The Biotechnology of High Rate Algal Ponding Systems in the Treatment of Saline Tannery Wastewaters.

THESIS

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Abstract

Salinisation has been identified as a major cause of the progressive deterioration in the public water system in South Africa. To deal with this problem Waste Stabilisation Ponding systems have been used by the Leather Processing Industry as zero-discharge wastewater evaporation disposal processes in water-limited inland regions of the country.

While effective in the evaporation disposal function these systems are plagued by the generation of serious odour nuisance creating intractable environmental problems relating to adjacent residential communities. High loading to ponds of organic compounds, sulphides and ammonia results in strongly reducing anaerobic conditions prevailing in early parts of pond cascades. These are characterised by bright red colours due to the predominance of purple photosynthetic bacteria. Sporadic microalgal blooms of *Spirulina sp.* and *Dunaliella sp.* had been previously noted to occur on the latter ponds in these cascades, and were associated with their conversion to facultative function, with aerobic surface layers, and a marked reduction in odour release.

This research programme undertook an investigation of the microbial ecology of a tannery waste stabilisation ponding system to describe factors which give rise to these blooms, and to determine whether microalgal growth may be manipulated to achieve a reliable oxygen-generating capping of the anaerobic ponds. The predominance of near pure cultures of *Spirulina platensis* was demonstrated for the blooms and factors restricting its growth in the system were described. These include the interaction of ammonia and sulphide toxic effects and laboratory studies were undertaken to show how effluent loading may be regulated to enable effective growth of the cyanobacterium. At appropriate dilutions of tannery effluent an enhancement of growth was noted, compared to growth in defined mineral medium. An investigation of this phenomenon provided preliminary evidence for organic uptake by the pond microalgae and a possible contribution to heterotrophic nutrition.

The manipulation of *Spirulina sp.* growth in a High Rate Algal Pond raceway was undertaken in outdoor pilot plant studies and the effect of microalgal capping of the anaerobic ponds in the cascade was demonstrated by activating a recycle loop from a blooming facultative pond.

Heavy metal contaminants were effectively eliminated by an optimisation of the primary anaerobic pond function and precipitation as metal sulphides. Biomass was harvested and dried, during which a range of methods were evaluated. Toxicological studies were undertaken on the dried biomass using *Artemia* and chick assays, and feed studies showed its useful application in rations for the abalone *Haliotus midae* and rainbow trout *Onchorhynchus mykiss*.

Based on positive independent assessment of research outcomes, a decision was made by the tanning company operating the Waste Stabilisation Ponding system, to proceed to the construction of a full-scale 2 500 m^2 High Rate Algal Pond raceway. This would be used for controlled *Spirulina* biomass production to effect a practical capping of the anaerobic ponds in the system, and to evaluate its commercial potential in the feed market. The Advanced Integrated Wastewater Ponding System described by Oswald (1991) provided the conceptual basis for the Algal Biotechnology process development undertaken.

The studies of the microbial ecology and the biotechnological potential of this system have shown that a *Spirulina*-based High Rate Algal Ponding process can be engineered in such a way that saline tannery effluents may be treated to effect a significant reduction in overall pollution load, that biomass may be recovered as a value added product of the treatment process and that the operational performance of Waste Stabilisation Ponding systems, and hence their immediate environment, may be improved by the use of the High Rate Algal Pond as a retrofitted upgrading unit operation.

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This work is dedicated to my parents for all they have done - thanks

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List of Abbreviations

ABP - Algal biomass and Oxygen Release potential

AIWPS - Advanced Integrated Wastewater Ponding System

APE - Anaerobic Pond Effluent

BOD - Biological Oxygen Demand

BSA - Bovine Serum Albumin

CCAP - Culture Collection for Algae and Protozoa

CFU - Colony Forming Unit

chl_a - $Chlorophyll_a$

COD - Chemical Oxygen Demand

DO - Dissolved Oxygen

EC - European Commission

dpm - Disintegrations per minute

DWA - Department of Water Affairs

FAO - United Nations Food and Agriculture Organisation

HPLC - High Performance Liquid Chromatography

HRAP - High Rate Algal Pond

HROP - High Rate Oxidation Pond

HSL - Hide Soak Liquor

ISO - International Standards Organisation

LIRI - Leather Industries Research Institute

MWL - Mossop Western Leathers

PAR - Photosynthetically Available Radiation

PE - Aerated Ponded Tannery Effluent

PTE - Physico-chemically Pre-treated Tannery Effluent

RSA - Republic of South Africa

SEM - Scanning Electron Microscopy

Spirulina - *Spirulina platensis* (used in this study)

SS - Settleable Solids

SRB - Sulphide Reducing Bacteria

TDS - Total Dissolved Solids

TDIS - Total Dissolved Inorganic Solids

TKN - Total Kjeldal Nitrogen

TEM - Transmission Electron Microscopy

UASB - Upflow Anaerobic Sludge Bed

UTE - Untreated Combined Tannery Effluent

WSP - Waste Stabilisation Ponds

Chapter One

The Leather Processing Industry and Algal Biotechnology.

1. Introduction

1.1. Leather Manufacture

Tanneries are by definition industrial processing operations in which raw or semi-processed animal hides and skins are converted into the end-product known as leather, by stabilising the collagen structure of the hide using natural or synthetic chemicals (Hart *et al.*, 1987). The actual tanning process varies greatly based on the type of raw material used and the technique employed. Consequently the effluents from tanneries vary equally widely in concentration of pollutants (Tadesse, 1993). The whole process can be divided into four major production stages namely: the Beamhouse, the Tan Yard, Post-tanning and Finishing. Each stage is accomplished through distinct steps, as outlined in **Figure 1.1.**

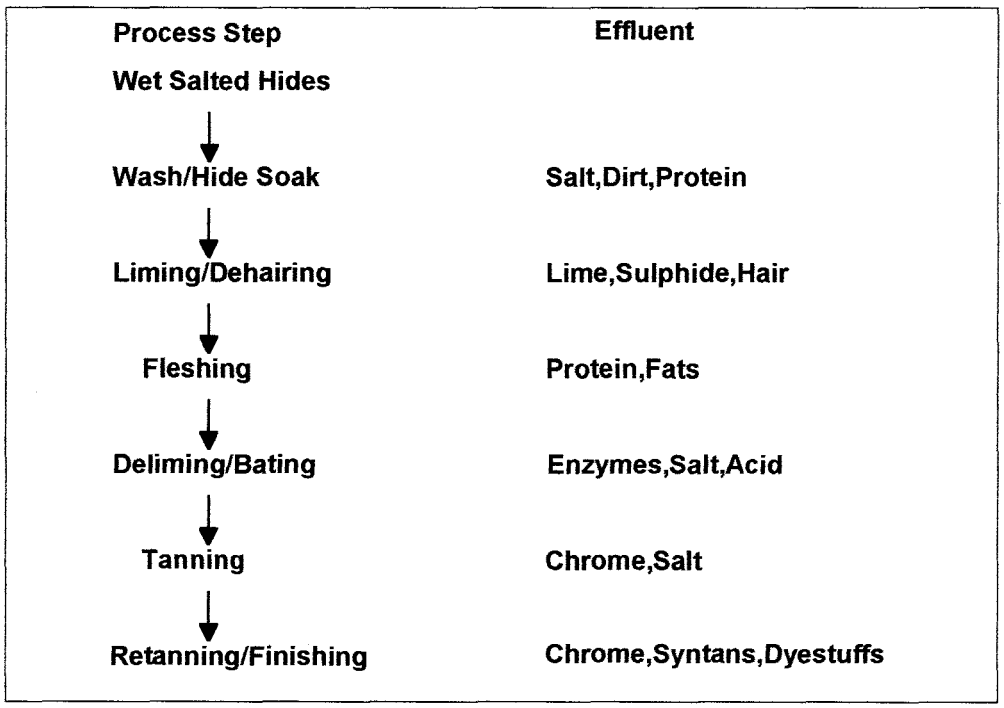


Figure 1.1. Schematic diagram of the tanning process (Laubscher, 1991)

Rawstock in the form of either salted or freshly slaughtered green hides and skins enters the process through a series of washing and soaking steps before passing to depilation which is usually effected in a form of the Lime-Sulphide Dehairing Process. Removal of the epidermis to expose the corium or grain enamel layer is completed in the Deliming and Bating Process where enzymatic reagents are used. Traditionally bating enzymes were sourced from dog faeces (Thorstensen 1984).

Hides may be fleshed and split at this stage and then pass to the Tan Yard where stabilisation of the collagen is accomplished with the use of any of a range of tannages such as chromium and vegetable tannins. In the case of chromium the product of this process is known as Wet Blue Leather. Completing the production of finished leather involves the further steps of Retanning with fatliqouring lubricating agents and the final application of a range of surface coating finishing chemicals (Thorstensen 1984).

1.2. Environmental Problems

Tanneries have been noted as producing the most polluting wastes of any industry (Tsotsos 1986) and Thorstensen (1984) has commented on their unenviable reputation for being "one of the filthiest, evil smelling of industries". In the early part of this century when production was scattered among a large number of small plants, tanneries had a relatively minor environmental impact, owing to the self-purification capacity of the receiving waters and to the statistical and geographical distribution of the plants (Carre *et al.*, 1983). The steady growth of tanneries and changes in processing methods; such as shorter processing times, lower water usage (especially relevant to a water-scarce country like South Africa) and more intensive chemical usage, have resulted in the generation of far more concentrated effluents which are increasingly difficult to treat (Hart *et al.*, 1987 ; Talinli, 1994 ; Genschow *et al.*, 1996).

It is estimated that about 40 million m^3 tannery wastes are discharged into the world's waterways annually (Macchi *et. al.*, 1991; Walsh and O'Halloran, 1996) with a pollution load calculated at 1600 population-equivalents per ton of hides processed (Carre *et. al.*, 1983). The South African Leather Industry processes around 1.8 million hides per annum and in

doing so uses an estimated 500 000 m^3 water through the various stages of the production process (Hart *et al.*, 1987 ; Nel 1997, pers. comm.).

Historically, tanneries were located alongside watercourses which provided both the supply and the route for effluent disposal. Today, any such effluent discharges must comply with either the General or Special Standards provided for by the Water Act No. 54 of 1956, and the Water Amendment Act No. 96 of 1984 (Neytzell-de Wilde *et. al.*, 1992), and thus river discharge no longer presents a practical route for effluent discharge for any South African tannery. The degree of on-site effluent treatment to be carried out at a tannery is determined by whether the treated effluent is to be recycled or discharged to a municipal sewer, evaporation ponds or to land irrigation disposal (Hart *et al.*, 1987).

In South Africa, salinisation, is regarded as the single most serious threat of pollution facing the public water system (Stander, 1987 ; Rose, 1991 ; Herold and Bailey, 1996). The Leather Processing Industry has been identified as one of the main contributors to the rising salinity in the South African public water system (Neytzell-de Wilde *et. al.*, 1992). At present, the only practical technology capable of achieving a final removal of dissolved solids is via membrane processes, such as reverse osmosis and electrodialysis. These are expensive to install and costly to maintain (Hart *et. al.*, 1987), and have not been found to be successful in the tannery effluent application (Neytzell-de Wilde *et. al.*, 1992).

Significant changes in approach to the disposal of tannery effluents and solid wastes by the industry have become necessary due to stricter environmental protection and control measures now being enforced world-wide, the increased costs for effluent treatment by local authorities, the reluctance of some local authorities to accept untreated tannery effluents for disposal and far more stringent requirements for the operation and control of such effluent disposal systems (Hart *et al.*, 1987 ; World Leather, 1996). The costs to the tannery involved in this situation are significant and can affect the overall profitability of the tanning operation. The regulation of pollution controls have resulted in the closing of tanneries in the United States (Thorstensen, 1984), and has contributed significantly to the shrinkage of the industry in many European countries (Rose 1997, pers. comm.). Hart *et. al.* (1987) have noted that the continued existence, or indeed any expansion, of the nationally important economic activities

of the Leather Industry will depend in significant measure on the development of more advanced scientific and technological methods for handling its solids and effluent wastes.

1.3. Cleaner Production Practices

In recent years, there has been a growing trend for manufacturing companies to regulate internal compliance to environmental regulation by implementing environmental and quality management systems such as those developed by the International Standards Organisation (ISO). Many of the local tanneries in South Africa have implemented the quality infrastructure and have obtained ISO 9002 quality management listing. More recently, ISO 14001 has been introduced as a guideline for sound environmental management (SLTC, 1996).

While environmental auditing relates to a specific site, eco-labelling is applied to products. The new European Commission (EC) eco-labelling scheme, aims to provide an official guide to consumers on goods that cause the least damage to the environment. Manufacturers or importers will have their product evaluated against an assessment matrix of the product's life cycle and environmental impact, e.g. water use, water/soil/air pollution, noise and consumption of energy and resources (Alexander *et. al.*, 1992).

The idea of 'clean' technology, although a fairly new concept, has been extensively reviewed over a number of years (World Leather, 1992 to 1996). The emphasis falls on the adoption of low-waste technologies in order to reduce pollution load effectively. The measures range from recycling of useful by-products, process modification and use of less polluting alternative chemicals for recovery of useful substances from spent liquors (Tadesse, 1993).

For example the use of fresh hides directly from the abattoir avoids disposal of salt (World Leather, 1992). The Liricure powder biocide composition for hide and skin preservation serves as another method to reduce salt levels (Russell *et. al.*, 1996). In dehairing, the amount of sulphur based chemicals can be reduced substantially and enzyme-based depilatories can be introduced (SLTC, 1996). Ammonia in deliming can be reduced or replaced with carbon dioxide (World Leather, 1994).

The potential toxicity of chromium based tannages has received significant focus in recent years. Consumption has grown from the first introduction of chromium salts as tanning agents in 1858 and it is now estimated that this application accounts for around 32 % of world trade in chromium compounds (Walsh and O'Halloran, 1996). Toxicity studies are, however, ambiguous indicating either no chromium-related harmful effects among tannery workers (Armienta- Hernendez and Rodrigues-Castillo, 1995) or weak correlation's with chromosomal aberrations (Sbrana *et. al.*, 1991).

Extensive studies have documented the cycling of chromium in soil and water and indicate the rapid biologically-mediated reduction of the highly toxic Cr VI species (not used in tanning) in the presence of sulphide and the fixing and immobilisation of Cr III in natural environments (Bartlett, 1991; Conzomo *et. al.*, 1994 ; Losi *et. al.*, 1994 ; Pettine *et. al.*, 1994).

Nevertheless, chromium remains an emotive environmental issue and eliminating or reducing quantities in the effluent is tackled in two ways. One is by using alternative tanning agents such as aluminium, titanium, and zirconium, with possibly reduced environmental impact. The other is the adoption of high chrome exhaustion and recovery systems where discharge into the effluent is reduced to extremely low values (World Leather, 1992, 1993). Mortalities have been linked to compounds such as benzidine-based leather dyes (Montanaro *et. al.*, 1997) and in finishing, solvent-based coatings and pigments can be replaced by aqueous-based alternatives (World Leather, 1992).

It has been noted, however, that some of the cleaner processing options may be capital-intensive and beyond the reach of tanneries in developing countries. Effective housekeeping measures on the production floor can achieve substantial reduction in pollution loads with little capital investment and also providing surprising cost savings (Tadesse, 1993).

1.4. Tannery Effluents

The composition of tannery effluent depends among many other things on the type of processes in use and on the level of water consumption (Tadesse, 1993). The operations involved in the transformation of hides into leather generate both wastewater and solid wastes as pollution loads at various processing stages (Carre *et. al.*, 1983). The majority of the

pollution load arises from the wastes discharged from the Beamhouse and Tan Yard sections of the tannery (Jackson-Moss, 1990).

Over 80 % of the organic pollution load in terms of Biological Oxygen Demand (BOD) emanates from the beamhouse, although it only comprises 30 % of the total volume of wastewater (Cooper *et. al.*, 1984). Of this, 10 % comes from the soak liquors and over 70 % from the unhairing/liming and deliming/bating liquors. The soak water provides 60 % of the salinity. The beamhouse liquid effluent, often called lime/sulphide liquor, is characterised by high alkalinity and high sulphide content (Tadesse, 1993).

Of special significance in tanneries is that the final, mixed tannery effluent arises batch-wise from various processing operations which generate effluent fractions with widely differing, and, in some cases, chemically interactive compositions (Jackson-Moss, 1990). The typical gross pollutant composition of combined tannery effluents (a mixture of lime/sulphide, chrome and wash liquors) are generally alkaline, contain high levels of organic pollutants, suspended solids, dissolved solids, sulphides, and in some cases chromium (Hart *et al.*, 1987). The principal components of the combined effluent are listed in **Table 1.1.**

1.5. Treatment of Tannery wastewaters

Tannery wastewater is not readily accepted into the domestic wastewater treatment plants of local municipal authorities without some form of pre-treatment, the extent of this being governed by costs and the local situation. In deciding to what degree a particular tannery should treat its effluent, the capital and running costs of its own treatment process need to be balanced against the ability of the local municipal authority to accept this wastewater, and the relative charges for local authority acceptance at the various possible levels of treatment (Rowswell *et. al.*, 1984). These authors have noted that in making long-term planning decisions it should be remembered that local authority charge rates generally escalate over time.

Table 1.1. Composition of typical untreated combined tannery effluent (Tadesse, 1993).

All values in $mg.L^{-1}$ (except pH)	Chrome Tannage	Vegetable Tannage
Ammonia nitrogen	70	70
Biochemical oxygen demand	900	1 700
Chemical oxygen demand	2 500	3 000
Chloride (Cl^{-1})	2 500	2 500
Chrome (Cr)	70	/
Ether extractable	200	200
pH	9.0	9.0
Phosphorus (P)	1.0	1.0
Settled solids (2h)	100	50
Sulphate (SO_4)	2 000	2 000
Sulphide	160	160
Suspended solids	2 500	1 500
Total ash	6 000	6 000
Total nitrogen	120	120
Total solids	10 000	10 000

On-site effluent treatment may be handled in pre-treatment, primary treatment and secondary treatment procedures. Effective pre-treatment prior to the primary effluent treatment processes can result in the installation of more simplified, more efficient and lower cost treatment plants. The three most important forms of pre-treatment are: (1) Removal or oxidation of sulphide ; (2) Chrome recovery and recycling ; (3) Grease removal.

Primary treatment refers to the physico-chemical separation of the suspended solids and some of the organic pollution, allowing these materials to be removed from the effluent. This may be achieved in part by mixing of the acidic and alkaline lime sulphide streams to effect the precipitation of chromium (Kabdasli *et. al.*, 1993). Depending on the type of primary treatment carried out, approximately 95 % of the suspended solids and between 40-70 % of the BOD can be removed (Garrote *et. al.*, 1995). The four major types of primary treatment are: Screening, Balancing and Equalisation, Sedimentation, and Air Flotation.

Tannery effluent, when treated efficiently by primary, physico-chemical processes, can be suitable for discharge to a municipal treatment plant where appropriate dilution is available. However, in circumstances where there is no municipal facility, or when suitable dilution is

not available, further treatment of the tannery effluent may be necessary and is normally effected by secondary biological treatment (Winters, 1986b).

In biological treatment processes, bacterial respiration is the primary method of oxidising organic substances present in the wastewater. This results in a substantial reduction of the effluent BOD and Chemical Oxygen Demand (COD). The four major types of secondary treatment in general use are: Biofiltration, Oxidation Ditches, Activated Sludge, and Ponding.

Overall, the combination of conventional primary physico-chemical and secondary biological treatment processes, when applied at suitable loading rates and in the correct sequences, can substantially reduce the nett pollutant load discharged from tanneries. The treated effluent quality may be good enough, in certain cases, to permit internal recycling of selected streams to some processes, thereby reducing the water and chemical usage and also the magnitude of the resultant effluent problem (Hart *et al.*, 1987).

However, secondary treatments generally do not produce an effluent purified sufficiently for direct discharge to public watercourses. In particular the inorganic dissolved solids content of the effluent remains well above the acceptable limit for discharge to watercourses (Rowswell *et. al.*, 1984). It is for this reason that further downstream processing options need to be considered. Dilution into domestic wastewaters, as has been practised, is not always available and is becoming a less favourable option for tanneries located in the inland regions of South Africa due to increasingly stringent control of inorganic solids addition to the water system. Membrane processes such as reverse osmosis, electrodialysis and ultrafiltration have been intensively evaluated, as has been noted, and found to function poorly in the tannery effluent application due to its colloidal and protein content (Neytzell-de Wilde *et. al.*, 1992). As with many desalination options disposal of the solids in the reject stream largely remains unresolved after treatment (Rose, 1991).

It is in the context of the saline wastewater disposal problem that Waste Stabilisation Ponds (WSP) have been used as zero-discharge evaporation disposal systems by the Tanning Industry in South Africa, and elsewhere in the world. While ponding, for organic load removal, presents a viable alternative to the physico-chemical and intensive biological

effluent treatment processes described, especially where land is available and in remote locations, it also provides one of the few practical options for final disposal of these effluents in circumstances where segregation of inorganic solids from the public water system is required.

1.6. Waste Stabilisation Ponds

Waste Stabilisation Ponds are used in all climatic zones of the world to treat domestic and industrial wastewaters (de Pauw and Salomoni, 1991). They have been defined as reactors constructed through excavation and compaction of earth to create reservoirs capable of holding water or wastewater for predetermined periods of time (Oswald, 1995). A complex interaction of algal photosynthesis, aerobic and anaerobic microbial activity, physical and chemical and climatological factors operate in these systems to degrade the organic load and to achieve a high quality final treated effluent (Pescod, 1996). WSP are operated in many different configurations from single mixed ponds to classic line lagoons where the constituent microbial, physical and chemical processes are separated into a sequence of anaerobic followed by facultative and maturation ponds (Gomez de Sousa, 1987).

Waste Stabilisation Ponds, in one form or another, seem to have a long history of use in many societies and Vuillot and Boutin (1987) note that, in Europe, the deliberate enrichment of fish ponds and farm dams with organic wastes is well documented since the Middle Ages. The first purpose-designed WSP, according to these authors, is the Fischteiche, a 233 ha ponding system built around 1920 as a tertiary wastewater treatment system for the city of Munich, and which still provides a regular harvest of Carp. Similar extensive ponds linking water treatment and fish production are in use in the Czech Republic. Oswald (1988a) notes that ponds came into use in California as early as 1915 as impoundments, in the first instance, preventing intrusion into unwanted locations.

The serious investigation of the role of WSP in wastewater treatment commenced after World War II with early studies reported by Caldwell (1946), Gotaas and Oswald (1954), Oswald *et. al.* (1957) and Oswald and Golueke (1960) identified the role of algal photosynthetic oxygen production and anaerobic microbial processes in the degradation and stabilisation of organic wastes. This resulted in rapid growth in the use of these systems, principally for the disposal

of domestic sewage, but also, increasingly, for the treatment of industrial wastewaters. Boutin *et. al.* (1987) record that, while the first plant was constructed in France in 1965, widespread use of the WSP in that country only followed Health Ministry approval in 1976. A 1987 survey reported some 3600 WSP in Europe and that 1500 plants had, by that time, been built in France alone (Vuillot and Boutin, 1987). At the same time Middlebrooks (1987) reported around 7000 WSP operating in the USA. By 1995 the technology had matured to the extent that attention was being focused on the procedures for decommissioning and rehabilitating ponds that had now become obsolete following construction in the 1960s (Lawty *et. al.*, 1995). Comprehensive symposia on WSP have been edited by Mara and Marecos Do Monte (1987) and Mara *et. al.* (1996).

Early attempts to establish rational criteria for the design of WSP, based on chemical reaction engineering principles, were pioneered in South Africa by Marais and Shaw (1961), Marais (1966) and design applications by Meiring *et. al.* (1968). More recently Mara (1976), Oron and Shelef (1980) and Wood (1986) have contributed to this development. However, by 1987 both Wood and Middlebrooks noted serious discrepancies between the expected and observed performance of these systems and the limitations of the first-order kinetics, mixed reactor assumptions that had been previously made. Banerji and Ruess (1987) compared 20 plants operating in the USA and could find no correlations between designed loading rates, hydraulic detention times and BOD removal in these systems. As a result, and over a wide spread of experience, rules of thumb for the design of WSP have been relied on, quite successfully, and operational input data have been based on empirical observation, the use of area loadings per population unit (Bucksteeg, 1987a/b) and both area and volumetric loading of total BOD (Boutin *et. al.*, 1987 ; Gomez de Souza, 1987).

Reasons for the problems encountered in the predictive mathematical modelling of these systems have been identified as the inadequate description of the complex variables operating in them such as hydraulic residence time distribution, mixing, thermal stratification, pond geometry, and dependence on physical, climatic and biological factors (Finney and Middlebrooks, 1980 ; Pruel and Wagner, 1987 ; Middlebrooks, 1987 ; de Pauw and Salomoni, 1991). Description of some aspects of these variables have been reported by Azov and Shelef (1982), de Pauw and van Vaerenbergh (1983) and Abeliovich (1986).

Middlebrooks (1987) has noted the importance of laboratory-scale studies used to estimate pond performance, especially in the light of the shortcomings of predictive models. Both Pearson *et. al.* (1987) and de Pauw and Salomoni (1991) have identified the critical need for further studies of the complex physical and biological factors operating in these systems in order to derive the simplifying assumptions needed for modelling purposes. A number of studies have reported recent progress in computational modelling approaches to the problem (Marques and d'Avila, 1995 ; von Sperling, 1995).

1.7. Algal Biotechnology

The modern origins of Algal Biotechnology may be dated from the early studies on algal cultivation sponsored by the Carneigie Institute of Washington (Burlew, 1953) and the work of Oswald and co-workers (Oswald *et. al.*, 1957) identifying the role of microalgae (both cyanobacteria and eucaryotic green algae) in the successful operation of WSP (de Pauw and Salomoni, 1991). These workers showed that in addition to providing oxygen for bacterial respiratory function, and also the production of possibly useful biomass, the waste-grown microalgae enhanced the sedimentation of solids, the removal of nutrients, heavy metals and toxic xenobiotics, and the successful disinfection of pathogens present in domestic sewage. Algae also convert incident light to heat at an efficiency approaching 90 %, with the warming effect impacting directly on the kinetics of the treatment process (Oswald, 1988a).

Attempts to intensify the operation of the algal component in a separate unit operation linked to a WSP system led to the development of the High Rate Algal Ponding (HRAP) concept (Oswald, 1963), where a high efficiency of algal performance is achieved in a shallow carousel- type raceway mixed by a slow paddle wheel action. This system is also known as the High Rate Oxidation Pond (HROP), and in a particular configuration of the WSP developed subsequently as the Advanced Integrated Wastewater Ponding System (AIWPS) (Oswald *et. al.*, 1994). (The term HRAP will be used here and the term algae follows conventional usage referring to both the cyanobacterial and eucaryotic microalgae).

Not only was the efficiency of wastewater treatment improved but the production of

microalgal biomass opened substantial possibilities for value-added bioproducts and downstream aquaculture developments. A prodigious research development of these concepts followed over the next three decades and the broad principles of Algal Biotechnology have been the subject of several comprehensive reviews (Shelef and Soeder, 1980 ; Richmond, 1986a/b/c ; Borowitzka and Borowitzka, 1988 ; Cresswell *et. al.*, 1989 ; Lembi and Waaland 1988).

The detailed theory of the operation of the HRAP has been reviewed by Shelef *et. al.* (1980), Azov and Shelef (1982), Abeliovich (1986) and Oswald (1988a/b). The relationship between the photosynthetic and heterotophic populations in these systems has been the subject of detailed reporting (Ganapati, 1975 ; Rodgers and De Pinto, 1981 ; Fallowfield and Garret, 1985 ; Oswald, 1988a) with the heterotrophic uptake of organics by the microalgae studied by Abeliovich and Weissman (1978) and Abeliovich (1980, 1986). Harvesting of microalgal biomass has been reported by Sandbank and Shelef (1987), Mohn (1988) and Rose *et. al.* (1992).

Grobbelaar *et. al.* (1988) have reported on the oxygen production in microalgal mass cultures and the impact on productivity of turbidity, shading and dark/light cycles (Grobbelaar, 1991 and 1994 ; Grobbelaar *et. al.*, 1996). Attempts to link microalgal biomass production and aquaculture have been reported by Ryther *et. al.* (1975), Sandbank and Hepher (1980), de Pauw and van Vaerenbergh (1983) and Mitchell (1986).

Progress in the development of the outdoor mass cultivation of microalgal monocultures has been limited to organisms for which stringent environmental selection can be readily manipulated. The cultivation of the extreme halophile *Dunaliella salina* for the production of β-carotene has been pioneered in Australia by Borowitzka *et. al.* (1984) and Israel by Ben-Amotz and Avron (1989). A novel two-stage cultivation system has been developed in South Africa by Rose and co-workers (Phillips, 1994) and has been commercially developed to pilot-scale. The cultivation of the saline alkalophilic cyanobacterium *Spirulina sp.* has also been commercialised with one of the major installations at Earthrise Farms in California, USA. Cultivation of *S. platensis* in closed photobioreactors has been reported by Cornet *et. al.* (1992 a/b), in winter and in temperate climates by Zitelli *et. al.* (1996) and in integrated culture systems by Pushparaj *et. al.* (1997).

A range of alternatives to the algal raceway as a bioreactor have been evaluated. The use of tubular photobioreactors has been reported by Richmond *et. al.* (1993), Richmond (1996) and thin layer reactors by Livansky and Doucha (1996).

The potential for linking useful microalgal biomass production with nutrient recycling and resource recovery from wastewater treatment focusing on sustainable development technologies and improving human nutrition has been evaluated by Oswald (1980), Fox (1983), Hall (1986), Shelef and Azov (1987), de Pauw and Salomoni (1991), Hung *et. al.* (1996) and Pushparaj (1997). Oswald (1995) has predicted that these objectives, together with the relatively low cost of the pond as a reactor for treating wastewaters, will ensure the continued growth and utilisation of pond technologies in the 21st Century.

The significant developments in Algal Biotechnology recorded above also contributed to further evolution of the WSP and its refinement through the independent optimisation of the component biological processes into, what Oswald (1991) has called, the AIWPS. In this format the anaerobic stage has been optimised in a fermentation pit constructed within the facultative pond, and earth berms are constructed to limit surface water inversions and hence obviating oxygen inhibition of methanogenesis. Carbon dioxide is captured in the upper layers of this pond and together with the partially treated effluent passes to the HRAP located downstream from this unit. High rates of algal growth are achieved and oxygenated water is recycled back to effect odour control over the surface of the primary facultative pond.

The development of the HRAP provides the efficient optimisation of the constituent processes operating within the WSP. Studies on the HRAP at the Haifa Technikon, Israel, have focused on developing the efficient operation of the water treatment function in combination with the production of valuable by-products. Shelef and Azov (1987) report that the biomass has been evaluated in the following applications: protein-rich animal feed, specialist rations for fish larvae and other aquaculture applications, and for the extraction of natural pigments, fatty acids and vitamins. A high quality effluent is produced and these workers have concluded that within environmental and economic constraints it is possible to design an HRAP that will achieve the goals of treatment and by-product recovery in a reliable manner.

1.8. Current Status of Algal Biotechnology

A consideration of developments described above, and possible new applications in Algal Biotechnology, needs to take into account problems identified relating to the slow progress made in certain aspects of the field (Richmond, 1996).

Whereas the use of WSP is in full expansion world-wide, with further development predicted for the next century (Oswald, 1995), the application of the HRAP for wastewater treatment has remained somewhat restricted with a limited number of plants constructed around the world (de Pauw and Salomoni, 1991). The early promise of Algal Biotechnology to provide inexpensive food, renewable energy resources and environmental reductions in CO_2 have also not yet materialised (Grobbelaar *et. al.*, 1996). Richmond (1996) has recalled one of the original objectives of Algal Biotechnology as using abundant solar energy, saline waters and sun-rich arid lands to develop a new mode of agriculture. While advances in the production of speciality microalgal products have been made this is confined to probably less than a dozen commercial enterprises, focusing on the exploitation of three species of microalgae, and mainly for the lucrative health food market (Richmond, 1996).

The reasons for the current status in the development of Algal Biotechnology in general, and the biological and technical constraints operating on the HRAP technology in particular, have been critically addressed by a number of authors (Shelef and Azov, 1987 ; de Pauw and Salomoni, 1991 ; Richmond, 1996). These all focus on the question which has tormented the fairly large group of scientists and engineers who have been studying and developing HRAP for the past forty years: why has such an ingenious process, which epitomises the principles of water and nutrient recycling, and which closes the cycle between waste to primary biomass more efficiently than any other outdoor process, had such a meagre performance in the field. Shelef and Azov (1987) note the further irony that the stripping of nutrients and production of protein rich biomass is accomplished by the HRAP without mechanical aeration and using only solar energy, and producing a high quality effluent not yet surpassed by any other biological or physicochemical water treatment process. The anticipated linkage between waste treatment and the production of speciality microalgal products has also not materialised on any significant commercial scale.

The following issues contributing to this situation have been identified by these authors:

1. The key problem of economic harvesting of biomass has not yet been satisfactorily solved and the total suspended solids may be higher in the final, than in the secondary, effluent due to algal growth (D'Sousa *et. al.*, 1997a);

2. The technical complications of harvesting algal biomass tend to label the HRAP a sophisticated operation requiring considerable investment. Engineers in the field do not consider this a low-technology option (de Pauw and Salomoni, 1991);

3. The process is limited to geographic regions with the appropriate climate and land availability.

4. The use of waste-derived by-products for animal feed has not found favour in countries such as the USA (Shelef and Azov, 1987);

5. The R&D investment in the field has been dispersed, short term and strangled because of lack of funds. Demonstration plants have not been erected and operated for long enough to establish engineering parameters and economical feasibility (Shelef and Azov, 1987);

6. The open raceway has become the standard device for the mass cultivation of microalgae outdoors and, while requiring simple technology to construct and maintain, its inefficiencies have impeded the further development of industrial microalgal culture. This relates to the lack of temperature control, the long light path of about 15 cm (which, in turn, limits production to very dilute culture suspensions, and yields far below those theoretically achievable) and problems of maintaining monoalgal cultures which restricts production to a few species which thrive in extreme aqueous environments (Richmond, 1996).

It is apparent that, while an attempt has been made to answer these difficult questions, not all of the above reasons advanced are entirely valid. For example in certain applications separation of algae can be simply accomplished by sedimentation, final removal of algal suspended solids is achievable using methods currently employed in large WSP

installations and waste-grown biomass is already utilised to a degree in some countries.

Additional factors need to be taken into account in deriving a balanced view of the state of what is, after all, a relatively young Biotechnological discipline. The field shares in common, with other areas of Biotechnology, the slow developments associated with the "technology-push" approach adopted in its early stages of research development. In most cases rapid expansion of technological applications has followed where the development of real "market-pull" forces have materialised. This has in many cases been separated from early research efforts by several decades and is not unnatural within the context of managing technology development life cycles (Rose 1997, pers.comm.)

The different areas of potential application of Algal Biotechnologies, although apparently related in some ways, need to be separately defined and independent objectives identified in each case. Although it developed first, the WSP may be seen as a particularly successful subset of the wastewater treatment Microalgal Biotechnology application. It will be argued in this thesis that the difficulty faced by the HRAP application in competing with the simpler WSP relates mainly to its use for domestic wastewater treatment. Where particularly difficult problems occur, such as happens in the treatment of industrial effluents, the HRAP finds an role as a "specialist application" of microalgal ponding treatment technologies. These applications require separate definition and independent focus in determining research and development focus.

It is also evident, as has happened in other areas of the Environment Industry, that market forces are moving to accommodate a fairly profound shift taking place from consumptive to sustainable modes of manufacturing production. As accounting practice is forced to take a broader Life-cycle Assessment approach to economic activity it is likely that "market-pull" forces will begin to operate on enabling technologies which efficiently couple nutrient recycling and resource recovery with the basic requirements of cost-effective wastewater treatment (OECD, 1996).

1.9. Algal Biotechnology and "Specialist" Effluents

The term "Specialist" effluent is used here loosely to describe mainly industrial streams or other concentrated process-derived wastewaters which may present significant challenges to conventional domestic wastewater treatment systems. These effluents are generally complex and composed of mixed process streams with conflicting physical, chemical and biological interactions. Where these need to be handled separately, and outside the domestic wastewater system, very specific treatment plant design criteria are required. Technologically complex, and often costly, treatment systems are designed and built and require high levels of operator competency in their management. These are often small units operating entirely independently from one another and without the cost, and operational, advantages of scale. Often the ability to operate these systems determines both the establishment and the continued trading of such enterprises.

The decentralised treatment of such wastewaters at low cost both in terms of capital investment and operating requirements seems to offer a specific niche application for the HRAP technologies in the water treatment field which is quite apart from the domestic wastewater treatment market. Oswald (1988b) reports cost comparisons for HRAP where construction and operating costs are respectively 40 % and 30 % of conventional activated sludge systems. These observations have been confirmed in independent studies of an Oswald-designed AIWPS constructed and operated in Grahamstown (Rose 1997, pers. comm.).

Studies on the application of the WPS with, or without, an associated HRAP operation and downstream biomass application, have been undertaken and scale evaluations reported for a range of "specialist" effluents including: abattoir (Duarte *et. al.*, 1987) ; cannery (Gaicher *et. al.*, 1982) ; cattle and dairy wastes (Mitchell and Richmond, 1988 ; Kilani, 1992) ; rettery wastewaters (Bartoszewski and Bilyk, 1987) ; piggery (Rodrigues and Oliviera, 1987a) and tomato concentrates (Rodrigues and Oliviera, 1987b).

1.10. Tannery Wastewaters as "Specialist" Effluents

It is in the context of the application studies outlined above that wastewaters produced by tanneries are termed "specialist" in this study. It will be argued that the tannery wastewater system provides a useful model for evaluating the potential for treatment of "specialist effluents" by microalgal wastewater treatment processes. It should be noted, however, that in the case of tannery effluents this is not merely a case of looking for some probable further practical application for algal technologies, but is driven by certain internal consistencies in the tannery WSP which will be identified in this report.

In South Africa the use of WSP for treating tannery effluent was proposed by Shuttleworth of the Leather Industries Research Institute (LIRI) at Rhodes University (Shuttleworth, 1978). One of several WSP which were constructed is that currently in operation at Mossop Western Leathers, Wellington in the Western Province of South Africa. The 13.7 ha ponding system was commissioned in 1964 and has been operated as a zero-discharge or containment evaporation process. By 1990 daily effluent production had nearly doubled and, together with a Department of Water Affairs (DWA) requirement that all rainfall and seepage runoff from the site should also be disposed to evaporation, the system was operating in a severely negative water balance. Accumulation of sludges in the receiving ponds and undegraded organics throughout the system was evidence of a poorly functioning biological process and one which, under certain weather conditions, gave rise to substantial odour release and serious offense to the local community.

