs

The cost of materials used in the construction of the 2500 m^2 HRAP at Wellington are based on 1996 Rand values as follows:

Marine ply for construction of paddle wheels	R 21 157
Cement building blocks (15 000)	R 15 486
Aluminium silicate sealer	R 46 178
Earth moving and labour costs	R 40 000
Harvesting equipment	R 20 000
Electric motor, pipes, cement, sand etc.	R 38 688
Total Cost	R 181 509

5.4 CONCLUSIONS

1. The scale-up exercise from the initial observations of *Spirulina* blooms in tannery WSP, through laboratory flask and photobioreactor studies, to outdoor pilot plant studies and the development of the S-HRAP, was undertaken to investigate the feasibility of the process as a separate stand-alone unit operation suitable for the treatment of tannery wastewaters.

2. It has been shown in the pilot plant studies that successful performance of the S-HRAP, in the tannery effluent treatment application, depends on the effluent volumetric loading and retention times applied. The treatment process operates under ammonia regulation of *Spirulina* growth and requires relatively low effluent loading rates for long-term stable operation.
3. Experimental results indicated that the implementation of a pond water

recirculation strategy, whereby alkaline waters are circulated to the S-HRAP from the latter ponds of the WSP, would serve to relieve the ammonia stress inherent in the tannery effluent treatment application. Hydraulic loading to the S-HRAP could be substantially improved in this way.

4. The above studies provided the application input data used in the design and construction of the full-scale 2500 m^2 S-HRAP built by Mossop-Western Leathers Co. on the WSP site at their Wellington tannery.

6 INTEGRATION OF S-HRAP AND WSP UNIT OPERATIONS IN TANNERY EFFLUENT TREATMENT

6.1 INTRODUCTION

Investigations which led to the conceptualisation and development of the S-HRAP treating tannery wastewaters had indicated two possible modes of operation - either as an independent unit operation in tannery effluent treatment, or as a unit integrated into the WSP process train. The construction of the full-scale S-HRAP, at the Mossop-Western Leathers Co. WSP site in Wellington, provided the opportunity to evaluate both configurations of IAPS approaches to effluent treatment. The evaluation of the full-scale system is described in this chapter.

The independent or stand-alone S-HRAP unit operation would require a system of pre-treatment similar to that in use at the Wellington tannery, and including physico-chemical pretreatments, flocculant-assisted settling of solids, and some form of biological treatment process to effect an initial reduction in the influent COD load. The availability of the primary anaerobic ponds at the Wellington site, as components of the WSP (Figure 3.1), provided the opportunity to evaluate the retrofit of these as properly configured PFP, based on the AIWPS concept outlined by Oswald *et al*. (1994).

The development of the S-HRAP as an integrated unit operation in the WSP system would provide the opportunity for follow-up studies on a number of issues which had emerged earlier in the research programme, including the potential advantages of recirculation of alkalised pond water to improve both organic loading and hydraulic detention in the unit. In addition to an improved performance in the S-HRAP from recirculation, the operation of the WSP could benefit from the integration of the S-HRAP unit with reduced organic loading to the ponds, possibly a reduced pond surface area requirement, improved evaporation performance and the production of a low organic brine more suitable for crystallisation than that currently produced. The location of the S-HRAP at the head of the WSP system would also provide algal-laden and oxygen-rich warm water to overlay the downstream ponds in the cascade. This capping effect on the anaerobic ponds could achieve the particularly effective odour control observed in the latter pond, as described in the pond ecology studies above.

The full-scale application studies planned for the integrated S-HRAP/WSP system would also provide an opportunity to evaluate more thoroughly the recovery of value addition from the treatment process in the form of harvested *Spirulina* biomass. Provisional studies on the toxicology of *Spirulina* biomass harvested from the WSP system, undertaken at the same time as the initial pond ecology studies, had shown that heavy metal contamination of the biomass could present a problem in the beneficiation exercise. Despite levels elevated slightly above those recommended for animal feeds, test organisms had not shown any toxicity response. (These toxicological evaluation studies are reported in Chapter 8). Nevertheless, it would be crucial to the beneficiation exercise to be able to demonstrate the reduction of heavy metal contamination in the harvested biomass. Heavy metal precipitation in the PFP thus became one of the additional objectives in designing the retrofitting of this unit. The process train for the full-scale implementation studies was thus configured as described in Figure 6.1.

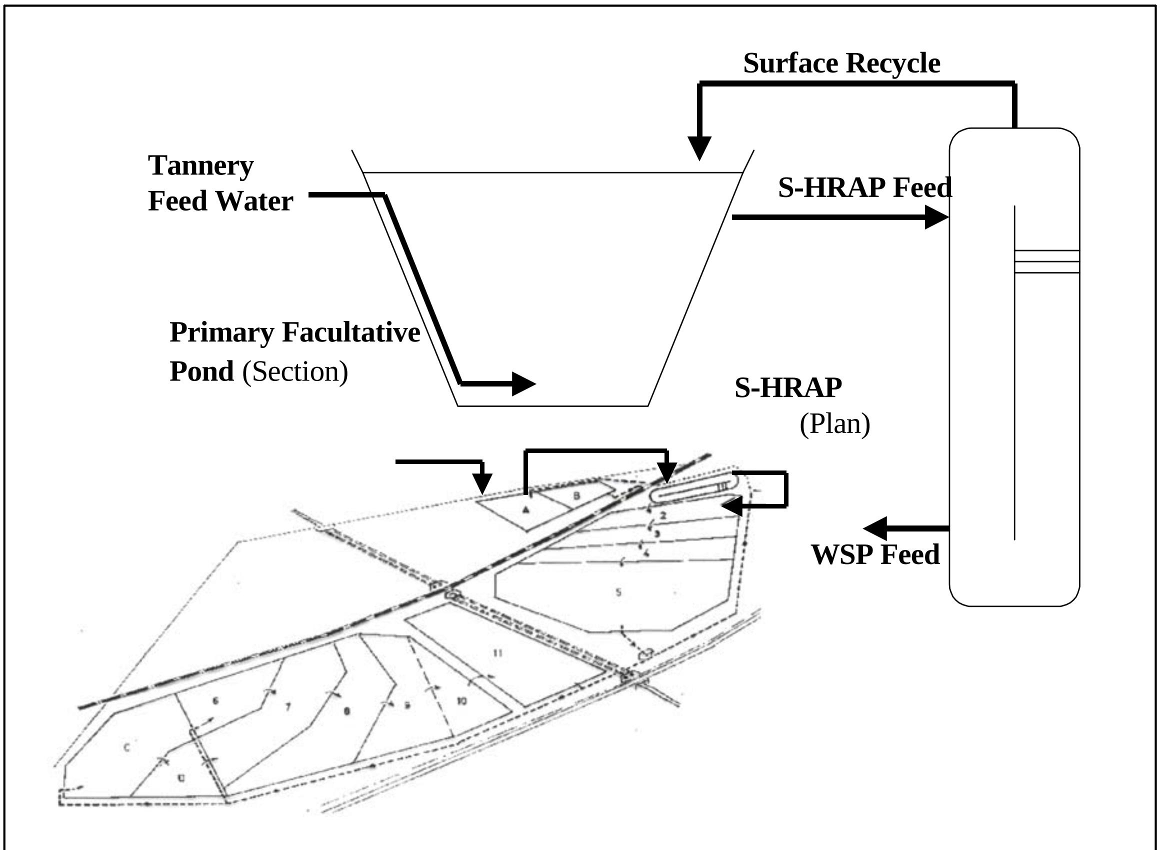


Figure 6.1 Configuration of the Primary Facultative Pond and *Spirulina*-High Rate Algal Pond (S-HRAP) as a component unit operations of an IAPS-based approach to tannery effluent treatment, and constructed at the Mossop-Western Leathers Co. tannery effluent WSP site in Wellington. Treated water from the S-HRAP may pass back to the tannery for reuse in certain processes or be discharged as partly treated to a WSP or other receiving water body.

6.2 METHODS

Methods used were as previously described.

6.3 RESULTS AND DISCUSSION

6.3.1 The Primary Facultative Pond

Oswald (1988a) has used the recirculation of oxygen-rich HRAP overflow to the primary pond in the AIWPS design in order to establish the PFP configuration of anaerobic pits overlaid by aerobic waters. Pipework constraints and the topography of the site at Wellington prevented the recirculation of HRAP water to the primary ponds A and B, and hence the evaluation of micro-algal capping of the primary anaerobic ponds to establish facultative conditions could not be investigated. It also proved impractical to shut down the WSP system in order to have fermentation pits retro-fitted as indicated by the AIWPS design. However, the underlying principles of the AIWPS were followed in the practical optimisation of the PFP performance, and these designs were based on discussions with Prof W. Oswald and Dr Bailey Green who visited the site in 1994. Mechanical surface aerators were used to effect the overlay of the PFP with oxygenated water and a stable oxypause was established at 0.5m in Pond A (total depth 4m). The inlet pipe was re-fitted to feed tannery effluent to the base of the pond and thus provide an upflow configuration in this pond. Table 6.1 reports the improvement in PFP effluent treatment performance effected by these interventions. Table 6.2 shows the performance of the dual compartment configuration established by the installation of the surface aerators. Biogas production was recorded (methane content around 20%) and a 99% reduction of sulphate in the lower compartment was observed indicating the active participation of both methanogens and sulphate reducing bacteria (SRB) populations. Sulphide produced in the lower compartment was substantially re-oxidised as the water moved up into the upper aerobic compartment, and thereby reducing much of the previously severe odour release from this unit. Sulphide oxidation was completed in the HRAP ensuring that only sulphates passed to the downstream ponds in the WSP.

Table 6.1. Tannery effluent treatment performance of the primary facultative pond before and after optimisation of the anaerobic and aerobic compartments. Standard deviation in brackets.

	Tannery Effluent	PFP Before	PFP After	% Improvement
COD ($mg.L^{-1}$)	2474(1810)	1722(325)	1216(93)	30%
Ammonia ($mg.L^{-1}$)	731(98)	764(93)	452(105)	40%
Sulphide ($mg.L^{-1}$)	285(422)	500(90)	76(16)	85%
Phosphate ($mg.L^{-1}$)	19(12)	7(1)	1.7(2)	76%
Suspended Solids ($mg.L^{-1}$)	243(196)	434(67)	0.5(0.5)	99%

An interesting observation at this point was the singularly effective removal of settleable and suspended solids (> 99%) associated with the establishment of effective anaerobic conditions in the PFP. This was a unit which had previously been chronically plagued with solids build-up, and major desludging exercises had been required from time to time. The efficient breakdown of particulate solids and the apparent utilisation of the organic carbon compounds present in the tannery effluent as electron donor sources by SRB, led to follow-up studies on the use of complex carbon sources for biological desalination of sulphate-saline wastewaters. These investigations became the substance of a number of subsequent WRC projects on the treatment of AMD wastewaters where sewage sludges were used as the carbon source for sulphate reduction, and associated heavy metal removal.

While these findings were not explored further in this study they did demonstrate a potential linkage of saline wastewater treatment and the disposal of organic solid wastes, such as sewage sludges. This result not only fulfilled an objective of project K4/495, indicating an additional utility for saline wastewater impoundments, but also provided an important additional indication for the development of 'integrated waste resource management' strategies for these wastes. The potential for linking sewage sludge disposal and AMD treatment has assumed specific relevance on the Witwatersrand gold fields where mine closure is expected to be followed by the decanting at the surface of large volumes of low grade waters (Scott, 1995). These studies are detailed in WRC report 'The Rhodes BioSURE Process®. Part 1:

Biodesalination of Mine Drainage Wastewaters' (Appendix 1).

Table 6.2 Performance of the Primary Facultative Pond and high rate algal pond treating tannery effluent showing sulphate reduction, sulphide oxidation and COD removal through the various stages of the process.

	Tannery effluent	Facultative pond anaerobic compartment	Facultative pond aerobic cap	HRAP
Sulphate as SO_4^{2-}	975	<1	98976.51216	8090.1394
Sulphide as Na_2S	285	1100		
COD	2474	1216		

6.3.2 Sulphate Reduction and Heavy Metal Precipitation

Tannery wastewaters usually contain a number of heavy metal contaminants, and since micro-algal biomass recovery, as a value-added component of saline wastewater beneficiation, formed an important objective of the overall programme, it was necessary that the potential accumulation of heavy metals by micro-algae be investigated and monitored. Although the major fraction of metals was removed from the raw effluent in the solids sedimentation pretreatment step, low levels of metal carry-over were measured in the primary tannery effluent passing to the WSP. These tend to precipitate in the ponding system and are present only in very low concentrations in the water column, where algal growth occurs (Table 6.3).