Few rigorous studies of WSP systems treating tannery wastewaters have been reported in the literature. Shuttleworth (1978) and Rowswell *et. al.* (1984) have reported on the operation of WSP treating leather wastewaters and Jackson-Moss (1990) undertook a study of the anaerobic digestion processes operating in them. Apart from severe odour nuisance identified with the operation of these systems, and which is related to sulphide release (Guidotti, 1996), problems include build up of solids and salts (Winters, 1986a/b ; Jackson-Moss, 1990) which renders the pond water unsuitable for recycle to beamhouse operations, as had originally been suggested by Shuttleworth in the 1950s. Lalitha *et. al.* (1994) has also reported on the anaerobic digestion of solid tannery wastes.

It was against this background that, in the middle 1980s, Rose and co-workers at Rhodes University, Grahamstown, undertook the further investigation of observations they had made of blooms of microalgae occurring at apparently irregular intervals in the Wellington WSP system (Rose *et. al.*,1992). These were found, on different occasions, to consist of nearly pure microalgal monocultures of either *D. salina* or *Spirulina sp.* (depending on location in the ponding cascade) and including a range of photosynthetic bacterial forms (Rose *et. al.*, 1992). The *Spirulina* isolate has been provisionally identified as *Spirulina platensis* and , unless otherwise noted, reference will be to this species. *Dunaliella* refers to *D. salina* unless otherwise noted. Harvesting of the *D. salina* using cross-flow ultrafiltration was evaluated and laboratory studies on the potential role of *D. salina* in a microalgal HRAP treating tannery wastewaters was reported by Laubscher *et. al.* (1990), Laubscher (1991), Rose (1991), and Rose *et. al.* (1992).

The studies on *D. salina* reported above gave rise to an investigation of the stress regulation mechanisms operating in this organism and their manipulation to enhance the productivity of β -carotene production (Cowan and Rose, 1991 ; Cowan *et. al.*, 1995 ; Logie, 1994 ; Phillips, 1995 ; Phillips *et. al.*, 1995). This led to the development and patenting of a two-stage open pond production process which has been scaled up to commercial pilot plant evaluation by Sasol Co. in Upington, South Africa (Rose *et. al.*, 1992 ; Phillips, 1994).

The above studies showed that despite the demonstrated technological competence of a *Dunaliella*-based HRAP for treating tannery effluents commercialisation of the final β-carotene product would be targeted on the pharmaceutical market and thus required cultivation in a pure culture medium. Production for the animal feed market remained a possible avenue but has not been persued. The use of the process purely for the treatment of hypersaline media to remove organic contamination is currently under evaluation by Laubscher in Botswana.

Maart (1993) undertook the evaluation of *Spirulina* biomass harvested from the Wellington WSP. Toxicological and feed studies in a range of animals indicated the suitability of its use in aquaculture and other feed rations (Rose *et. al.*, 1996) and Brits (1996) reported its use in the development of a novel ration for abalone (*Haliotus midae*) aquaculture. The use of this feed, which includes Wellington *Spirulina* biomass, has been commercialised in an abalone

cultivation operation in Hermanus, South Africa (Brits 1997, pers. comm.).

1.11. Research Objectives

The study to be reported here is based on the preceding work noted above relating to an Algal Biotechnological approach to the treatment of saline tannery wastewaters, and it concentrates, in the main, on the research and development of a *Spirulina*-based HRAP process. The commencement of this study was contingent upon a positive outcome to the technical evaluations of the *Spirulina* biomass recovered from the ponds (as reported by Maart 1993) to ensure that the quality of the waste-grown biomass would not be an impediment to the practical relevance, and a potential "market-pull" outcome, to the programme of scientific and technological development which was to follow.

Since no detailed studies of the biology, the hydrology and the performance of tannery WSP were found to be reported in the literature it was considered necessary to undertake such an investigation at the outset. This prerequisite was accorded particular importance in the light of the injunction of Pearson *et. al.* (1987) that the further development of a functional predictive approach to the management of the WSP is dependent on a detailed understanding of the complex factors that operate in them. It was considered necessary that such insights into pond operation should include an understanding of the growth behaviour of the principal organisms present in the system. The subsequent components of the investigation were to be determined by these research outcomes.

Hypothesis

It was against this background that the research hypothesis for this study was formulated as follows:

A *Spirulina*-based High Rate Algal Ponding system can be engineered in such a way that saline tannery effluents may be treated to effect a significant reduction in the overall pollution load, that biomass may be recovered as a value-added by-product of the treatment process and that the operational performance of WSP, and hence their immediate environment, may be

improved by the use of the HRAP as a retrofitted upgrading unit operation.

Objectives

The research objectives of the study were identified as follows:

1. To determine and attempt to describe the principal physical, chemical and biological factors operating in a WSP cascade treating tannery wastewaters. The generation of a functional descriptive model should provide an initial step for subsequent predictive mathematical modelling of these systems based on rational design criteria;

2. To determine the role played by the principal microorganisms growing in the system and to attempt to explain the adaptive mechanisms used by them to survive in the pond environment;

3. To demonstrate whether a *Spirulina*-based HRAP can be developed as a functional process for the treatment of tannery wastewaters. This would need to focus in the first instance on laboratory and pilot studies;

4. To determine whether such a system can be scaled-up to operate as a functional Algal Biotechnological waste treatment process in the tannery wastewater application.

Chapter Two

The Biology and Performance of Tannery Waste Stabilisation Ponds.

2.1. Introduction

The use of waste stabilisation ponds in the treatment of sewage has been well documented (Mara and Marecos Do Monte, 1987), and extensive studies of both performance and design criteria have been reported (Pearson *et al.*, 1987 ; Lansdell, 1987). However, there is a distinct lack of literature regarding both the performance and biology of waste stabilisation ponds used in the treatment of tannery effluent, especially where zero discharge systems are concerned. The construction of WSP have been recommended by LIRI for several South African tanneries where climatic conditions were favourable and sufficient land was available, as being a viable economical alternative to conventional treatment techniques for the disposal of untreated tannery effluent (Rowswell *et al.*, 1984).

The Mossop Western Leathers Company, located in Wellington, R.S.A., rely on a closed WSP for secondary biological treatment and final disposal of all effluent generated in their leather production processes (Rose, 1991). These ponds became the focus of investigations conducted over a number of years by researchers in the Department of Biochemistry and Microbiology at Rhodes University, Grahamstown. These studies were the first to suggest that either the *D. salina* or *Spirulina sp.*, found in these ponds, could possibly be used in an application of the HRAP process for the treatment of tannery effluent (Laubscher, 1991 ; Rose *et al.*, 1992 ; Rose *et al.*, 1995).

The initial observation that a salinity gradient existing across the treatment series allows for the growth of both purple and green sulphur bacteria, as well as, non-sulphur photosynthetic bacteria in the early stages of the ponding cascade, and prolific seasonal blooms of both *Spirulina* and *Dunaliella* in the more saline, alkaline latter stages (Rowswell and Rose, 1990 ; Rose, 1991 ; Maart, 1993), was the first attempt to understand the complex biology of this type of system. The observations suggest that the saline tannery effluent not only supports the growth of these micro-organisms but in fact provides the conditions required for the species

selection and dominance patterns noted by these authors. In order to proceed further with the potential development of the HRAP technology for the treatment of tannery effluent, a more comprehensive understanding of the function, and in particular the biology, of the existing WSP system was identified as requiring further detailed investigation.

2.2. Research Objectives

A survey of the WSP system in Wellington would be undertaken in order to describe the biology of the system and the operational factors which determine performance and function of the existing WSP.

2.3. Materials and Methods

2.3.1. Pond Sampling Procedures

Pearson *et. al.* (1987) have reported a comparative evaluation of the reliability of water column sampling and grab samples drawn at various points and at different times in WSP, they found that individual samples representative of the entire depth of the water column, taken at any time of the day, provided reasonably accurate mean daily effluent values for all the parameters measured. This was not found to be the case where individual grab samples were drawn.

Given the particular circumstances prevailing in the tannery WSP, and the need to take into account algal surface growth and pronounced rafting phenomena not considered in the Pearson's study, three different methods of drawing samples from the ponding system were used. These were executed as follows to take into account variations in the area and depth of the ponds together with constant changes in the surface rafting phenomenon and upwellings caused by wind action:

1. Representative samples were drawn at a number of points across each pond, at various depths, and then combined to form what was called the Composite Grab Sample. The sampling sites were selected subjectively with the aim of acquiring a representative sample of

the overall status of the pond at any one time, and taking into account the variable factors such as biomass rafting and surface and subsurface changes due to wind action.

2. Three fixed sampling points were also identified in each pond and vertically stratified points in the water column were sampled at the same place at various times of the year and disregarding any observed changes in the system. Each sample was separately analysed (as described below) and the averaged results reported. These were called the Pond Strata Samples.

3. Where photosynthetic productivity measurements were made the ponds were divided into a grid pattern, CO_2 fixation was measured (as described below) repeatedly at the same points in the grid throughout the study period. Each sample was separately analysed and the averaged results reported.

Unless indicated otherwise results are reported as the mean of at least 3 readings. Standard deviations have been calculated in each case and are reported where appropriate. Standard deviations have been excluded in multi-plot graphs to avoid confusion.

2.3.2. Analysis of Pond Samples

Pond samples were collected for analysis, throughout the three year study period providing measurement of physical and chemical parameters representative of the range of seasonal variations in Wellington. Both composite grab sampling and the drawing of pond strata samples were performed in all the WSP using an ELE International EL521-020 combination sampling and measuring kit. The following physical parameters were recorded: temperature, Forel-Ule Index number, odour, dissolved oxygen (DO), pH, salinity, and light intensity.

The temperature was measured using a standard thermometer which was placed in a chamber in the sampling device. The Forel-Ule Index number indicating colour changes across the system was monitored following the method and using the colour charts supplied by ELE International. The odour emission from the WSP was rated subjectively using the following scale: +++ seriously offensive, ++ noticeable, + not unpleasant, 0 none.

The level of DO in the wastewater was determined using a YSI Model 57 Dissolved Oxygen Meter. The DO readings were automatically temperature compensated for solubility of oxygen in water and permeability of the probe membrane. The standard tables for the prediction of equilibrium DO concentrations in salt lakes dominated by sodium chloride were used for applying a salinity compensation factor (Sherwood *et al.*, 1992).

The pH of the wastewater was determined using a Hanna Instruments, 8520 pH meter, standardised at pH 4.0 and 7.0 with SAARCHEM standard buffer solutions.

The salinity of the wastewater was determined using an Atago Co. Ltd. Cat. No. 2441, Salinity Refractometer. In certain instances the electrical conductivity of the wastewater was determined using a Hanna Instruments, HI 8633 conductivity meter. This instrument was calibrated with standard solution HI 7034 at 25 °C supplied by SAARCHEM.

The light intensity recorded as photosynthetically available radiation (PAR) was measured using a Skye Instruments Ltd. SKP 210 'Special' light sensor together with an intelligent base unit model SDL 2580 and Skye Instruments Ltd. computer software. The attenuation of light was determined using the Secchi disc method and the values for the light intensities at different depths calculated using the formulas as described by Ramus (1985).

Analysis of the following chemical parameters was undertaken on the WSP water samples using methods as described in A.P.H.A. Standard Methods (1989) and by Jackson-Moss (1990) : chemical oxygen demand (COD), chloride as Cl^{-1} , ammonia as NH_3, nitrate as NO_3, phosphorus as P_2O_5, settleable solids (SS), sulphate as SO_4, sulphide as Na_2S, total alkalinity, total dissolved solids (TDS), total dissolved inorganic solids (TDIS), total Kjeldal nitrogen (TKN). All samples were analysed following filtration through 0.45 μm GF/A filters (Whatmans) to remove suspended biomass and particulate matter.

Biological measurement included microscopic observation of all samples with cell counts being performed using an improved Neubauer haemocytometer. Bacteria present were tentatively identified to the genus level on gram stained samples and using morphological descriptions from Bergy's Manual (Stanley *et. al.*, 1989). Algae and cyanobacteria were identified to genus level using type strains from the Culture Collection for Algae and

Protozoa (CCAP), descriptions in A.P.H.A. Standard Methods (1989) and in sources referenced elsewhere in the text of this report. Chlorophyll$_a$ (chl$_a$) was measured according to the method of Lichtenthaler (1987) and total biomass content A.P.H.A. Standard Methods (1989).

Biogas production in pond A was measured using a specially designed inverted funnel with an attached tube connecting the funnel to the surface. Carbon dioxide and methane gas levels were measured by gas chromatography on a capillary column in a Chrompak 101.

2.3.3. Determination of Photosynthetic Productivity

Photosynthetic productivity studies were undertaken in the WSP at various times during the year. The productivity was measured over several seasons and at different depths to allow for both seasonal variation and vertical stratification of the photosynthetic biomass in the effluent ponds. Productivity was measured using a [^{14}C]-sodium bicarbonate labelled CO_2 fixation method as described in A.P.H.A. Standard Methods (1989) and modified according to Lewis and Smith (1983) and Oren (1992). Wastewater cultures (10 mL) from the ponds were dispensed into 15 mL screw-capped tubes to which [^{14}C]-sodium bicarbonate (Amersham) was added at a final concentration of 0.1 μCi.mL^{-1}. The sample tubes were suspended from polystyrene floats at different depths in the waste stabilisation ponds and exposed for 24 hours. On completion the samples were treated with formalin (0.3 mL) to stop any further biological reaction, acidified to remove any unfixed carbon, and 1 mL aliquots were filtered through 0.45 μm GF/A filters (Whatman). The filters were allowed to dry, following which 10 mL aqueous scintillation fluid (Packard) was added and measurements made using a Beckman LS3150T scintillation counter. As an indicator of productivity, the alkalinity of the ponded effluent was determined using a modified method as described in A.P.H.A. Standard Methods (1989).

2.3.4. Photosynthetic Bacterial Activity

The photoautotrophic and heterotrophic activity of the purple photosynthetic bacteria present in the WSP system was monitored. Samples of anaerobic pond effluent containing

photosynthetic bacteria were drawn in 100 mL bottles, filled to the brim and sealed to maintain anaerobic conditions. On transfer to the laboratory, samples were split into three 500 mL stoppered conical flasks and treated as follows: Air (21 % oxygen) was sparged continuously through the first flask, nitrogen was sparged continuously through the second flask, and finally a 10 % inoculum of *Spirulina* was added to the third flask to simulate cyanobacterial photosynthetic oxygen production. The flasks were incubated in a constant environment room at 25 °C, and a light intensity of 158 μmoles.m^{-2}.s^{-1}. The change in pigment concentrations of both the photosynthetic bacteria and the *Spirulina* were followed over 5 days by extracting their respective pigments in 100 % acetone and measuring the absorbance on a Shimadzu spectrophotometer. Plate counts were performed to establish the numbers of purple photosynthetic bacteria present using the method described by Malik (1983), while cell counts to monitor the *Spirulina* were made in an improved Neubauer haemocytometer.

2.4. Results

2.4.1. Waste Stabilisation Ponding System

Mossop Western Leathers operate a wet blue leather production plant currently processing 1500 hides.day^{-1}. All the effluent generated on site is collected and pumped to a physico-chemical pre-treatment facility. Several unit operations are conducted in order to reduce the pollution load including grease removal, sulphide oxidation, balancing/aeration and solids removal by flocculation and settling. A zero-discharge, closed facultative WSP system operates for secondary biological treatment and final disposal of all effluent generated in their leather production processes.

The ponding system commissioned in 1964 was designed by Civil Engineering Consultants Ninham Shand Inc., to accept an effluent flow of 270 m^3.day^{-1} which included a 33 % over-design as freeboard. In the late 1980's capacity was upgraded to accept 500 m^3.day^{-1}. The WSP (**Figure 2.4.1.**) constitute a surface area of 13.7 ha., have a total capacity of 197 165 m^3, and vary in depth from 0.5-4.0 m.

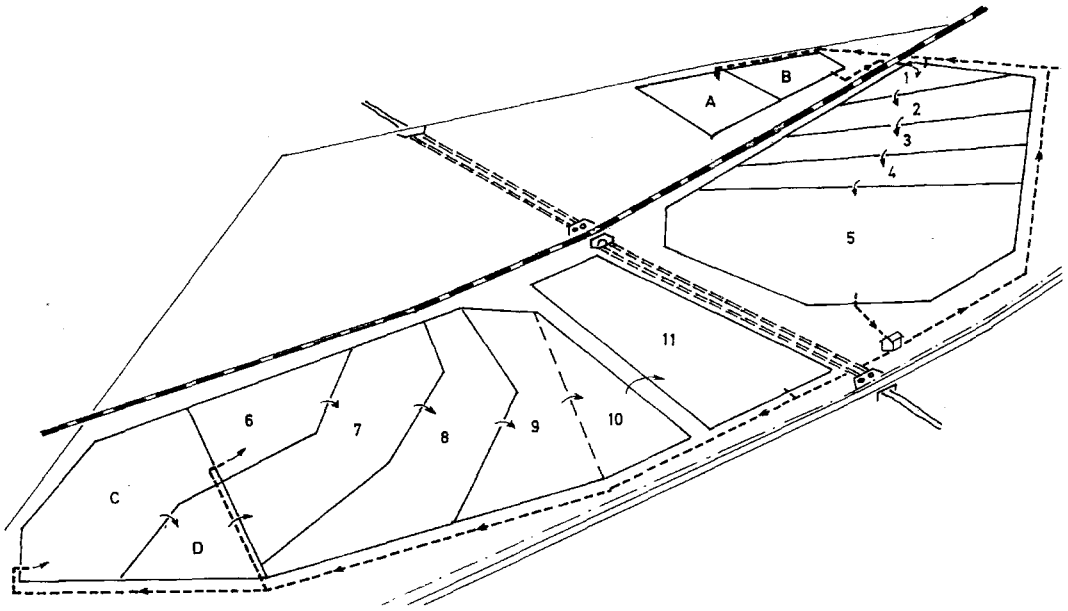


Figure 2.4.1. Schematic diagram illustrating the operation and direction of flow through the waste stabilisation pond cascade at Mossop Western Leathers, Wellington.

The physico-chemically pre-treated tannery effluent (PTE) is pumped initially to pond A, from where the effluent is circulated gravitationally through ponds B and 1-5 and to a sump. From this point it is pumped to pond 6 and from here the effluent is again circulated gravitationally through ponds 7-11. Ponds C and D serve as an additional side loop to the system. By the end of 1990 effluent production had risen to 455 $m^3.day^{-1}$ and together with a requirement by the DWA that all seepage and runoff from the entire waste management site as well as a significant portion of the 40 ha. tannery site be disposed of by evaporation, the system was operating in a severe negative water balance.

Although there is a certain level of treatment through the system (as reported in **Table 2.4.1.**), in general the overall performance is not consistent throughout the year. The current situation of severe hydraulic and organic overload, accumulation of sludge in the receiving ponds, highlevels of organics in the stabilisation ponds and odours providing offense to the local community over many years combine to indicate a poorly functioning biological process; and are typical problems experienced when using WSP to treat tannery effluent (Rose, 1991).

Table 2.4.1. Analytical values of effluent in composite grab samples drawn at various points across the Wellington waste stabilisation ponding system. Results reflect the mean of 3 sets of samples.

All values in $mg.L^{-1}$ except pH	Raw effluent	Settled effluent	Pond A effluent	Pond 5 effluent	Pond 6 effluent	Pond 11 effluent
Ammonia as NH_3	940	925	764	119	14	30
COD	8,044	3,173	1,722	522	450	1,677
DO	< 0.005	< 0.005	< 0.005	< 0.005	3.98	4.71
pH	7.4	7.4	8.3	8.6	9.2	9.5
Phosphate as PO_4	30	15	7	7	7	12
Sulphate as SO_4	975	364	<1	715	1,365	943
Sulphide as Na_2S	1,065	1,192	500	28	<1	6
Total alkalinity as $CaCO_3$	380	525	640	1,246	1,615	2,585

2.4.2. Overview of Waste Stabilisation Pond Operation

The first stage of the investigation of pond operation involved the collection of composite samples from each of the WSP over the three years of the study in order to establish a general overview of the function and performance of the system. Despite observable variation due to both the seasonal fluctuations, prevailing meteorological and environmental conditions, and the effluent pumping regime employed in the operation of the ponding cascade, a number of clearly definable trends emerged through the course of the study which are reported in the following tables and figures.

Physical performance data for the WSP, averaged over several seasons and based on the composite sampling programme, are reported in **Table 2.4.2.** The results of the Forel-Ule Index number measurement demonstrated a constant distribution of water colour through the system. The brown (No. XVIII) tannery effluent discharged from pond A is strongly anaerobic immediately below the first few millimetres of the surface layer, and assumes a pink to dark purple colour in ponds 1 to 5 (No. XXI) which correlates with the presence of the purple sulphur and non-sulphur bacteria *Chromatium* and *Rhodobacteria* species. From pond 6 onwards green colours (No. XIII) prevail which correlate with huge blooms of *Spirulina* and in places also *Dunaliella salina* and *Dunaliella viridis*. The colour profile correlates closely with changes in the pH, salinity and DO levels. The pH increases from 8.3 in pond A to 9.4 in pond 11, and in each individual pond remains relatively stable throughout the year (see **Figure 2.4.2.**).

Table 2.4.2. Analysis of waste stabilisation ponded effluent measured in composite grab samples drawn over a 3 year period. Results reflect the mean of at least 3 readings. Standard deviations in brackets.

Pond number	Forel-Ule Index number	Odour	pH	salinity ($g.L^{-1}$)	DO ($mg.L^{-1}$)
A	XVIII	+++	8.30 (0.33)	12.00 (2.00)	< 0.005
B	XIX	+++	8.46 (0.46)	11.71 (1.89)	< 0.005
1	XIX	+++	8.16 (0.17)	12.13 (3.00)	< 0.005
2	XXI	+++	8.25 (0.24)	13.71 (2.56)	< 0.005
3	XXI	+++	8.40 (0.29)	15.77 (5.34)	< 0.005
4	XXI	++	8.49 (0.31)	22.30 (19.44)	< 0.005
5	XXI	++	8.59 (0.26)	17.97 (5.28)	< 0.005
C	XII	+	9.23 (0.23)	25.10 (6.25)	4.07 (2.65)
D	XIII	+	9.37 (0.24)	37.68 (12.07)	-
6	XV	+	9.13 (0.45)	18.78 (7.05)	-
7	XIV	+	9.16 (0.29)	22.98 (8.96)	4.17 (3.11)
8	XIII	+	9.24 (0.27)	60.66 (44.91)	-
9	XII	0	9.42 (0.29)	55.70 (46.91)	-
10	XI	0	9.37 (0.28)	58.64 (51.83)	-
11	XIII	0	9.40 (0.22)	53.46 (12.50)	4.71 (1.06)

(+++ seriously offensive, ++ noticeable, + not unpleasant, 0 none.)

The salinity increases across the ponding system from 12 $g.L^{-1}$ in pond A to 53.5 $g.L^{-1}$ in pond 11. This is due in part to the remineralisation of the organic content of the tannery effluent by the metabolic activity of the microflora in the ponds, resulting in the production of salts such as bicarbonate (see pH elevation in **Figure 2.4.2.**), together with the high evaporation rates in the summer months which also leads to a rise in the level of inorganic compounds.

The nett result is an increase in the salinity and TDS of the tannery effluent through the WSP system. There is a fluctuation in the level of TDS in the ponds corresponding to seasonal changes (see **Figure 2.4.3.**) where lower TDS levels in July and October correspond with the winter rainfall season.

Degradation of the organic content of the tannery effluent by microbial and chemical action leads to a sequential decrease in COD through the WSP system, as illustrated by the July and October readings shown in **Figure 2.4.4.** However, COD levels are sustained over the summer period and a net increase occurs during the mid-summer period (January) when

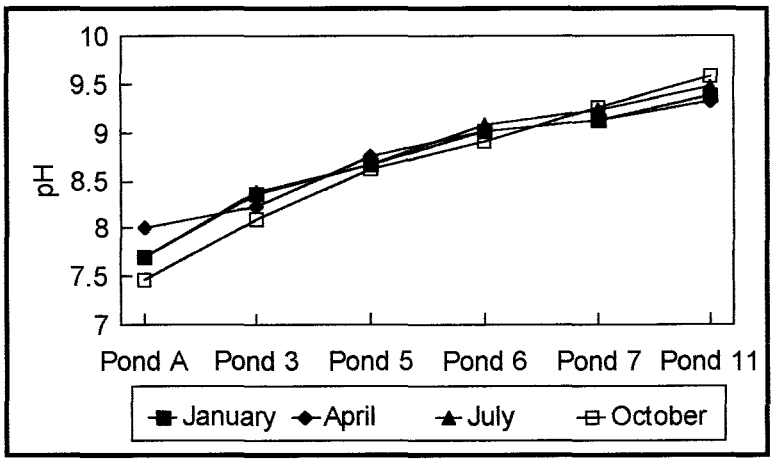


Figure 2.4.2. Changes in pH measured in composite grab samples drawn across the waste stabilisation ponds at different seasons over a 3 year period.

blooms of photosynthetic organisms are observed **Figure 2.4.4.** This provides a provisional indication of the release of organic compounds during bloom conditions. This change in the organic load correlates with the release of soluble nitrogen in the early part of the system which is then incorporated into microbial biomass leading to a subsequent decrease in soluble nitrogen in the latter stages of the system, as illustrated in **Figure 2.4.5.**

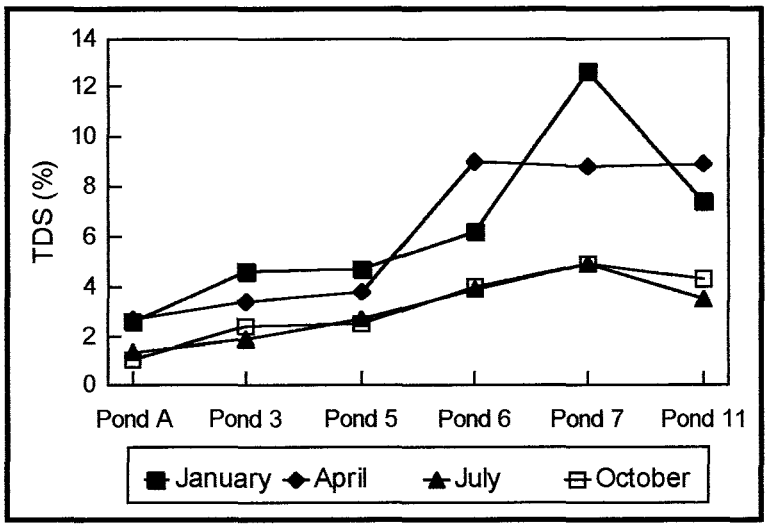


Figure 2.4.3. Changes in total dissolved solids measured in composite grab samples drawn across the waste stabilisation ponds at different seasons over a 3 year period.

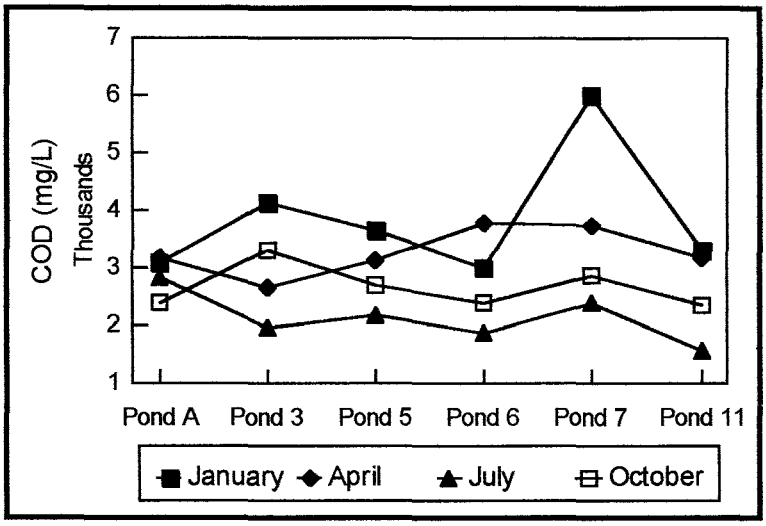


Figure 2.4.4. Changes in chemical oxygen demand measured in composite grab samples drawn across the waste stabilisation ponds at different seasons over a 3 year period.

The level of DO increases through the system from 0.005 $mg.L^{-1}$ in pond A through to 4.7 $mg.L^{-1}$ in pond 11, as shown in **Table 2.4.2.**, indicating increasing photosynthetic activity and leading to a change from anaerobic to aerobic conditions in the latter part of the system. This increase in the level of DO results in a commensurate decrease in the levels of reduced substances such as hydrogen sulphide in the tannery effluent (see **Figure 2.4.6.**). Odour

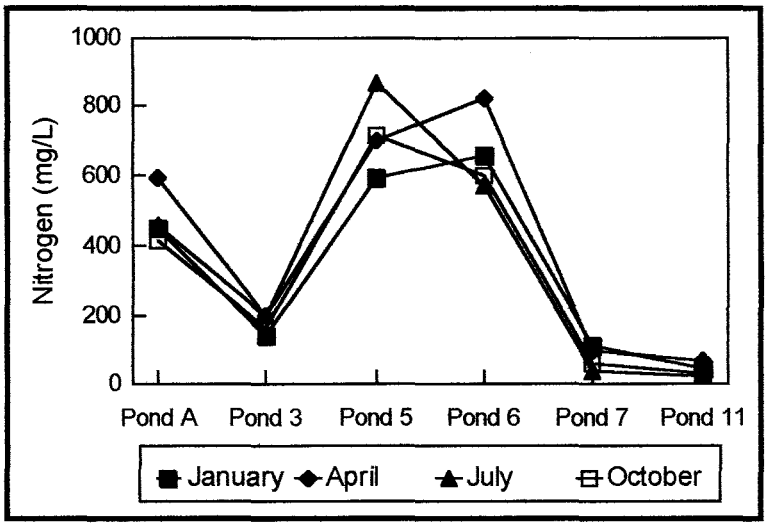


Figure 2.4.5. Changes in total soluble nitrogen measured in composite grab samples drawn across the waste stabilisation ponds at different seasons over a 3 year period.

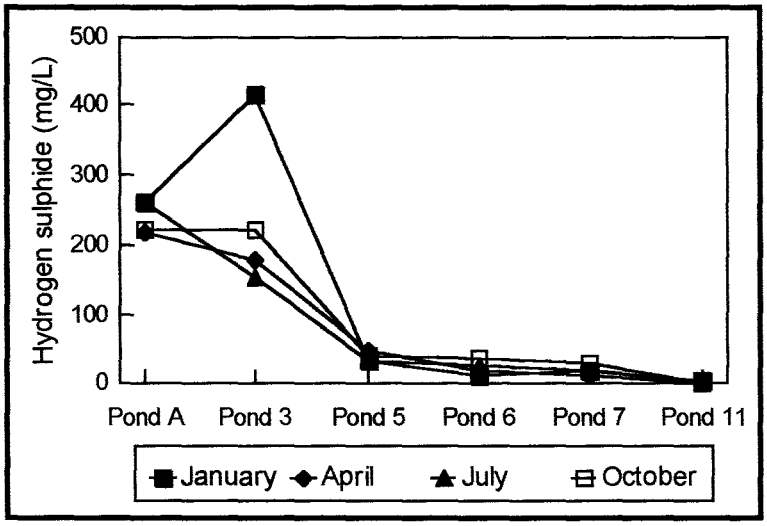


Figure 2.4.6. Changes in hydrogen sulphide measured in composite grab samples drawn across the waste stabilisation ponds at different seasons over a 3 year period.

emissions, mainly attributed to ammonia, hydrogen sulphide, and mercaptan release, decrease as the pH and DO concentration increases through the ponds with little or no odour noted towards the end of the system.

2.4.3. Water Column Sampling

The measurement of pond performance based on analysis of water column (pond strata) samples, drawn from fixed points through the course of the 3 year study, largely confirmed the picture that had emerged from the composite sample study. The entire ponding system is constantly in a state of dynamic flux as regards the microbial populations that inhibit this niche. A combination of factors lead to the dominance of certain species at certain stages and at various seasons in the WSP. The pond cascade may be divided into two distinct systems anaerobic and facultative based on the distribution of dissolved oxygen within the ponds. Results are reported below for the anaerobic and the facultative group of ponds.

2.4.3.1. Anaerobic Ponds

Ponds A to 5 are all highly anaerobic with dissolved oxygen limited to the air water surface

Table 2.4.3. Analysis of anaerobic pond strata. Data collected over a 3 year period and reported as the mean of at least 3 readings in each pond and then averaged across the anaerobic ponds. Standard deviations in brackets.

Depth (m)	pH	salinity ($g.L^{-1}$)	temperature (°C)	light (PAR) $moles.m^{-2}.s^{-1}$	DO ($mg.L^{-1}$)	$chlorophyll_a$ ($mg.L^{-1}$)
0	8.63 (0.20)	13.6 (2.19)	22.1 (7.9)	571.6 (216.2)	0.014	0.319 (0.158)
0.05	8.63 (0.19)	13.6 (2.19)	20.7 (6.3)	104.4 (39.5)	0.010	0.296 (0.185)
0.15	8.65 (0.20)	13.6 (2.19)	20.3 (5.4)	3.47 (1.31)	0.010	0.241 (0.123)
0.30	8.65 (0.19)	13.6 (2.19)	20.0 (5.1)	0.022 (0.008)	0.009	0.200 (0.054)
0.50	8.64 (0.19)	13.6 (2.19)	19.8 (5.1)	0	0.009	0.171 (0.07)
1.00	8.66 (0.22)	37.6 (30.66)	19.0 (4.8)	0	0.009	0.147 (0.01)
1.50	8.66 (0.22)	37.6 (30.66)	19.0 (4.8)	0	0.009	0.147 (0.01)

interface (**Table 2.4.3.** and **Table 2.4.4.**). The high organic loading rates received by these ponds results in the efficient microbial scouring of oxygen, and the anoxic conditions which prevail throughout the year. These anaerobic ponds are characterised by low pH and TDS, and high COD, SS, soluble N, Na_2S and SO_4 and they are well mixed with no significant physical or chemical stratification occurring in the water column.

The pH in the anaerobic ponds was found to range between 8.43 and 8.88. The salinity levels remain constant throughout the water column (13.6 $g.L^{-1}$) with the salts tending to concentrate in the sediments (up to 68 $g.L^{-1}$) at the base of the ponds. In these ponds temperature stratification is not well defined but does play a role during the very warm summer months (during summer the surface water temperature may be 30 °C while that of the water at 1.50 m below the surface is 23.8 °C).

Red photosynthetic bacteria predominate in the initial anaerobic ponds in the treatment system, giving the ponds a characteristic purple-red colour (**Figure 2.4.7.**). Tentative identification indicates that the photosynthetic microbial population includes the purple sulphur bacterium *Chromatium sp.*, the purple non-sulphur bacterium *Rhodospirillum sp.*, and the green sulphur bacterium *Chlorobium sp.* These micro-organisms utilise reduced inorganic compounds such as H_2S, thiosulphate and H_2, or organic compounds such as photosynthetic electron donors for photosynthetic growth under anaerobic conditions (Stanier *et al.*, 1978).

The anaerobic conditions together with an abundance of reduced sulphur compounds in the

Table 2.4.4. Analysis of anaerobic pond strata. Data collected over a 3 year period and reported as the mean of at least 3 readings in each pond and then averaged across the anaerobic ponds. Standard deviations in brackets.

All values in $mg.L^{-1}$	Surface	0.75 m	1.50 m	Average
Ammonia as NH_3	54 (26)	61 (30)	65 (27)	60 (5.6)
Chloride as Cl^{-1}	7106 (815)	7167 (869)	7198 (856)	7157 (47)
COD	760 (43)	841 (74)	1074 (241)	892 (163)
Nitrate as NO_3	44 (24)	41 (24)	35 (20)	40 (23)
Phosphate as P_2O_5	27 (5)	32 (3)	35 (3)	31 (4)
Sulphate as SO_4	759 (212)	719 (183)	667 (152)	715 (46)
Sulphide as Na_2S	20 (10)	25 (15)	30 (18)	25 (5)
SS	386 (353)	405 (348)	510 (480)	434 (67)
TDS	17687 (2573)	17906 (2498)	18071 (2467)	17888 (193)
TDIS	15528 (2291)	15637 (2347)	15692 (2367)	15619 (83)
Total alkalinity	4085 (685)	4135 (714)	4155 (708)	4125 (36)
TKN	109 (20)	116 (25)	122 (25)	116 (6.5)

initial ponds explains the prolific growth and dominance of this group of bacteria in the upper layers of this niche. Their presence is revealed macroscopically by flocculation of elemental sulphur which accumulates as a white-film which, at times, is blown into a thick windrow on the sides of the ponds.

In addition to the photosynthetic bacteria a large microbial population of non-photosynthetic bacteria are also present in the anaerobic ponds. Species of *Halobacteriaceae* were observed, generally located in the sediments where the salinity was highest, and showed a seasonal variation in numbers in response to changes in salinity. Representatives of the *Halobacteriaceae* have been reported to dominate in strongly saline environments, such as the alkaline saline lakes of East Africa (Larsen, 1974) and the Great Salt Lake, Utah, U.S.A. (Post, 1981 ; Rodriquez-Valera, 1992).

The euphotic zone extends to a depth of only 15 cm in the anaerobic ponds, which means that the major components of the microbial population in the pond experience dark conditions through at least a portion of their life cycle. A variety of chemolithotrophic bacteria, which occupy this niche, are responsible for bacterial mineralisation of the organic matter entering the initial WSP through sulphate reduction, nitrification/denitrification, and methanogenesis.

Figure 2.4.7. Photograph of the 'red' anaerobic ponds in the waste stabilisation ponds at Mossop Western Leathers, Wellington.

Methane in the gas liberated from the floor of pond A was measured and the biogas composition found to be 19 % CH_4 and 81 % CO_2. A COD reduction of over 50 %, in ponds A and B, suggests effective anaerobic digestion of the organic matter in the influent stream and an active microbial population even at elevated salt concentrations in the effluent.

Since effluent enters the system with the sulphur component largely in the oxidised form, the presence of both sulphide and elemental sulphur indicates the presence of sulphate reducing bacteria (SRB) and provides further evidence that a continuous sulphur cycle is in operation in the waste stabilisation ponds. The sulphates that enter the system are reduced by the SRB to H_2S, some of which precipitates as metal sulphide complexes and thus becomes part of the black sludge sediments observed at the base of the ponds. Some H_2S passes into the atmosphere giving rise, in part, to the odour nuisance associated with the system or it may be utilised as an electron donor by the photosynthetic bacteria to form elemental sulphur or other oxidised sulphur compounds.

The only algae and cyanobacteria observed in the initial waste stabilisation ponds was the

seasonal occurrence of a *Dunaliella sp.* along the perimeter of the anoxic ponds at the air/water interface, and an *Anacystis sp.* which was found at several depths throughout the ponds, but in very low numbers.

2.4.3.2. Facultative Ponds

Ponds 6 to 11 and C,D function facultatively, with an oxygen-rich upper layer of up to 15 cm and anoxic oxygen depleted bottom waters. These ponds are characterised by high pH and TDS, and low COD, SS, soluble N, Na_2S and SO_4 (**Table 2.4.5.**, and **Table 2.4.6.**). The ponds exhibit very pronounced physical and chemical stratification which allows for the development of distinct aerobic and anaerobic regions.