Nevertheless, a low level of metals accumulation was measured in *Spirulina* biomass harvested from the WSP in the initial stages of the study, and prior to the reconfiguration of the system (Table 6.4). It was clearly necessary to demonstrate the reduction and reliable maintenance of these values at levels below that required for the use of *Spirulina* biomass as a feed. (The standard values used in this study, for permissible levels of metals in animal feeds, were those outlined in the German Ministry of Agriculture and Nutrition Law Collection No 117-1987). This requirement applies equally to the wider objective of rendering saline water impoundments safe as wetlands and for wildlife habitation. Experiences in California at Kesterson Reservoir (Presser, 1994) and the Salton Sea (USBR, 2000), have shown that removal of metals and certain trace elements may be the determining factor in the entire success of a solar saltern. These case studies are summarised in Report 1 of this series (see Appendix 1).

In a number of experimental investigations undertaken on-site at the Wellington WSP, it was shown that the reconfiguration of the PFP, in a bottom fed upflow mode, and the controlled maintenance of the anaerobic compartment in this pond unit, encouraged the development of SRB populations, high rates of sulphate reduction (Table 6.2), and commensurately effective precipitation of metals in the system. The metal residuals in the effluent were shown to precipitate in this unit as metal sulphides, but also possibly as metal carbonates (Dunn, 1998).

Table 6.3 Heavy metal concentrations in tannery effluent at various stages of treatment in the Mossop-Western Leathers Co. Waste Stabilisation Ponding system at Wellington before the reconfiguration of this system. Values in $mg.L^{-1}$.

Element	Untreated Effluent	Treated Effluent	Sludges	Final WSP Water
Aluminium	17.2	1.2	456	0.5
Cadmium	0.08	0.02	0.21	0.03
Chromium	4.68	0.25	1405	0.6
Cobalt	0.35	0.18	1.7	0.16
Copper	0.03	0.02	0.32	0.02
Iron	39.9	0.41	336	0.41
Lead	0.76	0.11	6.2	0.41
Manganese	62.9	1.2	704	0.22
Nickel	0.41	0.21	4.95	0.18
Zinc	1.51	<0.1	8.1	<0.1

Metal levels measured in the *Spirulina* biomass produced in the S-HRAP, in the treated water from the reconfigured PFP, were reduced to the range prescribed by the German standard for animal feed-grade use (Table 6.4). Acceptable metal levels were also demonstrated in feeding studies (outlined in Chapter 8) using the Wellington produced *Spirulina* biomass.

Table 6.4 Heavy metal content in *Spirulina* biomass harvested before and after the metal precipitation step in the IAPS reconfiguration of the Waste Stabilisation Ponding system at Mossop-Western Leathers Co. Wellington. Standard feed values follow the German Ministry of Agriculture and Nutrition Law Collection No 117-1987. Values in $mg.L^{-1}$.

Element	Feed Standard	Before Metal Ppt.	After Metal Ppt.
Cadmium	1.0	5.96	<1
Chromium	10-15	25.8	<1
Cobalt	4-6	22.4	3.3
Iron	1250-2500	2012	795
Lead	15-20	219	2.3
Nickel	10-15	49.2	17.5
Zinc	250-500	218.5	22.5

The results of this study indicated that a biomass could be produced in a tannery effluent growth medium that complied with feed grade requirements, but also that sulphate reduction utilising complex carbon substrates as electron donor sources, provides a useful mechanism for delivering metal free brines to solar evaporation impoundments.

6.3.3 Comparative Effluent Treatment and System Performance

Initial evaluation of full-scale S-HRAP performance was undertaken without recirculation of alkaline WSP pond waters and start-up was undertaken through operation at increasing effluent volumetric loading rates (expressed as % HRAP $volume.day^{-1}$) from the PFP. These commenced at $3\%.day^{-1}$ and were increased through $15\%.day^{-1}$ over a period of time, and allowing at least 3 volume changes for each loading rate. Stable operating conditions could be sustained at a maximum loading rate around 10% HRAP $volume.day^{-1}$ enabling reductions in organic load of 78%, ammonia 94%, sulphides 99% and phosphates 92% (Table 6.5). This is nearly double the optimum loading previously achieved in the pond study and the increased surface area could account for the improvement in performance compared to photobioreactor and pilot S-HRAP studies reported earlier.

The quality of effluent treatment recorded in the S-HRAP exceeded that achieved in the 13.6 ha of the WSP cascade receiving the same effluent type. In a separate study (results not reported) the S-HRAP-treated effluent was shown to be of suitable quality for return to the tannery for use in certain production operations, thereby achieving a substantial improvement in the WSP water balance.

Table 6.5 Summary of the development and scale-up of the *Spirulina* HRAP from laboratory studies through piloting to design, construction and performance evaluation of a full-scale 2500m^2 HRAP unit operation treating tannery effluent. Percentage removal efficiencies refer to presented load removed by the HRAP compared to the total effluent load treated by the combined IAPS operation .

	Laboratory	Pilot	HRAP Design	HRAP Operation	IAPS
Hydraulic loading (%vol.day^{-1})	5	5-8	25	20-25	3
Hydraulic residence time (days)	20	13-20	4	4-5	34
Volumetric organic loading (kg COD.m^{-3}.day^{-1})	0.08	0.08-0.1	0.43	0.35-0.43	0.05
Areal organic loading (g COD.m^{-2}.day^{-1})	-	0.92	O.13	0.1-0.13	0.14
Organic load reduction (%)	79	76	80	77	84
Ammonia removal (%)	86	87	-	86	95
Sulphide removal (%)	99	99	-	99	99
Phosphate removal (%)	90-99	90-99	-	99	99
Photosynthetic productivity (g C.m^{-2}.day^{-1}) (season av.)	-	5	10.7	11	11

6.3.4 Recirculation

The toxic effects on *Spirulina* growth of the high ammonia levels in tannery effluent was clearly shown to influence the distribution pattern of the organism within the WSP cascade (confining blooms to the latter ponds), and also appeared to present the most important limitation on performance in the S-HRAP. The 731 mg.L^{-1} ammonia in the effluent is considerably higher than the 100 mg.L^{-1} toxic threshold reported by Azov and Goldman (1982) and Konig *et al.* (1987), and is some 18 times higher than the optimum measured in flask growth studies for this medium.

Table 6.6 reports the results of the 0.2:1 recirculation to the S-HRAP from the terminal pond in the WSP cascade (alkalinity 6500 mg.L^{-1} as $CaCO_3$). This procedure showed an 8% improvement in COD reduction achieved at twice the daily volumetric loading to the S-HRAP previously applied. This result indicated that the daily volumetric loading rate could potentially be increased to between 20% - 25% pond volume, which confirmed the estimates based on experimental data, and those used in the design of this unit. Reducing the HRT in the S-HRAP to 5 days or less represents a substantial advantage achieved, especially where HRT minima of 10 and 20 days had previously been indicated in the laboratory and S-HRAP pilot plant studies.

Table 6.6 Tannery effluent treatment in a 2500 m^2 *Spirulina* HRAP following a Primary Facultative Pond comparing an unamended feed regime and one diluted 0.2:1 with alkalinity recirculation and fed at 10% and 20% daily volumetric loading rates respectively. Standard deviation in brackets.

	Effluent	PFP	HRAP (10%without recycle)	HRAP (20% with recycle)
COD ($mg.L^{-1}$)	2474(1810)	1722(325)	539(147)	394(212)
Ammonia as NH_3 ($mg.L^{-1}$)	731(98)	764(93)	42(32)	36(33)
Sulphides as Na_2S ($mg.L^{-1}$)	285(422)	993(90)	1.3(5.2)	<0.1

6.3.5 Micro-algal Pond Capping and Odour Control

The initial WSP ecology studies had confirmed previous observations that blooms of *Spirulina* in the tannery WSP are both unpredictable, and also apparently unstable, forming and disappearing in an unreliable manner. Similar problems of unpredictability also plague recirculation strategies in domestic wastewater WSP (Pescod, 1996), making reliable use of these operations difficult to incorporate into system designs. Factors controlling *Spirulina* blooms in tannery ponds, and mechanisms available for their manipulation, had not been previously described.

Prior to construction of the S-HRAP, evaluation of recirculation to the first anaerobic ponds in the WSP was undertaken from the terminal Facultative Ponds in the WSP system. This would bring forward micro-algal biomass in addition to alkalinity, and operate a classic dilution process for odour control in the initial anaerobic ponds. While a degree of micro-algal growth may be achieved on the surface of the anaerobic ponds in this way, large dilution volumes are required which, in the system studied, would require several months of continuous pumping to move. This form of pond operation was attempted and proved difficult to sustain given the unreliable control of micro-algal growth on the surface of the anaerobic ponds.

While the conventional recirculation and blending exercise was found to be problematic in its execution in the tannery WSP application, the use of the warm, oxygenated and partly treated micro-algal-laden wastewaters from the S-HRAP was evaluated for effecting the capping of anaerobic ponds. Outflow from the S-HRAP was loaded to the surface of anaerobic Pond 2, and an effective dual compartment configuration was established converting these anaerobic Ponds 2-5 to facultative operation, and in this way producing an effective control of odour emission (Pond 2 became the first pond in the WSP following the construction of the S-HRAP on the site of the former Pond 1). However, the oxygenated *Spirulina* overlay was not self-sustaining and required continuous supplementation from the S-HRAP for the maintenance of the aerobic surface layer. While long-term evaluation of micro-algal capping is required, results to date indicate that an appropriately sized S-HRAP may be used for the effective control of odour nuisance in WSP treating tannery wastewaters.

6.4 CONCLUSIONS

Retrofitting of the PFP and construction of the S-HRAP provided the opportunity to evaluate IAPS configurations in tannery effluent treatment. Results showed that the S-HRAP could be used as an independent unit operation in tannery effluent treatment excluding the use of WSP. Although treatment was less effective without alkalinity recirculation from the WSP, and lower loading rates were required, a quality of effluent could be produced which was suitable for reuse in certain operations in the tannery.

Where a final discharge route for the S-HRAP treated wastewaters would not be available, the integrated S-HRAP/WSP configuration of an IAPS would provide a means for solar evaporation of treated water, and the generation of 'clean' brines suitable to pass to crystallisation operations. It was shown in the full-scale process application that the S-HRAP may be used to efficiently control odour nuisance in WSP treating tannery wastewaters.

The system also generated substantial quantities of *Spirulina* biomass, and the evaluation of value recovery, as an exercise in the beneficiation of tannery wastewater treatment, is detailed in the subsequent chapters.

7 THE HARVESTING AND RECOVERY OF EFFLUENT-GROWN *SPIRULINA* BIOMASS

7.1 INTRODUCTION

A major difficulty in the development of commercial micro-algal production has been the economic feasibility of the biomass harvesting processes (Abeliovich, 1986: Richmond, 1988). Costs associated with harvesting include the acquisition, installation and maintenance of the equipment, power consumption of the device, and high costs of thermal energy requirements for final product processing and drying (Mohn, 1988; De Pau and Salamoni, 1991).

Several widely differing technologies have been developed for the separation of a variety of micro-algae from their growth medium, and have been extensively reviewed (Golueke and Oswald, 1965; Mohn, 1980, 1988: Ben-Amotz and Avron, 1989). Micro-algal cell separation methods currently in use include centrifugation (Mohn, 1988), electro flocculation (Richmond and Becker, 1986), chemical flocculation (Golueke and Oswald, 1965; McGarry, 1970; Azov *et al*., 1980), sedimentation (Mohn, 1988) air flotation (Richmond, 1986; Oswald, 1988b), continuous belt filtration, vibrating and stationary screens (Richmond and Becker, 1986; Abeliovich, 1986; Richmond, 1988), sand bed filtration (Naghavi and Malone, 1986; Oswald 1988b), and a variety of membrane-separation technologies (Rose, 1991). Only a few of these systems, however, have potential as efficient, low-cost harvesting methods (Mohn, 1988).

Currently, in commercial *Spirulina* production, drying of the final product poses a problem of major economic importance, in that it may constitute up to 30% of the production cost (Oswald and Golueke, 1960; Richmond, 1988). The various systems used for drying differ both in the extent of capital investment and in energy requirements, and have a marked effect on the food value and the taste of the final product (Richmond, 1988).

The usual method for drying of *Spirulina* is spray-drying, although drum-drying is also used to produce a very good quality product (Soeder, 1986; Richmond and Becker, 1986 ; Richmond, 1988). Direct drying of the *Spirulina* slurry in the sun may offer an inexpensive solution acceptable for the production of animal feed (Oswald, 1988b ; Richmond and Becker, 1986; Richmond, 1988; Maart, 1993). However, sun-drying is not recommended for preparing a high quality product intended for human consumption (Richmond, 1988).

Several studies of the chemical composition of *Spirulina* have indicated its potential as a human food, animal feed and as a source of natural products (Clement *et al.,* 1967; Hudson and Karis, 1974; Narasimha *et al.,* 1982; Santillan, 1982; Richmond; 1988; Belay *et al.,* 1996). The cellular composition of *Spirulina* varies in relation to physiological conditions. The greatest variation is in protein content, which ranges from 50 % to 70 % of dry weight (Richmond, 1988). The amino-acid composition of *Spirulina* has been investigated (Clement *et al.,* 1967) and although it is generally well balanced it is, however, low in sulphur-containing amino acids and tryptophan (Richmond, 1988; Dillon *et al.,* 1995).

Spirulina appears to have the highest vitamin B_{12} content of any unprocessed plant or animal food (Richmond, 1988). The blue pigment phycocyanin, which may constitute up to 20% of *Spirulina* dry weight, has been identified as an anti-tumour agent (Mathew *et al.* ,1996) and may also stimulate the immune system, providing protection against a variety of diseases (Hayashi and Okuwaki, 1994 ; Quereshi and A1i, 1996 ; Yang *et al.,* 1997).

The carotenoids, which are the other commercially important pigments present in *Spirulina,* specifically β-Carotene has attracted interest for anti-cancer properties (Richmond, 1988). Many other attributes of *Spirulina* are also of nutritional significance, for example iron and essential unsaturated fatty acids, the most important of which is gamma-linolenic acid. S. *platensis* is unique among photo autotrophic organisms so far studied, containing substantial quantities of gamma-linolenic acid (Cohen and Vonshak, 1991).

Certain species of *Spirulina* can contain up to 6% dry weight of the commercially valuable biodegradable plastics precursor, poly-β -hydroxybutyrate (Campbell *et al.*, 1982 ; DePhilippis *et al*., 1992). The nutritive value of waste-grown algae has also been reported (Cook, 1962; Maart 1993).

An evaluation of the biomass produced in the pilot plant studies reported above was undertaken as a final step in the decision making process to proceed to the construction of a full-scale plant at Mossop Western Leathers in Wellington. The research objectives included the evaluation of a number of procedures for harvesting and drying the *Spirulina* biomass. An analysis of the *Spirulina* biomass produced in the S- HRAP was undertaken to ensure that the product complies with nutritive and toxicological criteria for sale as a specialised animal feed. Toxicological and feeding studies are reported in the following chapter.

7.2 METHODS

7.2.1 Harvesting

Harvesting of the *Spirulina* biomass was conducted on WSP C, D, 6, 7, and also the two pilot-scale HRAP. The harvesting was performed firstly by hand, which involved manually scooping the rafts off the pond surface. A small-scale screen harvester was then constructed and operated according to Mitchell (1986). Finally a technical-scale screen harvester, designed by R. Rowswell of LlRI, and constructed by R. Smith, engineer at Mossop Western Leathers, was used to harvest *Spirulina* biomass from pond C (Figure 7.1). A screen with a pore size of 80 m was used for the harvesting, while the optimum pumping rate was found to be 100 $l.minute^{-1}$. Biomass determinations were performed on a dry weight recovery basis.

Figure 7.1 Screen harvester and drying racks used for the recovery of *Spirulina* biomass from the WSP at Wellington

7.2.2 Drying Biomass

Several methods were investigated with the aim of developing an inexpensive method for drying the harvested *Spirulina* biomass, possibly incorporating existing technology and equipment used in the tannery. These included heated plates, a rotating heated drum, sand drying beds, and conventional leather crust drying tunnels. The best results were achieved using sun-drying. Spray drying facilities were not available to the project. Algal biomass harvested either by hand or by screen harvester was placed on a drying bed covered with a plankton screen (pore size 80 μ) for approximately 2 hours, resulting in an algal slurry of about 10 % solids. This slurry was then spread out on muslin cloth, stretched over wooden pallets, to a thickness of 15-20 mm, and allowed to dry to a final water content of 20-25 %.

7.2.3 Electro-Osmotic Dewatering

Two samples of harvested algal biomass were used to evaluate the Electro-Osmotic dewatering technique developed at the C.S.I.R in Stellenbosch. The system operates using a modified belt press which not only dewaters the product through the action of pressure rollers but also as a result of the voltage applied. The first sample was an unwashed amount of screen harvested *Spirulina* biomass from pond 6. For the second sample the screen harvested *Spirulina* biomass was first washed with water in an attempt to lower the conductivity and hence the operating voltage.

7.2.4 Biomass Analysis

The method used for the determination of total kjeldahl nitrogen (TKN), and the calculation of total protein (expressed as a percentage) by multiplication of the TKN value by 6.25 was as described by Maart (1993). The amino acids were analysed according to the gas-liquid chromatographic protocol by Beckman in the System

6300 application notes, and was carried out in conjunction with the University of Natal, Animal and Poultry Science Laboratory.

Total carbohydrates were determined according to the Phenol-Reaction method and also lipids using the method outlined by Gerhardt *et al.,* (1981). The levels of Carotenoids and Xanthophylls were determined using reverse-phase HPLC (Maart, 1993). The procedure for the quantification of the phycobiliproteins was essentially that of Bennet and Bogorad (1973). The pellet remaining after buffer extraction of the phycobiliproteins was re-extracted in 80 % acetone (v/v) and the absorbance determined in order to quantify the chl a concentrations. The formulas described by Lichtenthaler (1987) were used. For the ash determinations, porcelain crucibles were heated at 100 ˚C overnight, dried in a desiccator (2 hrs), and weighed. Three dried biomass samples (60 ˚C/3 hrs) of known weights were added to the crucibles and ashed (600 ˚C/24 hrs), burning off all the organics. Crucibles were again placed in a desiccator (2 hrs), and reweighed. The difference between the starting biomass weight and the ashed weight gave the ash content of the different samples. Triplicate quantities of milled *Spirulina* were weighed into pre-dried and pre-weighed crucibles, and dried at 105 ˚C until no further weight loss was observed (approximately 5 hrs). The moisture content was then determined by subtraction.

The amount of energy present in the *Spirulina* biomass was determined using a DDS CP400 bomb calorimeter. Benzoic acid was used as a calibration standard. Gelatin capsules of known energy values were filled with milled *Spirulina,* and fired in the calorimeter. Each determination required a temperature equilibration of 8 minutes and a reading stabilisation of 4 minutes.

7.3 RESULTS AND DISCUSSION

7.3.1 Harvesting

Harvesting of *Spirulina,* by hand, small-scale screen harvester and technical-scale screen harvester, on WSP C, D, 6, and 7, resulted in a total recovery of 546 kg dried *Spirulina* biomass during the research programme.

Harvesting of the autoflocculated *Spirulina* biomass by hand was conducted over an 8 day period spread over the months of February, March and April 1993. A total of 271 kg of *Spirulina* was recovered in this manner. The method, however, proved to have some limitations, being labour intensive and weather dependent. A further disadvantage of this method, was that the *Spirulina* rafts present on the pond surface represent mostly biomass in the stationary phase of growth, the quality of the biomass harvested was thus low when compared to *Spirulina* biomass harvested when the cells were still actively growing (as is the case with screen harvesting). The small-scale screen harvest of the autoflocculated biomass (2.2 % solids) yielded a thick cell slurry of 21.4 % solids concentration. Screen harvesting thus resulted in a 10-fold harvesting efficiency. A total of 25 kg dried weight of *Spirulina* was harvested in this manner. Theoretical calculations, based on the *Spirulina* distribution and concentration in the entire water column, show that a near 100- fold concentration of biomass is possible if a mechanical pumping device is used, utilising *Spirulina* present in the water column instead of the autoflocculated biomass.

Figure 7.1 shows the technical-scale screen harvester on-site at Wellington. The total biomass harvested by the screen was 250 kg, at an average yield of nearly 10 $g.L^{-1}$, $hour^{-1}$. Initially using the screen harvester as designed, approximately 20 % of the algae passing over the screen was removed. Subsequently, improvements were made which included supporting the screen with a woven nylon mesh to lessen the drag of the water through the screen. The screen harvester, with the pump head located below the surface, extracted algal biomass from the water body and not from the surface. Rafting biomass was thus not taken up, which meant the quality of the biomass was higher compared to hand harvested autoflocculated biomass.

7.3.2 Drying

7.3.2.1 Sun Drying

This method formed the basis for the drying process used, and with relative success. Algal biomass harvested either by hand or by screen harvester was placed on a drying bed covered with screen cloth (pore size 80 μ) for approximately 2 hours, resulting in an algal slurry of about 10 % solids. The biomass was then dried over a period of 5 days (Figure 7.2). Problems associated with this method were that the drying was weather dependent, labour intensive, required a large surface area and long periods of drying time.

Figure 7.2 Sun drying of *Spirulina* biomass harvested from the WSP at Wellington.

7.3.2.2 Electro-Osmotic Dewatering

Two applications of the Electro-Osmotic dewatering technique were evaluated for the concentration of harvested algal biomass. In the first a sample of unwashed screen harvested *Spirulina* biomass from pond 6 was passed through the dewatering process with the application of a 5 volt electric current. For the second sample the screen harvested *Spirulina* biomass was washed with water in an attempt to lower the conductivity, in order to lower the energy demand and to improve the dewatering result. The conductivity was reduced from 4150 $mS.m^{-1}$ to 1700 $mS.m^{-1}$ and the concentrated biomass was then passed through the process a second time. The results for biomass concentration by Electro-Osmotic dewatering are shown in Table 7.

Table 7.1 Screen harvested *Spirulina* biomass concentrated by Electro-Osmotic dewatering**.**

Stage/Sample	SolidConcentration (%) 5 volts (unwashed)	Solids Concentration (%) no voltage (washed)	Solids Concentration (%) 5 volts (washed)
Before dewatering	13.74	11.78	11.78
First Stage	18.74	14.82	15.16
Second Stage	19.36	16.82	18.7

The current efficiencies and energy requirements for the dewatering of the washed sample are shown in Table 7.2.

Table 7.2 Current efficiencies and energy requirements in the use of Electro-Osmotic dewatering for concentrations of *Spirulina* biomass.

Stage	Current efficiencies ($A.L^{-1}.h^{-1}$)	Energy requirements ($W.L^{-1},h^{-1}$)
First Stage (no voltage)	0.03	192.98
Second Stage (5 volts)	0.02	263.42

With this method, the best results obtained were an increase in total solids concentration from 11.78 % to 18.70 % after two cycles at 5 volts for the washed *Spirulina* biomass. The energy requirements were considered to be high, and the resultant product of this process in any event required further drying. Further investigation of this method was not pursued beyond this point.

7.3.3 Analysis of the Harvested *Spirulina* Biomass.

The tannery effluent-grown *Spirulina* biomass was harvested by filtration from the pond using the technical-scale screen harvester. Autoflocculated cyanobacterial biomass was not used as it was found to be of a lower quality due to the cells already deteriorating to some extent. The algal slurry (10% solids concentration) was initially dried on sand beds, in the sun, before final drying in drying tunnels. The dried biomass was milled into a fine powder which had a dark blue-green colour, a salty taste and a sea weed-like odour.

An evaluation of the chemical composition of harvested *Spirulina* biomass was undertaken. The tannery effluent-grown *Spirulina* biomass was found to contain 57 % protein, which is lower than reported values for commercially grown *Spirulina* : 60-71 % protein, (Durand-Chastell, 1980), 60% (Tel-Or *et al.,* 1980), 71 % (Richmond, 1988) and 60- 70 % (Earthrise Farms, California, USA), but compared well with values reported for sewage grown *Spirulina* 50-55% protein (Saxena *et al.,* 1983).

The reason for the lower protein levels has been ascribed to the sun-drying technique employed, which is known to lead to degradation of the protein due to the length of time required to dewater the biomass. Although the lack of a cellulose cell wall seems to favour the sun-drying of *Spirulina* without any loss of digestibility (Venkataraman *et al.*, 1980), the protein-loss factor seems to override this advantage. Even with the loss of some of the protein content the *Spirulina* biomass compares well with other feed products (fishmeal and seaweed) with regard to protein content (Table 7.3).

The relatively low protein content of harvested *Spirulina* biomass is correlated with the relatively high ash content (17 %), when compared to *Spirulina* biomass from pure-culture grown biomass: 9% (Richmond, 1986), 7-13% (Earthrise Farms, California, USA) and 6.4-9 % (Durand-Chastell, 1980). The ash value represents the inorganic content, and includes absorbed salts and minerals. The ash content depends primarily on the composition of the medium. Micro-algae usually contain less than 10% of their dry weight as ash, and the ash content only marginally affects the nutritional quality of the biomass (Becker, 1986).