Table 2.4.5. Analysis of facultative pond strata. Data collected over a 3 year period and reported as the mean of at least 3 readings in each pond and then averaged across the facultative ponds. Standard deviations in brackets.

Depth (m)	pH	salinity ($g.L^{-1}$)	temperature (°C)	light (PAR) $\mu moles.m^{-2}.s^{-1}$	DO ($mg.L^{-1}$)	$chlorophyll_a$ ($mg.L^{-1}$)
0	9.19 (0.19)	27.8 (13.3)	22.2 (5.8)	571.6 (216.2)	16.7 (1.27)	3.95 (0.270)
0.05	9.19 (0.19)	28.0 (13.6)	20.9 (5.7)	104.4 (39.5)	11.0 (4.26)	4.23 (0.693)
0.15	9.20 (0.19)	28.0 (13.6)	20.1 (6.4)	3.47 (1.31)	3.28 (1.26)	3.73 (0.810)
0.30	9.19 (0.19)	28.1 (13.6)	19.6 (5.7)	0.022 (0.008)	0.848 (0.693)	3.35 (0.403)
0.50	9.18 (0.19)	28.1 (13.5)	18.9 (5.1)	0	0.276 (0.164)	2.63 (0.319)
1.00	9.13 (0.24)	29.8 (11.7)	18.8 (3.5)	0	0.019	2.33 (0.352)
1.50	9.20 (0.18)	34.3 (13.7)	18.6	0	0.01	1.63 (0.007)
2.00	9.08 (0.22)	39.5 (13.4)	17.9 (2.1)	0	0.01	1.39 (0.198)
2.50	9.07 (0.20)	49.0 (17.8)	18.0 (3.5)	0	0.01	0.84 (0.348)
3.00	8.98 (0.36)	49.0 (17.8)	17.8 (3.3)	0	0.01	0.75 (0.402)

The pH in the facultative ponds was much higher than that in the anaerobic ponds and was found to range between 8.62 and 9.37. The salinity levels remained relatively constant throughout the water column in each pond, with the salts tending to concentrate in the sediments (up to 66.8 $g.L^{-1}$) at the base of the ponds. Note that although the salinity in the water column of the facultative ponds was higher than the anaerobic ponds, as may be anticipated at this latter stage in the evaporation process, the levels of the salts in the sediments were both high.

Table 2.4.6. Analysis of facultative pond strata. Data collected over a 3 year period and reported as the mean of at least 3 readings in each pond and then averaged across the facultative ponds. Standard deviations in brackets.

All values in $mg.L^{-1}$	Surface	0.75 m	1.50 m	Average
Ammonia as NH_3	22 (1)	25 (2)	30 (4)	26 (2)
Chloride as Cl^{-1}	14170 (7435)	14633 (7810)	15983 (8968)	14928 (8069)
COD	557 (58)	623 (70)	730 (135)	636 (86)
Nitrate as NO_3	15 (4.6)	14 (2.3)	19 (14)	16 (5.3)
Phosphate as P_2O_5	25 (2)	26 (2)	29 (1)	27 (2)
Sulphate as SO_4	1066 (547)	923 (402)	832 (348)	943 (430)
Sulphide as Na_2S	9 (5)	14 (5)	76 (92)	28 (24)
SS	91 (56)	153 (132)	211 (169)	152 (115)
TDS	50520 (16942)	51124 (17172)	54747 (18671)	52179 (17492)
TDIS	46539 (14913)	47414 (15463)	50653 (16623)	48202 (15546)
Total alkalinity	6074 (2142)	6270 (2162)	6827 (2285)	6340 (2241)
TKN	51 (14)	56 (18)	65 (20)	57 (17)

In the facultative ponds a pronounced thermocline develops and plays a role during the very warm summer months when the surface water temperature may be 30 °C while that of the water at 3.00 m below the surface is 21 °C. At the same time a thermocline of only 5 °C was measured in the anaerobic ponds. The DO concentration decreases vertically from 17.97 $mg.L^{-1}$ at the surface to 0.01 $mg.L^{-1}$ at the bottom, and may be correlated with a decrease in chl_a concentration from 3.95 $mg.L^{-1}$ at the surface to 0.75 $mg.L^{-1}$ at the bottom. A euphotic zone of 15 cm in depth is similar to that observed in the anaerobic ponds.

Chemical stratification in the water column of the facultative ponds correlates with the DO and chl_a concentration. While in the oxygen-rich surface waters the levels of ammonia, hydrogen sulphide, and COD are low these values increase with an increase in depth and, as may be anticipated from the observations on the operation of a sulphur cycle reported earlier, the levels of nitrate and sulphate show an inverse relationship with depth.

The predominant photosynthetic species in the latter ponds are the cyanophyte *Spirulina* and the chlorophyte *Dunaliella* with the two representative species being *D. salina* and *D. viridis*. The *Spirulina* tends to autoflocculate to the surface and is often wind-concentrated at the pond edges, giving rise to the characteristic "bloom raft" (**Figure 2.4.8.**) The *Spirulina*

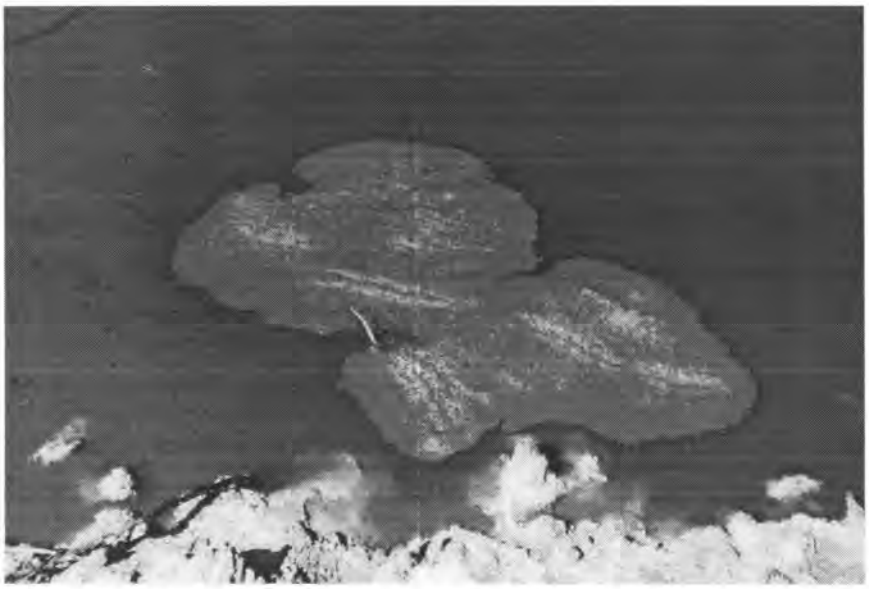


Figure 2.4.8. Photograph of the 'green' facultative ponds in the waste stabilisation ponds at Mossop Western Leathers, Wellington.

population appears to consist of a number of morphological variants of the same species.

In these facultative ponds the *Spirulina* migrate through the entire water column as a result of daily and seasonal fluctuations in the light regime and the prevailing chemical and physical conditions. The movement of the *Spirulina* would appear to occur as a result of both cell buoyancy and the gliding motility of the cyanobacteria. The changes in chl_a concentrations in facultative pond 6 is mainly attributed to the vertical migration of *Spirulina* and serve to illustrate the variations that occur in cell numbers at various depths both over the day and from season to season.

Figure 2.4.9. shows summer chl_a levels 2.5 times higher than winter readings with variation between morning and afternoon readings not showing as dramatic a change. However, a tendency for the photosynthetic stratum to broaden through the day was observed. These changes in chl_a concentration can be correlated with the light attenuation profile reported in **Figure 2.4.10.**

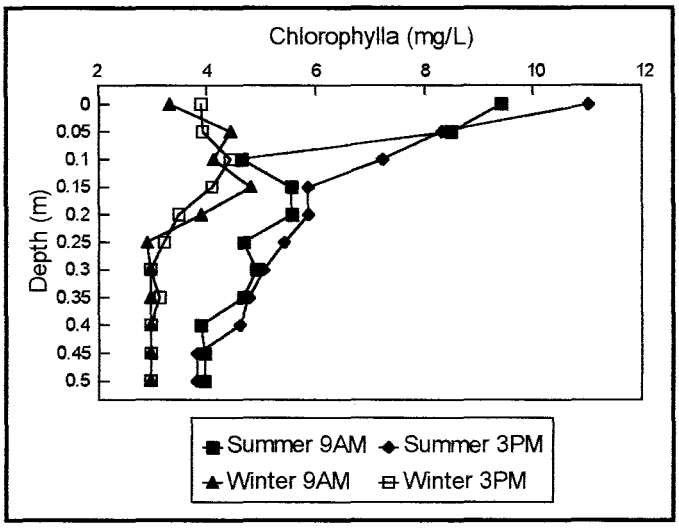


Figure 2.4.9. Depth profile of chlorophyll$_a$ concentration in the facultative ponds. Results are reported as the mean of 3 sets of observations made at each point in pond strata samples and at 3 sampling points in each pond over a 3 year period.

The amount of light that penetrates the water column increases over the day as the sun moves across the ponds with higher intensities observed in summer months. Even though the light intensity in summer is higher than winter at the surface, the light attenuation is greater because of the higher concentration of *Spirulina* biomass. This results in a similar light

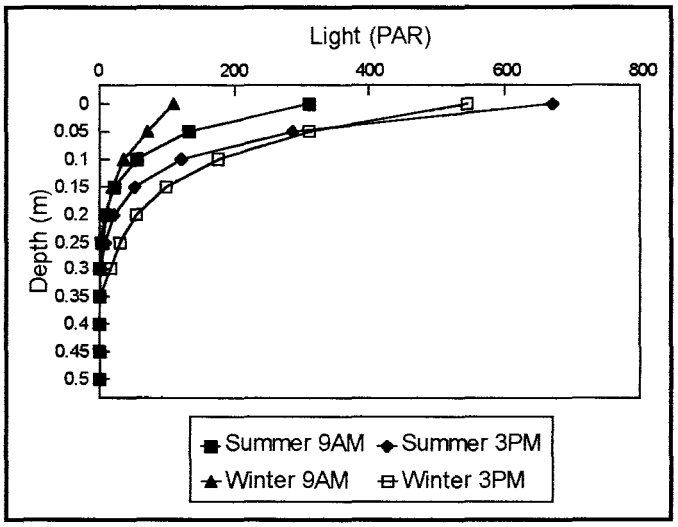


Figure 2.4.10. Depth profile for light attenuation in the facultative ponds. Results are reported as the mean of 3 sets of observations made at each point in pond strata samples and at 3 sampling points in each pond over a 3 year period.

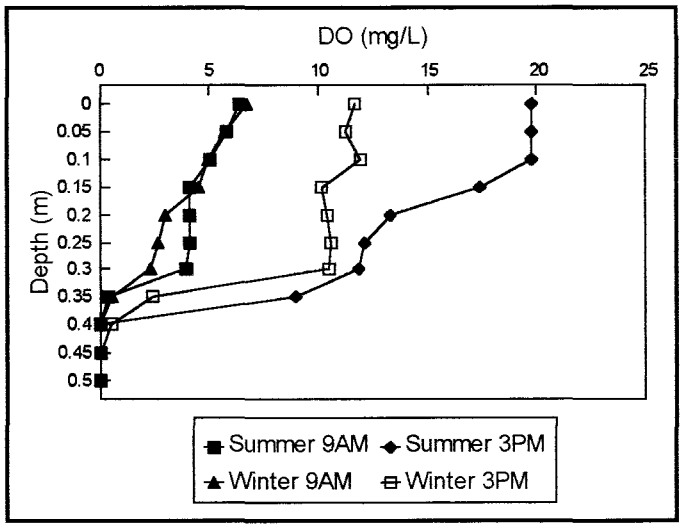


Figure 2.4.11. Depth profile for dissolved oxygen in the facultative ponds. Results are reported as the mean of 3 sets of observations made at each point in pond strata samples and at 3 sampling points in each pond over a 3 year period.

penetration profile for both seasons.

The chl_a stratification and light depth profiles correlate well with the characteristic stratification of the DO concentration in the WSP, as illustrated in **Figure 2.4.11.**

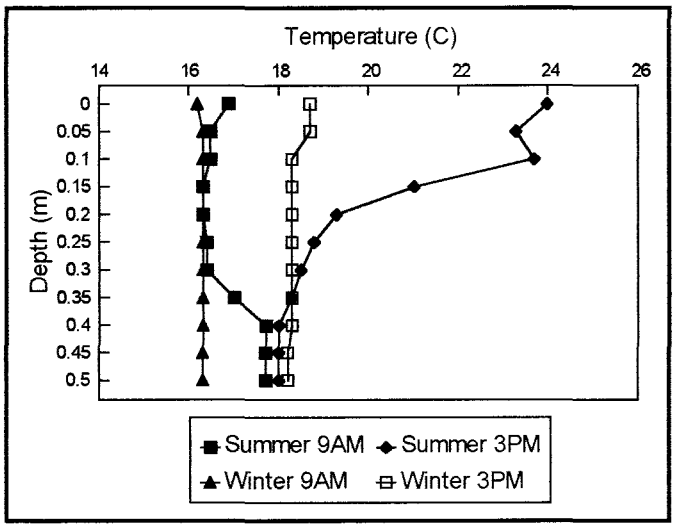


Figure 2.4.12. Depth profile for temperature in the facultative ponds. Results are reported as the mean of 3 sets of observations made at each point in pond strata samples and at 3 sampling points in each pond over a 3 year period.

The decrease in the DO from summer to winter can be attributed to the decreased productivity of the *Spirulina* as a result of the lower temperatures (**Figure 2.4.12.**), and possibly also decreased light intensities. Increased temperatures through the course of the day correlate well with both chl_a and DO measurements suggesting that light attenuation may play the lesser role.

The pronounced thermal stratification observed during the course of daylight hours provides temperature differentials of up to 9 °C within the first 0.5 m of pond depth during summer. While there is a pronounced diurnal temperature variation between the 9 am and 3 pm readings during both the summer and winter seasons the thermal gradient through the water column is most strongly developed during summer.

The photosynthetic growth of *Spirulina* is limited to the euphotic zone which, in the case of the facultative ponds, is approximately 30 cm in depth. Even within this narrow band there exists a certain degree of variation in productivity in response to the attenuation of light. The ability of light to penetrate the tannery effluent in the facultative WSP is a function of the strength of the incident light at the surface, the turbidity of the medium, and the concentration of the *Spirulina* biomass. The productivity of the *Spirulina* in this zone was measured as CO_2 fixation rate in facultative pond 6. The results are reported in **Table 2.4.7.**, and show the importance of light attenuation due to chl_a containing biomass in the summer months. These results also suggested that since carbonate was thought not to be limiting, temperature probably plays the principal constraining role on CO_2 fixation at the surface during winter, but not at lower levels, while light attenuation is the principal limiting factor at the lower levels during summer. Nevertheless, total productivities through the water column are comparable for both seasons.

The influence of wind on the ponds results in a breakdown of the stratification patterns which has been described and leads to inversions and mixing of the pond strata and the release of odiforous gasses to the atmosphere. In winter, when the prevailing north-west wind is accompanied by low pressure systems and rain, the odour problem is at its most offensive and draws the strongest response from the local community.

Table 2.4.7. Productivity of *Spirulina* in the euphotic zone measured as CO_2 fixation rate in Pond 6 strata samples.

Depth (m)	Summer (mg $C.m^2.Day^{-1}$)	Winter (mg $C.m^2.Day^{-1}$)	Average (mg $C.m^2.Day^{-1}$)
0	4787	1756	3271
0.05	3922	1972	2947
0.10	3987	3217	3602
0.15	2394	2630	2512
0.20	2074	2695	2384
0.25	1339	3188	2263
0.30	713	2212	1462
Total Productivity	19216	17670	18441

The facultative ponds also support a varied population of birds, including ducks, waders, and ground plover. Large flocks of the Lesser Flamingo, *Phoeniconaias minor*, periodically visit the WSP and feed on the *Spirulina.* This phenomenon of Flamingos feeding on *Spirulina* has been well documented for certain lakes in the Rift Valley of Kenya (Fox, 1983) and at salt pans in Botswana but not in WSP.

2.4.4. Determination of Photosynthetic Productivity.

In addition to the pond strata productivity studies reported above, productivity was also measured in composite grab samples collected over a period of several months from the facultative ponds. Results are reported in **Table 2.4.8.** The productivity of the *Spirulina*, as the major representative species in the facultative ponds, varies throughout the year in response to environmental changes. The productivity decreases in winter in response to the decrease in temperature and light intensity which relates to the rainy overcast conditions that prevail during this period.

The total area of facultative ponds currently containing *Spirulina* in the WSP system is approximately 4 hectares. Thus the total *Spirulina* biomass generated through the system may be calculated on the basis of productivity measurements to reach a level of 109 tons dry $wt.annum^{-1}$.

Table 2.4.8. Productivity of *Spirulina* in the facultative ponds measured as CO_2 fixation rate in composite grab samples.

Pond No.	February mg C. m^{-2}. Day^{-1}	March mg C. m^{-2}. Day^{-1}	April mg C. m^{-2}. Day^{-1}	May mg C. m^{-2}. Day^{-1}	June mg C. m^{-2}. Day^{-1}	Average mg C. m^{-2}. Day^{-1}
Pond C	10095	8040	5490	4875	3900	6480
Pond 7	12690	6150	6990	4671	4017	6904
Pond 11	14970	9000	9180	7269	4833	9050
Average	12585	7730	7220	5605	4250	7478

2.4.5. Photosynthetic Bacterial Activity.

The high levels of photosynthetic oxygen produced by *Spirulina* in the facultative ponds (**Figure 2.4.11.**), may play a decisive role in its dominance over the other micro-organisms in this particular niche, and correspondingly low DO may explain the dominance of purple bacteria in the anaerobic ponds. To investigate this phenomenon the absorbance spectrum of the extracted pigments of *Spirulina* and purple photosynthetic bacteria were used to establish the interaction between these two species in the anaerobic and facultative WSP.

When the purple photosynthetic bacteria were grown anaerobically and without the presence of *Spirulina*, in culture flasks sparged with nitrogen, there is an increase in cell number (results not shown) and a concomitant increase in the concentration of bacterial pigments. This can be seen in the results shown in **Figure 2.4.13.** The reverse effect is true for purple photosynthetic bacteria grown aerobically, in culture flasks sparged with air, or where grown in combination with *Spirulina* inoculated into the flask. There is an increase in cyanobacterial cell numbers accompanied by an increase in oxygen and a decrease in the concentrations of bacterial pigments. Interestingly, however, while there is a decrease in purple bacterial pigment concentration due to aeration the cell numbers do not decrease significantly. This confirms previous observations that these bacteria may undergo a switch in their metabolism in response to aeration which enables them to grow heterotrophically.

2.5. Discussion

The two sampling procedures used (subjective composite grabs and objective pond strata sampling) showed results which were quite closely comparable over the course of the

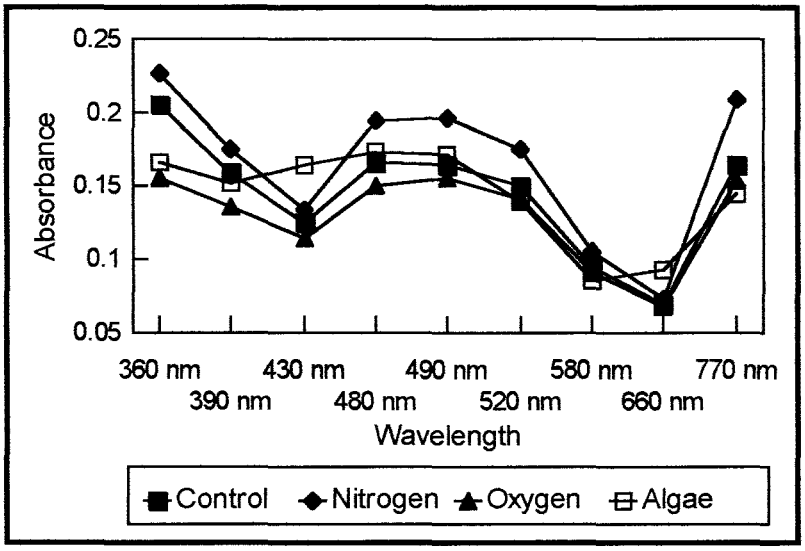


Figure 2.4.13. Changes in the absorbance of bacterial pigments in response to aerobic and anaerobic growth conditions. Results reflect the mean of 3 experiments.

three-year study. Although this contradicts the finding of Pearson *et. al.* (1987) that grab sampling is unreliable, it is evident that a certain degree of common sense is required in drawing the subjective sample, and that accuracy is enhanced where this is done, in each instance, by the same person. Nevertheless, the comparability of the two sets of results lends confidence to the observations recorded in the WSP system, and also to conclusions which emerge from the study relating to the biological dynamics and physical and chemical mechanisms operating in the pond ecosystem.

A shift in both chemical composition of the effluent and the dominant microbial populations is clearly apparent through the course of the WSP cascade and gives rise to the operation of two trophic systems. The microbiological investigation has shown that well defined, aerobic and anaerobic populations are located in the stratified micro-environments that establish throughout the ponding system.

The striking red to purple colour of the initial ponds in the system (No. 1-5) are indicative of purple photosynthetic bacteria. These organisms can function in the photoautotrophic mode, under anaerobic, surface illuminated conditions but also in the heterotrophic mode, in the dark water column and in the sediments of the ponds, utilising amino and organic acids as the carbon source and sulphide as electron donor (Brock and Madigan, 1988 ; McGarth and Harfoot, 1997). Due to the high influent sulphur content converted to sulphides by SRB, and

protein degradation in the initial ponds, the stringent conditions required by the purple photosynthetic bacteria appear to be satisfied. The organic load (COD) is at its greatest in the initial ponds, as are the levels of the other elements essential for facilitating halobacterial and sulphur-bacterial heterotrophic growth.

The initial ponds in the system are also characterised by the occurrence of elemental sulphur production by large numbers of photosynthetic and non-photosynthetic sulphur bacteria. At times these organisms appear to 'bloom' and float on the surface of the first ponds as a white, paint-like film of sulphur granules. The purple non-sulphur bacteria are the only photosynthetic bacteria in the system that are not strict anaerobes (Stanier, 1977). The response of these bacteria to the introduction of oxygen is a progressive decline in the cellular content of bacteriorhodopsin and carotenoids, resulting in the cells becoming decolorized and increasing reliance on heterotrophic growth. These changes induced by oxygen in purple non-sulphur bacteria are, however, reversible (Stanier, 1977 ; Zviagintseva *et. al.*, 1995 ; D'Souza *et. al.*, 1997).

A full sulphur cycle with oxidation and subsequent anaerobic reduction in the bottom sediments, therefore, appears to be in active operation in these ponds. Anaerobic digestion of the organic compounds in the tannery effluent plays a crucial role in the functioning of the sulphur cycle in the anaerobic ponds and this is discussed in greater detail at a later stage.

Gu and Stefan (1995) have reported studies on stratification in a WSP in Minnesota and noted the effect of a pronounced thermocline in preventing mixing of the hypolimnion and the epilimnion, and which was in effect for up to 75 % of observations made in that system over a 600 day period. They observed that while stratification is related to heating, destratification is linked to wind action. Observations of pronounced stratification in the WSP contradict early modelling efforts based on mixed reactor assumptions (Marais and Shaw, 1961 ; Pearson *et. al.*, 1987 ; Ellis and Rodrigues, 1995) and Torres *et. al.* (1997) suggest that a lack of understanding of the internal hydraulic performance of WSP, relating to factors such as mixing and stratification, frequently accounts for the malfunction of these systems. The results of this study show a direct relationship between effective stratification and control of the odour problem.

It was apparent that heating of the upper layers of the ponds is not only a function of the absorption of infra-red radiation by the surface water, as was observed in the more pronounced thermocline that establishes in the microalgal-dominant facultative ponds. Oswald (1988a) reports that microalgal photosynthesis converts light to heat energy at an efficiency approaching 90 % and he has used this heating effect to cap the facultative ponds, in the AIWPS configuration, with warm oxygenated water from the HRAP (Oswald 1991). While the dark-green colour of the microalgae probably plays a significant role in the establishment of the more intense thermocline in the facultative ponds the contribution of metabolic heat to the system should also be taken into account.

Observations of odour release patterns in the Wellington WSP correlate directly with the relationship between a microalgal cap and the presence of thermal and DO gradients in the ponds (see **Figure 2.4.12.** and **Tables 2.4.3.** and **2.4.5.**). Odour complaints in Wellington were most pronounced during North Westerly wind conditions in the winter season when the thermocline and hence stratification is least developed in the system. Under these conditions even limited mixing results in severe odour release from the anaerobic red ponds. While odour release from these ponds is less severe in summer, the absence of an oxygenated surface layer ensures the continuous low level production of odour but, once again, severe release with mixing caused by strong winds. In the microalgal capped facultative ponds, however, both the thermal and DO gradients are well developed and, together with a strongly oxidised upper layer, mixing of the bottom layers and hence odour release are minimised.

Odour production shows a pronounced inverse correlation with the rising oxygen and alkalinity gradients through the course of the ponding cascade. De Pauw and Salomoni (1991) describe a mechanism whereby H_2S, mercaptans and volatile fatty acids, the primary odour causing compounds, are chemically trapped at elevated alkalinity levels. These are then filtered out and oxidised by phototrophic bacteria growing in the euphotic zone (Almasi and Pescod, 1995).

The characteristic green colour of the latter ponds (No. 6-11) is a result of the dominance of large populations of photosynthetic cyanobacteria and green alga. The predominant cyanobacteria in these ponds, is *Spirulina*, which occurs in large seasonal 'blooms' which form wind-concentrated rafts on the sides of the ponds. *Spirulina*, a mesohaline species,

dominates at salt concentrations < 30 $g.L^{-1}$, while the green algal *D. salina* (a halophilic chlorophyte) predominates at higher salt concentrations. This occurs regardless of the pH gradient (Paerl, 1996). The other *Dunaliella* species to appear was identified as *D. viridis* by comparing type strains from the Culture Collection for Algae and Protozoa (CCAP). The *Dunaliella* appeared in all the latter ponds at low concentrations and exhibited a circadian rhythm, rising and falling through the water column during the day. At times, wind concentration would produce apparent 'blooms' at pond ends. Wind concentration of the *Dunaliella* in open ponds has been reported by Borowitzka *et al.* (1984). The pronounced salinity gradient in the pond medium is the principal factor limiting microbial growth largely to these two halophilic types.

The *Spirulina* present in the system has been provisionally identified as *S. platensis* and appears to occur in two of the three distinct morphological variants which have been documented (van Eyklenberg, 1979, 1980 ; Richmond, 1988). These different morphological types are thought to occur in response to the prevailing physical conditions, especially light intensity and temperature. It is thus possible that the different forms noticed in the effluent are caused by the vertical stratification of light penetration and temperature, and are not different species of *Spirulina*.

The characteristic change in microbial species dominance in the WSP, which results in the dominance of *Spirulina* in the latter ponds is the result of a number of interacting factors including salinity, elevated alkalinity, increased resistance to sulphide toxicity, nutrient variation in the water column, along with buoyancy and other concentrating mechanisms as described for *Anaebaena flos-aquae* in eutrophic lakes (Schanz *et al.*, 1979). Particular tolerance to sulphide has been demonstrated in cyanobacterial mats in hypersaline ponds by Frund and Cohen (1992). Certain *Spirulina*, optimise nutrient and light gradients by alternate sinking and floating, accomplished by the formation and collapse of gas vesicles (Walsby and Klemer, 1974). The gliding motility although still poorly understood, has been extensively studied (Holm-Hansen, 1968 ; Halfren and Castenholz, 1971 ; Stanier and Cohen-Bazire, 1977). High solar irradiation and artificial UV irradiation have been shown to affect the percentage of motile filaments and impair the linear velocity of movement in several cyanobacteria (Donkor and Hader, 1991).

The high levels of sulphide and ammonia occurring in the earlier stages of the ponding cascade (see **Table 2.4.1.** and **Figure 2.4.4.**) provide a dual-factor, interacting toxicity effect which almost certainly limits the natural growth of microalgae in the early part of the system (Houghton and Mara, 1992 ; Almasi and Pescod, 1995). Pearson *et. al.* (1987) have observed that sulphide is probably the major toxicant affecting the performance of heavily loaded facultative ponds and have suggested the algal species succession, and patterns of dominance, seen in WSP may serve as indicators of the sulphide and ammonia status in these systems (Pearson, 1996). They have shown for fresh water WSP that *Chlamydomonas* dominates under high sulphide conditions while *Chlorella* is the major species present where ammonia levels are high.

It has been noted that it is the unionised form of both sulphide and ammonia which passes most readily across the cell membrane causing the uncoupling of photosynthesis. In this regard sulphide toxicity is more pronounced at lower pH values (with the H_2S species predominating), while ammonia toxicity rises with increasing pH (the NH_3 species predominating), providing stringent regulation of photosynthetic activity across the alkalinity gradient in the system (Howsley and Pearson, 1979 ; Abeliovich and Azov, 1976). However, in addition to shifting sulphide equilibrium to the HS^- species, the volatilisation and stripping of ammonia also occurs as pH is elevated through the system (O'Brien *et. al.*, 1986) tending to relieve the problem in the later ponds.

It appears that the interaction of these toxic effects may not only provide a basis for explaining the microbial species distribution, and biological dynamics, operating in the Wellington WSP but also an indication of a mechanism whereby the system may be rationally managed. This argument will be expanded at a later stage in this report.

Sulphur is an essential element in algal nutrition which is provided as sulphate under natural conditions. Sulphate uptake has been studied in *S. platensis* (Menon and Varma, 1982 ; Frund and Cohen, 1992). In addition, many cyanobacteria have been shown to photoassimilate CO_2, coupled with an oxidation of H_2S to elemental sulphur, which indicates an ability to grow photosynthetically under highly reducing conditions in the presence of sulphide (Stanier and Cohen-Bazire, 1977). Chlorophyll and sulphide levels reported in **Figure 2.4.9.** and **Table**

2.4.6. and carbon fixation at lower levels of the water column, particularly during winter provide an indication that such a process could operate in the tannery WSP system.

The role of microbial competition between micro-organisms occupying the same niche has been studied by Fredrickson and Stephanopoulos (1981). Although it seems convenient to assume localisation of nutritional modes, this probably does not occur. Overlapping of both auto- and heterotrophy occurs in a single population as is shown for the purple non-sulphur bacteria. A great deal of information is available on the symbiotic relationship between algae and bacteria in oxidation ponds (Ganapati, 1975 ; Oswald, 1988a). Generally, the principle products of bacterial organic oxidation are CO_2, NH_3 and H_2O, which constitute the main requirements for algal photosynthesis together with the additional requirement for light energy.

However, utilisation of organic compounds, initially thought to be due entirely to bacterial oxidation, may also possibly be affected via *Dunaliella* and *Spirulina* heterotrophic activity. The phenomenon of heterotrophy has been demonstrated in a variety of cyanobacteria (Smith *et al.*, 1967 ; Diakoff and Scheibe, 1975 ; Hoare *et al.*, 1967 ; Rippka, 1972 ; van Baalen *et al.*, 1971) and in *Dunaliella* (Oliviera and Huynh, 1989, 1990). The possibility therefore exists that components of the organics present in the effluent may account, at least in part, for the enhanced growth of *Dunaliella* and *Spirulina* in the tannery wastewater growth medium. Follow-up studies of these observations are described in chapter 3 of this report.

Palmer (1969) has suggested that algal species dominance in these systems depends primarily on the organic load. Development of cyanobacteria 'blooms' is generally indicative of a low organic load. This is apparently the case in this WSP system, where *Spirulina* blooms dominate in ponds with the lowest concentrations of organics.

The prevailing high daytime air temperatures in Wellington, during the summer, ensure near-optimal growth conditions for *Spirulina*. The optimal temperature for growth has been documented between 35 and 37 °C. While Richmond (1988) has noted 18 °C as the minimum permissible daytime temperature that allows some growth active carbon fixation was measured in this WSP *Spirulina* isolate at least 2 °C below this level in winter studies. It is

also known that *Spirulina* can tolerate relatively low night temperatures if the day temperature rises above the minimum threshold (Richmond *et al.*, 1980).

Light intensity has been documented as one of the primary factors influencing the biomass output rate Vonshak *et. al.* (1996). The relatively low average photosynthetic carbon fixation rate of 7478 mg $C.m^{-2}.day^{-1}$, may be related to poor light penetration in the unoptimized WSP and to temperature effects. It was noted in these studies that a 4 °C increase in temperature and a 10 % increase in light during the summer period resulted in a doubling of the oxygen production, 2.5 times the quantities of $chlorophyll_a$ and twice the level of CO_2 fixation. These observations suggest that temperature plays the constraining role on CO_2 fixation and productivity at the surface during winter, but not at deeper levels, while light attenuation and not temperature is the principal limiting factor at deeper levels during summer.

Although the average productivity in the facultative ponds of 7478 mg $C.m^{-2}.day^{-1}$ over the year is much lower than some reported values for productivity of *Spirulina* in optimised, well-mixed ponds (Richmond in 1988 reported 30 g $C.m^{-2}.day^{-1}$), this value compares well with the 8-12 g $C.m^{-2}.day^{-1}$ cited for outdoor culture basins by Fox (1983). At present, the 109 $tons.annum^{-1}$ estimated *Spirulina* biomass production in the unoptimised Wellington WSP would, nevertheless, have a significant monetary value on the specialist animal feed market if shown to be nutritionally acceptable. The identification of the commercial potential of the Wellington WSP system, provided an incentive for the subsequent investigations of biomass production in the system.

The principal outcome from the findings relating to the WSP biology and performance reported in this chapter, however, concerned the questions whether microalgae observed to flourish, at times, in the ponds could be manipulated in such a manner as to provide a sustainable microalgal capping of the entire system and hence effective odour control ; and also whether the use of these organisms in established algal biotechnological processes such as the HRAP might offer an intensive alternative to the extensive WSP process for treating tannery effluents. In this regard it was important to determine the possible role played by, and extent of, heterotrophic nutrition in the organic load reduction effect observed together with microalgal growth.

Chapter Three

The Contribution to Organic Load Reduction by Algal Heterotrophic Nutrition.

3.1. Introduction

The role of dissolved organic nitrogen in determining the primary productivity of marine and freshwater ecosystems has received considerable attention and its function in the nutrition and cell biology of phytoplankton has been reviewed by Flynn and Butler (1986), Kaplan *et al.* (1986), Kerby *et al.* (1989), and Anita *et al.* (1991). While controversy surrounds the question of whether organic compounds are released by micro-algal cells (Hellebust, 1970, 1985 ; Hellebust and Lewin, 1977 ; Sharp, 1977), there is a considerable body of evidence demonstrating the uptake of amino acids (Nielson and Larsson, 1980 ; Raven, 1980 ; Eddy, 1982 ; Flynn and Butler, 1986, Hammer, 1993) ; peptides (Lang *et al.*, 1979 ; Storey and Wagner, 1986) ; protein (Oliviera and Huynh, 1989) ; purines and pyrimidines (Shah and Syfrett, 1984) and hypoxanthine (Oliviera and Huynh, 1990).

The ecological survey described in chapter 2 revealed that the latter ponds in the WSP system were characterised by seasonal blooms of *Spirulina* and *Dunaliella.* Subsequent studies demonstrated that both *Dunaliella* (Rose, 1991 ; Laubscher, 1991) and *Spirulina* (this study) not only grew in tannery effluent but, in certain of these specific effluent streams, grew very well indeed, with a growth stimulation effect producing higher cell yields than complete, semi-defined media. This surprising observation of increased growth was accompanied by a reduction in the organic load which these authors had suggested may possibly indicate uptake and heterotrophic utilisation of the organic constituents by the photosynthetic organisms.

3.2. Research Objective

To determine whether *Dunaliella* and *Spirulina* growing in the WSP system utilise organic compounds present in the tannery effluent.

3.3. Materials and Methods

3.3.1. Organic Nutrition

Spirulina cells isolated from the WSP system were washed and cultured in either defined Zarrouk's medium (Zarrouk, 1966) or effluent medium. Effluent medium was prepared by filter sterilisation of pre-treated tannery wastewater collected at the inflow to pond A, and diluted to 20 % strength using tap water. The *Spirulina* cells were filter washed (x3) with sterilised Zarrouk's media to reduce bacterial numbers to $< 1x10^2$ mL^{-1}, determined as colony forming units (CFU) on defined media plate-counting agar.

Colony Forming Units 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 release of labelled 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 a concentration range of unlabelled glycine and glucose (Merck) respectively (excluded in the controls), and incubated at 25 °C in a constant environment room. One set of cultures, for each treatment, was exposed to continuous illumination at 158 $\mu mol.m^{-2}.s^{-1}$, while the other set was kept in total darkness.

At time intervals of 5 minutes, 3 hrs., 6 hrs., 12 hrs., 24 hrs., and 48 hrs., 1 mL of culture medium was removed, filter washed with sterile defined medium (x3) and the cells resuspended in 100 µL distilled water and transferred to aqueous scintillant (Packard). Culture supernatant was likewise transferred in 100 µL aliquots to aqueous scintillant and counted in a Beckman LS3150T scintillation counter and results adjusted to disintegration per minute (dpm) values. The results reflect a triplicate mean.

3.3.2. Ultrastructural Investigation of both *Dunaliella* and *Spirulina*

3.3.2.1. Entrapment and Embedding

Cultures of *D. salina* and *Spirulina* were grown in either defined or effluent media under conditions as reported by Laubscher (1991) and described in chapter 4. Samples of cells (10 mL) were centrifuged at 4000 rpm. for 10 minutes in a Heraus centrifuge. The pellets were transferred to 1 mL microfuge tubes (Eppendorf), to which 6 % low melting point agarose was added to entrap the cells. After solidification, the agarose pellet was cut into 2 mm cubes and fixed in 2.5 % glutaraldehyde in 0.1 M phosphate buffer.

3.3.2.2. Preparation for Electron Microscopy

The samples were prepared for electron microscopy following the methods of Cross (1979). For transmission electron microscopy (TEM), following primary fixation in glutaraldehyde, this involved washing the samples in 0.1 M phosphate buffer followed by post-fixation for 90 minutes in 1.0 % phosphate buffered osmium tetroxide. Following two further buffer washes the samples were dehydrated by transfer through a series of ascending concentrations of ethanol (30 %-100 %). This was followed by two washes of propylene oxide and transition to a resin medium through three propylene oxide:epoxy resin mixtures (75:25, 50:50, 25:75) and finally pure epoxy resin. Samples were then transferred to pure epoxy resin in embedding moulds, and polymerization was allowed to take place over 36 hours at 60 °C. Ultra-thin sections were cut using a LKB 111 Ultramicrotome and collected onto alcohol -washed grids after which they were post-stained with 5 % aqueous Uranyl Acetate (30 minutes), followed by Reynold's Lead Citrate (5 minutes). For scanning electron microscopy (SEM) cells were collected by filtration on to cellulose acetate filters. The cells on small pieces of filter were fixed in glutaraldehyde as for TEM, but after the posphate buffer washes were transferred directly to the first stage of the ethanol dehydration series. Following dehydration, critical point drying of the filters and cells was carried from liquid carbon dioxide. Dried cells on filters were mounted on specimen stubs and sputter-coated with a thin layer of gold.

3.3.2.3. Electron Microscopy

For TEM, ultrathin sections were examined using a JOEL JEM 100 CXII transmission electron microscope. For SEM, whole cells on cellulose acetate filters were examined using a JOEL JSM 840 scanning electron microscope.

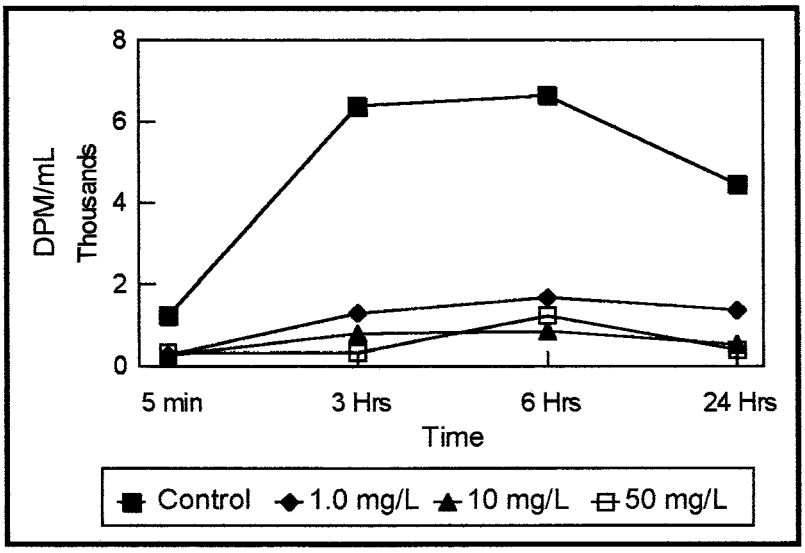


Figure 3.4.1. Uptake of D-[^{14}C]-Glucose by *Spirulina* incubated in the light, in defined medium without unlabelled glucose in the control and also together with increasing concentrations of unlabelled glucose. Results reflect the mean of 3 experiments.

3.4. Results

3.4.1. Organic Nutrition

In an attempt to determine whether *Spirulina* could effect the uptake and utilisation of organic compounds in a manner similar to that already demonstrated for *D. salina* growing in tannery effluent (Glaum, 1991 ; Rose, 1991) a series of experiments were conducted in which [^{14}C]-glycine and D-[^{14}C]-glucose, were added to the washed cultures containing a concentration range of unlabelled glycine and glucose.

The results reported in **Figure 3.4.1.** indicate assimilation of labelled carbon by *Spirulina* from the D-[^{14}C]-glucose source added to defined medium and grown in the light. A reduced uptake of label is noted as the label is diluted with the increasing concentration of unlabelled glucose. The cultures grown in the dark showed a reduced D-[^{14}C]-glucose-sourced carbon uptake by *Spirulina* (**Figure 3.4.2.**), however, the dilution effect of unlabelled glucose is still observable in these results. Uptake increased over a period of time with the use of higher label dilutions. Successful dark uptake of labelled glucose suggests bacterial mineralisation followed photoautotrophic uptake of labelled CO_2 may not be a significant factor in the light

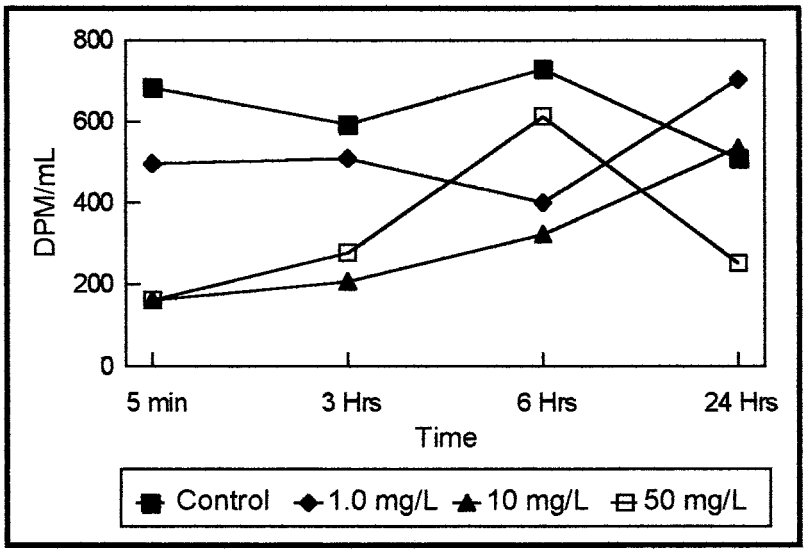


Figure 3.4.2. Uptake of D-[^{14}C]-Glucose by *Spirulina* incubated in the dark, in defined medium without unlabelled glucose in the control and also together with increasing concentrations of unlabelled glucose. Results reflect the mean of 3 experiments.

incubated studies. Increased uptake in higher label dilutions over time which, together with rapid uptake over a period of a few minutes by control cultures and extremely low bacterial CFU, indicates a real label dilution effect. Uptake results at incubation times of 24 and 48

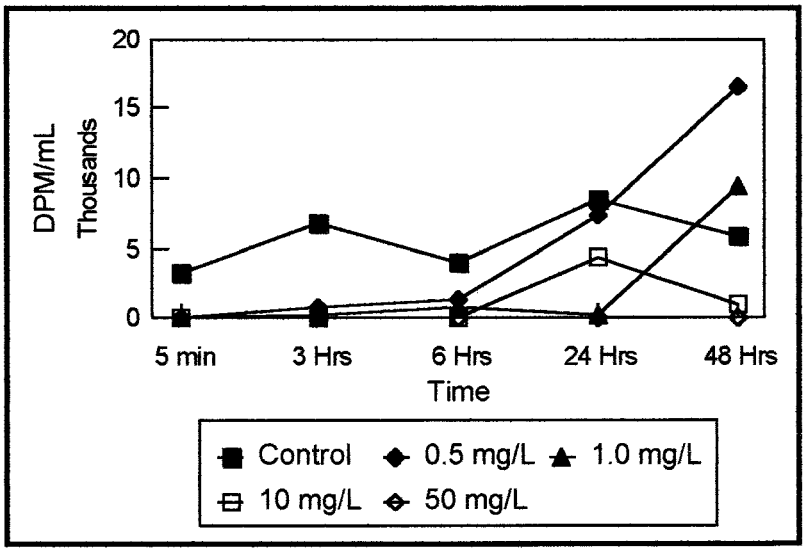


Figure 3.4.3. Uptake of [^{14}C]-Glycine by *Spirulina* incubated in the light, in defined medium without unlabelled glycine in the control and also together with increasing concentrations of unlabelled glycine. Results reflect the mean of 3 experiments.

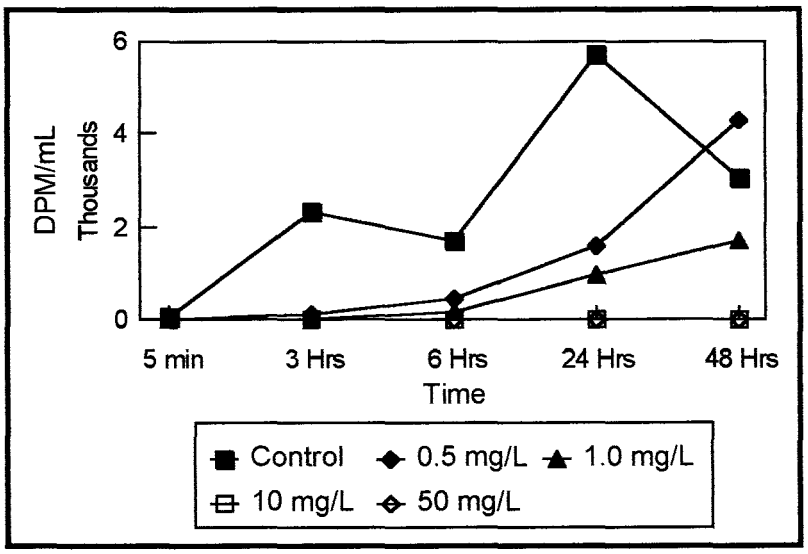


Figure 3.4.4. Uptake of [^{14}C]-Glycine by *Spirulina* incubated in the dark, in defined medium without unlabelled glycine in the control and also together with increasing concentrations of unlabelled glycine. Results reflect the mean of 3 experiments.

hours should be treated more cautiously as post incubation bacterial controls were not made and thus possible bacterial activity cannot be entirely ruled out. Results reported in **Figure 3.4.3.** show a similar response with [^{14}C]-glycine uptake studies in defined medium and in

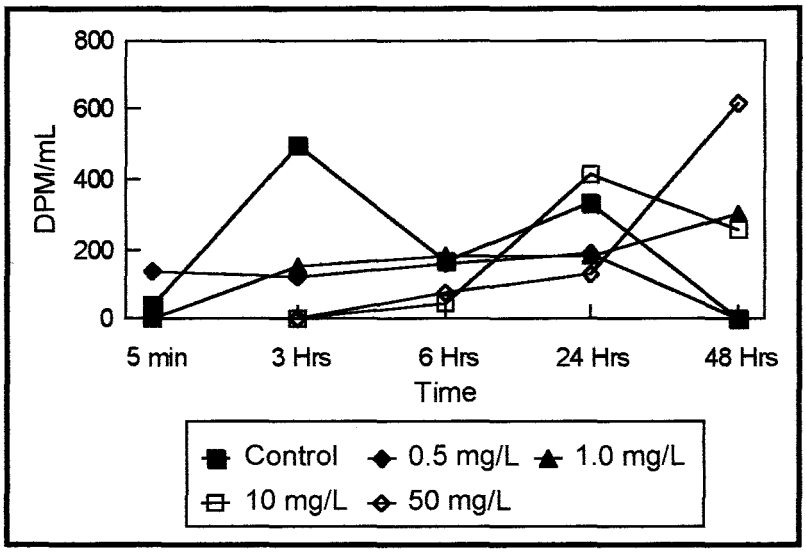


Figure 3.4.5. Uptake of [^{14}C]-Glycine by *Spirulina* incubated in the light, in effluent medium without unlabelled glycine in the control and also together with increasing concentrations of unlabelled glycine. Results reflect the mean of 3 experiments.

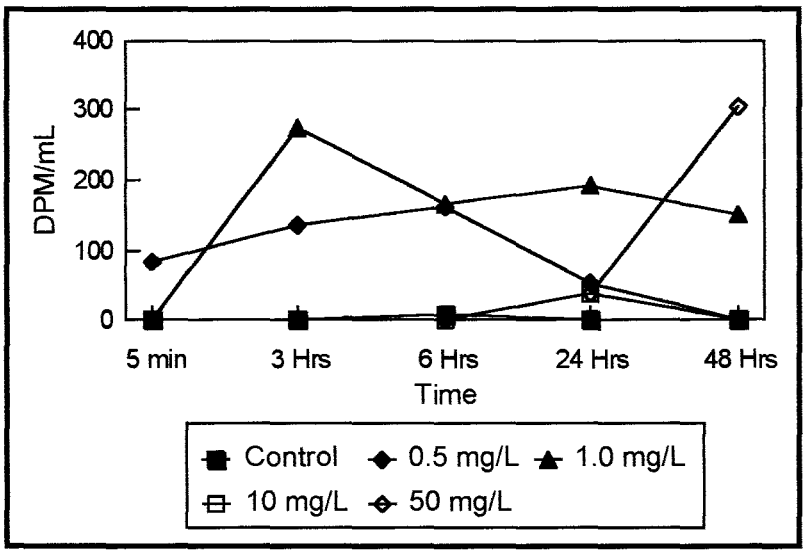


Figure 3.4.6. Uptake of [^{14}C]-Glycine by *Spirulina* incubated in the dark, in effluent medium without unlabelled glycine in the control and also together with increasing concentrations of unlabelled glycine. Results reflect the mean of 3 experiments.

samples incubated in the light. Again the rate of uptake was affected by the concentration of unlabelled glycine in the medium. Uptake of [^{14}C]-glycine-sourced carbon in the dark (**Figure 3.4.4.**) shows a similar picture except once again the rate is somewhat reduced.

Given the high levels of glycine to be anticipated in a collagen rich effluent medium the labelled glycine carbon uptake study was also undertaken to compare these observations in defined medium with uptake in tannery effluent medium. In both the light (**Figure 3.4.5.**) and the dark (**Figure 3.4.6.**) incubation studies with [^{14}C]-glycine label the uptake data are less clear-cut for both uptake by the controls and graded uptake in the presence of increasing concentration of unlabelled glycine. These results suggest a quenching effect due to the high levels of glycine present in the growth medium.

3.4.2. Ultrastructural Investigation

While uptake of labelled organic carbon had been demonstrated for *D. salina* in both light and dark (Glaum, 1991 ; Rose, 1991), associated ultrastructural changes had not been studied. *D. salina* cells grown in the high saline Hide Soak Liquor (HSL), which carries the salt

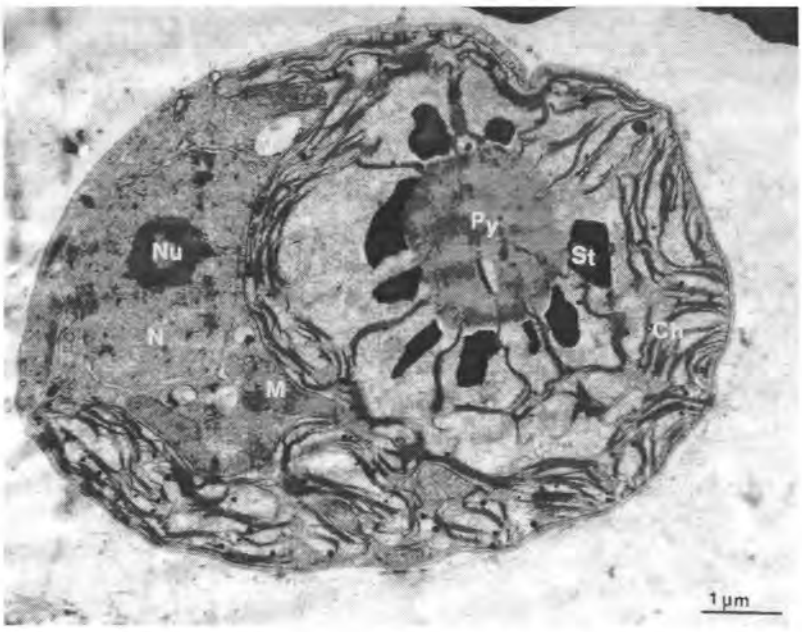


Figure 3.4.7. Electron micrograph of *Dunaliella salina* grown in defined medium.

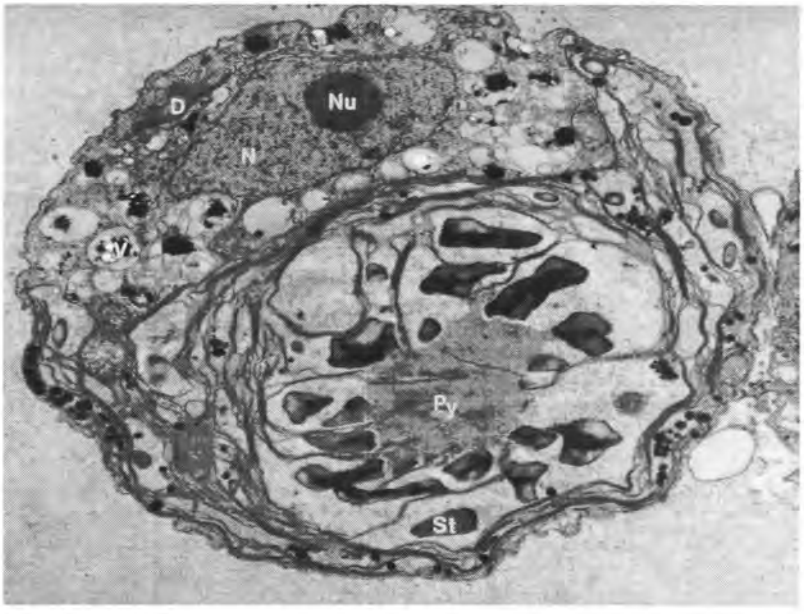


Figure 3.4.8. Electron micrograph of *Dunaliella salina* grown in effluent medium.

[Legend for Figures 3.4.7 to 3.4.10: Bc = beta carotene; Ch = chloroplast; D = dictyosome; M = mitochondrion; N = nucleus; Nu = nucleolus; p = pore; Py = pyrenoid; St = starch; V = vacuole; Vs = vesicle]

preservative load removed from the cured raw stock at the start of the manufacturing process, showed a number of noteworthy ultrastructural changes compared to cells grown in defined medium (**Figures 3.4.7.** and **3.4.8.**). The changes observed in response to salinity were similar to those reported by Oliviera and Huynh (1989).

Compared to the cells grown in defined media the HSL-grown cells showed greatly increased invagination of the cytoplasmic membrane, including large numbers of endocytotic pockets or pits, often in association with vacuoles just below the plasma membrane, as shown in **Figure 3.4.9.** and **Figure 3.4.10.** The size of both the vacuoles and pyrenoid centre (as % cell volume) were shown to undergo dramatic changes in response to the addition of effluent. Additional mitochondria noted in the endocytotic area suggested the function of an energy-requiring process not present in control cells. Together with the presence of multi-vesicular bodies, this strongly suggests the presence of a receptor-mediated pinocytotic (endocytotic) uptake mechanism in *D. salina* for the internalisation of large molecular weight organic molecules. This may explain the observed increased growth rates of the *D. salina* found when grown in effluent medium (Laubscher, 1991).

The high organic (COD) levels noted in some of the latter ponds in the WSP (see chapter 2) during 'bloom' conditions could result from photosynthate release by the cells. The ultrastructural investigation showed that membrane bound vesicles were present in both log and stationary phase cells of *D. salina* in response to salt and temperature stress, as shown in **Figure 3.4.10.** Although, not conclusive this study showed that the release of cellular photosynthate may result from the formation of these vesicles on the outer membrane of the *D. salina* cells.

An investigation was conducted to see whether ultrastructural changes could be observed in the effluent grown *Spirulina*, which could further clarify the observations suggesting the uptake and utilisation by this organism of organic components present in the tannery wastewater. The results obtained for the TEM showed that no clearly visible morphological differences could be distinguished in this preliminary study, between *Spirulina* cells grown in defined medium and those grown in effluent medium.

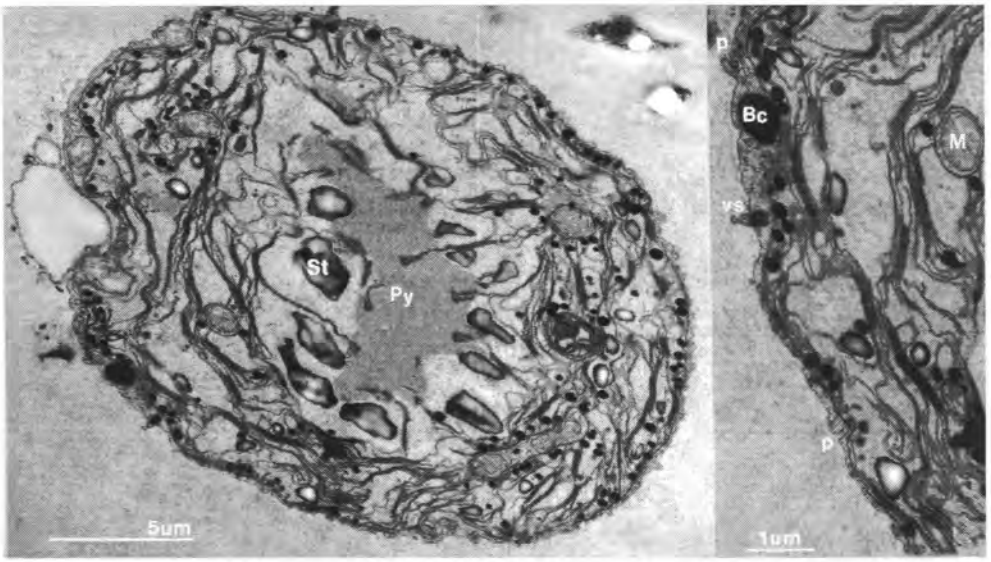


Figure 3.4.9. Electron micrograph of *Dunaliella salina* grown in effluent medium.

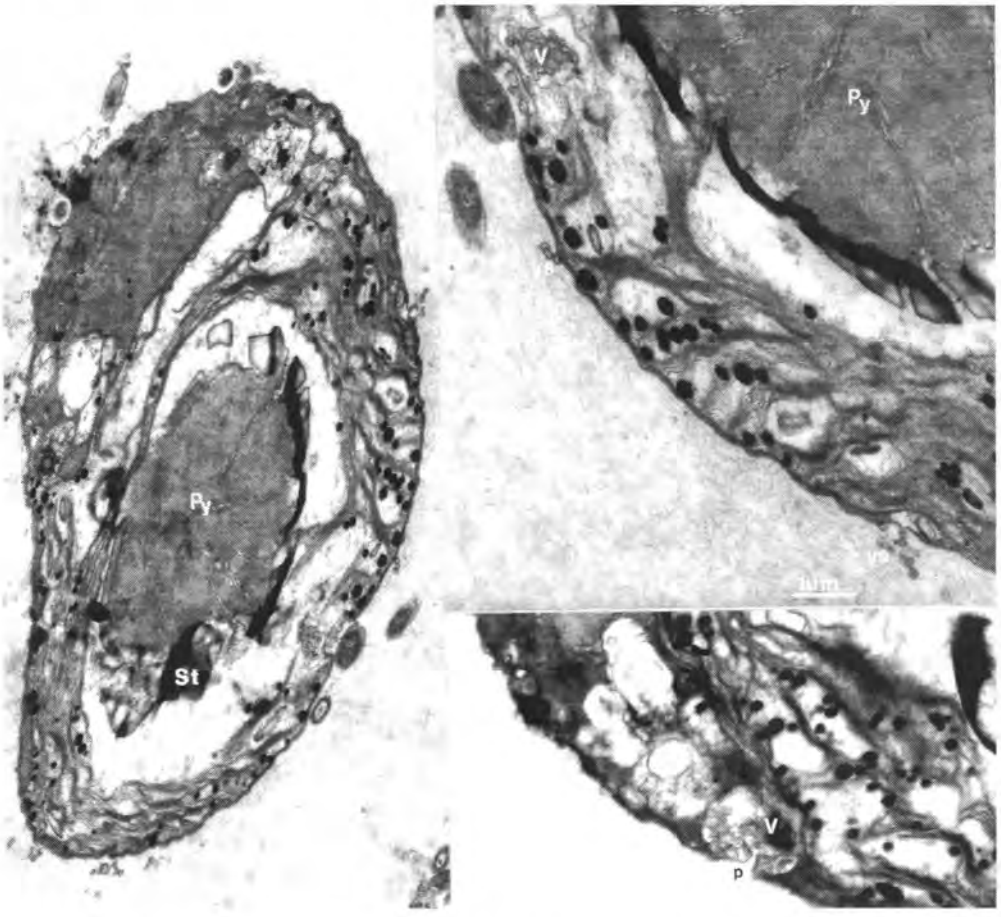


Figure 3.4.10. Electron micrograph of *Dunaliella salina* grown in effluent medium.

3.5. Discussion

There are numerous early reports of eucaryotic algal heterotrophic growth in the dark (van Baalen *et al.*, 1971). The role of heterotrophic nutrition in the growth of alga in the HRAP has been reported by Abeliovich and Weisman (1978). The concentration and variety of compounds in tannery wastewater raises the question of the role of algal nutrition in oxidation ponds treating these wastes. The increased growth of *Spirulina* in the latter ponds was accompanied by a reduction in the organic load which raised the possibility of uptake of organic constituents playing some role in the COD reduction process.

Although most evidence suggests that the saturation kinetics for micro-algal uptake of organics is low in natural aquatic ecosystems (Anita *et al.*, 1991), Abeliovich (1986) has found that the algal component may be responsible for up to 50 % of organics removed in the HRAP treating sewage effluent. Like most cyanobacteria, *Spirulina* has also been considered an obligate photoautotroph which cannot grow in the dark in media containing organic sources of carbon (Ciferri, 1983). The inability of cyanobacteria to grow in the dark at the expense of all substrates normally oxidised through the tricarboxylic acid cycle has been attributed to a specific enzyme deficiency, the absence of *a*-ketogluterate dehydrogenase (Pelroy and Bassham, 1972 ; Stanier and Cohen-Bazire, 1977). However, several studies have shown that with the addition of proper organic supplements and with the appropriate growth conditions some cyanophytes can grow in the dark (Rippka, 1972 ; Diakoff and Scheibe, 1975).

In the light, several cyanobacteria may utilise carbohydrates (Ciferri, 1983). The photoassimilation of glucose and acetate have been reported (Hoare *et. al.*, 1967 ; Smith *et al.*, 1967 ; Holm-Hansen, 1968 ; Miller *et al.*, 1971 ; Stanier and Cohen-Bazire, 1977 ; Anderson and McIntosh, 1991). In dim light, mixotrophic growth results in cell yields that are two to threefold higher than the corresponding yields obtained phototrophically (van Baalen *et al*, 1971 ; Ciferri, 1983). The existence of active transport systems operating in amino acid uptake in photoautotrophic cyanobacteria has been reported Labarre *et al.* (1987). Clearly microalgal uptake of organic molecules may play a greater role in nutrient cycling at the lower light limited layers of the euphotic zone.

Previously, the uptake of both [^{14}C]-glycine and [^{14}C]-bovine serum albumin (BSA) had been demonstrated for *D. salina*, with the amino acid internalised, possibly by an energy-requiring active transport process (Glaum, 1991 ; Rose, 1991). The result is in accordance with the observation that in a natural environment the micro-algal cell would need to alter its nitrogen uptake from a widely fluctuating supply. The quantification of direct glycine use, compared to the uptake of ammonium and other nitrogen sources was not determined, but a nutritional advantage was demonstrated for *D. salina* with the combination of glycine and inorganic nitrogen in the growth medium (Glaum, 1991 ; Rose, 1991).

The results of the current study provide a strong indication that the *Spirulina* is capable of assimilating glucose-sourced carbon in both the light and in the dark where the possible role of bacterial mineralisation as a factor has been effectively eliminated in control studies. Ciferri (1983) reports the utilisation of glucose, where about 50 % of the radiolabelled glucose was recovered in the cells after four days incubation with light. Photo-assimilation of acetate has been shown to contribute as much as 10-32 % to newly synthesised cell carbon under phototrophic conditions (Holm-Hansen, 1968 ; Stanier and Cohen-Bazire, 1977).

These initial studies indicate that the *Spirulina* assimilate small amounts of glycine under light and dark conditions. However, the rate of uptake is affected by the concentration of glycine in the medium where a pronounced quenching effect seems to operate. Dilution studies would have confirmed the quenching effect. But given the wider objectives of the investigation this was not persued further.

These observations contradict the traditional view that saprophytic bacteria are the only users of dissolved organic nitrogen, undertaking its mineralisation and releasing free ammonia, which is in turn utilised by phytoplankton in primary production. Bacteria clearly do play a role in this process and are thought to be able to out-compete phytoplankton for most organic substrates in marine ecosystems (Wheeler and Kirchman, 1986). Ducklow *et al.* (1986) have noted, however, that their role is more important in the mineralization than in the assimilation of organic matter into higher trophic levels.

The comparative quantitative roles of bacteria and micro-algae in the utilisation of organics is

not clear. It has been argued that the products of bacterial protein hydrolysis and deamination are largely consumed by the bacteria themselves, given their relative C:N ratios which indicate a higher nitrogen requirement for bacteria (3:1) than for micro-algae (6.6:1)(Wheeler and Kirchman, 1986). The process seems to be partly controlled by the C:N ratio of the substances being degraded (Billen, 1984). Where C:N > 10 the uptake and utilisation by bacteria of ammonium produced in deamination exceeds its release to the medium.

The ultrastructural investigation was conducted to establish whether morphological differences are apparent in cells grown in effluent compared to growth in defined medium and whether observed changes might further clarify the label uptake studies. While *D. salina* cells showed distinct changes associated with growth in organic medium, the results for the *Spirulina* were not clear. The ultrastructure of *Spirulina* cells has been extensively studied (Holmgren *et al.*, 1971) ; van Eykelenburg, 1979 ; van Eykelenburg, 1980 ; Golecki and Heinrich, 1990) and changes relating to organic nutrition have not been reported. It is suggested that further immunocytochemical studies could assist in determining to what extent organic uptake occurs and how this might be quantified in the operation of an HRAP.

At this point in the study it was noted that having demonstrated a probable role for organic nutrition in the system, confirmation of the effect and its quantification would form the basis of a complete investigation in itself, and one outside the scope of this study. It was therefore decided to pursue the further evaluation and development of an HRAP approach to pond management and tannery effluent treatment.

Chapter Four

A *Spirulina*-based High Rate Algal Ponding Process for the Treatment of Tannery Wastewater.

4.1. Introduction

The concept of linking the production of useful biomass and the treatment of wastewater involving algae has been recognised from the earliest developments in algal biotechnology (Burlew, 1953 ; Oswald *et al.*, 1957). The system offers the dual benefits of an effective treatment process and an economic medium for the production of algal biomass. The HRAP system has already been used extensively in the treatment of domestic sewage (Shelef, 1982 ; Oswald, 1988a). Also to treat animal wastes (Rodriques and Oliviera, 1987a ; Fallowfield *et al.*, 1992 ; Mitchell and Richmond, 1988) and food industry effluents (Rodriques and Oliviera, 1987b ; Ayala and Vargas, 1987). Recently Rose *et al.* (1996) described its use in the treatment of saline tannery effluent.

The pond survey detailed in chapter 2 showed that the tannery effluent in the final WSP supported the growth of large seasonal 'blooms' of both *Spirulina* and *D. salina* and their growth was associated with substantial reductions in COD load and odour nuisance. Several of the above studies have indicated that the filamentous cyanobacterium *Spirulina*, may be screen-separated from the medium with relative ease, indicating its promise as an organism for wastewater treatment and one with a substantial market value in the form of dried biomass. A previous investigation by Laubscher (1991) had shown the marginal practicability of a *Dunaliella*-based HRAP with the capability to treat the full salinity range of effluents produced by a tannery. The likely out-competition of *Dunaliella* by *Spirulina* in an open HRAP system was confirmed in this study and no further investigation of a *Dunaliella*-based approach was undertaken.

It was against this background that the following investigation was conducted into the feasibility of developing a saline *Spirulina*-based High Rate Algal Pond for the treatment of tannery effluent, using the strain isolated from the ponds in Wellington. Given the

observation of *Spirulina* growth in only the latter ponds of the WSP it was assumed at the outset that the study would have to include an investigation of factors giving rise to and constraining this distribution.

4.2. Research Objectives

The research objectives were to establish whether the Wellington *Spirulina* isolate could be successfully cultivated in combined tannery effluent discharged to the WSP system, and following only primary treatment as described in chapter 2. Also to determine what factors limit *Spirulina* growth in the early ponds in the WSP system, whether the growth of *Spirulina* results in organic load reduction effects, and whether growth performance would be sufficient to consider the HRAP model as an effective linkage between the functions of pond management, effluent treatment and the yield of a biomass product with commercial value.

4.3. Materials and Methods

4.3.1. *Spirulina* Culture

All experimentation reported in this study involved the use of an effluent-adapted culture of the *Spirulina* isolate from the WSP at Wellington.

4.3.2. *Spirulina* Culture Medium

4.3.2.1. Defined Medium

The defined Zarrouk's medium used in this study for the cultivation of the *Spirulina* isolate was prepared as described by Zarrouk (1966). The medium was made up with the appropriate volume of distilled water and filter sterilised through 0.45 μm GF/A Filters (Whatman).

4.3.2.2. Effluent-formulated Media

The effluent formulated media used in this study for the cultivation of *Spirulina* were

prepared by making up dilutions with tap water, of untreated combined tannery effluent (UTE), physico-chemically pre-treated combined tannery effluent (PTE) or aerated ponded tannery effluent (PE) drawn from several ponds. The tannery effluents were filtered through Whatman No.1 filters to remove suspended particles and through 0.45 μm GF/A filters (Whatman) where sterile conditions were required. The salinity and pH of the effluents were not adjusted. For certain experiments the effluent formulated medium was enriched with nutrients by adding NaOH and NaCl to adjust the pH and the salinity. For the grid experiments, sodium bicarbonate and ammonium chloride were added to set up a concentration range.

4.3.3. Effluent Analysis

Procedures for chemical analysis are as described in A.P.H.A. Standard Methods (1989). Sodium, calcium, iron, and chromium were determined using a Varian Atomic Absorption Spectrophotometer. Certain analyses of nitrate, ammonia and phosphate were undertaken using a Merck SQ118 Spectroquant apparatus. The salinity of the wastewater was determined using an Atago Salinity Refractometer.

Although the validity of COD values measured in high salinity media are open to question due to the oxidation of chlorides by chromate, Gocke and Hoppe (1977) have found that reliable comparisons are possible for brackish-water environments especially where there is not a great deal of variation in the salt content composition of the sample, and where appropriate controls are used (Rose, 1991).

4.3.4. Heavy Metal Removal

Heavy metal removal studies employed anaerobic tannery effluent sourced from the base of waste stabilisation Pond A, and incubated in a 200 L container ensuring that anoxic conditions were maintained throughout. This anaerobic effluent culture was then dispensed into 500 mL stoppered flasks to which pond A influent containing metals of known concentration was added. The cultures were then allowed to incubate for 168 hours, at room temperature. Following preparation procedures described in A.H.P.A. Standard Methods (1989) the

samples were analysed using Atomic Absorption Spectroscopy (Varian) for the levels of aluminium, cadmium, chromium, cobalt, copper, iron, lead, magnesium, manganese, nickel, potassium, sodium, and zinc.

4.3.5. Flask Growth Studies

Culture media was dispensed in cotton wool stoppered 500 mL conical flasks and after inoculation, incubated in a constant environment chamber - temperature 25 °C and a 8:16 hour Light:Dark cycle. Flasks were incubated on a light bench with light intensity of 158 μmoles. $m^{-2}.s^{-1}$ PAR. Each experimental result reflects a triplicate mean. Oswald (1988a) has described the use of preliminary flask growth tests to determine the compatibility of specific algal/effluent systems. The importance of using acclimated cultures in biological organic utilisation studies in environmental engineering has been noted by Gaudy and Gaudy (1980).

4.3.6. Estimation of Cell Growth

4.3.6.1. Cell Counts

All cyanobacterial cell counts were made in an improved Neubauer haemocytometer and results reflect a triplicate mean.

4.3.6.2. Determination of Chlorophyll$_a$

Cells were separated from growth medium by filtration through 0.45 μm GF/A filter discs (Whatman). The filtered cells were then placed in 80 % acetone for extraction of the chlorophyll$_a$ pigments. The absorbance was read at 660 nm on a UV-160A Shimadzu UV-Visible Spectrophotometer, while the levels of chlorophyll$_a$ were calculated using the formulas described by Lichtenthaler (1987).

4.3.7. Photobioreactor Simulation

A New Brunswick Bioflo III, microprocessor-controlled fermentation system was used to

conduct scale-up evaluations of the initial flask studies. The photobioreactor adaptation was achieved through the arrangement of a half circle of four 50 cm, cool white fluorescent tubes around the diameter of the vessel. Techniques involving the adaptation of fermentation vessels for algal modelling studies have been described (Markl, 1980 ; Rose, 1991). The use of photobioreactors for *Spirulina platensis* growth studies have been described by Cornet *et al.*, 1992a ; Cornet *et al.*, 1992b). The changes in DO, temperature, and pH were logged at 15 minute intervals using Advanced Fermentation Software (New Brunswick Instruments). The Ingold DO electrode was calibrated by sparging uninoculated medium with nitrogen and oxygen as described by the manufacturers. Headspace pressure was equilibrated with atmospheric pressure via a condenser vent which also provided the only source of possible gas exchange between the internal and external environments. The *Spirulina* culture and PTE used in the photobioreactor simulation were sourced from the Wellington ponds.

4.3.7.1. Optimisation Studies

A 10 % inoculum of the *Spirulina* culture was added to 5 L Zarrouks medium and placed in the photobioreactor with the following operating parameters; temperature 25 °C, salinity 30 $g.L^{-1}$, agitation 30 rpm, and light intensity 158 μmoles. $m^2.s^{-1}$ PAR. Samples were drawn daily via the sampling device and the levels of *Spirulina* biomass were monitored by analysis of the amounts of $chlorophyll_a$, as previously described.

4.3.7.2. Batch Cultures

In the Batch studies, 5 % PTE was added to the optimised effluent-adapted *Spirulina* culture. Analysis of the changes in levels of COD, NO_3 and NH_3, were performed using a Merck Spectroquant SQ118. The growth of *Spirulina* biomass was monitored by analysis of the amounts of $chlorophyll_a$, as previously described.

4.3.7.3. Fed batch Cultures

In the Fed Batch studies, 3 %, 5 % and 8 % PTE was added to the optimised effluent-adapted *Spirulina* culture. Again analysis of the changes in levels of COD, NH_3, NO_3 and PO_4

were performed using a Merck Spectroquant SQ118, while the level of *Spirulina* biomass was monitored by analysis of the chlorophyll$_a$ concentration. The oxygen uptake rate was calculated from the slope of the DO utilisation curve after the addition of the effluent and O_2 sparging as described by Gaudy and Gaudy (1980).

4.3.8. Ammonia Toxicity

Effluent-adapted *Spirulina* culture was removed from the photobioreactor and used in experiments to determine the levels at which ammonia becomes toxic to the cyanobacteria. The cultures were set up in 50 mL tissue culture flasks arranged in a grid formation, on a light box providing light intensity of 158 μmoles.m^2.s^{-1} PAR, and incubated at 25 °C in a growth room for 5 days. No agitation was provided. The change in chlorophyll$_a$ was used to determine growth rates, sodium bicarbonate (reagent grade, Merck) was added to flasks in a range of concentrations.

4.4. Results

4.4.1. *Spirulina* Culture

The species of *Spirulina* occurring in the WSP in Wellington appears as a near pure culture of one morphological type of *Spirulina*. A culture was isolated from the ponds and was tentatively identified as *Spirulina platensis* as previously noted (see **Figure 4.4.1.**). Some confusion exists in species taxonomy of *Spirulina*, because of the wide morphological variability which exists within this genus (Bai and Seshadri, 1980 ; Richmond, 1986a). Detailed studies of *Spirulina* species have been conducted by Fott and Karim (1973), while a revision of generic assignments, strain histories and properties of pure cultures of cyanobacteria has been conducted by Rippka *et al.* (1979). Detailed taxonomy is crucial for the selection and maintenance of *Spirulina* strains suitable for mass culture (Tomaselli *et. al.*, 1987).