Table 7.3 Amino acid composition of *Spirulina* biomass harvested in Wellington compared with seaweed, fishmeal and *Spirulina* from a number of literature reports. (g amino acid/16g N)

Amino Acid	Seaweed	Fishmeal	Richmond (1986)	Santillan 1982	Saxena *et al.*, 1983	This Study
Alanine	0.778	3.9604	5.82	5.82	9.76	3.5042
Aspartate	1.2447	6.3735	6.43	6.43	12.51	4.8974
Arginine	0.7005	3.5915	5.98	5.98	4.06	3.2573
Glutamate	1.7621	8.9076	8.94	8.94	10.44	8.8298
Glycine	0.7952	4.0623	3.46	/	7.78	2.4269
Histidine	0.1863	2.026	1.08	1.08	1.87	0.6876
Isoleucine	0.5494	2.7012	4.13	4.13	3.91	2.6144
Leucine	0.7162	5.0004	5.8	5.8	8.3	4.2521
Lysine	0.7707	5.4503	4	4	3.94	2.3757
Methionine	0.157	2.0025	2.17	2.17	1.52	0.9526
Phenylalan.	0.6949	2.7077	3.95	3.95	2.76	2.0562
Proline	0.5553	2.9488	2.97	2.97	5.35	2.0149
Protein	16.08	70.1	71	70	50.55	57.1
Serine	0.738	2.6318	4	3.18	7.21	2.2433
Threonine	0.5434	3.1001	4.17	4.17	5.41	2.5096
Tyrosine	0.3	2.24	4.6	/	2.1	1.94
Valine	0.6763	3.3336	6	6	6.86	3.1221

The high ash content seems to be an area of concern when considering the effluent-source of the medium. Minerals from the culture medium contribute to the ash content and the biomass may, therefore, possibly contain a variety of toxic inorganic minerals or compounds. It is also known that a high concentration of unutilised minerals in a feed results in a change in the proportion of the other major cellular constituents (Becker, 1986).

Harvested *Spirulina* biomass was treated with either fresh or acidified water before sun drying in an attempt to reduce the ash content. Washing *Spirulina* biomass with pH 4 acidified water, before drying, has been shown to reduce the levels of carbonates (Richmond, 1988). Analysis of the salts which often contribute to the elevated ash levels in dried *Spirulina* biomass was not performed. The following results indicate that the ash content of the *Spirulina* biomass can be reduced by washing with either fresh water or acidified water (Table 7.4).

Table 7.4 Various treatments of *Spirulina* biomass to reduce ash content.

Treatment Process	Ash content (5%)
HRAP sundried *Spirulina sp.* no wash	17
HRAP sundried *Spirulina sp.* water wash	14
HRAP sundried *Spirulina* sp.pH5 water	12
HRAP sundried *Spirulina sp.* pH2 water	8

An analysis of the amino acid composition of the biomass protein content was undertaken. Of special interest are the essential amino acids, which animals are incapable of synthesising. In order to compare the amino acid content of tannery effluent-grown *Spirulina* with those obtained by other authors, the amino acid content shown in Table 7.3 was converted to g amino acid.16 g^{-1} N, the conversion taking into account the TKN value of the biomass, as reported by Maart (1993).

When compared to the levels suggested by the United Nations Food and Drug Organisation (FAO), the tannery effluent-grown *Spirulina sp.* appears to be deficient in a number of essential amino acids, including isoleucine (25% deficiency), leucine (36 %), lysine (60 %), phenylalanine (72 %), methionine (71 %) and threonine (33 %). The other *Spirulina* biomass sources included for comparison are also deficient, in varying degrees, in the essential sulphur amino acids, especially lysine, phenylalanine and methionine. This deficiency in amino acids almost certainly arises, in part, from the lengthy drying time associated with sun-drying and may have been caused by leaching out in the gravity filtered medium, and/or from bacterial activity. The main practical consideration in mini mi sing protein loss is thus to investigate a faster, more efficient drying procedure.

Chemical analysis of the sun-dried *Spirulina* biomass showed that 14.9% of the dry weight was carbohydrate. This carbohydrate content of the *Spirulina* biomass is comparable to that of other sources: 16.5% (Richmond, 1986), 16% (Durand-Chastel, 1980),8-14% (Tipnis and Pratt, 1960), 15-25 % (Earthrise Farms, California, USA) and 17 % (Becker and Venkataraman, 1984). The energy content was 17.0 kJ.g^{-1} dry weight. Crude lipid content was 7.6% (dry weight), which is comparable to that of other sources: 6.0-7.0% (Durand- Chastel, 1980), 4-9 % (Tipnis and Pratt, 1960), 4-7% (Earthrise Farms, California, USA) and 3.0 % (Becker and Venkataraman, 1984).

The total Xanthophyll content amounted to 1.68 g.kg^{-1} (dry weight) and is comparable to that of other sources of *Spirulina:* 1.80 g.kg^{-1} (Richmond, 1986), 1.40-1.80 $g.kg^{-1}$ (Durand- Chastel, 1980). Carotenoids (β-carotene + lutein) amount to 2.90 $g.kg^{-1}$ (dry weight).

The chl a content of the dried *Spirulina* was 2.69 $g.kg^{-1}$ dried biomass. This is generally lower that the levels found by other authors: 6.1- 7.6 $g.kg^{-1}$ (Durand-Chastel, 1980) and 11 $g.kg^{-1}$ (Earthrise Farms, USA). Chlorophyll b content was 1.2 $g.kg^{-1}$. Because cyanobacteria, including *Spirulina*, only contains chl a, the presence of chl b indicates contamination of the surface floc by other green algae. This, in part, may help to explain the comparatively lower protein and amino acid content of the harvested biomass.

Total phycobiliproteins in the dried *Spirulina* biomass amount to 4.51 $g.kg^{-1}$ dry weight. This is much lower than the levels found by Earthrise Farms, USA (150 $g.kg^{-1}$), and which according to Tel-Or *et al.* (1980) should range between 10-30 $g.kg^{-1}$. It is known that the levels of phycobiliproteins fluctuate with the prevailing environmental conditions (Richmond, 1986), especially in response to various lighting regimes. The variable light-conditions in the effluent ponds caused by the continual shifts in vertical distribution of microbial populations may contribute to the phycobiliprotein content, but this aspect will need to be looked at in more detail in order to maximise the recovery of these light-harvesting pigments.

Quantitatively, the low amount of phycobiliproteins present in the *Spirulina* biomass does not correspond with the relatively high carotenoid levels (2.9 $g.kg^{-1}$ dry weight), which is higher than those quoted by other authors: 1.9 $g.kg^{-1}$ (Richmond, 1986), 1.5-19 $g.kg^{-1}$ (Durand-Chastel, 1980). This may be due to the fact that the *Spirulina* bloom traps cells at the surface of the pond, and subjects them to the danger of photodynamic stress. Healthy cells may thus counteract this danger by an increase in cellular carotenoid levels which screen out much of the harmful UV irradiation. This may also explain the lower levels of chlorophylls observed, which is usually concomitant with higher levels of carotenoids and lower levels of phycobiliproteins.

The toxicological properties of the dried and milled tannery effluent-grown *Spirulina* were evaluated. Analysis included nucleic acid, pesticide and heavy metal contents. No pesticides belonging to the organochloride, organophosphates or synthetic pyrethroids were detected.

The concentrations of heavy metals in the HRAP harvested biomass following optimisation of anaerobic pond A showed that nickel was the only heavy metal that remained present at slightly higher concentrations than is acceptable for the animal feed application.

7.4 CONCLUSIONS

Spirulina biomass grown in tannery wastewater was recovered, processed and evaluated. An investigation into the use of a screen harvesting unit for the recovery of *Spirulina* biomass was performed. The first small-scale harvester with a 100 ~m mesh was used successfully in a ten-fold concentration of *Spirulina*, yielding a solids concentration of 21.4 % (dry weight). The biomass was sun-dried, resulting in a yield of 25 kg. The subsequent design and evaluation of a scale-up, technical-scale model with an 80 µm mesh yielded similar cell concentrations, with a total biomass yield of 250 kg.

The experimental, small-scale and technical-scale screen harvest demonstrated that *Spirulina* can be successfully concentrated from the tannery effluent medium. These results indicate that an industrial-size, scaled-up, automated model of the screen harvester could be used in the optimisation of the harvesting operation. It is envisaged that the automated harvester would concentrate the water-column biomass, as opposed to the surface autoflocculated mat. A design modification in the recovery of the cell slurry from the collecting reservoir is also necessary, with direct transfer to the drying units. A conveyer-belt type mechanism would seem to be the most effective way of performing this function.

The harvested *Spirulina* was dried using various techniques, including sun-drying, heated plates, tunnel dryers and Electro-Osmotic dewatering to evaluate their efficiency as alternative methods to conventional spray and drum drying. For successful commercialisation of the procedure, storage and transportation of the final product it becomes necessary to completely dewater the biomass so as to minimise transport and preservation costs (Richmond, 1981). At present sun drying represents the only economically feasible processing step on site at Wellington. However, as the production increases, capital may be invested in more sophisticated drying equipment and spray drying units may be considered.

An evaluation of the chemical composition and toxicological properties of tannery wastewater- grown *Spirulina* biomass was undertaken. The chemical composition of the *Spirulina* biomass was found to be comparable to reported values for *Spirulina* and other protein feed products. A bioassay was performed using *Artemia salina*, the observed low biotoxicity coupled to the absence of toxins produced by *Spirulina*, leads to the conclusion that the tannery effluent-grown *Spirulina* biomass has no active biotoxic compounds. The biomass was used in a feeding trial with chickens, and intensive toxicological and pathological evaluations were performed, and results showed no toxicological effects (Maart , 1993 ; Rose *et al.*, 1996; Venkataraman *et al.*, 1994). The analysis of the biomass and the results of the feeding trial allowed a preliminary conclusion that *Spirulina* grown on tannery effluent had no decisive toxicological constraints.

Tannery effluent-grown *Spirulina* harvested at the Mossop Western Leathers WSP was used in aquaculture feeding studies on the abalone *Haliotus midae* and the rainbow trout *Oncorhynchus mykiss* (Maart, 1993). These findings showed that tannery effluent-grown *Spirulina* had a potential as a feed supplement comparable to fishmeal, and also that it may be used for the development of an economically viable artificial feed for the aquaculture production of *H midae* (Maart, 1993). Britz (1996) reported the development of an artificial feed for abalone culture containing the tannery *Spirulina* biomass. Manufacture of this feed has proceeded to commercial production stage and is utilised by an abalone farm in South Africa (Britz 1997, pers comm.).

The feasibility of incorporating effluent-grown *Spirulina* in the artificial diets of the rainbow trout (*Oncorhynchus mykiss*) was also investigated (Maart, 1993). Results followed the trend of other studies conducted with micro-algae, and indicated that lower concentrations of *Spirulina* supplementation does not alter the growth rates, and that there are no decisive pathological manifestations of toxicity .The main drawback regarding the use of micro-algae as fish feed has been the exorbitant production and processing costs. The positive toxicological assessment and low production cost of the tannery HRAP effluent-grown *Spirulina* recovered in this study, taking into account the opportunity-value and shared cost with the effluent treatment function, and the positive results achieved in the pilot plant study, provided strong inputs to the decision by Mossop Western Leathers Co. to proceed to the construction of a full-scale S-HRAP and IAPS for treating their wastewaters.

8 BENEFICIATION POTENTIAL OF WSP-GROWN *SPIRULINA* BIOMASS: TOXICOLOGY AND FEED EVALUATION STUDIES

8.1 INTRODUCTION

The utilisation of *Spirulina* biomass as a food source in human and animal rations has been well documented (Becker, 1986; De Pauw and Persoone, 1988), and there are early reports by Fra Toribio Bennevente in 1524, of its use as a staple food by Aztecs living around Lake Texacoco in Mexico (Richmond, 1986). The dried 'tecuitlatl' was probably similar to the dried 'dihe' cake eaten traditionally by the Kanembu people along the shores of Lake Chad in cental West Africa (Richmond, 1986). While the high protein content of 60-70% (dry mass) is of principal interest in feed formulations, the nutritional value of *Spirulina* also derives from carbohydrate values around 15-20% (dry mass), vitamin, fatty acid and pigment values, which has focussed interest on its use in pharmaceutical and specialist aquaculture ration applications (Ciferri, 1983; Richmond, 1988; Cohen and Vonshak, 1991).

Micro-algal biomass produced under controlled, pure culture conditions, and in defined growth media, has a largely predictable chemical content with a low risk of contamination from external sources. Production in industrial effluents, notwithstanding the organic origins of these media, involves an element of hazard due to the uptake of potentially toxic compounds and the incorporation of adulterants from a wide range of possible sources (Guzelian, 1990). In common with other sources of single cell protein (SCP), the nucleic acid content of harvested micro-algal biomass presents an additional risk of toxicity (Becker, 1986).

Despite the demonstration of unimpeded cell growth, effluent-grown micro-algal production, in all but the most carefully controlled food-grade wastes, is precluded from use for human consumption as a precautionary principle. However, this need not apply as a blanket proviso in animal feed applications, given adequate controls and the prior demonstration of appropriate nutritional values. Although largely biological in origin, ponded tannery wastewaters present a number of potential risk factors as growth media for animal feed-targeted *Spirulina* biomass production. These include chemicals used in the tanning process, high concentrations of salts, heavy metals, and possibly also pesticides.