4.4.2. *Spirulina* Cultivation

Following isolation, the *Spirulina* was incubated under controlled conditions.

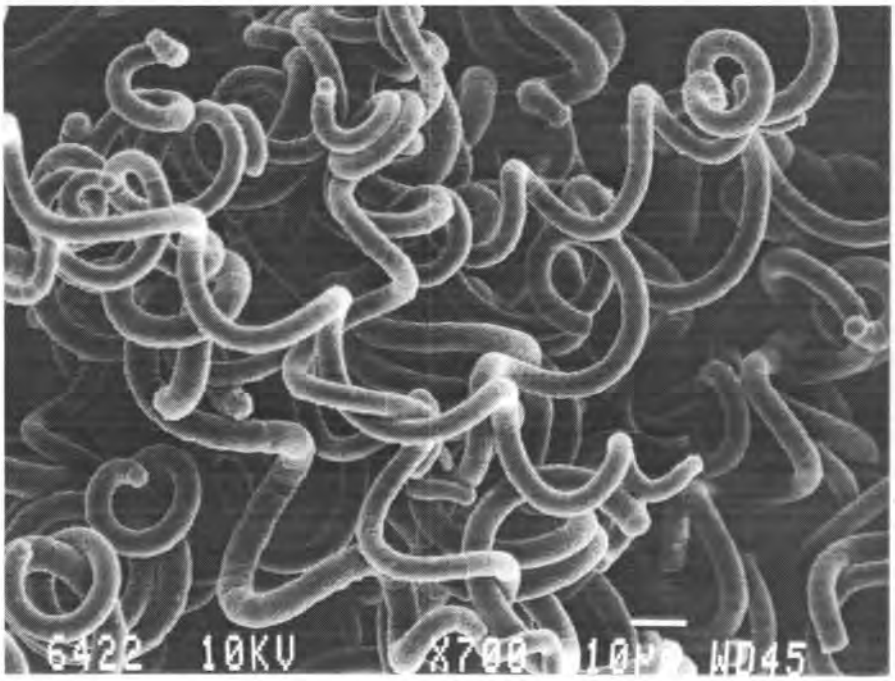


Figure 4.4.1. Scanning electron micrograph of the *Spirulina* isolate from the waste stabilisation ponds at Wellington.

The growth rate of the *Spirulina* was determined from daily cell counts (see **Figure 4.4.2.**). A generation time of 48 hours for growth in Zarouk's medium was noted for this strain under the conditions applied.

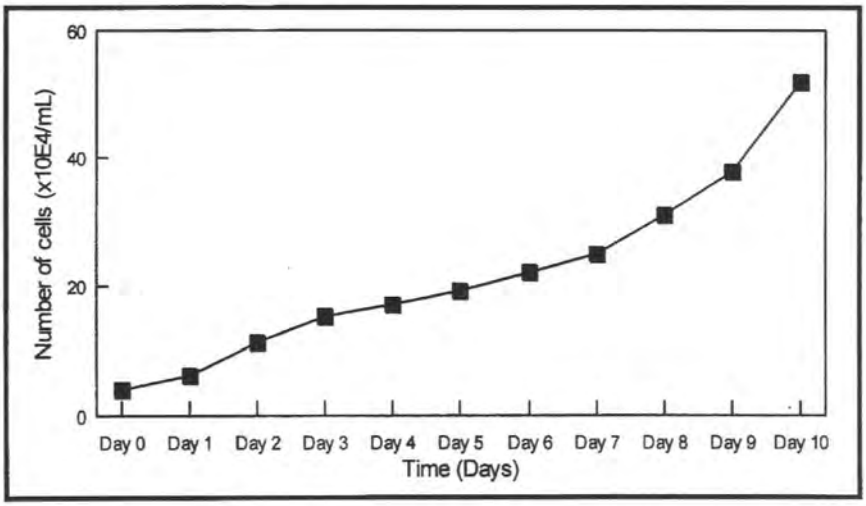


Figure 4.4.2. Growth curve for *Spirulina* in defined (Zarouk's) medium. Results reflect the mean of 3 experiments.

Under favourable conditions of illumination, temperature and adequate supply of nutrients the generation time of most cyanobacteria varies between 12 and 36 hours (Fay, 1983) and it is evident that the effluent-grown cultures require a period of about seven days to adapt to pure-culture growth conditions.

4.4.3. Untreated Tannery Effluent

Untreated combined tannery effluent obtained from the experimental tannery at LIRI, Grahamstown, was used for the initial studies on *Spirulina* growth in tannery effluent. The analysis of the UTE is reported in **Table 4.4.1.**

Table 4.4.1. Chemical analysis of untreated combined tannery effluent from the experimental tannery at LIRI.

All values in $mg.L^{-1}$ except pH or where specified	Untreated combined tannery effluent
Ammonia as NH_3	323
Chemical oxygen demand	17720
Nitrogen as soluble N	1540
pH	12.3
Sulphate as SO_4	364
Sulphide as Na_2S	1192
Suspended solids	1736
Total dissolved solids (%)	1.93
TDIS (%)	0.63

No cyanobacterial growth occurred even at 10 % dilution of UTE as shown in **Figure 4.4.3.** The high levels of COD appear to be strongly inhibitory to the *Spirulina* growth, while the high pH (12.3) and ammonia levels (323 $mg.L^{-1}$) resulted in chlorosis and photooxidative death of the cyanobacterial cells and cell lysis. The high SS (1736 $mg.L^{-1}$) results in a high turbidity and a dark colour resulting in limited light penetration. Based on the results which indicated the unsuitability of the raw UTE as a growth medium it was decided to evaluate whether physico-chemically PTE could be used as a medium to support the growth of *Spirulina*.

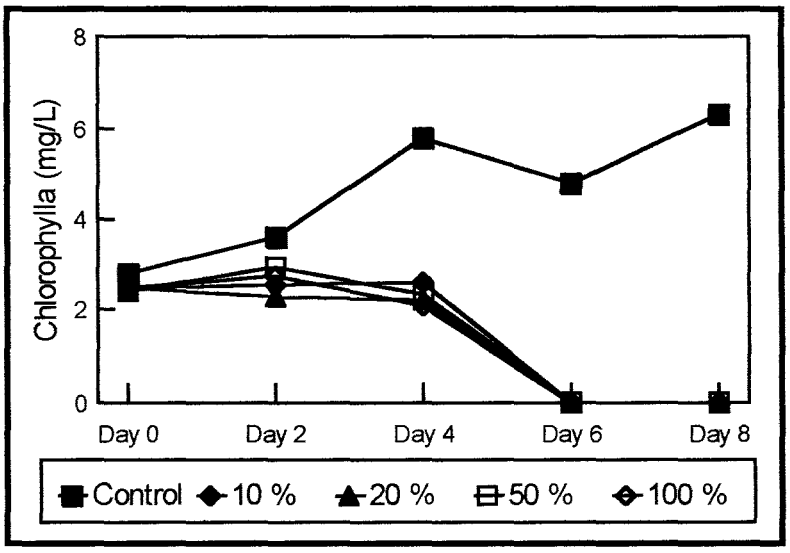


Figure 4.4.3. Growth of *Spirulina* in various concentrations of untreated combined tannery effluent medium. Results reflect the mean of 3 experiments.

4.4.4. Pre-treated Tannery Effluent

The combined process effluent used for formulating effluent media in this study was sourced from the Mossop Western Tanning, Wellington. This effluent is physico-chemically pre-treated on-site to remove suspended solids, and to reduce the levels of sulphide and ammonia. The average analysis of this effluent source based on samples collected and analysed over a period of several months, is reported in **Table 4.4.2.**

A comparison of the composition of PTE and various ponded effluents (PE) sourced from Wellington was undertaken including the calculation of the Algal Biomass Potential (ABP) reported by Oswald (1988a). This is a theoretical technique used to determine nutrient limitations in waste media based on the assumption that all nutrients are in forms available the algae and that algal composition remains constant. It is usually used together with a bioassay technique.

Suitability of these effluents as growth media was evaluated in comparison with the formula proposed by Zarrouk (1966) for the cultivation of *Spirulina*. The results of these studies are reported in **Table 4.4.3.** and **Table 4.4.4.**

Table 4.4.2. Chemical analysis of pre-treated combined tannery effluent from Mossop Western Leathers, Wellington. Standard deviation in brackets.

All values $mg.L^{-1}$ except pH	Pre-treated combined tannery effluent
Ammonia as NH_3	731 (98)
Chloride as Cl_2	4048 (1687)
Chemical oxygen demand	2474 (1810)
Nitrogen as soluble N	569 (106)
pH	8.2 (0.06)
Phosphate as P_2O_5	19 (12.5)
Potassium as K	127 (67)
Sulphate as SO_4	975 (788)
Sulphide as Na_2S	285 (422)
Sus pended solids	243 (196)
Total dissolved solids	11475 (3006)
Total dissolved inorganic solids	9097 (3061)

In general the PTE and PE each provide an adequate medium for the cultivation of *Spirulina*, when compared to defined medium. However, in the full strength PTE the 731 $mg.L^{-1}$ ammonia measured is some 7.3 times higher than the 100 $mg.L^{-1}$ toxic threshold level reported for *Spirulina* (Natarajan, 1970 ; Abeliovich and Azov, 1976 ; Abeliovich, 1983 ; Konig *et al.*, 1987).

The ABP as an indicator of the nutritional capacity of an effluent, can be calculated for the different effluents assuming a ratio of carbon 50 %, nitrogen 8 % and phosphorus 1 %, in dried cell biomass, described by Oswald (1988a). The calculated ABP carbon value in **Table 4.4.4.** indicates that the level of carbon available for growth may be limiting in the PTE when compared to defined medium, but that the level of carbon does increase through the ponding cascade. The calculated ABP nitrogen value indicates that the nitrogen levels would not be limiting in the PTE when compared to defined medium, but that these levels decrease through the effluent treatment process. The low levels of phosphate through the WSP system reported in **Table 4.4.3.** result in a low calculated ABP phosphate value which indicates that the levels of phosphate may be a limiting factor for cyanobacterial growth in all the tannery effluents.

Table 4.4.3. Comparison of the chemical compositions of defined medium and various tannery effluent media sourced from Mossop Western Leathers, Wellington.

All values in $mg.L^{-1}$	Defined (Zarrouk's) medium	Pre-treated tannery effluent	Pond 5 effluent	Pond C effluent	Pond 11 effluent
Calcium	13.3	226	/	/	/
Carbon	2800	957	1246	1615	2585
Chlorine	527	4048	7157	11651	24184
Iron	0.4	10.7	6.2	0.65	6.14
Magnesium	7.0	260	171	191	190
Nitrogen	500	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

Table 4.4.4. Comparison of the algal biomass and oxygen release potential of defined and various tannery effluent media, sourced from Mossop Western Leathers, Wellington.

ABP	Defined (Zarrouk's) medium	Pre-treated tannery effluent	Pond 5 effluent	Pond C effluent	Pond 11 effluent
Carbon	5600	1914	2492	3230	5170
Nitrogen	6250	8962	1562	787	675
Phosphorus	6250	1900	2400	2300	2400

The growth of *Spirulina* was evaluated in dilutions of the PTE (5 %, 10 %, 20 %, 50 %, and also in an undiluted sample). The growth response is reported in **Figure 4.4.4.**

The *Spirulina* cultures showed the following interesting phenomena: at low dilutions of PTE (namely 5 and 10 %) the growth rate of *Spirulina* was higher than that of the control (defined medium), at higher dilutions of the PTE (namely 20 and 50 %) the growth was lower than that for the control (defined medium), and finally at full strength (100 %) PTE the *Spirulina* became chlorotic, died and disappeared after 48 hours. It should be noted that the growth of *Spirulina* at dilutions of 20 and 50 %, although at first strongly inhibited, after day 5 began to show an increase in cell numbers. This would seem to indicate that the inhibitory factors present in the effluent were overcome either by removal or by an avoidance strategy employed by the *Spirulina*.

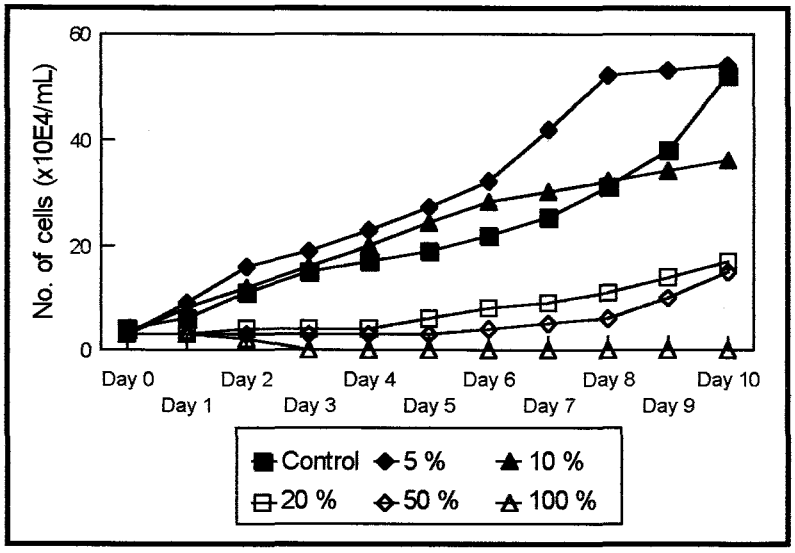


Figure 4.4.4. Growth of *Spirulina* in various concentrations of pre-treated combined tannery effluent medium. Results reflect the mean of 3 experiments.

An investigation of *Spirulina* growth in adjusted PTE was undertaken to evaluate the availability and completeness of the effluent as a growth medium. As mentioned previously, *Spirulina* is known to grow and live under alkaline conditions, thus unfavourably low pH values could possibly inhibit growth. Experiments were conducted to determine the optimum pH and concentration of salinity for the growth of *Spirulina* in PTE. The results for a set of three pH/salinty grid matrix experiments are reported in **Table 4.4.5.** and the trends are illustrated in **Figure 4.4.5.** These experiments, in which the pH and salinity for PTE were adjusted, showed that the *Spirulina* grows best at a pH of 9.5, while the growth was significantly reduced at pH 8.0 and pH 11.0. Similar results have been reported for pH sensitivity of *S. maxima* in synthetic media (Kosaric *et al.*, 1974).

Table 4.4.5. pH/Salinity grid matrix growth rate study for *Spirulina* in pre-treated tannery effluent, growth measured as mg $chl_a.L^{-1}.d^{-1}$ Results reflect the mean of 3 experiments.

Salinity/pH	8	8.5	9	9.5	10	11
0.25 M	0.34	0.54	0.64	0.61	0.17	0.12
0.50 M	0.19	0.23	0.41	0.37	0.36	0.25
0.70 M	0.17	0.24	0.35	0.34	0.34	0.18
0.90 M	0.15	0.22	0.34	0.31	0.42	0.04
1.00 M	0.05	0.09	0.19	0.25	0.19	0
2.00 M	0	0	0	0.02	0	0

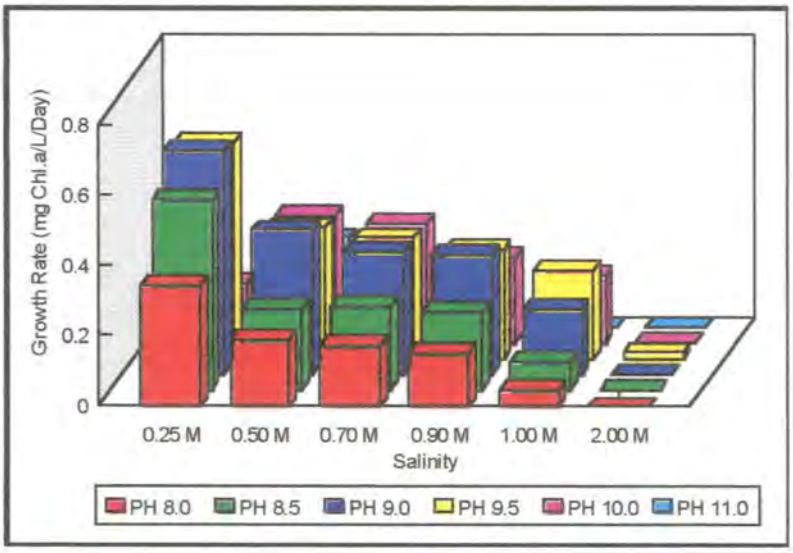


Figure 4.4.5. Three dimensional representation of a pH/Salinity grid matrix growth rate study of *Spirulina* in pre-treated tannery effluent.

The optimum salinity for the growth of *Spirulina* in PTE was found to be 0.25 M which would confirm the observation made in chapter 2 that the latter ponds in the WSP support large 'blooms' of *Spirulina*. However, where the salinity increases above 0.50 M the concentrations of *Spirulina* decline and are replaced by 'blooms' of *Dunaliella* in those ponds with extremely high salinity measurements.

Table 4.4.6. Phosphate/bicarbonate grid matrix growth rate study for *Spirulina* in pre-treated tannery effluent, growth measured in mg $chl_a.L^{-1}.d^{-1}$ Results reflect the mean of 3 experiments.

Bicarbonate/ phosphate	860 *ug*.L^{-1}	431 *ug*.L^{-1}	*251 ug*.L^{-1}	*172 ug*.L^{-1}	86 *ug*.L^{-1}	Control
Control	0.14	0.16	0.06	0.18	0.04	0.15
0.05 M	0.06	0.17	0.13	0.15	0.34	0.8
0.10 M	0.19	0.03	0.16	0.35	0.3	1.05
0.15 M	0.11	0.16	0.19	0.64	0.59	1.09
0.20 M	0.33	0.72	0.98	1.09	1.19	1.13
0.40 M	0.25	0.47	0.35	0.75	0.57	0.62

Several authors have reported the addition of sodium bicarbonate to enhance the growth of *Spirulina* in a range of wastewaters (Chaudhari *et al.*, 1980 ; Materassi *et al.*, 1984 ; Mitchell and Richmond, 1988). Experiments were thus conducted to determine the optimum sodium bicarbonate concentrations for the growth of *Spirulina* in adjusted PTE. The results for a set of three phosphate/bicarbonate grid matrix experiments are reported in **Table 4.4.6.**

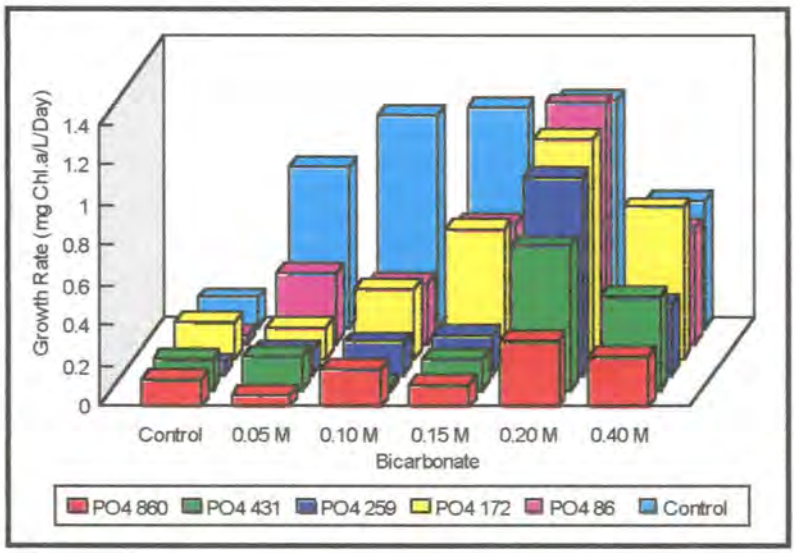


Figure 4.4.6. Three dimensional representation of a phosphate/bicarbonate matrix growth rate study of *Spirulina* in pre-treated tannery effluent. Phosphate recorded in $\mu g.L^{-1}$.

and trends are illustrated in **Figure 4.4.6.** The results show that the growth of *Spirulina* in PTE is enhanced when sodium bicarbonate is added up to a concentration of 0.4 M after which higher levels of bicarbonate appear to become inhibitory.

The effect of the sodium bicarbonate is to provide adequate buffering capacity preventing rapid changes in pH due to cell growth. In addition the sodium bicarbonate may also result in the removal of toxic components such as high levels of magnesium or calcium, via precipitation. The study indicated that the addition of phosphate at low concentrations enhanced the growth of the *Spirulina*, however, at increased concentrations phosphate exerted an inhibitory effect on growth.

4.4.5. Photobioreactor Studies

4.4.5.1. Optimisation

Following the initial flask studies, in which the optimisation of PTE as a growth medium was demonstrated, the growth response of the effluent-adapted *Spirulina* culture was measured in a photobioreactor study. A Bioflow III micro-processor controlled 2 L fermenter was set up

to operate as described in the methods section. A 500 mL culture of *Spirulina* was inoculated into 5 L of defined medium (Zarrouk's), and the growth followed over a 5 day period. The results, reported in **Figure 4.4.7.**, show that the *Spirulina* underwent a period of adaptation on transfer from the defined medium to the effluent medium. After this lag phase (in this case approx. 3 days) the *Spirulina* culture began to grow exponentially in the fermenter at the set operating parameters.

4.4.5.2. Fed Batch Culture

Organic load reduction of a 5 % PTE make-up was examined in a 5 L fed batch culture. Without emptying the reactor, 250 mL of the *Spirulina* culture in the defined medium was removed and replaced with 250 mL fresh PTE medium. 10 mL of the red bacterial culture present in the WSP was added to the fed batch culture to ensure that an appropriate inoculum of the bacteria which occur in the ponds was present in the culture. The culture was allowed to stabilise over the period of a day and then analytical readings commenced.

The fermenter was operated for 192 hours as a fed batch culture. The overall trend that emerged from this study indicated that the growth of the *Spirulina* in the 5 % PTE medium (shown in **Figure 4.4.8.**) could be correlated to a reduction in the pollution load (reported in **Table 4.4.7.**). The reduction in organic load (COD) over the 5 day period was 98 %, at a removal rate of 19.5 $\%.day^{-1}$. The rising levels of chl_a indicate continued cyanobacterial growth throughout the treatment period.

Table 4.4.7. Photobioreactor fed batch study of *Spirulina* in pre-treated tannery effluent.

All values in $mg.L^{-1}$	Effluent	Day 2	Day 3	Day 4	Day 5
Ammonia	731	15	25	28	29
COD	2474	964	456	99	51
Nitrogen	569	74	64	62	57

4.4.5.3. Continuous Culture

Following the initial 5 % fed batch culture investigation, experiments were conducted to

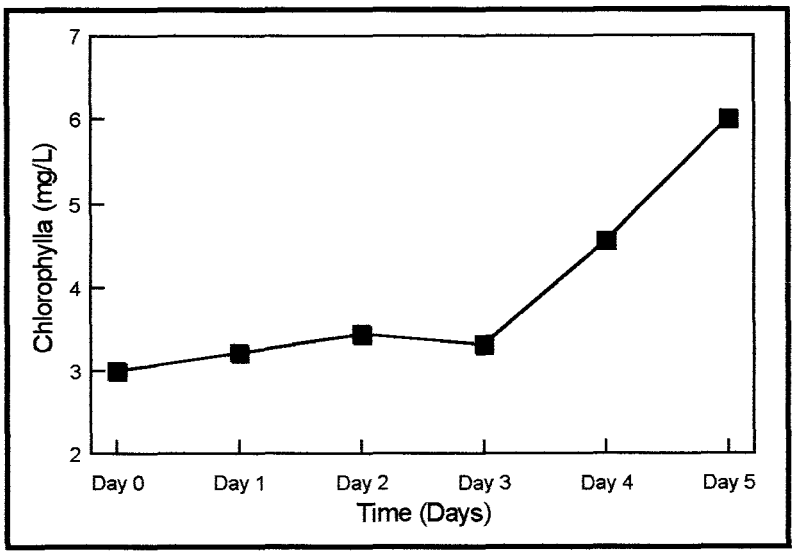


Figure 4.4.7. Growth of *Spirulina* in the Bioflow III photobioreactor recording growth as change in chlorophyll concentrations.

establish optimum loading rates. The *Spirulina* culture was run as a PTE continuous culture with daily loading rates of 3 %, 5 %, and 8 % of total reactor volume. Each percentage effluent loading regime produced approximate steady state conditions with regard to the various parameters measured. Results reported in **Table 4.4.8.** showed that 91 %, 86 %, and 77 % of the organic (COD) load was removed at loading rates of 3 %, 5 %, and 8 % respectively.

The nitrate levels were reduced by 97 %, 94 %, and 94 % at the loading rates used, while phosphate was completely removed at all loading rates.

Table 4.4.8. Performance of the continuous culture photobioreactor study at different daily loading rates. Standard deviations in brackets.

	Effluent	3 %	5 %	8 %
Biomass ($mg.L^{-1}$)	/	173 (42)	173 (43)	167 (11)
COD ($mg.L^{-1}$)	2474 (1810)	213 (102)	347 (50)	563 (85)
DO ($mg.L^{-1}$)	0.01	11.93 (1.84)	12.69 (1.25)	11.81 (1.23)
NH_3 ($mg.L^{-1}$)	731 (98)	41 (18)	61 (18)	135 (38)
NO_3 ($mg.L^{-1}$)	569 (106)	15 (9.6)	32 (13.3)	34 (27)
pH	8.2 (0.06)	10.0	10.0	10.0
PO_4 ($mg.L^{-1}$)	19 (12.5)	<1	<1	<1
Salinity ($g.L^{-1}$)	10.0 (1.6)	30.0	30.0	30.0

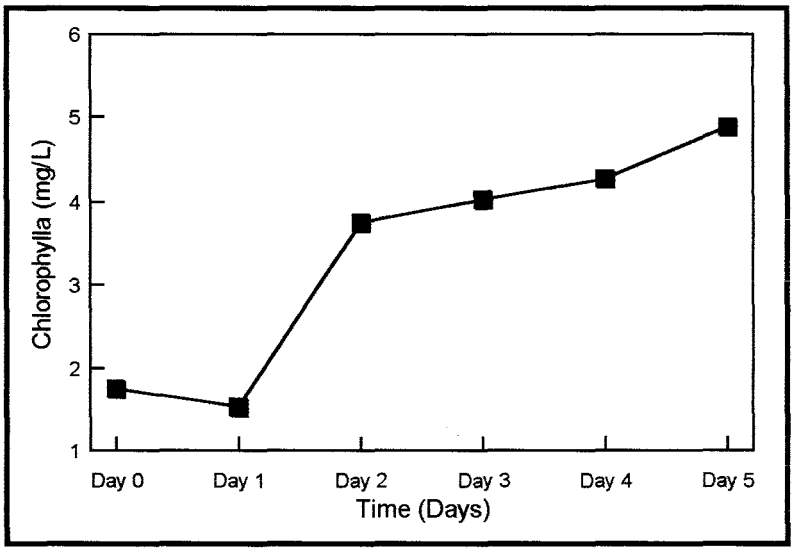


Figure 4.4.8. Fed batch study of *Spirulina* growth in pre-treated tannery effluent.

Although the ammonia levels were reduced by 94 %, 92 %, and 81 % respectively, ammonia showed a stepwise increase that correlated with increased effluent loading of the reactor. The cyanobacterial numbers showed a concomitant decline as the ammonia concentration exceeded approximately 80 $mg.L^{-1}$.

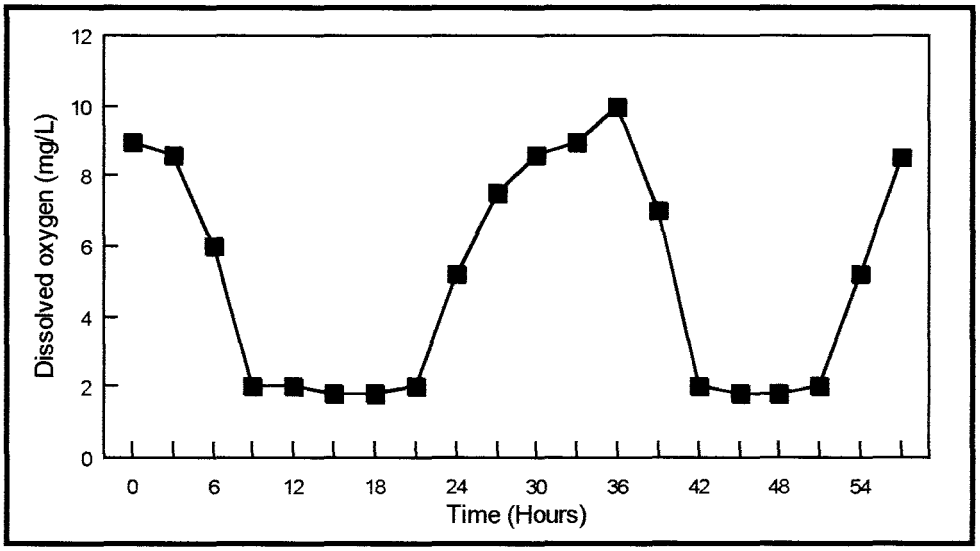


Figure 4.4.9. Dissolved oxygen in the photobioreactor study recording changes on the addition of pre-treated tannery effluent at a loading rate of 5 $\%.day^{-1}$.

The DO recovery period was determined for each loading rate. The oxygen demand was high during the initial stages of the process. Where photosynthetic oxygen production started to exceed heterotrophic consumption, this coincides with the removal of the major part of the organic load indicating a substantial respiratory consumption in the system. Results reported in **Figure 4.4.9.** show a 21 hour period for the 5 % loading. The 8 % loading rate required 42 hours for DO recovery (results not shown). This substantiates the observation of accumulating components at the 8 % daily loading rates followed by culture decline.

4.4.6. Ammonia Toxicity

Although there was an effective removal of the pollution load the *Spirulina* culture showed sub-optimal growth at higher loading rates. The lower growth rates of the *Spirulina* may be correlated with the higher volumetric loading rates, and it was proposed that this may be ascribed to the fact that the cultures were exposed to higher concentrations of ammonia and organic material. Coupled to the low phosphate levels in the pre-treated combined tannery effluent, this induces physiological stress conditions resulting in lower algal productivity at higher loading rates. The problem may be overcome by enriching the PTE with sodium bicarbonate. This has been reported for situations where *Spirulina* was grown in sewage waste (Chaudhari, *et al.*, 1980).

Adapted *Spirulina* culture was removed from the photobioreactor and used in experiments to determine the level at which ammonia becomes toxic to the cells, and also to investigate the mitigating effect of sodium bicarbonate addition. The change in chlorophyll$_a$ was used to determine the growth of *Spirulina* at different bicarbonate concentrations. The results reported in **Figure 4.4.10.** show that an increase in the bicarbonate level results in an increase in the growth rate of the *Spirulina* at ammonia levels that were found to be inhibitory in the previous experiment. This ability of the *Spirulina* to tolerate higher levels of ammonia, in the presence of bicarbonate in the closed bioreactor, and despite an equilibrium shift to toxic unionised form, indicates that the higher productivities achieved are linked to increased nitrogen consumption and that recirculation of alkaline pond water may allow increased loading rates to an HRAP. An evaluation of recirculation was undertaken and is reported in chapter 5.

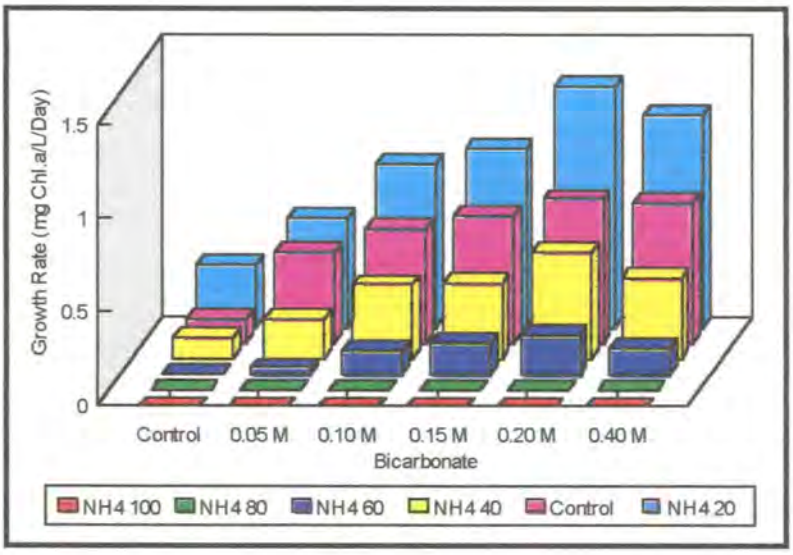


Figure 4.4.10. Three dimensional representation of an ammonia/sodium bicarbonate grid matrix growth rate study of *Spirulina* in pre-treated tannery effluent.

4.4.7. Heavy Metal Contamination

Accumulation of heavy metals by algae has been well documented (Becker and Venkataraman, 1980 ; Maart, 1993). This presents a very real problem where the biomass recovered in the proposed HRAP is targeted for use as a feed source. In the existing effluent treatment process no effluent stream separation or metal recovery/recycle process is in operation. Tannery effluents contain significant levels of heavy metals and to avoid the anticipated problem of heavy metal accumulation in the *Spirulina* biomass harvested from a tannery HRAP an investigation was conducted to establish means of reducing the levels of these metals in the tannery effluent. Analysis to trace the source and distribution of heavy metal contamination in the effluent sourced from the Wellington tannery are shown in **Table 4.4.9.**

These results indicate that although a substantial amount of the metals present in the tannery effluent are removed in the sludge component during the pre-treatment stage there was still a high enough level remaining in the PTE to cause concern over the use of the *Spirulina* grown in this medium as an animal feed. Thus an investigation was conducted to establish whether the metals present in the PTE could be removed by precipitation under anaerobic conditions.

Table 4.4.9. Heavy metal concentrations in effluents occurring at various stages of treatment at Mossop Western Leathers, Wellington.

All values $mg.L^{-1}$	Untreated Combined Tannery Effluent	Treated Sludge Values	Pre-treated Tannery Effluent	Aerated Ponded Effluent
Aluminium	17.2	456	1.2	0.5
Cadmium	0.08	0.21	0.02	0.03
Chromium	4.68	1405	0.25	0.6
Cobalt	0.35	1.7	0.18	0.16
Copper	0.03	0.32	0.02	0.02
Iron	39.9	336	0.41	0.41
Lead	0.76	6.2	0.11	0.02
Magnesium	688	1899	468	194
Manganese	62.9	704	1.2	0.22
Nickel	0.41	4.95	0.21	0.18
Potassium	117	120	111	104
Sodium	4390	1899	3090	2790
Zinc	1.51	8.1	0	0

Bacterial mineralization of organic matter in anoxic sediments proceeds by reduction of electron acceptors like nitrate (denitrification), bicarbonate (methanogenesis), and sulphate (sulphate reduction) Oremland *et al.* (1989). The activity of SRB has been demonstrated in the anaerobic tannery effluents and reported in chapter 2. Several reports have documented the use of SRB in the removal of metal contaminants from industrial wastewater with the precipitation of metal sulphides (Miller, 1950 ; Ilyaletdinov *et al.*, 1977 ; Whang, 1981). Metal precipitation experiments were conducted in the laboratory using sulphide rich anaerobic effluent sourced from the bottom of pond A (see **Figure 2.4.6.).** Results reported in **Table 4.4.10.** show that the heavy metals are removed by precipitation, probably as metal sulphides, but also possibly as metal hydroxides in the anaerobic pond A medium.

4.5. Discussion

The studies outlined indicate that *Spirulina* is able to grow in PTE. Indeed, at certain dilutions significantly higher cell yields were obtained in tannery effluent than in the defined Zarrouk's mineral medium, optimised for the growth of this cyanobacterium. This correlates with earlier observations relating to heterotrophic nutrition for this organism. The tannery effluent appears to provide complete nutritional requirements necessary for the growth of *Spirulina.*

Table 4.4.10. Experimental precipitation of heavy metals in anaerobic pond A water.

Metal	Metal added (corrected) ($mg.L^{-1}$)	Metal in Supernatant ($mg.L^{-1}$)	% Precipitated
Aluminium	107.83	< 1	99.16
Chromium	53.36	5.24	90.18
Cobolt	59.96	3.90	93.49
Copper	50.88	2.76	94.57
Lead	130.08	1.12	99.14
Manganese	107.47	0.34	99.68
Nickel	49.84	8.59	82.76
Iron	57.94	2.2	96.20

Alkalinity is mandatory for the growth of *Spirulina* as reflected in the pH optimum for its growth which ranges from pH 8.3 to 11.0 (Richmond, 1986c). *Spirulina* can readily tolerate progressive changes in the pH. The culture may, however, quickly deteriorate when the pH is changed abruptly, as may happen in a growth medium which is not well buffered (Richmond, 1986c). The 0.2 M $NaHCO_3$, which is the major salt component in the defined growth medium for *Spirulina*, provides a good buffering capacity (Richmond, 1986c) and reduces the level of contamination by other algae. High bicarbonate content and low concentrations of gaseous CO_2 were identified as major factors that prevented the contamination of *Spirulina* cultures by *Chlorella* (Richmond *et. al.*, 1982).

The pH and salinity of the tannery effluents need to be maintained at the highest possible values that still result in maximum *Spirulina* productivity and at the same time create the selective pressure for a near mono-culture of *Spirulina.* The poor control and lack of predictability of algal species occurring, has been widely recognised as one of the main problems associated with fresh water algal biotechnology systems (Benemann *et al.*, 1980 ; Richmond, 1986b ; Oswald, 1988a ; Rose, 1991).

It has been reported that high levels of Ca^{+2} and Mg^{+2} in water for *Spirulina* growth needs to be reduced, e.g. by precipitation with pH adjustment using sodium bicarbonate (Faucher *et. al.*, 1979 ; Richmond, 1981), as high levels of these ions may be toxic to *Spirulina* (Mitchell, 1986). The levels of both these ions in pre-treated tannery effluent appear to be at acceptable levels to allow for the growth of *Spirulina.* Both Na^{+2} and K^{+2} are indispensable in the

Spirulina growth medium. Inhibition of growth takes place when the K^{+2} to Na^{+2} ratio is >5. As long as this ratio is below 5, growth is uninhibited even at very high concentrations of 18 $g.L^{-1}$ Na^{+2}, as is found in the ponding system (Richmond, 1986c ; Mitchell, 1986).

The ABP carbon value indicates that the level of carbon available for *Spirulina* growth may be limiting in the pre-treated tannery effluent, due to slow breakdown of the organic compounds (Corning, 1978 ; Carre *et al.*, 1983), but that this limitation is removed further down the effluent treatment process as more carbon becomes available due to bacterial breakdown of the organic components. In outdoor cultures sufficient CO_2 can be taken up from the atmosphere to alleviate this problem and up to 5 g $C.m^{-2}.day^{-1}$ has been reported to be assimilated in this way by *Spirulina* (Richmond, 1986c).