Adequate procedures for the determination of the potential toxicity of harvested micro-algal biomass for animal feed applications are varied but depend ultimately on the acid test of intake by the particular organism concerned. Procedures followed in this study involved pre-animal feeding biomass toxicity studies including measurement of nucleic levels in tannery effluent grown biomass, heavy metal values, pesticide residues and *Artemia* bioassays. Animal feeding studies followed and included toxicological evaluations in newborn chicks, and growth performance studies in the South African abalone *Haliotis midae*, and the rainbow trout *Oncorhynchus mykiss.* The methods used in these studies are reported by Maart (1993), and the results relate to *Spirulina* biomass harvested as surface floating mats from the Wellington WSP prior to the retrofitting of the IAPS installation. Following concentration over an 80 μ plankton net screen the biomass was sundried (Figure

7.2) and the flakes then milled prior to use in the studies reported below. Subsequent harvesting of planktonic biomass from the water column produced the higher quality product noted in the previous chapter. However, the studies reported here were undertaken before this was available, and thus the results relate to the lower quality biomass harvested initially as the floating mats.

8.2 METHODS

Methods used in these studies are as previously described.

8.3 RESULTS AND DISCUSSION

8.3.1 Biomass Nucleic Acid Content

DNA values in the harvested biomass were measured by the diphenylamine reaction with a mean concentration of 3.7g DNA.kg^{-1} dry mass corresponding to 0.4% DNA content in the *Spirulina* biomass. RNA was measured by the orcinol reaction with 37.8g RNA.kg^{-1} dry mass corresponding to 3.8% RNA biomass content.

Total nucleic acid content therefore made up 4.2% of the total dry mass of the *Spirulina* sp. biomass harvested from the tannery ponds. This is in the range of 2.9 to 4.5% reported for *S. maxima* from Lake Texococo (Durand-Chastel, 1980), 2-5% in *S. platensis* (Tipnis and Pratt, 1960) and 4% in *Spirulina* sp. (Aaronson *et al*., 1980). These values compare favourably with other sources of SCP such as bacterial biomass (up to 16%), yeasts (6-11%) and filamentous fungi (2.6-6%) (Litchfield, 1979).

8.3.2 Pesticide Residues

Gas-liquid chromatography test results showed none of the following: DDT or its isomers at detection limits of 0.02 mg.kg^{-1} dry mass; organochloride, organophosphate and synthetic pyrethroid compounds at detection limits of 0.05 mg.kg^{-1} dry mass; Aldrin, Dieldrin, Endrin, Malathion, Parathion, Diazinon, Chlorpyrephos, Pyrenephos, Permethrin, Cypermethrin, alpha-Methrin and Fenvelarate.

8.3.3 Biomass Mineral and Heavy Metal Content

The mineral and heavy metal content of the harvested *Spirulina* biomass was determined by atomic absorption spectroscopy (Table 8.1). Although the components measured in this study relate to biomass harvested before the IAPS retrofit, and were used in the feeding studies reported here, the analysis did not include the toxicologically significant elements such as cadmium, cobalt, manganese, nickel and lead. These elements were measured in subsequent studies reported above, their presence measured at low levels in the untreated biomass, and reduced to acceptable norms in the post-treatment harvested material. The analysis of the *Spirulina* biomass harvested prior to the reconfiguration of the system, although presenting a probable worst-case scenario, nevertheless indicated that high salt levels and heavy metal content may present a potential problem in biomass recovery from ponded tannery effluents, and needed to be addressed. No toxic effects due to metal content were observed in the toxicology studies reported below.

Table 8.1. Mineral and heavy metal content of dried *Spirulina* biomass harvested from the Wellington Waste Stabilisation Ponds before the IAPS retrofitting of the system. Acceptable standards for heavy metal content of animal feeds follows the German Ministry of Agriculture and Nutrition Law Collection No 117-1987.

Element	***Spirulina* Biomass (mg.kg $^{-1}$ dry mass)**	**Heavy Metal Standard (mg.kg $^{-1}$ dry mass)**
Al	500	200-300
Cr	20	10-15
Cu	3	50-100
Fe	500	1250-2500
Zn	180	250-500
Ca	1390	-
Mg	5820	-
NaCl	7 800	-

8.3.4 Brine Shrimp Bioassay

The Brine Shrimp bioassay followed the method of Meyer *et al*. (1982) and using freshly hatched nauplii larvae of *Artemia salina*. Quadruplicate assays were undertaken for water soluble components using *Spirulina* whole cells, and for cell constituents using *Spirulina* biomass lysed by sonication. Water soluble components showed no observable toxic effects with >90% survival of larvae after 24 hours exposure. Lysed biomass showed a toxic effect at higher concentrations, and especially after 12 hours, as may have been expected. Results are reported in Figure 8.2.

8.3.5 Chick Feeding Study

Three groups of 7 day old chicks were fed chicken rearing mash supplemented with 10% and 50% harvested *Spirulina* biomass. After 21 days the feed conversion ratios (FCR) were calculated, organ dry weights, and liver mineral and heavy metal content were analysed, and histological examinations undertaken for heart, liver, kidney and small intestine tissue.

The feed consumption, growth rates and FCR values were found to be comparable in the three groups indicating the satisfactory supplementation of the chicken rearing mash with up to 50% *Spirulina* biomass and confirming previous observations by Shelef *et al*., 1980. Chicks fed the *Spirulina* supplemented ration showed a marked increase in body pigmentation, a result also previously observed by Lipstein and Hurwitz.(1980). Results for the FCR study are recorded in Figure 8.3

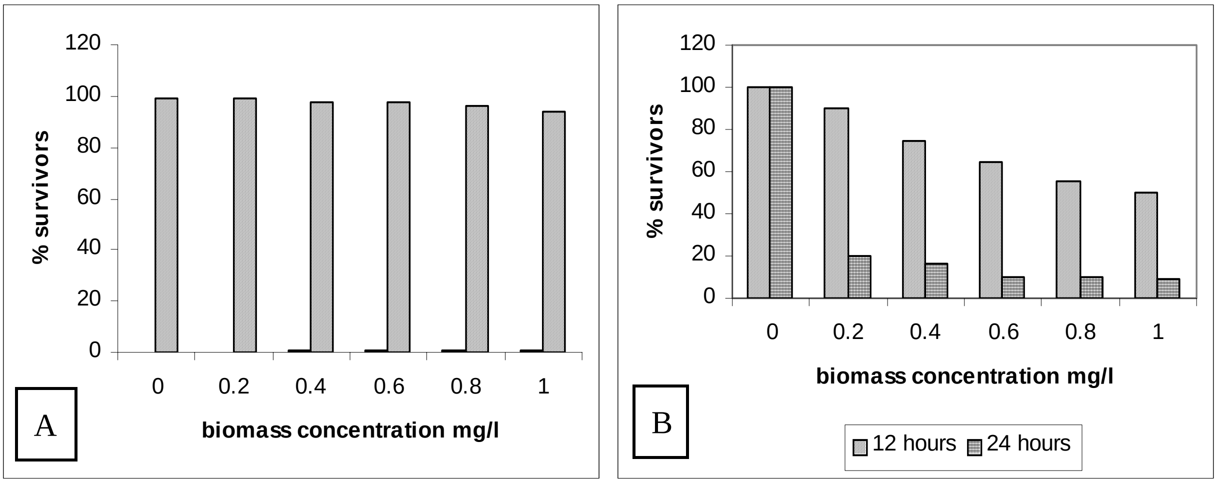


Figure 8.2 Survival of *A. salina* nauplii larvae exposed to whole cell (A) and lysed (B) cell solutions of tannery effluent-grown *Spirulina* biomass. Lysed cell exposures at 12 and 24 hours are compared.

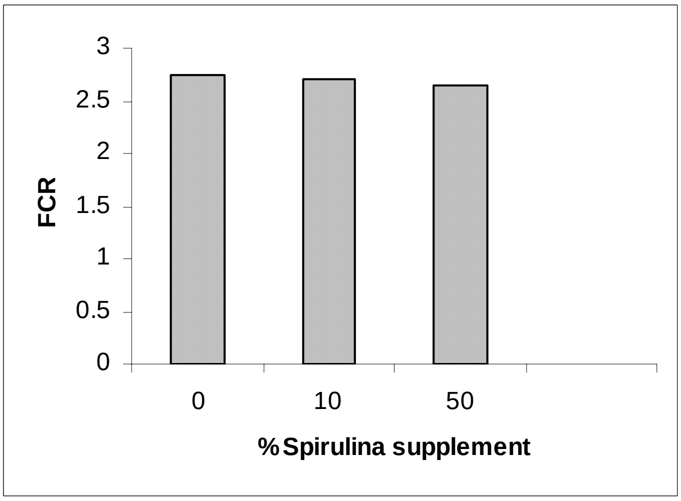


Figure 8.3 Feed conversion ratios (FCR) for three groups of chicks fed *Spirulina* supplemented chicken rearing mash at 10% and 50% compared to no addition controls

With the exception of kidney tissue which showed a slight increase in dry weight yield, the heart, liver and spleen tissue yields were slightly reduced for the supplemented feed groups. Histopathology findings showed little difference between the three groups with the exception of the kidney tissue in the *Spirulina*- fed groups where some renal tubular epithelial degeneration was noted. This could have been due to the increased salt intake in this group. Detailed histopathology results are recorded by Maart (1993).

The mineral and heavy metal content of chick livers was measured in acid digest samples. Both reduced and increased uptake patterns may be noted in the results reported in Table 8.2. but without particularly pronounced effects.

Table 8.2 Mineral and heavy metal content (mg.kg^{-1} dry weight) of liver acid digests from the three groups of chicks fed *Spirulina*-supplemented and unsupplemented chicken rearing mash.

Element	Control	10% Supplement	50% Supplement
Al	0.44	0.44	0.34
Cr	0.16	0.20	0.18
Cu	0.26	0.31	0.49
Fe	10.7	7.92	16.0
Zn	1.44	2.04	3.06
Ca	17.7	9.1	7.48
Mg	14.5	13.6	11.5
Na	562	366	294

8.3.6 Abalone Feeding Study

While abalone are ubiquitous along all the major continents and islands of the Pacific, Atlantic and Indian Oceans, and commercially significant species are found in the temperate waters off California, Japan, Australia, New Zealand and South Africa, most abalone fisheries based on natural harvest are now chronically over harvested, or have completely collapsed (Fallu, 1991). The continuing small but lucrative market for abalone meat has focussed interest in commercial aquaculture farming, with *Haliotis midae*, the predominant species along the South African coast, being the main focus of research development in this country (Britz, 1990).

H. midae feeds naturally off the brown alga kelp *Ecklonia maxima* as its principle food source and artificial diets have involved supplementation of agar-based diets largely with fishmeal and casein (Britz, 1996). As feed costs constitute the bulk of operating costs in intensive aquaculture systems the use of alternative protein sources has been considered, and in this study the tannery effluent-grown *Spirulina* was investigated as a supplement to artificial agar based diets. Questions to be answered included determining whether *H. midae* would accept an artificial diet including effluent-grown *Spirulina*, what would be the impact on growth rates of juvenile animals, and to what extent could *Spirulina* replace fishmeal without adversely affecting the growth rates. These studies were undertaken in the Rhodes University Department of Ichthyology and Fishery Sciences, under the supervision of Peter Britz. The detailed results of the study are reported by Maart (1993).

Juvenile *H. midae* were collected from the East Cape coast, and artificial diets formulated to compare fishmeal, casein and *Spirulina* biomass added as 5%, 10%, 50% and 100% supplements to the base agar ration formulation. Figure 8.4 shows both good acceptance and feed consumption values for the *Spirulina* supplemented diets, with an increased feed intake related directly to increasing levels of supplementation. This is most likely related to the lower level of protein present in the feed compared to casein and fishmeal, and this explanation of the observed results is supported by the increase in the FCR noted.

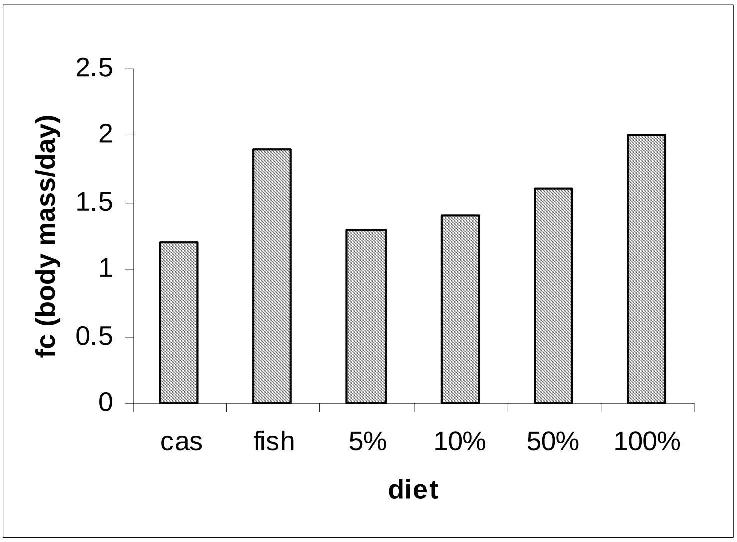


Figure 8.4 Feed consumption by abalone fed a range of test diets including supplementation with casein, fishmeal, and *Spirulina* biomass. Results are expressed as food consumed as a % of body weight.day^{-1}.

Specific growth rates (SGR) reported in Figure 8.5 were also comparable for the *Spirulina* supplemented feeds. However, growth measured as mass increase was better than growth as increase in length.

The study showed that in terms of overall consumption efficiency, *H. midae* was able to utilise fishmeal more efficiently than *Spirulina* biomass, but that supplementation at a 5% level produced results comparable to a pure fishmeal diet. Where the cost of effluent-grown *Spirulina* was to fall substantially below that of fishmeal, replacement at a higher level would become feasible. Subsequent harvesting studies which excluded floating mats of *Spirulina* biomass, and drew on planktonic organisms in first the WSP and then in the S-HRAP, showed that protein levels around 60% could be achieved for well managed systems using a tannery effluent growth medium. It is likely that improved feed conversion values for abalone cultivation would be found with the use of this source of improved quality biomass.

The above studies contributed to the development at Rhodes University in the Department of Ichthyology and Fishery Sciences of an artificial abalone diet formulated as a solid ribbon and commercialised under the name ‘Abfeed’ (Britz, 1996). This formulation has now become widely used in abalone farming both in South Africa and abroad (Figure 8.6).

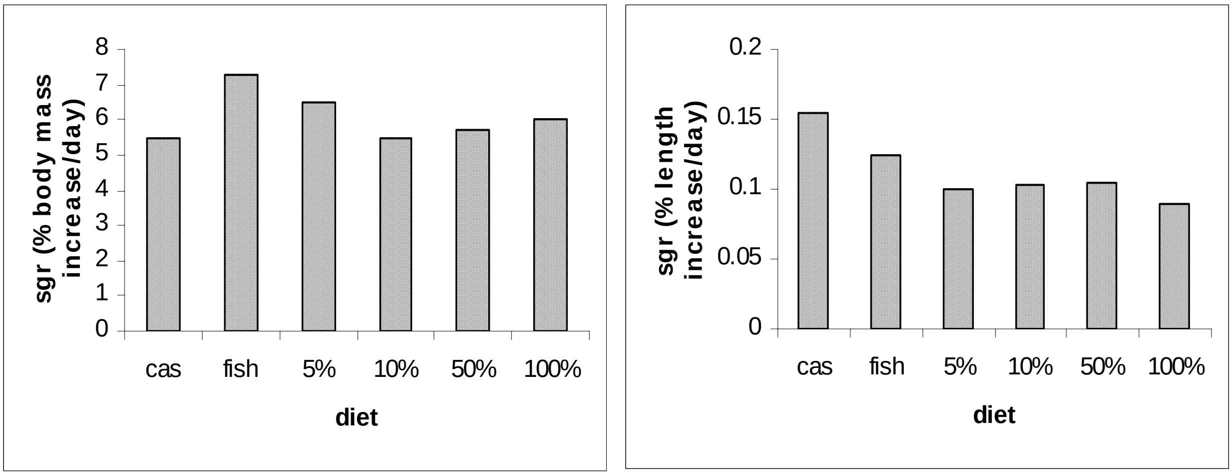


Figure 8.5 Specific growth rates values for abalone fed a range of diets including supplementation with casein, fishmeal, and *Spirulina* biomass. Results are expressed as % increase in mass.day^{-1} and % increase in length.day^{-1}.

8.3.7 Rainbow Trout Feeding Study

In common with most aquaculture systems, the feed cost in production of the rainbow trout *Oncorhynchus mykis* accounts for more than half the operating costs of the fish farming enterprise (Logan and Johnston, 1992).

Protein constitutes the major component in feed formulation and the quality of the protein source has an important impact on the productivity of the system. While fishmeal has traditionally filled this role, and constitutes up to 65% of commercial salmonid feed formulations, considerable research effort has focussed on the development of alternative protein sources (Murai, 1992). A factor of particular importance in artificial feed development is the effect on fish pigmentation, with flesh and skin colour being important in market acceptance of the artificially reared fish (Gall, 1992). Henson (1990) has reported that dietary *Spirulina* supplementation of the sweet smelt *Plecoglossus altivelis* produced a superior product, with nearly double the market value of conventionally fed animals, and also reduces fry mortality and medication expenses. Although this form of supplementation is expensive he reported favourable cost/performance ratios.

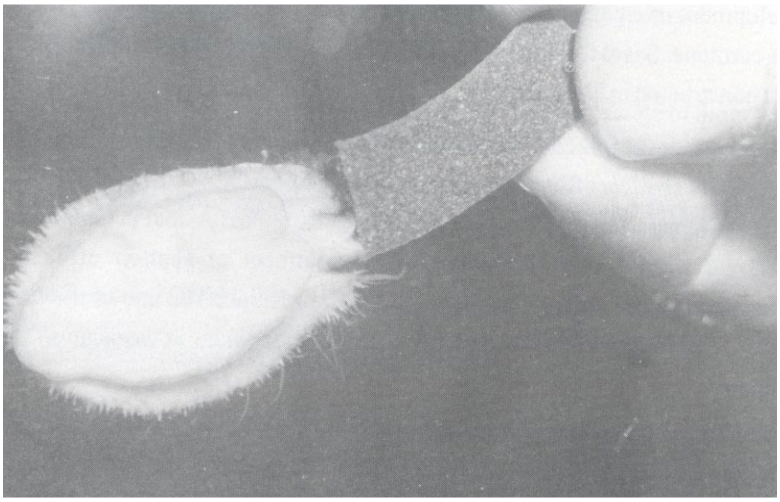


Figure 8.6 Young abalone feeding on the commercial artificial diet ‘Abfeed’ developed in the Department of Ichthyology and Fishery Sciences at Rhodes University.

In this study feeding trials were undertaken investigating the incorporation of the effluent-grown *Spirulina* biomass in rainbow trout feed rations. Juvenile trout were hatched in the hatchery of the experimental fish farm of the Department of Ichthyology and Fishery Sciences at Rhodes University, and the feeding studies supervised by Martin Davies. The results of this study are reported by Maart (1993).

Four diets were evaluated including the standard fishmeal diet and also its supplementation with effluent-grown *Spirulina* at 5%, 20% and 53%. Thirty juvenile trout were included in each study group. In addition to analyses of growth performance, histopathological studies were undertaken at the end of the study on heart, liver, kidney, spleen and intestine tissue from each group, and pigment content of skin and flesh was measured.

Feed consumption values determined in the study for 4 and 8 week feeding periods showed that the diet containing the effluent-grown *Spirulina* was well accepted by the fish (Figure 8.7). Specific growth rate values (Figure 8.8) showed an improved performance for trout fed on the 5% supplemented diet, but reduced growth at the higher levels of supplementation. This trend has been observed previously and Okada *et al*. (1991) reported similar results for *Spirulina* supplementation in feed studies of the striped jack *Caranx delicatissmus*.

Figure 8.7 Feed consumption by rainbow trout fed a standard fishmeal based diet supplemented with effluent-grown *Spirulina* biomass. Performance is recorded at 4 and 8 weeks with feed consumption determined as % body mass consumed.day^{-1}.

Figure 8.8 Specific growth rate of rainbow trout fed a standard fishmeal based diet supplemented with effluent-grown *Spirulina* biomass. Performance is recorded at 4 and 8 weeks with growth determined as increase in % body mass.day^{-1}.

While the feed conversion ratio for the *Spirulina*-supplemented diets (Figure 8.9) improved with the higher supplementation of the micro-algal biomass, this was probably due to an increased intake related to the somewhat lower protein values.

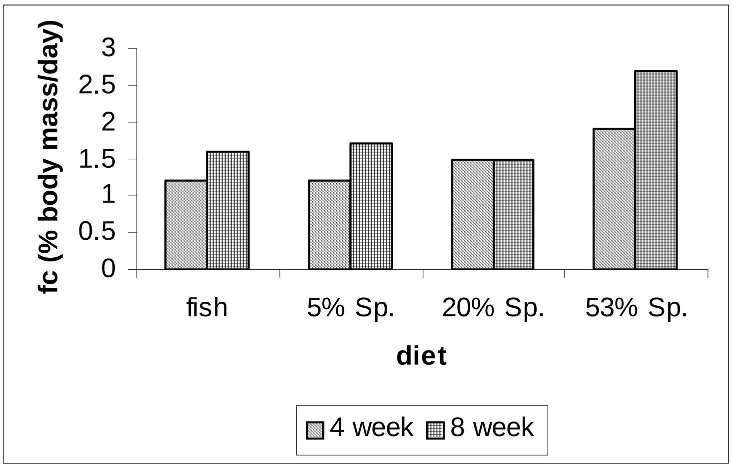


Figure 8.9. Feed conversion ratio for rainbow trout fed a standard fishmeal based diet supplemented with effluent-grown *Spirulina* biomass. Performance is recorded at 4 and 8 weeks with feed conversion measured as % body mass consumed.day^{-1}.

Enhanced pigmentation of both skin and muscle was noted for the trout groups fed the effluent-grown *Spirulina* supplemented feeds. Increasing levels of supplementation was found to be directly related to increased pigmentation of both skin and muscle in the test groups compared to the fishmeal fed group (Figure 8.10).

Figure 8.10 Pigmentation of skin and muscle tissue in rainbow trout fed a standard fishmeal-based diet supplemented with effluent-grown *Spirulina* biomass. Pigmentation was measured as total carotenoid levels.kg^{-1} skin and muscle at 4 and 8 weeks.

Histopathological examination of tissue samples recovered after the study period at 8 weeks showed no adverse effects from the *Spirulina* supplemented rations for all specimens with the exception of kidney samples. Early symptoms of nephrocalcinosis were noted particularly in the groups supplemented with the higher levels of *Spirulina.* The observation of basophilic lamellated calculi formations in the kidney tubules of these specimens may be related to the increased salt load and alkalinity of the effluent-grown biomass. Nephrocalcinosis has been reported in humans with increased alkali intake (Heptinstall, 1966).

8.4 CONCLUSIONS

The surprisingly high productivity measured for *Spirulina* growth in the tannery effluent WSP in Wellington, and the increased productivity levels subsequently observed in the retrofitted S-HRAP unit operations described above, raised the question of a potential utility value for the biomass produced as an exercise in the beneficiation of tannery wastewater treatment. While the high market price for *Spirulina* biomass, particularly in aquaculture and animal specialist feed applications, indicated the potential of a high opportunity value for the effluent-grown product, its removal from the system was also important in linearising effluent treatment in the zero discharge ponding system. The basic questions to be answered, however, if a beneficiation development was to be undertaken profitably, related to the potential toxicity of the effluent-grown biomass for target organisms, and the growth performance results which could be anticipated compared to more conventional feed supplements.

Both the toxicology and feed evaluation studies were largely positive, while indicating, in common with a number of other previous studies, that *Spirulina* biomass constitutes a valuable feed additive rather than a complete feed replacement for protein sources such as fishmeal. The studies did show, however, that potential problems may arise at high levels of dietary supplementation due possibly to the alkalinity and salt content of the sundried dried biomass. The problem has been previously noted by Richmond (1988) for *Spirulina* biomass produced in sea water media. Biomass washing studies showed that carbonate, heavy metal and ash content of the harvested biomass could be substantially reduced by acid washing prior to drying (Dunn, 1998).

The incorporation into the biomass of heavy metals concentrated within the WSP is clearly a potential hazard noted in the study, and although relatively low levels of contamination were observed this should be constantly monitored where the risk is apparent. The retrofitting of the WSP with an IAPS unit as a first stage in the effluent treatment train was shown to provide an effective mechanism, not only as a unit operation of the treatment process, but also in ensuring that *Spirulina* biomass did not accumulate metals at higher than acceptable levels. This step is important not only where biomass harvesting for feed utilisation is contemplated but also to ensure that metals do not enter the wider food chain via wildlife occupation of saline wastewater impoundments, and especially waterfowl.

The overall observation which may be drawn from these studies is that the *Spirulina*-based IAPS provides an effective system for treating tannery wastewaters, and that the biomass produced in the course of this operation is suitable for use as a product in animal feed process beneficiation applications. It may be noted that the Mossop-Western Leathers Co. undertook detailed market studies on the exploitation of the *Spirulina* biomass resource and the marketing of the dried product to a number of abalone and fish farming enterprises. Currently (2001) a private company is still harvesting the system commercially and selling dried *Spirulina* biomass.

9 CONCLUSIONS AND RECOMMENDATIONS

The research programme reported here has investigated aspects of the segregation option for the management of saline wastes. An integrated management of wastes through their beneficiation as resources, and the feeding of economically effective downstream production operations, had been identified as a strategy through which sustainability might be achieved. While the concepts of ‘integrated waste resource management’, waste recovery, recycle and reuse, emerged early as processes for operationalising sustainability objectives, these depend in the last resort on technological capacity for their implementation. Little attention has focussed on sustainable technologies in the management of saline wastes, or in the integration of the saline and sanitation waste systems using IAPS.