The ABP nitrogen value indicates that the nitrogen levels are not limiting in the pre-treated tannery effluent, but that these levels decrease through the effluent treatment process. An analysis of the tannery effluent conducted by Rose (1991), showed that the effluent has sufficient nitrogen, to support cyanobacterial growth. Mainly nitrates are assimilated by *Spirulina*, as a nitrogen source, but ammonium salts may be used. Levels of ammonia need to be carefully controlled as this compound is toxic to *Spirulina* at levels greater than 100 mg $N.L^{-1}$ (Abeliovich, 1986).

Urea has also been used as a nitrogen source for the mass culture of *Spirulina* (Richmond, 1986c). Phycocyanins and biliproteins, involved in the light harvesting reactions, may also serve as a nitrogen storage material since the phycocyanin concentration is highest when *Spirulina* is cultivated under favourable nitrogen conditions. When the level of available nitrogen in the medium decreases, or the cultures are completely deprived of nitrogen, a corresponding specific decrease in the cell content of phycocyanin is observed (Boussiba and Richmond, 1979 ; Boussiba and Richmond, 1980 ; Cifferi and Tiboni ,1985).

The ABP phosphate value indicates that the levels of phosphate may be a limiting factor for cyanobacterial growth. The low phosphate levels are due firstly to the low levels entering the effluent stream and secondly to the precipitation of the phosphate as calcium-phosphate (Moutin *et al.*, 1992) due to the high pH and the high levels of lime used in the leather

production process.

A reduction in organic and inorganic nutrient loading occurs during cyanobacterial growth, and offers the potential advantage of an effluent treatment operating simultaneously with the production of cell biomass.

Two apparently contradictory effects appear to operate on the cells grown in the tannery effluent medium. On the one hand full strength (undiluted) effluent can be strongly inhibitory, but on the other hand, at lower dilutions, there are pronounced growth stimulatory effects. The toxic effect can be correlated with high ammonia levels in the effluent.

In the full strength PTE, where cell growth was severely inhibited, the 731 $mg.L^{-1}$ ammonia measured is some 7.3 times higher than the (100 $mg.L^{-1}$) toxic threshold level reported for *Spirulina* (Natarajan, 1970 ; Abeliovich and Azov, 1976 ; Abeliovich, 1983 ; Konig *et al.*, 1987). Where cell growth in effluent has been successful, this can, in turn, be correlated with dilution factors that bring the ammonia concentration within a non-toxic range. Increasing alkalinity offers a growth advantage in addition to the ammonia dilution response noted.

Ammonia toxicity has been shown to be a major factor limiting algal growth in fresh water HRAP, used in the treatment of organic wastes such as sewage (Abeliovich and Azov, 1976; Abeliovich, 1980 ; Azov and Goldman, 1982 ; Konig *et. al.*, 1987 ; Soares *et. al.*, 1995). Ammonia is a potent uncoupler of photosynthesis and Abeliovich (1986) reports that when it exceeds 2 mM, at a pH 8.1-8.2, photosynthesis is inhibited and the system must rely on respiration for ammonia removal, which occurs at a 5-6 times slower rate. Algal photosynthesis is thus effectively under ammonia control in the HRAP system. As the pH exceeds 9, the NH_3 form predominates and this, according to Chevalier and de la Noue (1985), penetrates the cell by membrane diffusion rather than active transport, and thus is toxic at very low levels (Rose, 1991).

Of special significance when using a HRAP to treat wastewater is to establish the correct loading rates and retention times (Miller *et. al.*, 1977 ; Abeliovich, 1980). Results from the reported studies indicate that these parameters can be used to regulate ammonia toxicity in the

HRAP. Cultures pre-adapted to effluent growth do, however, perform better than inocula grown in defined medium. If ammonia is the crucial component causing the toxic effect, it is possible that adaptation may be the combined result of an avoidance mechanism acquired by cyanobacteria and the control of a bacterial population competent to deal more effectively with the proteolytic, deamination, ammonification and, possibly, deammonification functions involved in the organic degradation processes.

While the cause of the inhibitory effect is uncertain, these studies have, nevertheless, shown that inhibition of cyanobacterial growth can be overcome. Either dilution, resulting in increased retention times, or addition of sodium bicarbonate, apparently make this a reversible phenomenon. The substantial increase in alkalinity through the ponding system noted in chapter 2 suggests that the recirculation of carbonate rich water, together with the dilution of ammonia by manipulating loading rates, may offer effective strategies for operating a *Spirulina* HRAP receiving the pre-treated tannery effluent.

The operation of the AIWPS configuration developed by Oswald (1991) provides for an anaerobic unit operation ahead of the algal HRAP. The reduction in organic load demonstrated across pond A which operates anaerobically through most of its 4 m depth provided an opportunity, at the tannery ponding site in Wellington, to evaluate an Oswald-type configured system. The anaerobic step was also shown to provide a potentially important function in facilitating the removal of problematic heavy metals by precipitation as metal sulphides in this unit. The successful operation of this step is especially important where microalgae are to be grown in industrial effluents which might contain heavy metals or where the potential for spills in the production process indicates the need for a wide margin of protection.

Borowitzka and Borowitzka (1989) have commented on the value of scale-up studies in designing large-scale algal operations. The results outlined in this laboratory study, designed to demonstrate the initial feasibility of the *Spirulina*-based HRAP for the treatment of tannery effluent, were used as the basis for the subsequent design and operation of an integrated algal ponding system incorporating an 80 m^2 pilot-scale HRAP and is reported in chapter 5.

Chapter Five

Pilot-scale Evaluation of an Integrated Algal Ponding System Treating Tannery Wastewater.

5.1. Introduction

The outdoor cultivation of *Spirulina* today is generally carried out in raceways, mixing being provided by a paddle wheel (Richmond and Preiss, 1980 ; Cardenas and Markovits, 1985 ; Dodd, 1986 ; Richmond and Becker, 1986 ; Shimamatsu, 1987 ; Oswald, 1988a ; Oswald, 1988b ; Richmond, 1996). The most crucial challenge in the commercial production of *Spirulina* biomass is the maintenance of a mono-algal culture throughout the year. The basic demand in this respect is to provide stable growth conditions which will not deviate too much from the optimum for *Spirulina* (Richmond, 1986a ; Richmond, 1987). Macro-models have thus been developed for outdoor algal mass production, (Grobbelaar, 1980 ; Guterman *et al.*, 1989 ; Guterman and Ben-Yaakov, 1990), which predict optimal biomass concentrations for outdoor ponds.

The laboratory feasibility studies described in chapter 4 demonstrated that the growth of the Wellington *Spirulina* isolate in PTE was accompanied by a reduction in the organic load and thus providing an indication of the potential feasibility of a *Spirulina*-based HRAP as an engineerable process for the treatment of these wastes. In order to evaluate the practical performance of such a system it was decided to undertake the scale-up investigation of the proposed process in an outdoor pilot-plant study at the Mossop Western Leathers site in Wellington. This would not only enable the practical evaluation of the results derived from down-scaled laboratory studies but would also offer the opportunity to investigate the integration of anaerobic and photosynthetic aerobic stages in conceptualising an Oswald-type Integrated Algal High Rate Oxidation Ponding approach to the design of a treatment process (Oswald, 1991).

5.2. Research Objectives

The objectives of this study included the design and construction of two 80 m^2 pilot-scale HRAP located at the Wellington ponding site so as to be able to receive feed waters in sufficient quantities directly from the pre-treatment plant, from the anaerobic pond A and also high alkalinity waters recycled from pond 11.

The performance of the proposed process would be evaluated using this pilot facility and specifically to determine the following:

1. The operation of existing pond A as an anaerobic treatment stage prior to the tannery wastewater entering the HRAP;
2. Appropriate loading rates to the HRAP allowing effective organic load reduction and also providing adequate dilution of the ammonia toxicity effects;
3. The additive role of high-alkalinity waters recycled from pond 11 in regulating the ammonia toxicity effect;
4. *Spirulina* biomass productivities during effluent treatment and also the production of sufficient biomass to undertake toxicity and feed evaluation studies.

5.3. Materials and Methods

5.3.1. Pilot plant Design and Construction

Two 80 m^2 pilot-scale HRAP were designed using literature cited specifications (Oswald, 1988b) and in collaboration with R. Rowswell of LIRI. These two HRAP were constructed at the Wellington site in association with R.W. Smith (plant engineer) at Mossop Western Leathers, with funding provided by the Water Research Commission. The two pilot-scale ponds were located alongside the existing pond B, **Figure 5.3.1.** Refer to **Appendix 1** for full design plans for the pilot ponds.

The initial construction of the two 80 m^2 HRAP required the preparation of the site area. The chosen area located adjacent to pond B was cleared of all vegetation and rocks before the

ground was levelled using a front end loader. The site was then built up to a height of 50 cm above the surrounding ground level. This was done by working in natural clay as fill and compacting to 90 % to ensure a firm base on which to build.

The outer walls of the two HRAP were then built up to a height of 50 cm above the base. The walls were compacted and shaped with a slope of 1:3 to give the HRAP its characteristic rectangular form with rounded ends.

A UV stabilised PVC plastic liner was used to seal the HRAP. This liner was placed directly in the shaped pond and care was taken to avoid folds. The edges of the liner were folded into a shallow trench and a small amount of earth was added to keep the liner in place, to prevent the edges being lifted by the wind during construction of the centre wall.

The central dividing walls of the ponds were constructed out of concrete cast on paving slabs placed directly on the plastic liner. These tapered walls were 50 cm in height, while around the paddle wheel the central walls were built up to 1 m. Underneath the paddle wheel a 2 cm concrete base was cast to ensure a level surface between the base of the pond and the blade of the paddle, thus maximising the mixing effect of the paddle wheel.

The paddle wheel 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, which had 8 evenly spaced paddles, was driven by an electric motor which was placed alongside.

The level of the water in the HRAP was controlled by an overflow box with slats placed at one end of the HRAP. The overflow boxes were constructed out of concrete and the plastic liner was held in place by a metal frame to prevent leaking. The tannery effluent was fed into the HRAP from pond 6 using a pump.

5.3.2. *Spirulina* Culture

All experimentation reported in this study involved the use of an adapted culture of

Figure 5.3.1. Photograph of the two 80 m^2 pilot high rate algal ponds at Mossop Western Leathers, Wellington.

Spirulina drawn from pond 6.

5.3.3. Estimation of Cell Growth

The estimation of cell growth was determined by cell counts and determination of chlorophyll$_a$ following methods described in chapter 4. In addition biomass content was determined as described in A.P.H.A. Standard Methods (1989). The productivity of the *Spirulina* was determined as outlined in chapter 2.

5.3.4. Effluent Analysis

All physical and chemical analytical procedures used methods as outlined in chapters 2 and 4, or otherwise as described in A.P.H.A. Standard Methods (1989).

5.3.5. Ammonia Toxicity Experiments

Recirculation of high-alkalinity water from pond 11 to the HRAP was investigated in the laboratory in 500 mL conical flasks in a constant environment room at 25 °C, with a light intensity of 158 μmoles.m^{-2}.s^{-1} PAR, and with volumetric loading rates of 5 %, 15 % and 25 % (total flask volume) PTE. Analysis of the physical and chemical parameters and the estimation of cell growth and productivity are as outlined in chapters 2 and 4. Results of this study were used to establish the recirculation rates to the HRAP.

5.3.6. Heavy Metals Analysis

For heavy metals concentrations, *Spirulina* biomass (corresponding to 1.5 g dried weight) was placed in a 100 mL volumetric flask with a few glass beads. 10 mL nitric acid was added, followed by the addition of 30 mL mixed perchloric-sulphuric acid (3:1). Digestion was continued over heat until the thick, white acid-fumes lifted from the pale yellow remaining liquid. This remaining solution was cooled to room temperature, followed by the addition of 50 mL distilled H_2O. The solution volume was reduced to 25 mL by boiling, and then made up to 50 mL with distilled H_2O. Heavy metal content was then determined by Atomic Absorption Spectroscopy (Varian) and results reported within the range of detection limitations.

5.4. Results

5.4.1. Anaerobic Pond

The existing pond A, receiving PTE directly from the tannery, was operated together with surface inflow and with surface aeration, as a mixed facultative system in the upper layers and with a large dead volume below. Water passes rapidly through pond B to pond 1 without substantial treatment effect. It was found that pond A (**Figure 5.4.1.**) could be successfully converted to function anaerobically in an upflow mode by directing inflowing PTE to enter at the bottom of the pond by means of a pipe. Surface aerators operate continuously and ensure no mixing with subsurface layers in this 4 m pond.

Figure 5.4.1. Anaerobic pond A at Mossop Western Leathers, Wellington.

Odour release is controlled and an oxypause is established at about 0.5 m depth. Outflow now leaves the pond by passing through this well-defined aerated zone ensuring oxidising conditions prevail in effluent reaching the HRAP, via pond B.

Results obtained for the operation of the converted anaerobic pond (**Table 5.4.1.**) indicate an overall reduction in the organic load (COD) of 51 %, together with a reduction in ammonia and sulphide levels of 38 % and 73 % respectively. There was a 99 % removal of settleable solids. Oswald (1995) has reported that 60-80 % of the influent BOD and all suspended solids are removed by the anaerobic stage in the AIWPS treating sewage wastewaters, with which results reported here are comparable.

Analysis of samples of substantial biogas production within the base of the pond following its performance optimisation showed 19 % methane and 81 % carbon dioxide, and indicated an operational methanogenic process. Green *et al.* (1995) have reported values of 54 % methane in the AIWPS.

Table 5.4.1. Analysis of pond A effluent. Standard deviations in brackets.

All values in $mg.L^{-1}$ except pH	Combined tannery effluent entering pond A	Primary Anaerobic Pond outflow
Ammonia as NH_3	731 (98)	452 (105)
Chemical Oxygen Demand	2474 (1810)	1216 (93)
pH	8.17 (0.06)	8.10 (0.707)
Phosphate as P_2O_5	19 (12.5)	1.65 (2.33)
Sulphate as SO_4	975 (788)	989 (90)
Sulphide as Na_2S	285 (422)	76.5 (16)
SS	243 (196)	0.45 (0.49)
TDS	11475 (3006)	17320 (339)

5.4.2. Heavy Metal Removal

The presence of heavy metals in tannery effluents, and the potential problems associated with possible accumulation in harvested biomass, was noted in chapter 4, together with the results of a laboratory evaluation of the extent of precipitation, and hence sequestering, which may be anticipated in the effective anaerobic ponding of these wastewaters (see **Tables 4.4.9.** and **4.4.10.**). The optimisation of the anaerobic pond reported above provided an opportunity for the field evaluation of these findings to be undertaken on-site at Wellington.

Table 5.4.2. Metal concentrations in harvested biomass from high rate algal pond and waste stabilisation ponds.

All values in $mg.Kg^{-1}$	Standard Acceptable	Stabilisation Pond	HRAP
Aluminium	200-300	273.5	1540
Cadmium	1.0	6.72	5.96
Cobalt	4-6	18.41	22.36
Chromium	10-15	9.69	25.8
Copper	50-100	6.71	1.99
Iron	1250-2500	308.7	2012
Potassium	/	20820	15898
Magnesium	/	1038	1481
Manganese	200-300	14.42	23.35
Sodium	/	45140	7452
Nickel	10-15	30.11	49.19
Lead	15-20	210	218.6
Zinc	250-500	33.6	218.5

The concentrations of heavy metals were measured in biomass harvested from both the pilot HRAP and the facultative ponds prior to, and following, the optimisation intervention. The results are reported in **Tables 5.4.2.** and **5.4.4.** This change to the routing of effluent led to a more stabilised anaerobic region at the bottom of pond A and reduced levels of heavy metals entering the evaporation ponding system have been demonstrated. With the exception of nickel, the concentration of which was close to the standard required, all metals were reduced to acceptable levels in the biomass.

Table 5.4.3. Heavy metal concentrations in biomass exposed to a number of treatments.

All values in $mg.Kg^{-1}$	Unwashed	Fresh water	pH 5 water	pH 2 water
Cadmium	<1	1.4	1.4	<1
Cobalt	1.5	2.8	5.7	3.1
Chrome	<1	1.4	<1	<1
Iron	540	648	585	1408
Lead	6.2	<1	<1	<1
Nickel	17	23.7	18.4	11
Zinc	18.5	23.7	19.7	28.3

Richmond (1988) had reported the reduction in carbonate levels in harvested *Spirulina* biomass by washing with acidified and fresh tap water. This procedure was applied to harvested biomass and the results are reported in **Table 5.4.3.** With the possible exception of lead, the concentrations of heavy metals were not successfully lowered by the post-harvesting washing process.

Table 5.4.4. Heavy metal concentrations in high rate algal pond harvested biomass before and after the introduction of the metal precipitation step in pond A.

All values in $mg.Kg^{-1}$	Standard Acceptable	HRAP (before)	HRAP (after)
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

5.4.3. Pilot HRAP-Batch Operated

Following construction the HRAP were filled to a depth of 0.3 m with anaerobic pond effluent (APE) pumped from pond B and then drained. This process was repeated a number of times to ensure removal of any residual contaminants from the concrete and plastic used in the construction of the HRAP.

Subsequently, the ponds were filled to a depth of 0.3 m with a 50 % dilution of APE from pond B and inoculated on day 1 and again on day 3 with 10 % (volume) screen harvested *Spirulina* biomass from pond 6. The two HRAP were initially operated as batch cultures at a depth of 0.30 m and paddle mixing at a linear water flow velocity of 6.6 $cm.s^{-1}$. The results for the initial start-up of the HRAP are listed in **Table 5.4.5.**

Table 5.4.5. Analytical results reporting the start-up of the pilot HRAP operated in batch mode and fed anaerobic pond effluent. Results reflect the mean of 3 experiments. (All values except pH in $mg.L^{-1}$)

Day	1	2	3	4	5	6	7	8	9	10	11	12
Bio	322	360	520	390	322	322	300	368	306	580	510	596
Chl_a	4.8	5.4	7.8	5.8	4.8	4.8	4.5	4.9	4.6	7.8	7.6	8.9
COD	1707	1069	607	859	790	753	362	315	311	300	280	250
DO	0.05	0.05	0.05	0.05	0.06	0.06	1.20	2.70	3.0	6.8	6.8	9.9
NH_3	140	160	130	130	150	130	130	110	70	50	50	10
pH	7.91	7.96	8.05	8.06	8.10	7.94	7.61	7.57	7.90	7.94	7.70	8.13

The low initial levels of DO persisted until day 7, after which there was an increase in the level of DO. This correlated with an increase in the level of chl_a, *Spirulina* growth and a reduction in the organic load (COD).

A degradation of 85 % of the organic load (COD) was demonstrated in the 12 day study at a removal rate of 7 $\%.day^{-1}$. Ammonia was reduced by 93 % at a removal rate of 8 $\%.day^{-1}$ and *Spirulina* biomass increased from 322 $mg.L^{-1}$ to 596 $mg.L^{-1}$ over the study period.

The results of the start up investigation indicated that, although there was a significant reduction in the organic load (COD) the growth of the *Spirulina* in the HRAP had not achieved the levels demonstrated in the laboratory photobioreactor study.

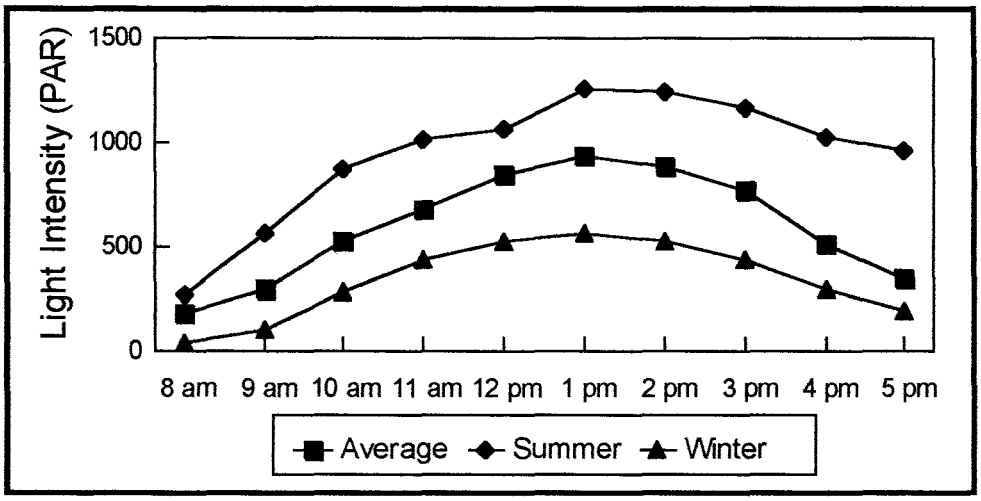


Figure 5.4.2. Changes in light intensity over the day at Wellington measured as photosynthetically active radiation (PAR).

The solar irradiation data collected for the duration of the study period, which reflect daily and seasonal variations, are shown in **Figure 5.4.2.** The chemical composition of tannery effluent is such that as a growth medium for the cultivation of *Spirulina*, it remains turbid even after pre-treatment and passage through the anaerobic ponds (see results reported in chapter 4). The result is that incident light only penetrates to a depth of approx. 15 cm as shown for the pond profile studies in chapter 2.

The HRAP were initially operated during the start-up at a water depth of 30 cm. Following measurements of the solar irradiation and a Secchi disc light extinction depth of (5 cm), the optimal pond depth that ensures sufficient light penetration was calculated to be 15 cm based on the method described by Ramus (1985). The productivity of ponds operating at both 15 cm and 30 cm was measured in start-up cultures. The changes in the *Spirulina* concentration in the HRAP, as a result of the change in operating depth is reported in **Figure 5.4.3.** The concentration of the *Spirulina* culture in the HRAP at a depth of 30 cm showed a net decrease over the 6 days, while the concentration of the *Spirulina* culture in the HRAP at a depth of 15 cm showed an increase over the same period and the commencement of a sustained 'bloom' response.

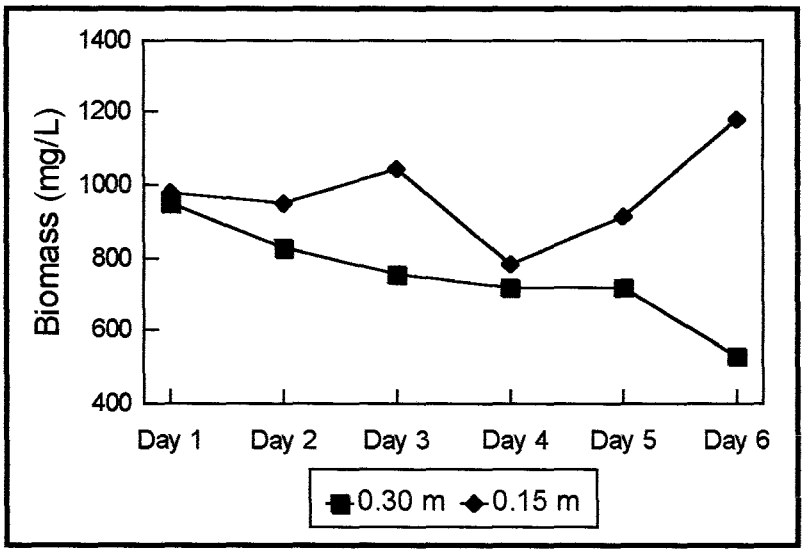


Figure 5.4.3. Changes in *Spirulina* concentration due to variations in the operating depth of the high rate algal pond.

At optimum cell density, defined as that density of biomass which results in the highest output rate per unit area, light limitation is extremely keen. At this density, solar irradiance penetrates to only a fraction of the pond's depth, leaving most of the cells in the culture in complete darkness at any given instant (Richmond, 1980 ; Richmond, 1988). This is why a turbulent flow (mixing) in the pond is the key factor for obtaining high output through a better utilisation of the irradiant flux which reaches the pond surface (Richmond, 1986c ; Richmond and Becker, 1986).

Although the velocity of flow in an open raceway of *Spirulina* should not be less than 5 to 7 $cm.s^{-1}$ (Richmond, 1988), it was found that when the pilot-scale HRAP was initially operated at a water velocity of 6.6 $cm.s^{-1}$, this resulted in the formation of dead spaces in the HRAP. In an attempt to avoid this phenomenon and to improve the mixing characteristics of the HRAP, the water velocity was thus increased to 18 $cm.s^{-1}$. Fallowfield *et al.* (1992) reported an optimum water velocity of 20 $cm.s^{-1}$ in algal raceways.

5.4.4. Pilot HRAP-Continuous Feed

When using the HRAP to treat wastewater it is essential to establish the correct loading rates

Table 5.4.6. Comparison of various operating loading rates of anaerobic pond effluent to the pilot-scale high rate algal pond. Standard deviation in brackets.

All values in $mg.L^{-1}$ except pH	Effluent	3 %	5 %	8 %	10 %
Ammonia	731 (98)	22.5 (15.4)	58.4 (22.1)	120 (40.2)	199 (39.8)
Biomass	/	720 (68.9)	625 (92.8)	234 (5.7)	173 (6.7)
COD	2474 (1810)	330 (66.2)	413 (81.8)	781 (70.7)	924 (87.8)
DO	0.01	10.5 (1.65)	9.5 (0.693)	8.2 (0.590)	5.0 (0.610)
pH	8.2 (0.06)	8.4 (0.24)	8.2 (0.21)	8.4 (0.13)	8.4 (0.13)

and retention times (Miller *et al.*, 1977). These parameters can be used to control the levels of ammonia build up which is reported to be toxic to *Spirulina* at levels above 100 mg $N.L^{-1}$ (Natarajan, 1970 ; Abeliovich and Azov, 1976 ; Abeliovich, 1983 ; Konig *et al.*, 1987).

A series of experiments were conducted over a two year period to determine the optimum loading rate for the HRAP operated as a continuous feed culture. APE from pond B was pumped to the HRAP at volumetric loading rates of 3 %, 5 %, 8 %, and 10 % per day respectively. Performance for the fed batch cultures are reported in **Table 5.4.6.** and reflect averages measured at steady state operation over a 28 day period in each case.

The pilot-scale HRAP demonstrated the ability to reduce the organic load due to dilution and the simultaneous growth of *Spirulina* when operated over a four week period at a hydraulic retention time of 33 days (3 % volumetric loading rate of the HRAP). Results representing means of daily readings showed an average pH of 8.4, the DO averaged 10.5 $mg.L^{-1}$ throughout the 28 day period. The organic load was reduced by an average of 87 % at a removal rate of 2.6 $\%.day^{-1}$, while the level of ammonia was reduced by an average of 97 % at a removal rate of 2.9 $\%.day^{-1}$. The *Spirulina* biomass concentration in the HRAP stabilised at an average of 720 $mg.L^{-1}$ at the 3 % loading rate.

The results for the 5 % volumetric loading rate to the pilot-scale HRAP (20 day hydraulic retention time) showed an average pH of 8.2, the DO was maintained at 9.5 $mg.L^{-1}$ throughout the four week period. The organic load was reduced by 83 % at an average removal rate of 4.2 $\%.day^{-1}$, while the level of ammonia was reduced by 92 % at an average removal rate of

4.6 %.day^{-1}. The *Spirulina* biomass concentration in the HRAP averaged 625 mg.L^{-1} through the course of the 5 % loading study.

The results for the 8 % volumetric loading rate to the pilot-scale HRAP (12.5 day hydraulic retention time) showed an average pH of 8.4, and that the DO was maintained at 8.2 mg.L^{-1} throughout the four week period. The organic load was reduced by 68 % at an average removal rate of 5.5 %.day^{-1} , while the level of ammonia was reduced by 83 % at an average removal rate of 6.7 %.day^{-1}. The *Spirulina* biomass concentration in the HRAP averaged 234 mg.L^{-1} .

The results for the 10 % volumetric loading rate to the pilot-scale HRAP (10 day hydraulic retention time) showed an average pH of 8.4, and DO of 5.0 mg.L^{-1} . The organic load was reduced by 63 % at an average of 6.3 %.day^{-1} , while the level of ammonia was reduced by 73 % at an average removal rate of 7.3 %.day^{-1}. At the 10 % volumetric loading rate the *Spirulina* biomass could only be maintained at 173 mg.L^{-1} for short periods after which there was a washout due to increased ammonia concentrations causing photooxidative death of the cyanobacteria.

These results obtained for the outdoor pilot-scale HRAP indicate that the system can be successfully used as a secondary treatment process to the primary processes, including pre-treatment and anaerobic digestion in the facultative ponding step, by reducing the organic load by 87 %, 83 %, 68 %, 63 % at volumetric loading rates of 3 %, 5 %, 8 %, and 10 % respectively.

The growth rate of the *Spirulina* in the HRAP was measured and gross photosynthetic productivity of the *Spirulina* recorded for the different effluent loading rates for both summer and winter conditions. The results are reported in **Table 5.4.7.** The general decreasing trend for the productivity of the *Spirulina* in the pilot-scale HRAP, occurs with increased volumetric loading rates and from summer to winter conditions. At best, the productivity of the *Spirulina* in the pilot-scale HRAP was found to be lower than data previously reported for pond 6 in chapter 2 or for the laboratory studies reported in chapter 4.

Table 5.4.7. *Spirulina* productivity in the pilot-scale high rate algal ponds fed anaerobic pond effluent at various loading rates. Results reported as mg $C.m^{-2}.day^{-1}$. Standard deviation in brackets.

	Ave. for the Waste Ponds	3 %	5 %	8 %	10 %
Summer	12585 (2439)	10374 (1096)	7194 (750)	5141 (290)	4450 (199)
Winter	4250 (508)	3671 (287)	2866 (317)	1998 (245)	1242 (219)
Average	7478 (1378)	7022 (4740)	5030 (3060)	3569 (2222)	2846 (2268)

5.4.5. Ammonia Toxicity

Two laboratory investigations were undertaken to establish whether the toxic effect of the high levels of ammonia could be reduced thus allowing increased loading to the HRAP. These studies were based on reports of ammonia removal by aeration in WSP (Pano and Middlebrooks, 1982) and by stripping-reabsorption from deliming effluent (O'Brien *et al.*, 1986).

First PTE was aerated by vigorous air sparging and the results reported in **Table 5.4.8.** show a 42 % reduction in ammonia levels at a rate of 14 $\%.day^{-1}$. The indication that the high-alkalinity water from the end of the ponding system could be used to reduce the ammonia toxicity (reported in chapter 4) was evaluated in a simulation exercise. PTE was diluted with 20 % pond 11 water (analysis reported in chapter 2) and subjected to the same aeration regime used above. Results in **Table 5.4.8.** show 100 % ammonia removal by sparging at a rate of 33 $\%.day^{-1}$ taking into account the dilution effect.

Table 5.4.8. Ammonia stripping of pre-treated tannery effluent by vigorous aeration and addition of alkaline pond water in flask studies.

Ammonia in $mg.L^{-1}$	Day 1	Day 2	Day 3
Pre-treated Combined Tannery Effluent	730	448	420
20 % Recirculation of Pond 11 water	146	50	0

Based on these promising results a more comprehensive dilution series was evaluated using pond 11 water but added to water drawn from the anaerobic pond A. The results reported in **Table 5.4.9.** show an increased buffering capacity with increasing concentration of pond 11

Table 5.4.9. Changes in pH and total alkalinity due to recirculation of pond 11 water measured in flask studies. Standard deviation in brackets.

Percentage Pre-treated Tannery Effluent	pH	total alkalinity (mg $CaCO_3.L^{-1}$)
100	8.10 (0.290)	2900
90	8.83 (0.099)	2900
80	9.07 (0.099)	3240
75	9.16 (0.113)	3560
65	9.35 (0.078)	4300
50	9.57 (0.106)	4640
25	9.79 (0.099)	5600
20	9.83 (0.099)	5800
0	9.90 (0.092)	6500

addition. The 20 % recirculation results in an increase in total alkalinity from 2900 $mg.L^{-1}$ to 5800 $mg.L^{-1}$, while the pH increases from 8.10 to 9.83.

5.4.6. Increased Loading Rates

Laboratory studies were undertaken to establish whether the ammonia toxicity reduction effect, achieved by regulation of pond 11 water, could be used to operate a higher loading rate

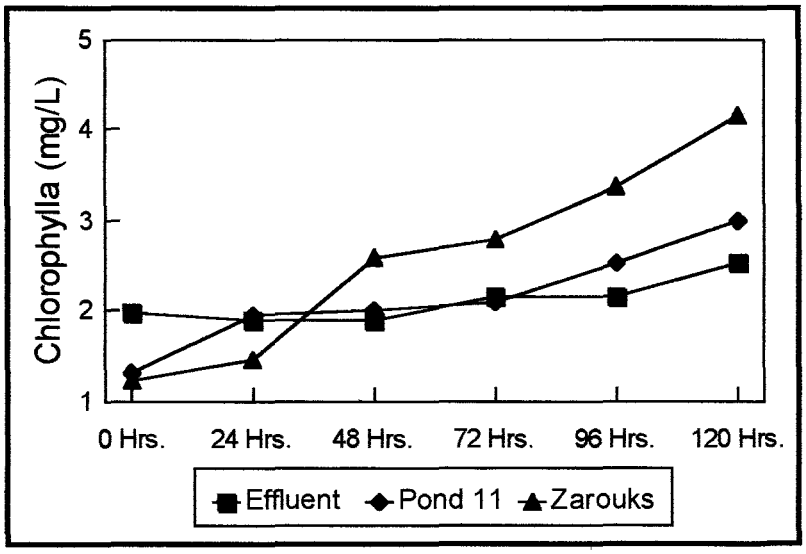


Figure 5.4.4. Growth of *Spirulina* in flask studies fed anaerobic pond effluent at 5 % loading rate. day^{-1} including recirculation from pond 11.

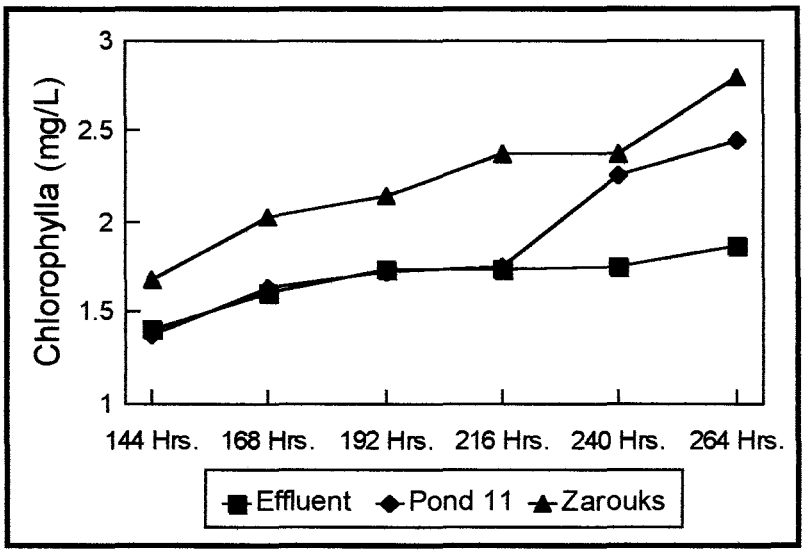


Figure 5.4.5. Growth of *Spirulina* in flask studies fed facultative pond effluent at 15 % loading rate. day^{-1} including recirculation from pond 11.

to the HRAP. The growth of *Spirulina* in defined medium, APE, and 20 % pond 11 water added to APE at volumetric loading rates of 5 %, 15 % and 25 %.day^{-1} was compared by changing operating conditions in a continuous culture. The results show that the recirculation of 20 % pond 11 water may allow the HRAP to be operated at higher loading rates because of

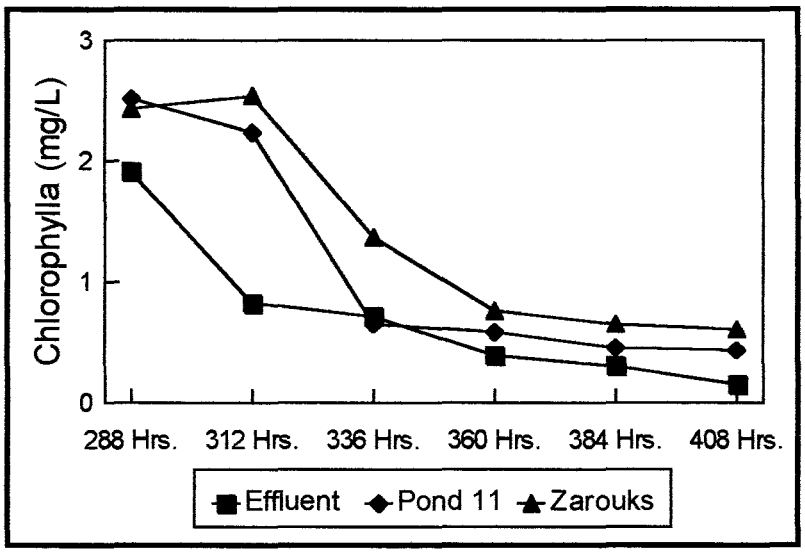


Figure 5.4.6. Growth of *Spirulina* in flask studies fed anaerobic pond effluent at 25 % loading rate. day^{-1} including recirculation from pond 11.

increased growth of *Spirulina* when compared to APE (**Figures 5.4.4., 5.4.5. and 5.4.6.**). At a volumetric loading rate of 5 %.day^{-1} the growth of *Spirulina* in defined media is higher than either of the other treatments. After a period of adjustment the growth of *Spirulina* in 20 % pond 11 water added to APE is comparable to that of defined media, at a volumetric loading rate of 15 %.day^{-1}. At a volumetric loading rate of over 25 %.day^{-1} the growth of *Spirulina* is insufficient to maintain a stable culture and washout results.

In addition to following the growth of the *Spirulina* in response to increasing volumetric loading rates using chl_a (as detailed above), the fixation of carbon was used to allow comparison of each treatment with the productivity measurements made in the waste pond (**Table 5.4.10.**). The productivity of the *Spirulina* in the studies was always higher in the treatments where defined media was added. The addition of 20 % recirculated pond 11 water resulted in an increase in the productivity of the *Spirulina* grown in APE. The productivity of the *Spirulina* was 8306 mg C.m^{-2}.day^{-1}, when 20 % recirculated pond 11 water was added compared to 4349 mg C.m^{-2}.day^{-1} at the 5 % volumetric loading rate. This is higher than the productivity recorded for the existing pond 6 (7478 mg C.m^{-2}.day^{-1}). At higher volumetric loading rates the productivity of the *Spirulina* was considerably higher in the 20 % recirculation treatments than in the treatments with APE alone. For example the productivity of *Spirulina* at the 15 % volumetric loading rate was 5173 mg C.m^{-2}.day^{-1}, which is higher than the value of 2846 mg C.m^{-2}.day^{-1} recorded at a volumetric loading rate of 10 % in the outdoor HRAP (see **Table 5.4.7.**).

Table 5.4.10. Productivity of *Spirulina* measured in flask studies fed anaerobic pond effluent at various loading rates including recirculation from pond 11. Results measured mg C.m^{-2}.day^{-1} and reflect the mean of three values. Standard deviation in brackets.