This study investigated options in the beneficiation of saline wastewaters based on developments in algal biotechnology, and the establishment of an economic basis for their long-term sustainable management in the production of high-value algal bio-products. A critical path research methodology was followed in which specific technological constraints were identified and investigated, in order to demonstrate a provisional feasibility for the overall project. Functionality would need to be demonstrated here, in the first instance, in order to proceed with a broader-based initiative. In this regard the tannery effluent WSP was chosen as a useful study model, in many ways representing a worst-case scenario for what might be encountered in saline wastewater impoundment. The WSP at the Mossop-Western Leathers Co. tannery in Wellington was used for this study.

9.1 FINDINGS

Anecdotal observations of massive micro-algal blooms occurring on the Wellington WSP, from time to time, were confirmed in a three-year study, which represents a first detailed report of the microbial ecology of these WSP systems. Factors influencing micro-algal growth and production in the WSP were described, and it was shown that the meso-saline and hyper-saline conditions established in the separate parts of the system provided potential for the cultivation of *Spirulina* and *Dunaliella*, as near mono-species cultures. The potential development of IAPS for the linkage of wastewater treatment and the capture of nutrient values in algal biomass production, particularly of high-value products, has been constrained by the ability to establish such mono-species growth in outdoor conditions. This objective was pursued in these studies.

The S-HRAP concept was developed through laboratory, pilot plant studies to its full-scale application as a novel and patented method for the treatment of tannery wastewaters. At the same time harvesting of *Spirulina* biomass and its toxicological assessment were undertaken, and it was shown that animal and aquaculture feed-grade micro-algal products could be recovered from the system.

The development of the hyper-saline D-HRAP application was also undertaken, involving the growth and recovery of *Dunaliella* spp. These studies are detailed in Part 2 of this report ‘Integrated Algal Ponding Systems and the Treatment of Saline

Wastewaters. Part 2: Hyper-saline Wastewaters - The *Dunaliella* Model' (Appendix 1).

The study achieved the principal objectives of the project and it was shown that:

- ❑ the HRAP may be developed as a useful unit operation in the treatment of saline wastewaters, and may be used for the reduction of both organic load and the removal of heavy metal contaminants;
- ❑ reduction of organic load in saline wastewater improves evaporation rates and would enable a reduced pond surface area requirement;
- ❑ removal of contaminating organic and heavy metal compounds enables the safe impoundment of saline wastewaters and eliminates the environmental threat where these become occupied as wildlife refuge sites;
- ❑ the S-HRAP was developed as a free-standing tannery wastewater treatment operation. The S-HRAP provides an IAPS configuration which may be used for the improved operation of WSP-based tannery wastewater treatment, and also to reduce environmental impacts such as odour nuisance;
- ❑ a utility was demonstrated for saline wastewater impoundments with the growth, and recovery of value, in the form of *Spirulina* biomass;
- ❑ the production of an algal bio-product, and the demonstration of a value-adding function for a saline effluent, indicated a possible model system for larger-scale application in sustainable management strategies for saline wastewaters;
- ❑ the enhanced hydrolysis of complex organic compounds observed in the sulphate reducing compartments of the tannery WSP indicated potential linkages for the saline and sanitation wastewater systems. The IAPS would not only provide a 'core technology' wastewater treatment in both systems, but disposal of sanitation system wastes in saline wastewaters could provide a basis for the capture of the substantial nutrient values in these wastes, in the form of high-value algal bio-products.

9.2 RECOMMENDATIONS

A number of recommendations followed from the research programme:

1. The segregation of saline wastewaters should remain an acceptable option as an alternative to their disposal by dilution in the fresh water system;

2. Active research should be undertaken on problems associated with the engineering and management of saline waste disposal facilities, such as impoundments, and on the technologies required to effect the appropriate handling of these wastes;
3. Development of value-adding operations whereby the long-term management of these wastes is placed on a sustainable economic footing, such as algal biotechnology-based production, should be pursued to commercial-scale;

4. Enhanced hydrolysis of complex organic carbon substrates in the sulphate reducing compartments of the sulphate-saline WSP system should be

subjected to follow-up investigation, especially where sanitation wastes might provide an electron donor source in the biological treatment of high-sulphate wastewaters such as acid mine drainage (AMD);

5. Potential linkage of the saline and sanitation wastewater systems should be actively pursued. 'Integrated waste resource management' strategies should become the subject of more intensive research and innovation, and further research and development should be encouraged in this area as a basis for promoting sustainability and sustainable development objectives in wastewater management.

9.3 RESEARCH PRODUCTS

The WRC studies reported here led to a number of follow-up studies based on the above recommendations. These are the subject of separate project reports which are listed in Appendices 1 and 4.

Student training in IAPS treatment of saline wastewaters has included 1 Post-Doctoral Fellow, 6 PhD and 6 MSc students. Publication of the results of these studies is ongoing but currently includes 7 patents, 13 papers in refereed journals and 6 articles of general scientific interest. Publication in conference proceedings includes 3 plenary and key note lectures, 16 international and 29 local conference presentations. Student training and publication outputs are reported in Appendix 2.

A number of industrial technology transfer exercises have been undertaken, involving the products of these studies, and are noted in Appendix 3. In addition, research spin-off developments which have resulted in associated follow-up research projects have been noted in Appendix 4 of this report.

9.4 FOLLOW-UP ACTIONS

In addition to the specific objectives for this project, it had also been required at the outset that the wider implications of a biotechnological approach to the saline wastewater problem should be borne in mind. In this regard a number of potential developments arose during the course of the project, and these became the subjects of follow-up WRC projects.

In implementing IAPS technology, the trade-marked Advanced Integrated Wastewater Ponding Systems (AIWPS), developed over many years by Professor William Oswald, University of California, Berkley, (Oswald, 1988 a&b;1991;1995), was identified as providing many of the engineering design aspects required to effectively manipulate algal-based wastewater treatment and biomass production operations. While this system had been principally developed in the domestic wastewater treatment application, it was considered the best model to effect the technology transfer necessary to place algal biotechnology applications development on a sound engineering basis in South Africa. A technology transfer exercise was undertaken in which a technical-scale AIWPS plant was constructed, as a component of an IAPS demonstration and research plant, at the Rhodes University

Environmental Biotechnology Experimental Field Station in Grahamstown. This was based on designs by Prof Oswald and Dr Bailey Green. They also provided valuable inputs into the Wellington developments. This initiative was undertaken in a follow-up WRC project and the results are detailed in the report 'Integrated Algal Ponding Systems and the Treatment of Domestic and Industrial Wastewaters. Part1: The AIWPS Model' (Appendix 1).

A number of industrial applications of the IAPS technology were also investigated as follow-up WRC projects including abattoir, winery and distillery wastewaters (Appendix 1). The studies on hyper-saline wastewaters were followed up in a joint WRC/EBG project with Sasol Ltd., and resulted in the development of a process for β-carotene production from *Dunaliella*, scaled-up to industrial-scale operation by Sasol Ltd. in Uppington. The development of the *Dunaliella*-HRAP (D-HRAP) for treating hyper-saline wastewaters was evaluated in the treatment of soda ash brines at Botswana Ash Co., Sua Pan, Botswana. These studies are detailed in Part 2 of this report 'Integrated Algal Ponding Systems and the Treatment of Saline Wastewaters. Part 2: Hyper-saline Wastewaters - The *Dunaliella* Model' (Appendix 1).

The potential for the linkage of the saline and sanitation wastewater systems, which had emerged from the tannery IAPS investigation, was followed up in a number of WRC studies. The construction of the IAPS demonstration and research plant treating domestic wastewater in Grahamstown, and the IAPS treating saline tannery wastewater in Wellington, had established the basis of the system as a 'core technology' linking the treatment of these wastes. The observations of enhanced hydrolysis of complex and particulate organic carbon had demonstrated a mechanism whereby the nutrient values in these wastes might be recovered in the form of value-added algal bioproducts. The use of sewage sludges (and other waste organic carbon substrates) as reagent feedstocks for the biological treatment of sulphate-saline wastewaters, such as AMD, was investigated and resulted in the development of the Rhodes BioSURE Process®. The production of treated water from AMD, as a product of the saline and sanitation linkage, is an important target given the large volumes anticipated following mine closures, and the very long time periods over which the problem is likely to affect the South African environment.

Besides environmentally sustainable advantages in a cost-effective co-disposal of these waste systems, the use of wastes as resources, an the basis of value-adding beneficiation operations, has potential to provide inputs to the wider sustainable development project. This is particularly the case in the developing world context where salinisation and desertification have been identified as primary agents in constraining human development.

The objective of establishing linkages in saline and sanitation wastewater treatment based on studies in environmental biotechnology was pursued by the EBG in a number of WRC projects and became the subject of 13 separate reports in the series 'Salinity, Sanitation and Sustainability: A Study in Environmental Biotechnology and Integrated Wastewater Beneficiation in South Africa' (Appendix 1).

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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 the findings of which have been detailed in the reports as outlined above:

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 Watewaters

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.

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APPENDIX 2

RESEARCH PRODUCTS

The results of the WRC study, and the research products which have been generated, have been the subject of detailed report in student theses, journal articles, patents and other publications.

2.1. STUDENTS TRAINED

2.1.1 Post-Doctoral Fellow

Dr. O. Shipin (1992-1993) Rate Determinants in Hyper-saline Anaerobic Digestion.

2.1.2 PhD Students

T. Phillips (1994) Stress manipulation in *Dunaliella salina* and dual - stage β-carotene production.

M. Logie (1995) Physiological signal transduction from the photosynthetic apparatus in the green alga *D.salina.*

L. Phillips (1995) Constituent processes contributing to stress induced β-carotene accumulation in *D.salina.*

K. Dunn (1998) The biotechnology of high rate algal ponding systems in the treatment of saline tannery wastewaters

G. Boshoff (1998) Development of integrated biological processing for the biodesalination of sulphate- and metal-rich wastewaters.

L. Dekker (current) - Integrated algal ponding systems and the treatment of saline winery and distillery wastewaters.

2.1.3 MSc Students

R.K. Laubscher (1992) The culture of *Dunaliella salina* and the production of β-carotene in tannery effluents.

B. Maart (1993) The biotechnology of effluent-grown *Spirulina* and application in aquaculture nutrition. (Degree awarded with distinction)

D. Glaum (1994) A process for the detanning of chrome leather wastes using tannery effluents.

R. Emmett (1996) Glycerol production by *Dunaliella* in saline wastewater treatment.

P. Masemola (2000) The nature and control of organic compounds in soda ash evaporite production.

S. Clarke (2002) The independent high rate pond as a unit operation in tertiary wastewater treatment.

2.2. PUBLICATIONS

2.2.1 Patents

1. Rose,P.D. 1992. Treatment of saline effluent.
   RSA 91/5069 (final)
   Namibia F0791 (final)
2. Rose,P.D., Cowan,A.K. 1992. A process for the production of useful products from saline media.
   RSA 91/5070 (final)
   Australia 19345/92 (final)
   Namibia F07913 (final)
   USA 906536 (final)
   EPO 92306077.4 (final)
   Israel 102357 (final)
3. Rose,P.D., Cowan,A.K. 1992. A process for the treatment of saline effluents.
   RSA 92/4865 (final)
   Australia 19344/92 (final)
   Namibia F07911 (final)
   USA 906535 (final)
   EPO 92306078.4 (final)
4. Rose,P.D. Cowan,A.K. 1992. Treatment of saline media with *Spirulina.*
   SA 92/4159 (final)
5. Rose,P.D., Glaum,D., Rowswell,R.A. 1992. A process for the removal of chromium from tanned leather.
   RSA VO 8584 (final)
6. Rose,P.D., Phillips,T.D., Sanderson,R. 1993. A process for the membrane-based extraction of β-carotene.
   RSA 93/0953 (final)
7. Rose P.D., Boshoff, G.A., Hart, O.O., Barnard, J.P. 1997. The double deck trench reactor.
   RSA 97/4165 (final)
   Australia 711069(final)

2.2.2 Papers

1. Cowan, A.K. and Rose, P.D. 1991. Abscisic acid metabolism in salt stressed cells of *Dunaliella salina*; a possible interrelationship with β-carotene accumulation. Plant Physiology 97: 798-803.
2. Cowan, A.K. and Rose, P.D. 1991. Is there a role for abscisic acid in the response of the green alga *Dunaliella salina var*. Bardawil to salinity stress? Abstract - Plant Physiology May 1991.
3. Rose,P.D.,Maart,B.A.,Phillips,T.D.,Tucker,S.L.,Cowan,A.K. and Rowswell,R.A. 1992. Cross-flow ultrafiltration used in algal high rate oxidation pond treatment of saline organic effluents with the recovery of products of value. Water Science and Technology 25: 319- 327.
4. Cowan,A.K., Rose,P.D. and Horne,L.G. 1992. *Dunaliella salina*: A model system for studying the response of plant cells to stress. Journal of Experimental Botany 43:1535-1547.

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APPENDIX 3

TECHNOLOGY TRANSFER ACTIVITIES

Notwithstanding the primary exploratory objectives of the study, and the focus of the investigation on potential available in an environmental biotechnology approach to salinity and sanitation sustainability, the project brief included a specific requirement to pursue ‘technology- push’ opportunities where these arose. A number of technology transfer activities were undertaken during the course of the programme to make known findings and results, and to assist in the diffusion of technology to potential users.

3.1 Official Opening of the Mossop-Western Leathers Co. Industrial-scale IAPS

Following studies on the performance and operation of the tannery WSP in Wellington, the full-scale implementation of the IAPS development was undertaken by Mossop Western Leathers Co. This involved the reconfiguration of the water treatment installation including the construction of the 2500 m^2 S-HRAP unit operation, the retrofitting of the PFP, and the incorporation of these units into the WSP system.

The new IAPS plant was officially opened at a ceremony in Wellington by the Hon. Minister of Water Affairs and Forestry, Prof Kader Asmal, on 28 November, 1997. The event was attended by 250 local people, engineers, scientists and senior government officials.

3.2 Site Visits

The Wellington IAPS plant has been visited by hundreds of people since its official opening in 1997. In this regard the full-scale plant has played an important role in disseminating knowledge about IAPS technology, and has also informed decisions to proceed with a number of follow-up studies, which are noted below.

3.3 Technical Tour

A technical tour of inspection of developments in the WRC study relating to IAPS Technology in South Africa was undertaken 24 - 26 January, 1996 (Figure A3.1). Members of the Technical Committee included: Prof C.T. Johnson (Chairman WRC); Mr P.E. Odendaal (Executive Director WRC); Mr D.S. van der Merwe (Deputy Director WRC); Mr Z. Ngcakani (Research Manager WRC); Mr J.R. Muller (Abakor); Dr A. Jarvis (Sasol Ltd.); Dr O.O. Hart (Rhodes University); Prof P.D. Rose (Rhodes University).

Sites visited included:

1. SASOL β-carotene production technical scale plant in Upington.
2. WRC/Mossop Western Leathers commercial scale IAPS plant in Wellington.
3. WRC demonstration plant IAPS sewage treatment in Grahamstown

4. WRC/Abakor demonstration IAPS plant at Cato Ridge Abattoir.
5. De Beers pilot plant for treatment of diamond wastes at De Beers Diamond Research Laboratory, Johannesburg.

Figure A3.1 Technical tour party which undertook the inspection of WRC AIPS project installations. From left to right Prof P.D. Rose (Rhodes University); Mr D.S. van der Merwe (Deputy Director WRC); Mr Z. Ngcakani (Research Manager WRC); Prof C.T. Johnson (Chairman WRC); Mr J.R. Muller (Abakor); (Pilot); Dr O.O. Hart (Rhodes University); Dr A. Jarvis (Sasol Ltd.); Mr P.E. Odendaal (Executive Director WRC).

3.4 Commercial *Spirulina* Biomass Production

Following the completion of this project, a study on commercialising *Spirulina* biomass production was undertaken by Mossop-Western Leathers Co. Harvesting and marketing of dried product as an animal and aquaculture feed has commenced as a private enterprise operated by former EBG student Len Dekker (Figure A3.2). This enterprise provides a model system in which the economics of saline wastewater beneficiation may be more fully investigated.

3.5 *Dunaliella salina* and β-carotene Production

The WRC project K5/495 also investigated the treatment of hyper-saline wastewaters, utilising the halophilic micro-alga *Dunaliella salina,* and linkage to β-carotene recovery as a value-added by-product of treatment. These studies have been detailed in WRC project 'Integrated Algal Ponding Systems and the Treatment of Saline Wastewaters. Part 2: Hyper-saline Wastewaters - The *Dunaliella* Model'.

Investigations in the optimisation of β-carotene production by *D.salina* led to the development and patenting of the Dual Stage Process. Research was partly funded by

Figure A3.2. Former EBG student Len Dekker and assistant Manie Jacobs harvesting *Spirulina* biomass on a Sweco screen, an operation in the commercial production of *Spirulina* biomass at Wellington.

Sasol Ltd. who also undertook the industrial scale-up development of the process at Sastech in Sasolburg, and technical-scale production studies in Uppington. At this time (2002) full-scale commercialisation is being planned in Uppington.

Development of a *Dunaliella*-based HRAP (D-HRAP) for the treatment of hyper-saline wastewaters was scaled-up and evaluated in the treatment of organic contamination in saline carbonate brines at the Botswana Ash Co. soda ash production facility at Sua Pan, Botswana (Figure A 4.3)

Figure A3.3. The *Dunaliella*-HRAP pilot plant treating saline carbonate brines at Botswana Ash Co. Sua Pan, Botswana.

APPENDIX 4

RESEARCH SPIN-OFF DEVELOPMENTS

A specific objective of this project, and the preceding WRC Project K5/410, which initiated the study series, was to investigate technology options which demonstrated a utility value for saline wastewaters and thus providing an economic base for waste resource beneficiation development. The studies described here have resulted in a number of follow-on 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.

4.1 INTEGRATED ALGAL PONDING SYSTEMS TECHNOLOGY TRANSFER PROGRAMME

It was evident from the initial studies that development of 'integrated wastewater 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), developed for domestic sewage treatment by Prof William Oswald in California, USA. Following a visit to California by the author, and then by WRC Research Manager Dr Oliver Hart, Prof Oswald was invited to visit South Africa in 1993. This led to the WRC Project K5/651: 'Appropriate low-cost sewage treatment using the advanced algal high rate oxidation pond', which commenced in 1994. This project undertook the technology transfer exercise, in collaboration with Prof Oswald and Dr Bailey Green, both of UC Berkley, California, USA, whereby an AIWPS design was implemented in the construction of a demonstration and research plant at the Environmental Biotechnology Experimental Field Station in Grahamstown (Figures A4.1 and 4.2). The IAPS demonstration and research plant and the Environmental Biotechnology Experimental Field Station in Grahamstown was officially opened by the Minister of Water Affairs and Forestry Prof Kader Asmal on 18 April 1997.

Figure A 4.1. The High Rate Algal Ponds of the Grahamstown IAPS demonstration plant treating domestic wastewaters.

Figure A 4.2 Minister of Water Affairs and Forestry, Prof Kader Asmal handing over the keys of the plant to the Mayor of Grahamstown Cnlr. Mpahlwa. Background from left to right: Dr D Woods, Vice Chancellor Rhodes University; Dr O Hart and Prof P Rose, Rhodes University EBG.

4.2 APPLICATIONS OF INTEGRATED ALGAL PONDING SYSTEMS

The investigation of the IAPS as a ‘core technology’ in an integrated beneficiation approach to saline and sanitation wastewater treatment was followed up in an IAPS development programme. In addition to domestic sewage wastewaters, applications of the technology were studied in the treatment of a number of industrial wastewater types. These technology development studies were undertaken in a number of WRC projects which are noted below.

4.2.1 The Independent HRAP in Tertiary Treatment Operations

While diffuse sources of contamination due to inadequate sanitation is well described, another contributing cause to the poor quality of rural surface waters is the small sewage treatment works, handling domestic and/or industrial wastewaters. Periodic malfunction in these works is common, often due to overload and operational problems, and results in an inability to meet surface water discharge standards. Where neither plant replacement, nor upgrading of the existing facility can be afforded, the problems of surface water quality may go largely unaddressed with severe consequences for downstream users.

A study was undertaken to develop the HRAP as an independent unit operation for nutrient removal. This work resulted in the development of the Independent HRAP (I-HRAP) for nitrogen and phosphate removal (Figure A 4.3), and is detailed in WRC report ‘ Integrated Algal Ponding Systems and the Treatment of Domestic and Industrial Wastewaters. Part 4: System Performance and Tertiary Treatment Operations’.

Figure A 4.3. Pilot plant of the Independent High Rate Algal Pond (I-HRAP) unit operation for tertiary treatment and the renovation of raw water at the Environmental Biotechnology Field Station, Grahamstown. The I-HRAP is in the foreground with the Algal Denitrification Bioreactor (ADB) column and the algal settling tank behind.

4.2.2 Nutrient Removal in Diamond Sorthouse Wastewaters

De Beers Co. have undertaken the evaluation of the I-HRAP as a free-standing unit operation for nitrogen removal from diamond sorthouse wastewaters. A 100 m^2 HRAP pilot plant was constructed at the De Beers research laboratories at City Deep, Johannesburg.

4.2.3 Treatment of Abattoir Wastewaters

A trend in South Africa away from centralised controlled slaughtering to small rural abattoirs has required a low-cost response to treatment of these wastewaters to deal with a diffusion of the potential pollution problems. The abattoir also presents a case study for the application of IAPS as an upgradeable ‘core technology’, in the sustainable development context. Here the initial investment by a community in sewage treatment technology should be upgradeable as its economic development unfolds. In generating a high-strength agro-industrial wastewater the abattoir provides a practical example to evaluate the flexibility of the 'core technology' investment.

Following laboratory studies on the IAPS application in abattoir wastewater treatment, a pilot plant was constructed at on-site at the Cato Ridge Abattoir in Kwa Zulu-Natal (Figure A 4.4). The results of this study is the subject of WRC Report 'Integrated Algal Ponding Systems and the Treatment of Domestic and Industrial Wastewaters. Part 2: Abattoir Wastewaters'.

Figure A 4.4 The HRAP unit operation of the IAPS plant constructed at the Cato Ridge Abattoir.

4.2.4 Treatment of Winery and Distillery Wastewaters

The investigation of high organic load saline wastewater treatment using IAPS technology was extended in studies on wine lees and distillery wastewaters. The saline IAPS was evaluated as an alternative to the existing practice of disposal to land irrigation. Final disposal via evaporation ponds, and associated micro-algal production, was investigated as a basis for both environmental and social sustainability in this agro-industrial application in the rural economy. In addition to a further evaluation of the upgradeability of IAPS as a 'core technology', a specific focus of this programme involved an evaluation of the beneficiation potential in transforming these agriculturally-derived wastewaters into a resource, with downstream production of algal bioproducts providing the basis for an 'integrated wastewater resource management' approach to the problem.

These studies commenced in the EBG laboratories, and involved the use of the anaerobic baffle reactor as an initial unit pre-treatment operation to reduce the organic load fed to the IAPS. Findings were then subjected to scale-up pilot study at the Brennokem (Pty) Ltd. wine lees plant in Worcester, South Africa (Figure A4.5).

The results of this study are detailed in WRC report 'Integrated Algal Ponding Systems and the Treatment of Domestic and Industrial Wastewaters. Part 5: Winery and Distillery Wastewaters'.

Figure A 4.5. Pilot plant at Brennokem Co., in Worcester, South Africa, evaluating the IAPS in the treatment of wine lees and distillery wastewaters.

4.2.5 The ASPAM Process

The observations of an enhanced hydrolysis of complex organic substrates present in tannery effluents, and their use as carbon and electron donor sources supporting high rates of sulphate reduction, provided an indication that ponding systems might themselves be used as bioreactors for the biological treatment of large-volume AMD flows. While WSP technology has been developed over the past 40 years for a wide range of wastewater treatment applications, little attention, if any, has focussed on the use of these systems for AMD remediation.

This application of IAPS was investigated in WRC Project K5/869: 'Biological sulphate desalination and heavy metal precipitation in industrial and mining effluents using the IAPS', and the use of tannery effluent and sewage sludges as effective electron donors in sulphate-salinity reduction applications was demonstrated. These studies resulted in the conceptual development of the Algal Sulphate Reducing Ponding Process for Acid Metal Wastewater Treatment (ASPAM) and are detailed in WRC report ' Integrated Algal Ponding Systems and the Treatment of Domestic and Industrial Wastewaters. Part 3: Mine Drainage Wastewaters - The ASPAM Model'.

4.3 THE RHODES BIOSURE PROCESS®'

4.3.1 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, and 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'.

4.3.2 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 (Ewat) 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 4.7) 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'.

Figure A 4.6 Headgear at the No.4 shaft Grootvlei Mine with the Rhodes 'BioSURE' Process pilot plant in the foreground. Reactors were constructed in shipping containers in Grahamstown, and then transferred to site.

Figure A 4.7. 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.

4.4 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.

4.5 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'.

4.6 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.

4.7 MEMBRANE BIOREACTORS

Studies undertaken in Project K5/495, using membranes for algal cell harvesting and also in the separation and recovery of β-carotene led to a WRC programme which undertook the development of membrane bioreactor applications. This developed into a joint study together with Prof Ron Sanderson and Dr Ed Jacobs of the Polymer Institute, Stellenbosch University, and Drs Stephanie Burton, Winston Leukes and Peter Rose of Rhodes University. Both novel membrane and membrane bioreactor developments have emerged from this initiative. The research involved a number of follow-up projects including an ESKOM TESP programme, and the participation of Peninsula Technikon, Cape Technikon, and the ML Sultan Technikon in a multidisciplinary R&D initiative.

4.8 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.

4.9 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.