	Waste Pond	5 %	15 %	25 %
Zarrouks	/	12690 (1655)	6758 (2919)	615 (130)
20 % Pond 11	/	8306 (2649)	5173 (4816)	519 (113)
Raw Effluent	7478 (1378)	4349 (1751)	2752 (2020)	379 (300)

The optimum volumetric loading rate from the above experiments appears to lie somewhere between 15 and 25 % (i.e. a retention time of 5 to 6.6 days). Above this volumetric loading rate the retention time is not adequate to allow optimum *Spirulina* growth in the HRAP.

Table 5.4.11. Analysis of effluent treatment in flask studies fed anaerobic pond effluent at various loading rates including recirculation from pond 11. Results reflect the mean of three values. Standard deviation in brackets.

All values in $mg.L^{-1}$ except pH	Effluent	5 %	15 %	25 %
Ammonia	731 (98)	44 (10.8)	149 (18.8)	269 (12.4)
Biomass	/	155 (34.4)	114 (30.0)	66 (38.6)
COD	2474 (1810)	342 (11.05)	704 (74.3)	1131 (34.8)
DO	0.01	9.25 (5.33)	7.55 (3.69)	5.16 (3.92)
pH	8.17 (0.06)	10.09 (0.53)	10.19 (0.62)	9.05 (0.09)

The growth of the *Spirulina* in the HRAP simulation experiments was accompanied by a significant organic load reduction effect, see **Table 5.4.11.** It was found that at a retention time of 20 days (5 % volumetric loading rate) the organic load was reduced by 86 % at a removal rate of 4.3 $\%.day^{-1}$, while the ammonia level was reduced by 94 % at a removal rate of 4.7 $\%.day^{-1}$. The pH was 10.1 and the DO was 9.3 $mg.L^{-1}$. The *Spirulina* biomass concentration was maintained at 155 $mg.L^{-1}$.

The results for the 15 % volumetric loading rate indicated that at a retention time of 6.6 days the organic load was reduced by 71 % at a rate of 10.8 $\%.day^{-1}$, while the level of ammonia was reduced by 80 % at a removal rate of 12.1 % $.day^{-1}$. The pH was 10.2 and the DO was 7.5 $mg.L^{-1}$. The *Spirulina* biomass concentration was maintained at 114 $mg.L^{-1}$.

At a volumetric loading rate of 25 % (4 day retention time) the organic load was reduced by 54 % at a removal rate of 13.6 $\%.day^{-1}$, while the ammonia level was reduced by 63 % at a removal rate of 15.8 $\%.day^{-1}$. The pH was 9.1 and the DO was 5.2 $mg.L^{-1}$. The *Spirulina* biomass concentration was not constant at 66 $mg.L^{-1}$ and eventually the culture was washed out at this volumetric loading rate.

These results showed COD reduced by 86 %, 71 %, 54 % at volumetric loading rates of 5 %, 15 %, and 25 % respectively. This indicates that the recirculation of pond 11 water to the HRAP system may allow increased volumetric loading rates of the pre-treated tannery effluent to enter the HRAP, thus resulting in higher organic load reductions (probably due to a combination of ammonia dilution and the enhanced growth rate of the *Spirulina*).

Following the successful laboratory demonstration of the effect of the recirculation of 20 % pond 11 water on the growth of *Spirulina* in APE, the experiments were repeated outdoors in the pilot HRAPs. The results (not reported) broadly follow those obtained for the laboratory investigation, with sustainable *Spirulina* growth at volumetric loading rates between 5-10 $\%.day^{-1}$. However due to practical limitations in the design of the pilot ponds which did not allow accurate recirculation directly from pond 11 a stable *Spirulina* culture could not be maintained for periods of more than two weeks at volumetric loading rates higher than 15-20 $\%.day^{-1}$.

5.4.7. Microalgal Capping.

The practical operation of recirculation in the WSP, the ability to establish a microalgal cap of *Spirulina* growth on the surface of anaerobic ponds and the contribution to odour control were evaluated by pumping pond 11 water to pond B, which then flowed through ponds 1 to 5. Apart from increased alkalinity a large inoculum of *Spirulina* was applied to the surface of the anaerobic ponds and **Figure 5.4.7.** shows the result of the capping effect achieved. At the time of this photograph pond 1 had been taken out of commission. The green colour of *Spirulina* growth is evident in ponds 2 to 4 replacing the normal red colour associated with anaerobic conditions as seen in pond 5 and a substantial improvement in odour was noted. However, the bloom conditions are not self sustaining and continuous recirculation is required to maintain the capping effect.

5.5. Discussion

In field-scale studies based on previous laboratory findings it was found that the lower zones of existing pond A could be successfully converted to function anaerobically and could thus be effectively used as the anaerobic pond component for an integrated algal ponding system for treating tannery wastewater. The pipe which directed the inflowing PTE to the bottom of the pond had the effect of establishing a stable anaerobic region at the bottom of pond A and, together with a mechanically aerated stable surface layer, effected an overall reduction in the organic load of 51 %, and a reduction in ammonia and sulphide levels of 38 % and 73 % respectively. There was a 99.8 % removal of settleable solids, while the stable anaerobic conditions led to the removal of a high percentage of heavy metals present in the PTE. As

Figure 5.4.7. Aerial photograph of the WSP at Mossop Western Leathers, Wellington, showing the pronounced colour difference between anaerobic (red) and aerobic (green) ponds, and also the early effects of establishing a microalgal capping of the anaerobic ponds by recirculation.

noted previously, the demonstration of an effective mechanism whereby possible heavy metal contamination of microalgal biomass may be effectively controlled, is a critical requirement in commercial harvesting of feed-grade biomass from Algal Biotechnology-linked treatments of industrial effluents. In the investigation undertaken here the function of the anaerobic ponding step, as a heavy metals sequestering operation, has been demonstrated together with a wide margin of protection afforded against accidental spillage within production areas. Low levels of chromium in the PTE indicate this element was not a factor impacting on the anaerobic digestion rates (Alkan *et. al.*, 1996).

Oswald (1995) has reported that 60-80 % of the influent BOD and all suspended solids are removed by AIWPS treating sewage wastewaters. The operation of the adjusted pond A, while not having the Oswald-type improved anaerobic pit system with constructed berms, nevertheless, effects a comparable level of treatment at somewhat longer hydraulic residence times per kg COD loaded. In this particular configuration anaerobic conditions were sustained, without a pit or protection berms inside the pond, by the action of the surface

aerators maintaining an effective oxypause at about 0.5 m below the surface. Separate optimisation of the anaerobic unit operation using an Upflow Anaerobic Sludge Bed (UASB) has been proposed by van Haandel and Catunda (1995) but this seems unwarranted in terms of an extensive WPS system, where relatively high throughput rates can be achieved, at low cost, in pond-designed reactors. Previous studies reported from this laboratory indicate that high rates of anaerobic digestion may be achieved in saline media where appropriately selected halophilic methanogenic cultures are established (Shipin *et. al.*, 1994). Khnieleniva *et. al.* (1997) have reported on adapted halophilic, alkalophilic methanotrophs and Colleran *et. al.* (1995) have describes the anaerobic treatment of sulphate containing wastewaters.

A successful demonstration of technological potential was achieved with the studies on the growth of *Spirulina*, in APE in the outdoor pilot-scale HRAP. The results of the pilot-scale HRAP studies broadly follow the predicted trends previously observed for the flask and photobioreactor simulation studies reported earlier and thus confirming the potential for *Spirulina* growth in tannery effluent and raising confidence in the practicality of using integrated ponding systems for treating these wastewaters.

A basic issue in the production of photoautotrophic organisms in general, and *Spirulina* in particular, is to maintain a continuous culture with an optimum population density. For *Spirulina* it has been found in all seasons that maintaining the culture at a concentration of approximately 300-500 mg dry weight per litre at a depth of 12 to 15 cm results in substantial increases in output rates compared to either lower or higher densities (Richmond and Vonshak, 1978 ; Vonshak *et al.*, 1982 ; Richmond, 1986c ; Richmond, 1987 ; Vonshak and Guy, 1992). While all concentrations were lower in this study, medium colour probably prevents photooxidative damage.

Spirulina is a mesophilic alga, i.e., the optimal temperature for its growth being relatively high, between 35-40°C (Richmond, 1988). Outdoors, when the maximal day temperature declines to below 18 °C, the culture deteriorates (Richmond, 1988). In contrast to day temperature, *Spirulina* can tolerate relatively low night temperatures (Richmond *et. al.*, 1980). The temperature trend in the existing WSP (described in chapter 2) is that stratification exists in the summer months but breaks down in the colder winter months. Although the

temperature can support cyanobacterial growth throughout the year, the *Spirulina* are subject to a temperature fluctuation of approx. 10 °C resulting in a reduced productivity in winter. As ambient water temperature cannot be controlled outdoors the pilot-scale HRAP will need to be operated at a depth of approx. 15 cm.

The nutrient requirements for the cultivation of *Spirulina* were investigated and are discussed in chapter 4. The pre-treated combined tannery effluent was shown to be capable of supporting the growth of *Spirulina*.

Richmond (1988) has noted that when temperature and nutrients are not limiting to the growth of *Spirulina*, light availability to the average cell becomes the dominant limiting factor. The availability of light to each cell in a phototrophic culture is a function of the intensity and duration of light irradiance, the concentration of cells or population density that affects the extent of mutual shading (Tamiya, 1957 ; Raven, 1988 ; Richmond, 1988), and in addition the turbidity of the growth medium.

The high level of ammonia in the PTE was identified as a limiting factor in the development of the *Spirulina*-based saline HRAP for the treatment of tannery effluent. The level of ammonia in the PTE was extremely high 730 $mg.L^{-1}$, compared to 9-30 $mg.L^{-1}$ in raw municipal wastewater (Konig *et al.*, 1987). At these elevated levels the ammonia is toxic to *Spirulina* causing photo-oxidation of the cyanobacteria, resulting in the observed suboptimal growth rates in the outdoor HRAP.

Under normal conditions the ammonia entering the existing WSP system may, over time, be converted to nitrate by nitrifying bacteria which coexist in the HRAP with *Spirulina.* These bacteria have a slow growth rate and the situation develops in the continuous HRAP culture where the retention time, due to the higher volumetric loading rate, may be too short to allow for their adequate growth. A build up in the levels of ammonia in the HRAP occurs resulting in reduced *Spirulina* growth due to photooxidation and a washout at a loading rate above 10% per day.

A second reason for the lower growth rates for both the *Spirulina* and the nitrifying bacterial

community in the continuous HRAP culture is that the alkalinity of the PTE is rather low (2900 $mg.L^{-1}$). This may result in a carbon limitation at certain times, however, more importantly leads to a low buffering capacity of the effluent, and this has been shown to have a negative effect on the growth of *Spirulina* (Chaudhari, *et. al.*, 1980 ; Richmond, 1988). The nitrifying bacteria, similarly, require a high bicarbonate content in their growth media to prevent drastic changes in the effluent pH. The problem may be overcome by enriching the pre-treated combined tannery effluent with sodium bicarbonate and this has been reported for situations where *Spirulina* was grown in sewage waste (Chaudhari, *et. al.*, 1980). Preliminary studies were discussed in chapter 4.

Results from the studies reported indicate that the toxic effect of high ammonia levels, at higher volumetric loading rates, can be reduced by either ammonia stripping (O'Brien *et al.*, 1986) or the recirculation of bicarbonate-rich water from pond 11 to the HRAP. These findings are supported in a report by Shelef *et. al.* (1980) that up to 27 % of nitrogen loaded can be lost by ammonia stripping in open ponds and Koopman *et. al.* (1980) note that this is increased by both effective mixing and elevating pH and temperature.

The *Spirulina* productivities recorded for the optimised pilot-scale HRAP compared well with those measured in the latter ponds of the WSP. The values for the productivity of the *Spirulina* in the pilot-scale HRAP were found to be suboptimal when compared to peak productivities of 30 g dry matter.m^{-2}. day^{-1}, which have been reported by DePauw *et al.* (1978), and with the more sustainable productivities between 15-20 g dry matter.$m^{-2}.day^{-1}$ reported by Oswald (1988a) and Fallowfield *et al.* (1992). However they do compare well with long-term yields of 7.3-9.5 $g.m^{-2}.day^{-1}$ reported by Saxena *et. al.* (1983) which have been attained during summer, and which decrease to about 5 $g.m^{-2}.day^{-1}$ during winter. Fox (1983) has described a system that is based on collecting human excrement in collective latrines, and digesting in a high temperature (55°C) digester, and 100 m^2 ponds linked to this system produced 8-12 g $C.m^{-2}.day^{-1}$.

A significant reduction in organic and inorganic nutrient loading was shown to occur during cyanobacterial growth. The results for organic load reduction derived from the pilot-scale HRAP study compare well with results for fresh water HRAP previously reported in the

literature. Kilani (1992) has reported soluble COD removal of 80 % in waste stabilisation ponds treating yoghurt wastes at a detention time of 7.9 days, while Silva *et al.* (1987) have shown a 69 % COD removal at a detention time of 25 days for sewage. Rodrigues and Oliviera (1987a) have reported COD removal of between 68-95 % at a detention time of 5 days in a sewage HRAP, while Azov and Shelef (1982) have reported a 93 % and 86 % reduction of BOD in HRAP operated in summer and winter respectively. Abeliovich (1986) records a 90-99 % removal of soluble BOD fraction from domestic wastewater with a 10-30 day retention time in a sewage HRAP.

Benemann *et al.* (1980) have reported COD removals in a 0.25 Ha experimental pond, treating municipal sewage effluent. The results are largely comparable to those recorded above for the continuously fed study - a 55 % COD removal at 7 days compared to a 87 %, 83 %, 68 %, 63 % COD removal at 33.3, 20, 12.5, and 10 days hydraulic retention times respectively.

The results obtained for the outdoor pilot-scale HRAP were found to be broadly comparable to literature values for fresh water HRAP systems and showed that *Spirulina* biomass could be reliably produced in PTE influent to the WSP system. It was also shown that loading rates to the HRAP could be improved with the recirculation of alkalinity from pond 11 water and that *Spirulina* biomass could be used to establish an effective capping of the anaerobic ponds. Since the capping effect is not a self-sustaining process a reliable source of biomass would be required, and it was proposed that an HRAP inserted into the ponding cascade between pond A/B and pond 2 could provide this function. It was thus with an increased level of confidence that these results were used as the basis for the design and construction of a full-scale 2 500 m^2 HRAP, to be reported in chapter 7. However, the commercial value potential of the *Spirulina* biomass to be produced formed an important component of the up-scaling decision-making process and hence an evaluation of the recovery and quality of harvested biomass was undertaken and results of this study are reported in chapter 6.

Chapter Six

Recovery and Quality of Tannery Effluent-grown *Spirulina* Biomass.

6.1. 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 Pauw and Salomoni, 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, 1980, 1988), electroflocculation (Richmond and Becker, 1986), chemical flocculation (Golueke and Oswald, 1965 ; McGarry, 1970 ; Azov *et al.*, 1980), sedimentation (Mohn, 1988), air flotation (Richmond, 1986c ; 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, 1965 ; 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 (Iijima *et al.*, 1986 ; Hayashi and Okuwaki, 1994 ; Quereshi and Ali, 1996 ; Quereshi *et. al.*, 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, e.g., iron and essential unsaturated fatty acids, the most important of which is gamma-linolenic acid. *S. platensis* is unique among photoautotrophic 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).

6.2. Research Objectives

Following the demonstration, in the pilot plant studies reported in chapter 5, of the potential of the *Spirulina*-based HRAP as a process in the treatment of tannery wastewaters, an evaluation of the biomass produced 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 possible means of harvesting and drying the *Spirulina* biomass. Also to undertake an evaluation of the *Spirulina* biomass produced in the tannery effluent HRAP to ensure that the product complies with nutritive and toxicological criteria for sale as a specialised animal feed.

6.3. Materials and Methods

6.3.1. Harvesting of the *Spirulina* Biomass.

Harvesting of the *Spirulina* biomass was conducted on waste stabilisation ponds 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, using a scraper device. A small-scale screen harvester was then constructed and operated according to Mitchell (1986). And finally a technical-scale screen harvester, designed by R. Rowswell of LIRI, and constructed by R. Smith engineer at Mossop Western Leathers, was used to harvest *Spirulina* biomass from pond C (**Figure 6.4.1.**). A screen with a pore size of 80 μm 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.

6.3.2. Drying of the *Spirulina* 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. However, the best results were

achieved using sun-drying. Algal biomass harvested either by hand or by screen harvester was placed on a drying bed covered with screen cloth (pore size 80 μm) 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 %.

6.3.3. Electro-Osmotic Dewatering.

Two samples of harvested algal biomass were used to evaluate the Electro-Osmotic dewatering technique developed by M. Smollen at the Council for Scientific and Industrial Research (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.

6.3.4. Evaluation of the Harvested *Spirulina* Biomass.

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 suggested 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 chlorophyll$_a$ 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.

6.4. Results

6.4.1. Harvesting

Harvesting of *Spirulina*, by hand, small-scale screen harvester and technical-scale screen harvester, on waste stabilisation ponds C, D, 6, and 7, resulted in a total recovery of 546 kg dried *Spirulina* biomass.

Harvesting of the autoflocculated *Spirulina* biomass by hand was conducted over an 8 day period during the months of February, March and April 1993. A total of 271 kg of *Spirulina* was recovered in this manner. This 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 is thus low when compared to *Spirulina* biomass harvested when the cells are 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 6.4.1. 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 0.100 $g.L^{-1}$. $hour^{-1}$. 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 is thus not taken up, which means the quality of the biomass was higher compared to hand harvested autoflocculated biomass.

6.4.2. Drying

6.4.2.1. Sun Drying

This method formed the basis for the drying process used, 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 μm) for approximately 2 hours, resulting in an algal slurry of about 10 % solids. **Figure 6.4.2.** shows the sun drying beds. The biomass dried over a period of 5 days. Problems associated with this method were that the drying was weather dependent, labour intensive, required a large surface area and long time periods.

Figure 6.4.1. The technical-scale screen harvester on-site at Mossop Western Leathers, Wellington.

Figure 6.4.2. The sun drying beds used at Mossop Western Leathers, Wellington.

6.4.2.2. 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 the concentration of biomass by Electro-Osmotic dewatering are shown in **Table 6.4.1.**

Table 6.4.1. Screen harvested *Spirulina* biomass concentrated by Electro-Osmotic dewatering.

Stage/Sample	Solids Concentration (%) 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	18.70

The current efficiencies and energy requirements for the dewatering of the washed sample are shown in **Table 6.4.2.**

Table 6.4.2. 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.

6.4.3. 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 being in some form of deterioration. 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 6.4.3.**

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 1986a), 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 6.4.3. 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 (1986a)	Santillan *et al.* (1982)	Saxena *et al.* (1983)	This Study
Alanine	0.7780	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	2.0260	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	5.80	5.80	8.30	4.2521
Lysine	0.7707	5.4503	4.00	4.00	3.94	2.3757
Methionine	0.1570	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	70.10	71.0	70.0	50-55	57.10
Serine	0.7380	2.6318	4.00	3.18	7.21	2.2433
Threonine	0.5434	3.1001	4.17	4.17	5.41	2.5096
Tyrosine	0.3000	2.2400	4.60	/	2.10	1.9400
Valine	0.6763	3.3336	6.00	6.00	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 6.4.4.**).

Table 6.4.4. Various treatments of *Spirulina* biomass to reduce ash content.

Treatment Process	Ash content (%)
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* to those obtained by other authors, the amino acid content shown in **Table 6.4.3.** was converted to g amino acid/16 g 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 surely 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 due to bacterial activity associated with the lengthy drying period. The main practical consideration in minimising 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, 1986a), 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 amounts to 1.68 $g.kg^{-1}$ (dry weight) and is comparable to that of other sources of *Spirulina*: 1.80 g.kg-1 (Richmond, 1986a), 1.40-1.80 $g.kg^{-1}$ (Durand-Chastel, 1980). Total carotenoids (β-carotene + lutein) amount to 2.90 $g.kg^{-1}$ (dry weight).

The $chlorophyll_a$ content of the dried *Spirulina* is 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 $chlorophyll_a$, the presence of $chlorophyll_b$ 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 substantially 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, 1986a), 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, 1986a), 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 phenomenon was observed by Paerl *et al.* (1983) with the cyanobacterium *Microcystis aeruginosa*. 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 (discussed in chapter 5) showed that nickel was the only heavy metal that remained present at slightly higher concentrations than is acceptable for the animal feed application.

6. 5. Discussion

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 μm 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 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, scale-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 apposed 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* (Pohland *et al.*, 1990), 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, all results showed no toxicological effects (Maart , 1993 ; Ross *et. al.*, 1994 ; Venkataraman *et. al.*, 1994). The analysis of the biomass and results of the feeding trial allows a preliminary conclusion that *Spirulina* grown on tannery effluent has 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* has potential as a feed supplement comparable to fishmeal and 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 follow the trend of other studies, conducted with micro-algae, and indicate 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 Mossop Western Leathers Board decision to proceed to the construction of a full-scale Integrated Algal Ponding Process for treating their wastewaters. This scale-up development of the research results discussed up to this point in the study is detailed in chapter 7 of this report.

Chapter Seven

Process Design for a *Spirulina*-based Integrated High Rate Algal Pond System for Treating Tannery Wastewater.

7.1. Introduction

7.1.1. Case study: Mossop Western Leathers

The problem of odour nuisance at the tannery in Wellington, has been a persistent issue facing management over many years. The odour nuisance is caused chiefly by the gaseous emission of sulphides, ammonia, and mercaptans into the atmosphere as a result of either the production methods used in the process of leather manufacture, or chemical and biological reactions taking place within the effluent in the ponds. These gasses are then carried, by the prevailing winds, over the residential areas of Wellington, causing serious offence to the local community.

The management of Mossop Western Leathers decided to adopt a holistic planning approach to ensure the successful reduction of odour from all sources including: changing to 'clean' production methods to be used in the manufacture process, implementing good 'housekeeping' practices on-site, upgrading of the physico-chemical pre-treatment facility and upgrading the operation of the WSP system.

In this regard the results of the operation and performance of the tannery WSP, undertaken and reported here, were used as the basis of the decision by Mossop Western Leathers to proceed with an Algal Biotechnology solution to the odour problem. It had been demonstrated that microalgal capping with *Spirulina* growth, which is associated with effective odour control, provides a stratification of aerobic over anaerobic pond water during summer and sustained surface oxygenation during winter. It was shown that growth of *Spirulina* in the PTE influent to the WSP system may be manipulated within an HRAP to achieve production of a feed-grade quality biomass product, and also effective treatment of the effluent at appropriate loading rates.

Spirulina biomass-containing treated water could be used to overlay the odour producing anaerobic ponds and the functionality of a microalgal capping process has been demonstrated. The feasibility of proceeding to implementation of a microalgal solution to the WSP problem, and the results of the investigations completed, were evaluated by Prof. William Oswald who undertook a visit to site during 1994. Based on a positive assessment of research development up to this point Mossop Western Leathers decided to proceed with the construction of a 2 500 m^2 full-scale HRAP. This was to be a first step in the possible implementation of a full AIWPS process and further developments would depend on the outcomes of this phase of the project. At the outset the HRAP would be used to subject the microalgal capping proposal to a long-term practical evaluation and also to undertake an assessment of marketing the biomass harvested from the system.

7.2. Research Objective

Following the successful demonstration, both in the laboratory and pilot-scale investigations, of the feasibility of a *Spirulina*-based saline HRAP which, together with the anaerobic pond and the WSP, would serve as a component of an integrated approach to the ponding treatment of tannery effluent, the design, construction and evaluation of a full-scale 2 500 m^2 HRAP unit would be undertaken.

7.3. Materials and Methods

All methods used in this chapter are as previously described.

7.4. Results

The following reasoning was followed in developing the design of the full-scale HRAP.

7.4.1. Pre-treatment

The pre-treatment that most commonly precedes a municipal AIWPS is screening and grit removal. The pre-treatment at Wellington prior to the wastewater being introduced into the

proposed HRAP, consists of a series of physico-chemical processes designed to remove solids and extended aeration to convert H_2S to sulphate. On-site wastewater treatment may be improved by further segregating specific process effluent streams, upgrading screening (solids removal), and the implementation of catalytic sulphide oxidation to reduce the release of sulphide odours. The introduction of 'clean' production technology, such as an ammonia "limited" delime process, would further reduce the levels of atmospheric emissions and the nitrogen load to the HRAP.

7.4.2. Primary Anaerobic Pond

It was found that the existing pond A (**Figure 5.4.1.**) could be successfully converted and thus be used as the equivalent of the anaerobic pit as described for AIWPS primary facultative pond, for the anaerobic treatment of the tannery wastewater. The mechanical aerators in use provided effecting oxygen capping of the pond's surface. Results obtained for the operation of the reconfigured primary anaerobic pond (**Table 5.4.1.**) indicate an overall reduction in the organic load of 51 %, together with a reduction in ammonia and sulphide levels of 38 % and 73 % respectively. There was a 99.8 % removal of settleable solids, while stable anaerobic conditions led to the removal, to low levels, of any heavy metals present in the tannery wastewater. Oswald (1995) has reported that 60-80 % of the influent BOD and all suspended solids are removed by AIWPS treating sewage wastewaters. This indicated that the reconfigured pond A would function sufficiently close to this level of efficiency without further modification being required in the first instance.

The converted primary facultative pond thus would appear to offer adequate treatment of the tannery effluent, however, it should be noted that the implementation of the AIWPS fermentation pits could further enhance the treatment performance.

7.4.3. High Rate Algal Pond

7.4.3.1. Design

The successful design of a large-scale algal treatment system requires the consideration of,

not only the application desired, but also a host of factors, many of which are uncontrollable in the natural environment (Oswald, 1988b). Some factors requiring consideration in design are: the specific application, media requirements, and local climatological conditions.

The general sequence for the scale-up of a biotechnological programmes (Hacking, 1986 ; Trilli, 1986 ; Borowitzka and Borowitzka, 1989) was adhered too through this stage of the project. This included the initial technical study which involved a detailed evaluation of the biology of the existing process (refer to chapters 2 and 3), establishment of broad parameters for growth medium constituents (refer to chapter 4), and an assessment of suitable harvesting techniques and evaluation of the product (refer to chapter 6). With the establishment of biological and technical feasibility, the scale-up phase was undertaken through a number of stages (refer to chapter 5). The design criteria used for the design and construction of the HRAP closely follow those described by Oswald (1988b).

At the Mossop Western Leathers tannery the daily processing of 1500 hides generates about 600 m^3 of combined pre-treated tannery effluent.

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

The BOD_{ULT} of the combined pre-treated tannery effluent is 1124 $mg.L^{-1}$ (based on a COD of 2474 $mg.L^{-1}$ and BOD:COD ratio of 1:2.2 for tannery effluent, Rowswell 1997, pers comm.). So the organic loading rate to the system (L_O) will be:

$$\textbf{Organic Loading Rate } (L_O) = 675 \text{ kg.day}^{-1} \qquad \textbf{(2)}$$

High Rate Ponds are designed on the basis of organic loading rate and solar energy availability. Assuming a conservative BOD_{ULT} in the influent waste-water of 1124 $mg.L^{-1}$ and assuming an eventual removal in the Primary Anaerobic Pond of 60 %, the HRAP influent BOD_{ULT} (I_{HRP}) will be:

$$I_{HRP} = 0.4 \times 1124 \text{ mg.L}^{-1} \text{ BOD}_{ULT} = 450 \text{ mg.L}^{-1} \text{ BOD}_{ULT} \qquad \textbf{(3)}$$

The operation of the HRAP will 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 minimise the toxic effect of the ammonia. Therefore, the influent BOD_{ULT} (I_{HRP}) will be:

$$\mathbf{I_{HRP} = 360\ mg.L^{-1}\ BOD_{ULT}} \qquad \mathbf{(4)}$$

In order to oxidise the influent organic load, the concentration of algal cells (Cc) in an 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 their cell dry weight mass. Using an empirically derived formula, the depth of light penetration (d_L) expressed in centimetres can be determined by dividing 6000 by the influent BOD.

$$\mathbf{d_L = 6000 / 360\ mg.L^{-1}\ BOD_{ULT} = 16.66\ cm} \qquad \mathbf{(5)}$$

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:

$$\mathbf{d_{HRP} = 3/2 \times 16.66 = 24.99\ cm} \qquad \mathbf{(6)}$$

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:

$$\mathbf{hCc = SAF\theta} \qquad \mathbf{(7)}$$

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 litre in the HRAP expressed in cm^2, and F is the photosynthetic efficiency. Therefore,

$$\theta = \mathbf{(5.5\ cal.mg^{-1} \times 360\ mg.L^{-1})/(268.36\ cal.cm^{-2}.day^{-1} \times 1000\ cm^3/24.99\ cm \times 0.035)}$$
$$\mathbf{= 5.26\ days} \qquad \mathbf{(8)}$$

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

$$\mathbf{v_{OVERFLOW} = d_{HRP}/\theta = 0.2499\ m / 5.26\ days = 0.0475\ m.day^{-1}} \qquad \mathbf{(9)}$$

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

$$\mathbf{L_{HRP} = 0.0475\ m.day^{-1} \times 360\ mg.L^{-1}\ BOD_{ULT} = 17.10\ g\ BOD_{ULT}.m^{-2}.day^{-1}} \qquad \mathbf{(10)}$$

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

$$\mathbf{Cc = 17.10\ g\ O_2.m^{-2}.day^{-1} / 1.6 = 10.68\ g.m^{-2}.day^{-1}} \qquad \mathbf{(11)}$$

Assuming the flow to the high rate pond (Q_{HRP}) is 720 $m^3.day^{-1}$, the maximum area required for the HRAP (A_{HRP}) will be:

$$\mathbf{A_{HRP} = Q_{HRP} / V_{OVERFLOW} = 720\ m^3 / 0.0475\ m.day^{-1} = 15157.89\ m^2} \qquad \mathbf{(12)}$$

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

$$\mathbf{CL = A_{HRP} / 10\ m = 15157.89\ m^2 / 10\ m = 1515.78\ m} \qquad \mathbf{(13)}$$

The calculations indicate that HRAP covering an area of 1.5 Ha. would effectively treat the total effluent produced. Based on the information gathered, it was decided to proceed with the design of a 2500 m^2 HRAP capable of processing about 25 % of the daily effluent volume. **Figures 7.4.1.** to **7.4.3.** show the final construction and design plans are illustrated in **Appendix 2.**

7.4.3.2. Construction

Initial construction of the 2500 m^2 HRAP involved the preparation of the site area. Pond 1

Figure 7.4.1. The 2 500 m^2 high rate algal pond at Mossop Western Leathers, Wellington.

was drained, allowed to dry out and then the base was cleared of sludge and rocks before being levelled. The site was then built up to ground level and compacted to 90 % to ensure a firm base on which to build. Floating foundations of reinforced concrete were cast for the outer and central dividing walls of the 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 sealant. Clay walls were built by back filling to surround the HRAP walls, to provide additional support and to raise the ground level adjacent to the HRAP. The floor of the 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. Flow regulating walls (**Figure 7.4.2.**) were constructed at both ends of the HRAP, these had the effect of forming channels which result in improved movement of the

Figure 7.4.2. Flow regulating walls of the 2 500 m^2 high rate algal pond at Mossop Western Leathers, Wellington.

Figure 7.4.3. Paddle wheel of the 2 500 m^2 high rate algal pond at Mossop Western Leathers, Wellington.

water in the HRAP reducing the formation of dead spaces. The area around the paddle wheel was built so as to form a funnel which results in more efficient turbulent flow from the paddles and thus improves the mixing in the HRAP.

The paddle wheel (**Figure 7.4.3.**) 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, this was to ensure that the paddles were off set, and by eliminating the 'pulsing' action so reducing the size of the waves in the HRAP. The paddle wheel was driven by an electrical motor which was mounted alongside and was controlled by an electrical box. The level of the water in the 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 HRAP via a splitter box from the primary facultative pond. Harvesting was performed using a vibrating screen to recover the *Spirulina* biomass which was then dried.

7.4.3.3. Operation of the HRAP

On completion the HRAP was filled with tannery wastewater from the Pond A. This process was repeated a number of times, to ensure proper sealing of the aluminium silicate layer, before *Spirulina* culture from pond 6 was introduced. The HRAP was then operated at between 1- 3 % volumetric loading rate for a period to allow the culture to stabilise. The performance of the system was then monitored over a 6 month period and operational data recorded. The results obtained (**Table 7.4.1.**) indicated that the HRAP system was indeed capable of successfully treating the tannery effluent by reducing the organic load (COD) by up to 78 % at a volumetric loading rate of 10 %.day^{-1}. However, once this level was exceeded performance of the *Spirulina* culture rapidly declined.

There was an overall reduction in the settleable solids of 99 %, a decrease in the levels of ammonia of 94 %, phosphate of 92 %, and sulphide of 99 %. The treatment effect of the HRAP system thus followed the trend predicted by the laboratory and the 80 m^2 pilot-scale investigations (outlined in chapters 4 and 5). However, it was again demonstrated that the growth of the *Spirulina* was retarded at volumetric loading rates above 10 %.day^{-1}.

Table 7.4.1. Results for the large scale high rate algal pond at Mossop Western Leathers, Wellington. Standard deviation in brackets.

All values except pH in $mg.L^{-1}$	Combined tannery effluent	HRAP effluent
Ammonia as NH_3	731 (98)	42 (32)
Chemical oxygen demand	2474 (1810)	539 (147)
pH	8.17 (0.06)	8.98 (0.57)
Posphate as P_2O_5	19 (12.5)	1.5 (0.33)
Sulphate as SO_4	975 (788)	1097 (150)
Sulphide as Na_2S	285 (422)	1.3 (5.2)
SS	243 (196)	0.1 (0.29)
TDS	11475 (3006)	13656 (1400)

7.4.3.4. Effect of Recirculation on HRAP Performance.

The effect of a 20 % recirculation of pond 11 water on the performance of the HRAP was investigated. The results reported in **Table 7.4.2.** indicate that with the 20 % recirculation, the performance of the HRAP system was significantly improved resulting in the reduction of the organic load (COD) by 84 % at a volumetric loading rate of between 15-20 $\%.day^{-1}$ total volume.

Table 7.4.2. Comparison of high rate algal pond performance with changes to pond A and recirculation from pond 11. Standard deviation in brackets.

All values in $mg.L^{-1}$ except pH	Pretreated effluent	Pond A effluent before change	Pond A effluent after change	WSP (Pond 11) effluent	HRAP effluent
Ammonia asNH_3	731 (98)	764	452 (105)	30	36 (33)
COD	2474 (1810)	1722	1216 (93)	1677	394 (212)
pH	8.17 (0.06)	8.3	8.10 (0.70)	9.5	8.83 (0.58)
Posphate as P_2O_5	19 (12.5)	7	1.65 (2.33)	12	0.01
Sulphate as SO_4	975 (788)	<1	989 (90)	943	809 (135)
Sulphide as Na_2S	285 (422)	500	76.5 (16)	6	0.1
SS	243 (196)	434 (67)	0.45 (0.49)	152 (115)	0.3 (0.21)
TDS	11475 (3006)	17888 (193)	17320 (339)	52179 (17492)	17080 (1652)

There was an overall reduction in the organic load of 84 %, settleable solids of 99 %, a decrease in the levels of ammonia of 95 %, phosphate of 99 %, and sulphide of 99 %. However, the recirculation results in an increase in dissolved solids levels. The treatment effect of the HRAP system thus broadly followed the trend predicted by the laboratory and pilot-scale investigations (outlined in chapters 4 and 5) where pond 11 water was recirculated

and thus enabling increased photosynthetic productivity. The productivity of the *Spirulina* averaged 11 g.biomass.m^2.day^{-1} over the evaluation period at effluent volumetric loading rates maintained at between 15-20 %.day^{-1}.

7.4.3.5. Scale-up

An overall comparison of system performance through the scale-up exercise, from the initial laboratory studies and pilot-scale HRAP through to design values and finally the full-scale HRAP is reported in **Table 7.4.3.** This illustrates a broadly linear trend through the scale-up process.

Table 7.4.3. Comparison of high rate algal pond performance through the scale-up exercise from laboratory through pilot-scale studies to the full-scale high rate algal pond operated at loading rates of 15-20 %.day^{-1}.

	Laboratory Study	Pilot-scale Study	Design values	Full-scale Study
Ammonia removal (%)	80 - 94	73 - 92	100	95
Carbon production (g C.m^{-2}.d^{-1})	12	3 - 8	10.68	11
Hydraulic loading rate (%.d^{-1})	15 - 20	5 - 10	15 - 20	15 - 20
Hydraulic residence time (days)	5-6.7	10 - 20	5 - 6.7	5 - 6.7
Organic loading rate (gCOD.m^{-2}.d^{-1})	74	43	67	67
Organic load reduction (%)	86 - 71	87 - 68	80-90	84
Phosphate removal (%)	90 - 99	90 - 99	100	99
SS removal (%)	90 - 95	90 - 95	100	99
Sulphide removal (%)	90 - 99	90 - 99	100	99

7.4.3.6. Cost for the Construction of the 2 500 m^2 HRAP.

The cost of materials used in the construction of the 2 500 m^2 HRAP at Wellington are based on 1996 Rand values as follows:

Marine Ply for the paddle wheels (56 sheets, 16mm, 2500 x 1220 mm)	R 21 157
Cement building blocks (15 000 blocks)	R 15 486
Aluminium Silicate sealer (265 x 40kg bags)	R 46 178
Earth moving costs and labour costs	R 40 000
Harvesting equipment	R 20 000
Electrical Motor, plastic pipes, cement, filling sand, etc.	R 38 688

7.5. Discussion.

The problems such as odour, and lowered performance efficiencies in winter for the WSP system in operation at Wellington are typical of many such systems employed at tanneries, and located in rural areas, for the disposal of partially treated effluent. Although the performance of the treatment system currently results in the removal of up to 85 % of the organic load by the end of the cascade, and during optimal environmental conditions, the main problem with this system is that the effluent can neither be discharged nor reused in the tannery, due to the slow build up of inorganic salts. In addition, at a loading rate of 0.25 % total volume addition.day^{-1} the 13.7 ha. WSP system has fully occupied existing land availability thus limiting any further expansion of the manufacturing operations of the tannery.

It is evident from this study that the problems associated with the WSP system may be overcome through the implementation of an integrated algal ponding approach to the problem. The AIWPS in an adapted form, originally designed by Oswald for the treatment of sewage wastewaters, could offer an affordable solution to the environmental problems experienced at Mossop Western Leathers.

Although little data has been published regarding the cost of micro-algal systems, the cost advantages of AIWPS, over more conventional processes, results mainly from lower construction costs and from lower costs for operations and maintenance (Oswald, 1991). It is frequently asserted that HRAP depend upon the utilisation of the algal biomass for economically efficient operation (Fallowfield *et. al.*, 1992). Oswald, argues that these systems operate at a fraction of the cost of conventional waste treatment systems, without factoring into the calculation the value of the biomass produced during treatment (Fallowfield *et. al.*, 1992).

The capital cost for the AIWPS is 1/2 to 1/3 that of conventional treatment plants (Oswald, 1991). The low construction costs for the AIWPS results mainly from the minimisation of the use of reinforced concrete structures by using formed earth ponds (Oswald, 1991). The AIWPS needs limited design and construction time, thus decreasing inflationary costs. In addition, the AIWPS reduces the land area requirements over conventional waste stabilisation

ponding systems. The recent construction of an activated sludge treatment plant at a tannery of comparable size to Mossop Western Leathers, at a different location in South Africa, at a capital cost of around R 5 million provides a useful benchmark for comparison. The 2 500 m^2 HRAP unit has demonstrated a capability of processing 1/4 of the daily effluent production, thus the total daily effluent production could be accommodated in an area of approximately 1 ha. (compared to the area of 13.7 ha. currently required for the waste stabilisation ponding system).

The operation and maintenance costs for the AIWPS are 1/2 to 1/5 lower than conventional treatment plants (Oswald, 1991). These low operation and maintenance costs result from a number of factors unique to the AIWPS. One major saving is the elimination of day-to-day sludge handling. Another factor which lowers the cost of operations and maintenance is decreased energy requirements, particularly for aeration. Further energy savings result from omitting the energy usually required for day-to-day sludge transfer, mixing, heating and disposal. Minimisation of mechanical equipment is an integral part of the system design. This reduces the personnel required to operate and maintain equipment. Also, the specialisation level required of personnel is not as great as that needed to maintain mechanical equipment. The AIWPS provides an environmentally-sound opportunity for reclamation of water, nutrients, and in some cases energy (Oswald, 1995).

The studies undertaken here have shown that the *Spirulina*-based HRAP not only operates as an efficient method for treating tannery wastewaters as a stand-alone process, but also presents a mechanism whereby odour malfunction in the WPS could be managed. A dual action of the capping mechanism provides for microalgal-induced alkalinity trapping of odour-causing substances (with their subsequent biological oxidation), and the conversion of the system to aerobic operation (with removal of residual organics). The active harvesting and removal of biomass will also serve to linearise nutrient flow through the system and offers a partial solution to the long term remediation of such sites. The potential commercial value of the *Spirulina* biomass, which is recoverable from the tannery wastewater application in an aquaculture feed-grade form is an added bonus to an otherwise environmentally embattled industry.

Chapter Eight

Concluding Remarks

It has been noted that water, more than any other factor, determines the ultimate population capacity of a geographic province (Skinner, 1969). In South Africa the combination of rising demand and declining quality has been quantified and the critical impacts on this country of the intersection of these trends early in the next century has been noted (WRC, 1996). Given the identification of salinisation as the singular major factor responsible for the progressive decline in the quality of the country's public water system the research and development of appropriate methods for dealing with saline wastewaters has been the focus of considerable research investment. The Leather Industry presents a useful paradigm for the study of industrially generated, complex saline effluents, and in particular, in treatment applications where segregation and containment in WSP is implemented as the method of choice.

Oswald (1995) has argued that ponds provide the most cost-effective reactors for liquid waste management and a large body of literature details the development of the WSP technology over the past 50 years. However, few studies of tannery WSP have been reported and little is known about the biology of these systems; and hence also the limited availability of management guidelines and technical options whereby malfunction may be corrected. The efficiency of ponding systems in the capture of solar energy has been noted and Oswald (1995) records that multifaceted treatment-reclamation processes have attained solar energy conversion efficiencies of 3-4 %. In this regard it has been predicted that coupling of the wastewater treatment function with resource recovery and nutrient recycling will be increasingly driven by environmental issues relating to nutrition, the global human population and sustainability (de Pauw and Salomoni 1991). Here again no literature is available on the possible role tannery wastewater ponds might play in the recovery and recycling of resources.

In this study the ponding system at Mossop Western Leathers, in Wellington, which has been in operation for over 30 years, has been used as the basis for the investigation. The results provide first detailed reports of the occurrence of massive blooms of the halophilic microalgae *Dunaliella* and *Spirulina* in these systems together with a description of the

biological dynamics which appear to determine these phenomena.

It was demonstrated in this study that *Spirulina* is able to grow, and grow particularly well in certain tanning effluents, with an enhancement of cell production compared to defined media systems. This is accompanied by a substantial reduction in the organic load of the medium and indications of organic nutrition has presented a new perspective on what had, until recently, been regarded as an obligate photoautotrophic organism. While it has already been established that several species of cyanobacteria are capable of heterotrophic nutrition, the uptake of organics by *Spirulina* may account, in some part, for the decrease in organic load across the ponding system.

The role of *Dunaliella* in the system has been shown to be transient and relatively minor in terms of the total photosynthetic productivity of the WSP.

The study has shown that a number of factors within the system operate, probably both synchronically and antagonistically, to produce the dominant patterns of biological distribution that have been described. These include high levels of sulphide and ammonia in the influent to the system and also to the first ponds in the WSP cascade. These components are derived from process chemicals used in leather production and, in the case of ammonia, also significantly from the anaerobic degradation of collagen and keratin.

A rising, probably photosynthetically-driven, pH gradient across the cascade provides for the chemical trapping of sulphide and the commensurate reduction in its phytotoxic effects. Phototrophic microbial oxidation to either elemental S or SO_4 follows and the sulphur remains in the oxidised state in the aerobic compartment of the latter ponds. However, the conditions of rising pH also serve to increase reduction of ammonia to the uncharged NH_3 species and thus enhancing its toxicity to aquatic microorganisms. A pronounced thermocline in the ponds, with a temperature differential of up to 9 °C in the first 0.5 m of pond depth in summer, provides for the additional effect where for every 10 °C elevation of temperature the biologically toxic activity of ammonia is doubled (Ruffier *et. al.*, 1981). In this regard the toxic effects of ammonia on *Spirulina* production have been demonstrated in some detail in this study together with its tolerance to relatively high levels of sulphide.

At the same time, and notwithstanding the above effects, the elevation in pH in the system also serves to reduce the vapour pressure of ammonia and, in this way, facilitates its stripping from the water and its loss from the system. Results indicate the use of ammonia by *Spirulina* as its principal nitrogen source in the pond environment and, together, these mechanisms effect a high level of TKN reduction in the system.

Superimposed on the pH gradient is a very abrupt change in DO between ponds 5 and 6 which accounts for a shift from anaerobic to aerobic-facultative conditions. This is achieved primarily by *Spirulina*-generated photosynthetic oxygen production. This also marks the point at which the effects described above interact in such a way as to enable this organism's growth rate to exceed the dilution rate, and thus overcoming washout from the system. Under these conditions intense blooms of *Spirulina* are observed. An aerobic-anaerobic interface is established within the water column and, together with the thermocline, which is twice as pronounced in the presence of the microalgae, a strongly stratified system is established. Effective control of mixing in the facultative ponds in this way ensures the efficient regulation of the odour problem. Active biological sulphur and nitrogen cycles continue to operate vertically through the water column.

It was against this background that it was proposed to investigate an Algal Biotechnological approach to the management of the WSP, the development of the HRAP application as a potential stand-alone process for the treatment of these effluents, and the coupling of biomass resource recovery as a component of the exercise.

Borowitzka and Borowitzka (1989) have commented on the value of scale-up studies in designing large-scale algal operations. The results outlined in this study, designed to demonstrate the initial feasibility of the *Spirulina* based saline HRAP for the treatment of tannery effluent, were used as the basis for the subsequent design and operation of the pilot-scale HRAP. A successful scaling-up of the system was achieved by the demonstration of the growth of *Spirulina*, in pre-treated tannery effluent, in outdoor pilot-scale HRAP. The pilot-scale studies broadly followed the trends previously observed for the laboratory flask and photobioreactor simulations and indicated that *Spirulina* does, indeed, grow in pre-treated tannery effluent. The productivities for *Spirulina* that were obtained in optimised pilot-scale

HRAP compared well with those measured in the existing waste stabilisation ponds. A substantial reduction in organic and inorganic nutrient loading was shown to occur during cyanobacterial growth in the HRAP.

The variability of conditions associated with the outdoor studies reported resulted in a somewhat wide range in some of the data sets derived. Nevertheless, these findings allowed a demonstration of the feasibility of a saline HRAP based on *Spirulina* which provided a waste treatment function similar to its fresh water counterpart and the production of biomass, which in turn, demonstrated a utility for saline wastes. Finally, the results obtained in the laboratory and outdoor pilot-scale HRAP were used as the basis for the scale-up design and construction of a full-scale 2500 m^2 HRAP to treat tannery wastewaters.

Subjecting the effluent water to anaerobic conditions before being fed into the HRAP or stabilisation ponds results in precipitation and thus removal of the contaminating heavy metals, probably largely as metal sulphides, before the effluent reaches the HRAP. This was shown to be a critically important step where industrial effluents are to be used for algal biomass production. The optimised Primary Anaerobic Pond A also resulted in conditions that allow a more rapid degradation of the organic load, resulting in less COD stress being placed on *Spirulina* growth in the HRAP or WSP.

Given a basis for understanding the role of *Spirulina* in the operation of the WSP, and why it does not naturally occur in the earlier parts of the ponding cascade, led to the attempt to engineer its establishment at the start of the system, and hence to effect a control of the biological processes, including rates of organic load reduction and odour production by the system. Results derived to date suggest the *Spirulina*-based HRAP can be operated successfully as a full-scale unit operation, receiving pre-treated tannery effluents at the start of a pond cascade. Also that it will prove to be an effective mechanism for establishing an oxygen-generating cap on the anaerobic WSP, and for the accelerated conversion of the system to aerobic operation. This not only demonstrated the basis for a retrofitted unit operation to manage malfunction in the long-established WSP, but also that harvesting of pond biomass would serve to linearise nutrient flows and thus presenting a method for the long term bioremediation of such sites.

An evaluation of the chemical composition and toxicological properties of tannery wastewater-generated *Spirulina* biomass was undertaken. The chemical composition of the *Spirulina* biomass was found to be largely comparable to reported values for *Spirulina* and other protein feed products. The analysis of the biomass, and results of the feeding trials undertaken, allow a preliminary conclusion that *Spirulina* grown on tannery effluent in the systems as configured in this study has no demonstrated toxicological constraints.

One of the main drawbacks in the development of micro-algae production for animal and fish feeds has been the high production and processing costs of defined medium cultivation. In this regard the results of the study have a bearing on wider issues relating to the development of Algal Biotechnology and, in part, address certain long-term, and futuristic, objectives identified for the field and noted earlier in this report. Micro-algal production, as it has currently developed, is a high-cost operation and only a few speciality products have warranted large-scale commercial exploitation. The potential cost credits associated with waste disposal have been identified as an important advantage of the HRAP process which, it has been shown, could have a decisive influence on the problem of algal production costs; and this especially where the production of marketable products can be accomplished.

The saline HRAP, demonstrated here, offers the additional advantage over its fresh water counterpart, that salinity can be manipulated to ensure the dominance of a single algal species, with the benefits of stable and reliable production. This is an essential prerequisite for the commercial viability of any algal production process. Given the opportunities presented for the scale-up evaluation of both laboratory and pilot studies conceptualising a *Spirulina*-based HRAP for the effective treatment of tannery wastewaters, it proved possible to demonstrate, not only the biomass production potential of the system, but also possibilities for its use as a unit operation for managing malfunction in the WSP; and in this way the investigations undertaken here have provided verification of the research hypothesis on which the study was based.

List of Publications

The research outlined in this thesis, has been published and presented at scientific conferences as follows:

Dunn, K.M. 1991. Release of Photosynthate by *Dunaliella salina* leakage or excretion ? BSc Hons. Thesis, Rhodes University, Grahamstown.

Dunn, K.M. 1995. Experiences with HRAP at Western Tanning. Proc. Society of Leather Technologists and Chemists Annual Conference (South Africa section). Midrand, South Africa.

Dunn, K.M., Shipin, O.V., and Rose, P.D. 1993. Tannery effluent treatment and the production of *Spirulina sp.* Proc. Biotech SA 93 Conference, Grahamstown, South Africa.

Rose, P.D., Maart, B.A., Dunn, K.M., Rowswell, R.A., and Britz, P. 1993. Treatment of tannery effluent in algal high rate oxidation ponds. Proc. IAWQ conference on wastewater stabilisation ponding. Berkley, USA.

Rose, P.D., Maart, B.A., Dunn, K.M., Rowswell, R.A., and Britz, P. 1995. Ponding presents potential. Leather, Vol. 197, No. 4643.

Rose, P.D., Maart, B.A., Dunn, K.M., Rowswell, R.A., and Britz, P. 1996. High Rate Algal Oxidation Ponding for the treatment of tannery effluents. Wat. Sci. Tech. 33(7): 219-227.

Shipin, O.V., Dunn, K.M., Shipin, V.Y., and Rose, P.D. 1994. Saline anaerobic digestion in advanced algal high rate oxidation ponding for the treatment of organics in saline effluents. Proc.7th International Symposium on Anaerobic digestion, Cape Town, South Africa.

References

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

Abeliovich, A. 1983. The effects of unbalanced ammonia and BOD concentrations on oxidation ponds. Wat. Res. 17(3):299-301.

Abeliovich, A. 1986. Algae in waste-water oxidation ponds. In: A. Richmond (ed.), CRC Handbook of Microalgal Mass culture. p. 331-338. CRC Press Inc., Boca Raton, USA.

Abeliovich, A. and Azov, Y. 1976. Toxicity of ammonia to algae in sewage oxidation ponds. Appl. Environ. Microbiol. 31: 801-806.

Abeliovich, A. and Weisman, D. 1978. Role of heterotrophic nutrition in growth of the alga *S. obliquus* in HRAP. Appl. Environ. Microbiol. 35:32-37.

Alexander, K.T.W., Corning, D.R., Cory, N.J., Donohue, V.J. and Sykes, R.L. 1992. Environmental and Safety Issues - Clean Technology and Environmental Auditing. J. Soc. Leather Technol. Chem. 76(1):17-23.

Alkan, U., Anderson, G.K. and Ince, O. 1996. Toxicity of trivalent chromium in the anaerobic digestion process. Wat. Res. 30(3):731-741.

Almasi, A. and Pescod, M.B. 1995. Wastewater treatment mechanisms in anoxic stabilisation ponds. 3rd IAWQ International Specialist Conference and Workshop; Waste Stabilisation Ponds: Technology and Applications, Brazil.

Anderson, S.L. and McIntosh, L. 1991. Light-activated heterotrophic growth of the cyanobacterium *Synechocystis sp.* strain PCC 6803 : a blue light requiring process. J. Bact. 173(9): 2761-2767.

Anita, N.J., Harrison, P.J. and Oliviera, L. 1991. The role of dissolved organic nitrogen in phytoplankton nutrition, cell biology and ecology. Phycologia 30:1-89.

A.P.H.A. 1989. Standard methods for the examination of water and wastewater. 17th ed. Washington, USA.

Armienta-Hernandez, M.A. and Rodrigues-Castillo, R. 1995. Environmental exposure to chromium compounds in the valley of Leon, Mexico. Environ. Health Perspect. 103(1): 47-51.

Ayala, F. and Vargas, T. 1987. Experiments on *Spirulina sp.* culture on waste-effluent media and at the pilot plant. Hydrobiol., 151/152:91-93.

Azov, Y. and Goldman, J.C. 1982. Free ammonia inhibition of algal photosynthesis in intensive cultures. Appl. Environ. Microbiol. 43:735-739.

Azov, Y. and Shelef, G. 1982. Operation of HROPs: Theory and experiments. Wat. Res. 16:1153-1160.

Azov, Y., Shelef, G., Moraine, R. and Levy, A. 1980. Controlling alga genera in high rate wastewater oxidation ponds. In: G. Shelef and C.J. Soeder (eds.), Algae biomass production and use. p. 245-254. Elsevier, Amsterdam, Netherlands.

Bai, J. and Seshadri, C.V. 1980. On coiling and uncoiling of trichomes in the genus *Spirulina*. Archiv. Fur Hydrobiologie, Beihefte Ergelonisse der Limnologie. 60:32-47.

Banerji, S.K. and Ruess, B. 1987. Evaluation of waste stabilisation pond performance in Missouri and Kansas, U.S.A. Wat. Sci. Tech. 19(12):39-46.

Bartlett, R. 1991. Chromium cycling in soils and water : links, gaps and methods. Environ. Health Pers. 92: 17-24.

Bartoszewski, K. and Bilyk, A. 1987. Pond treatment of rettery wastewaters. Wat. Sci. Tech. 19(12):79-83.

Becker, E.W. 1986. Nutritional properties of microalgae: Potentials and constraints. In: A. Richmond (ed.), CRC Handbook of Microalgal Mass culture. p. 339-420. CRC Press Inc., Boca Raton, USA.

Becker, E.W. and Venkataraman, L.V. 1980. Production and processing of algae in pilot plant scale experiences of the Indo-German project. In: G. Shelef and C.J. Soeder (eds.), Algae biomass production and use. p. 35-50. Elsevier, Amsterdam, Netherlands.

Becker, E.W. and Venkataraman, L.V. 1984. Production and utilisation of the blue-green alga *Spirulina* in India. Biomass 4:105.

Belay, A., Kato, T. and Ota, Y. 1996. *Spirulina* (Athrospira): potential application as an animal feed supplement. J. Appl. Phycol. 8(4/5):303-311.

Ben-Amotz, A. and Avron, M. 1989. The biotechnology of mass culturing *Dunaliella* for products of commercial interest. In: R.C. Cresswell, T.A.V. Rees and N. Shah (eds.), Algal and Cyanobacterial Biotechnology. p. 91-114. Longmans, Harlow, UK.

Benemann, J.R., Koopman, B.L., Weissman, J.C., Eisenberg, D. and Goebel, R.P. 1980. Development of micro-algae harvesting and high-rate pond technologies in California. In: G. Shelef and C.J. Soeder (eds.), Algae biomass production and use. Elsevier, Amsterdam, Netherlands.

Bennet, A. and Bogorad, L. 1973. Complementary chromatic adaptation in a filamentous blue-green alga. J. Cell Biol. 58:419-435.

Billen, G. 1984. Heterotrophic utilisation and regeneration of nitrogen. In: J.E. Hobbic, P.J. Lee and B. Williams (eds.), Heterotrophic Activity in the Sea. Plenum, New York, USA.

Borowitzka, M.A. 1994. Large-scale algal culture systems: the next generation. Australas Biotechnol. 4(4): 212-215.

Borowitzka, M.A. and Borowitzka, L.J. (eds.) 1988. Micro-algal biotechnology. Cambridge University Press, UK.

Borowitzka, M.A. and Borowitzka, L.J. 1989. Industrial Production : methods and economics. In: R.C. Cresswell, T.A.V. Rees and N. Shah (eds.), Algal and Cyanobacterial Biotechnology. Longmans, Harlow, UK.

Borowitzka, M.A., Borowitzka, L.J. and Moulton, T.P. 1984. The mass culture of *Dunaliella salina* for fine chemicals: from laboratory to pilot plant. Hydrobiologia 116:115-134.

Boussiba, S. and Richmond, A.E. 1979. Isolation and characterisation of phycocyanins from the blue-green alga *S. platensis*. Arch. Microbiol. 120:155-159.

Boussiba, S. and Richmond, A.E. 1980. C-Phycocyanin as a storage protein in the blue-green alga *Spirulina platensis*. Arch. Microbiol. 125:143-147.

Boutin, P., Vachon, A. and Racault, Y. 1987. Waste stabilisation ponds in France: overall review. Wat. Sci. Tech.19(12):25-31.

Britz, P.J. 1996. An investigation into the suitability of selected protein sources for inclusion in artificial diets for the South African abalone, *Haliotus midae*. Aquaculture 140:63-73.

Brock, T.D. and Madigan, M.T. 1988. Biology of micro-organisms. Prentice-Hall, London, UK.

Bucksteeg, K. 1987a. German experiences with sewage treatment in non-aerated and in artificially aerated ponds. Seminar, Ardon, Germany.

Bucksteeg, K. 1987b. German experiences with sewage treatment ponds. Wat. Sci. Tech. 19(12):17-23.

Burlew, S. (ed.) 1953. Algal culture from laboratory to pilot plant. Carnegie Institution of Washington, Pub. No. 600. Washington, USA.

Caldwell, D.H. 1946. Sewage oxidation ponds - performance, operation, and design. Sewage Works J. 18(3):433-458.

Campbell. J. III., Stevens, S.E. and Balkwill, D.L. 1982. Accumulation of poly-β-hydroxybutyrate in *Spirulina platensis*. J. Bacteriol. 149:361-363.

Cardenas, A. and Markovits, A. 1985. Mixing and power characteristics of a mixing board device in shallow ponds. Applied Phycology Forum 2(3):1-4.

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

Chaudari, P.R., Krishnamoorthi, K.P. and Vittal Rao, M. 1980. Growth potential of *Spirulina*, a blue green algae in sewage. In: Indian Academy of Sciences. 89(3). Bangalore, India.

Chevalier, P. and de la Noue, J. 1985. Wastewater nutrient removal with micro-algae immobilised in carrageenan. Enzyme Microb. Technol. 7:621-624.

Cifferi, O. 1983. *Spirulina sp.*, the edible micro-organism. Microbiol. Rev. 47(4):551-578.

Cifferi, O. and Tiboni, O. 1985. The biochemistry and industrial potential of *Spirulina sp.* Ann. Rev. Microbiol. 39:503-526.

Clement, G., Giddey, C. and Menzi, R. 1967. Amino acid composition and nutritive value of the alga *Spirulina maxima*. J. Sci. Food Agric. 18:497-500.

Cohen, Z. and Vonshak, A. 1991. Fatty acid composition of *Spirulina* and *Spirulina*-like cyanobacteria in relation to their chemotaxonomy. Phytochemistry 30(1):205-206.

Colleran, E., Finnegan, S. and Lens, P. 1995. Anaerobic treatment of sulphate-containing waste streams. Antonie Van Leeuwenhoek 67(1):29-46.

Conzomo, V., Di Riso, C., Tudino, M., Ballsells, R.E. and Cirelli, A.F. 1994. Cadmium and chromium interactions with aquatic humic substances from Argentine ponds. Fresenius Environ. Bull. 3:1-5.

Cook, B.B. 1962. The nutritive value of waste-grown algae. A.J.P.H., 52(2):243-250.

Cooper, D.R., Russell, A.E., Shuttleworth, S.G. and Boast, D.A. 1984. Closed systems for salt and saline wastewater in curing and tanning. LIRI Research Bulletin No. 877. LIRI, Grahamstown, South Africa.

Cornet, J.F., Dussap, C.G. and Dubertret, G. 1992a. A structured model for simulation of cultures of the cyanobacteria *S. platensis* in photobioreactors: 1. Coupling between light transfer and growth kinetics. Biotech. Bioeng. 40:817-825.

Cornet, J.F., Dussap, C.G., Cluzel, P. and Dubertret, G. 1992b. A structured model for simulation of cultures of the cyanobacteria *S. platensis* in photobioreactors: 2. Identification of kinetic parameters under light and mineral limitations. Biotech. Bioeng. 40:826-834.

Corning, D.R. 1978. The biodegradability of tannery chemicals. J. Soc. Leather Technol. Chem. 62:63-67.

Cowan, A.K. and Rose, P.D. 1991. Abscisic acid metabolism in salt stressed cells of *D. salina*. Plant Physiol. 97:798-803.

Cowan, A.K., Rose, P.D. and Horne, L.G. 1995. *D. salina*: a model system for studying the response of plant cells to stress. J. Experimental Botany 43(257):1535-1547.

Cresswell, R.C., Rees, T.A.V. and Shah, N. (eds.) 1989. Algal and Cyanobacterial Biotechnology. Longmans, Harlow, UK.

Cross, R. 1979. The Preparation of Biological material for Electron Microscopy. Rhodes University, Grahamstown, South Africa.

de Pauw, N. and van Vaerenbergh, E. 1983. Micro-algal wastewater treatment systems: potentials and limits. In: Phytodepuration and the employment of biomass produced. Crento Pricerce Produzione Animali, Perugia, Italy.

de Pauw, N. and Salomoni, C. 1991. The use of microalgae in wastewater treatment: achievements and constraints. In: P. Madoni (ed.), Biological Approach to Sewage Treament Processes: Current Status and Perspectives. p. 329-352. Perugia, Italy.

DePhilippis, R., Shili, C. and Vincenini, M. 1992. Glycogen and poly-β-hydroxybutyrate synthesis in *S. maxima*, J. Gen. Microbiol. 138:1623-1628.

Diakoff, S. and Scheibe, J. 1975. Cultivation in the dark of the blue-green alga *Fremyella diplosiphon*. A photoreversible effect of green and red light on growth rate. Physiol. Plantarum. 34:125-128.

Dillon, J.C., Phuc, A.P. and Dubacq, J.P. 1995. Nutritional value of the alga *Spirulina*. World Rev. Nutr. Diet. 77:32-46.

Donkor, V. and Hader, D-P. 1991. Effects of solar and ultraviolet radiation on motility, photomovement and pigmentation of filamentous, gliding cyanobacteria. FEMS Microbial Ecology 86:159-168.

Dodd, J. 1986. Elements of pond design and construction. In: A. Richmond (ed.), CRC Handbook of Microalgal Mass culture. CRC Press Inc., Boca Raton, USA.

D'Souza, S.E., Altekar, W. and D'Souza, S.F. 1997. Nutritional value of the algae *Spirulina*. World Rev. Nutr. Diet. 77:32-46.

D'Souza, S.E., Altekar, W. and D'Souza, S.F. 1997. Adaptive response of *Haloferax mediterranei* to low concentrations of NaCl (<20 %) in the growth medium. Arch. Microbiol. 168(1) 68-71.

Duarte, A.C., Arroja, L.M., Diegues, P.F., Rosada, I., Hall, A. and Oliviera, J.B. 1987. Treatment of slaughterhouse waste-water in stabilisation ponds. Wat. Sci. Tech. 19(12):85-91.

Ducklow, H.W., Purdie, D.A., Williams, P.J. and Davies, J.M. 1986. Bacterioplankton: a sink for carbon in a coastal marine plankton community. Science 232:865-868.

Durand-Chastel, H. 1980. Production and use of *Spirulina* in Mexico. In: G. Shelef and C.J. Soeder (eds.), Algae biomass production and use. p. 51-64. Elsevier, Amsterdam, Netherlands.

Eddy, A.A. 1982. Mechanisms of solute transport in selected eukaryotic micro-organisms. Adv. Microb. Physiol. 23:1-78.

Ellis, K.V. and Rodrigues, P.C. 1995. Developments to first-order, complete-mix design approach for stabilisation ponds. Wat. Res. 29(5):1343-1351.

Fallowfield, H.J. and Garret, M.K. 1985. The treatment of wastes by algal culture. J. Appl. Bact. Sym. Suppl. 187S-205S.

Fallowfield, H.J., Svoboda, I.F. and Martin, N.J. 1992. Aerobic and photosynthetic treatment of animal slurries. In: J.C. Fry (ed.), Microbial control of pollution. Cambridge University Press, Cambridge, UK.

Faucher, O., Coupal, B. and LeDuy, A. 1979. Utilisation of seawater-urea as a culture medium for S. *maxima*. Can. J. Microbiol. 25:752-759.

Fay, P. 1983. The Blue-Greens (Cyanophyta-Cyanobacteria). Edward Arnold Publ., London, UK.

Finney, B.A. and Middlebrooks, E.A. 1980. Facultative waste stabilisation pond design. J. Wat. Pollut. Control Fed. 52:134-147.

Flynn, K.J. and Butler, I. 1986. Nitrogen sources for the growth of marine micro-algae : role of dissolved free amino acids. Mar. Ecol. Prog. Series 34:281-304.

Fott, B. and Karim, A.G.A. 1973. *Spirulina sp.* plankton community in a lake in Jebel Marra, Sudan. Archiv. fur Protistenkunde. 115:408-418.

Fox, R.D. 1983. Algoculture. PhD Thesis, University of Louis Pasteur, Strasbourg, France.

Fredrickson, A.G. and Stephanopoulis, G. 1981. Microbial competition. Science 213:972-979.

Frund, C. and Cohen, Y. 1992. Diurnal cycles of sulphate reduction under oxic conditions in Cyanobacterial mats. Appl. Environ. Microbiol. 58:70-77.

Gaicher, I.G., Cloete, T.E. and Toerien, D.F. 1982. Preliminary studies on the treatment of canning factory effluent with an integrated bacterial-algal-fish system. Water SA. 8(2):97-100.

Ganapati, S.V. 1975. Biochemical studies of algal-bacterial symbiosis in high-rate oxidation ponds with varying detention periods and algae. Arch. Hydrobiol. 76(3):302-367.

Garrote, J.I., Bao, M., Castro, P. and Bao, M.J. 1995. Treatment of tannery effluents by a two step coagulation/flocculation process. Wat. Res. 29(11):2605-2608.

Gaudy, A.F. and Gaudy, E.T. (eds.) 1980. Microbiology for Environmental Scientists and Engineers. McGraw-Hill Inc., London, UK.

Genschow, E., Hegemann, W. and Maschke, C. 1996. Biological sulphate removal from tannery wastewater in a two-stage treatment. Wat. Res. 30(9):2072-2078.

Gerhardt, P., Murray, R.G.E., Costilow, R.N., Nester, E.W., Wood, W.A., Krieg, N.R. and Phillips, G.B. 1981. Manual of Methods for General Bacteriology. American Sociecty for Microbiology, Washington, USA.

Glaum, R.A. 1991. The role of bacterial association in the utilisation of the amino acid glycine by *Dunaliella salina*. BSc Hons. Report, Rhodes University, Grahamstown, South Africa.

Gocke, K. and Hoppe, H.G. 1977. Determination of organic substances and respiration potential. In: G. Rheinheimer (ed.), Microbial ecology of a brackish-water environment. Springer, Berlin, Germany.

Golecki, J.R. and Heinrich, U-R. 1990. Ultrastructural and electron spectroscopic analysis of cyanobacteria and bacteria. J. Microscopy. 162:147-154.

Golueke, G. and Oswald, W.J. 1965. Harvesting and processing sewage-grown planktonic algae. J. Wat. Pollut. Control Fed. 37:471-498.

Gomes de Sousa, J.M. 1987. Waste-water stabilisation lagoon design criteria for Portugal. Wat. Sci. Tech. 19(12):7-16.

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

Green, F.B., Bernstone, L.S., Lundquist, T.J. and Oswald, W.J. 1995. Methane fermentation, submerged gas collection, and the fate of carbon in advanced integrated wastewater pond systems. Nat. Sci. Tech. 31(12):55-65.

Grobbelaar, J.U. 1980. Potential of algal production. Water SA. 8(2):79-85.

Grobbelaar, J.U. 1991. The influence of light/dark cycles in mixed algal cultures on their productivity. Bioresource Technology 38:189-194.

Grobbelaar, J.U. 1994. Turbulence in mass algal cultures and the role of light/dark fluctuations. J. Appl. Phycol. 6:331-335.

Grobbelaar, J.U., Nedbal, L. and Tichy, V. 1996. Influence of high frequency light /dark fluctuations on photosynthetic characteristics of microalgae photoacclimatised to different light intensities and implications for mass algal cultivation. J. Appl. Phycol. 8(4/5) :335-343.

Grobbelaar, J.U., Soeder, C.J., Groeneweg, J., Stengel, E. and Hartig, P. 1988. Rates of biogenic oxygen production in mass cultures of micro-algae, absorption of atmospheric oxygen and oxygen availability for waste-water treatment. Wat Res. 22(11):1459-1464.

Gu, R. and Stefan, H.G. 1995. Stratification dynamics in wastewater stabilisation ponds. Wat. Res. 29(8):1909-1923.

Guidotti, T.L. 1996. Hydrogen sulphide. Occup. Med. 46(5): 367-371.

Guterman, H. and Ben-Yaakov, S. 1990. On-Line optimisation of biotechnological processes: 1. Application to Open Algal Pond. Biotech. Bioeng. 35:417-426.

Guterman, H., Ben-Yaakov, S. and Vonshak, A. 1989. Automatic on-line growth estimation method for outdoor algal biomass production. Biotech. Bioeng. 34:143-152.

Hacking, J. 1986. Economic Aspects of Biotechnology. Cambridge University Press, Cambridge, UK.

Halfren, L.N. and Castenholtz, R.W. 1971. Gliding motility in the blue-green alga *Oscillatoria Princeps*. J. Phycol. 7:133-145.

Hall, D.D. 1986. The production of biomass: A challenge to our society. In: A. Richmond (ed.), CRC Handbook of Microalgal Mass culture. CRC Press Inc., Boca Raton, USA.

Hammer, K.D. 1993. Do soluble organic nitrogen compounds cause unusual blooms in seawater ? Zentralbl Hyg Umweltmed 194 (4):321-341.

Hart, O., Cooper, D.R., Shuttleworth, S.G., Rowswell, R.S., Dorrington, L.S., Squires, R.C., Young, T.P.M. and Steenveld, G.N. 1987. A guide to waste-water management in the Tanning and Fellmongering Industries. WRC Project No. 41, TT27/87. Pretoria, South Africa.

Hayashi, O. and Okuwaki, Y. 1994. Enhancement of antibody production in mice by dietary *Spirulina paltensis*. J. Nutr. Sci. Vitaminol. 40(5):431-441.

Hellebust, J.A. 1970. The uptake and utilisation of organic substances by marine phytoplankton. Occasional Publications of the Institute of Marine Science, University of Alaska Collection 1:225-256.

Hellebust, J.A. 1985. Mechanisms of response to salinity in halotolerant micro-algae. Plant and Soil 89:69-81.

Hellebust, J.A. and Lewin, J. 1977. Heterotrphic nutrition. In: D. Werner (ed.), The Biology of Diatoms. Blackwell, Oxford, UK.

Herold, C.E. and Bailey, A.K. 1996. Long-term salt balance of the Vaalharts Irrigation Scheme. Water Research Commission Report 420/1/96. Pretoria, South Africa.

Hoare, D.S., Hoare, S.L. and Moore, R.B. 1967. The photoassimilation of organic compounds by autotrophic blue-green algae. J. Gen. Microbiol. 49:351-370.

Holmgren, P.R., Hostetter, H.R. and Scholes, V.E. 1971. Ultrastructural observation of cross-walls in the blue-green alga *S. major*. J. Phycology 7:309-311.

Holm-Hansen, O. 1968. Ecology, physiology, and biochemistry of blue-green algae. Ann. Rev. Microbiol. 22:47-70.

Houghton, S.R. and Mara, D.D. 1992. The efects of sulphide generation in WSP on photosynthetic populations and effluent quality. Wat. Sci. Technol. 26:1759-1768.

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

Hudson, B.J.F. and Karis, L.G. 1974. The lipids of the alga *Spirulina sp.* J. Sci. Fd. Agric. 25:759-763.

Hung, K.M., Chiu, S.T. and Wong, M.H. 1996. Sludge-grown algae for culturing aquatic organisms: Part I. Algal growth in sludge extracts. Environ. Manage. 20 (3):361-374.

Iijima, N., Fugii, H., Shimamatsu and Katoh, S. 1986. Microalgae of economic importance. In: A. Richmond (ed.), CRC Handbook of Microalgal Mass culture. CRC Press Inc., Boca Raton, USA.

Ilyaletdinov, A. N., Enker, P.B. and Loginova, L.V. 1977. Role of sulphate-reducing bacteria in the precipitation of copper. Mikrobiol. 46(1):113-117.

Jackson-Moss, C.A. 1990. An investigation into the use of anaerobic digestion for the treatment of tannery wastewater. PhD Thesis, Rhodes University, Grahamstown, South Africa.

Kabdasli, I., Tunay, O. and Orhon, D. 1993. The treatability of chromium tannery wastes. Wat Sci. Technol. 28 (2):97-105.

Kaplan, D., Richmond, A.E., Dubinsky, Z. and Aaronson, S. 1986. Algal nutrition. In: A. Richmond (ed.), CRC Handbook of Microalgal Mass culture. CRC Press Inc., Boca Raton, USA.

Kerby, N.W., Rowell, P. and Stewart, W.D.P. 1989. The transport, assimilation and production of nitrogenous compounds by cyanobacteria and micro-algae. In: R.C. Cresswell, T.A.V. Rees and N. Shah (eds.), Algal and Cyanobacterial Biotechnology. Longmans, Harlow, UK.

Khneleniva, V.N., Kalyuzhnaya, M.G., Starostina, N.G., Suzina, N.E. and Trotsenko, Y.A. 1997. Isolation and characterisation of halotolerant alkaphilic methanotrophic bacteria from Tuva soda lakes. Curr Microbiol. 35(5):257-261.

Kilani, J.S. 1992. Studies on the treatment of dairy wastes in an algal pond. Water SA. 18(1):57-62.

Konig, A., Pearson, H.W. and Silva, S.A. 1987. Ammonia toxicity to algal growth in waste stabilisation ponds. Wat. Sci. Tech. 19(12):115-122.

Koopman, B., Benemann, J.R. and Oswald, W.J. 1980. Pond isolation for control of suspended solids concentration in sewage oxidation pond effluents. In: G. Shelef and C.J. Soeder (eds.), Algae biomass production and use. Elsevier, Amsterdam, Netherlands.

Kosaric, N., Nguyen, H.T. and Bergougnou, M.A. 1974. Growth of *Spirulina maxima* algae in effluents from secondary wastewater treatment plants. Biotech. Bioeng. 16:881-896.

Labarre, J., Thuriaux, P. and Chauvat, F. 1987. Genetic analysis of amino acid transport in the facultatively heterotrophic cyanobacterium *Synechosystis sp.* strain 6803. J. Bact. 169(10):4668-4673.

Lalitha, K., Swaminathan, K.R. and Bai, R.P. 1994. Kinetics of biomethanation of solid tannery waste and the concept of interactive metabolic control. Appl. Biochem. Biotechnol. 47(1):73-87.

Lansdell, M. 1987. The development of lagoons in Venezuela. Wat. Sci. Tech. 19(12):55-60.

Lang, W.C., Blatt, D. and Plapp, R. 1979. Proteolytic enzymes in *Clamydomonas*. A survey on the aminopetidase pattern in synchronous vegetative cells of *Clamydomonas reinhardii*. Plant Cell Physiol. 20:657-665.

Larsen, H. 1974. Halobacteriaceae. In: Bergy's Manual of Systematic Bacteriology. 3:261. Williams and Wilkins, London, UK.

Laubscher, R.K., Rose, P.D. and Aken, M.E. 1990. Saline tannery effluents as growth media for the halophilic alga, *D. salina*. Proc. Sixth Congress SA Soc. Microbiol., Stellenbosch, South Africa.

Laubscher, R.K. 1991. The culture of *Dunaliella salina* and the production of β-carotene in tannery effluents. MSc Thesis, Rhodes University, Grahamstown, South Africa.

Lawty, R., de B. Ashworthy, D. and Mara, D.D. 1995. Waste stabilisation pond decommissioning: a painful but necessary decision. Wat. Sci. Tech. 31(12):1-8.

Lembi, C.A. and Waaland, J.R. (eds.) 1988. Algae and Human Affairs. Cambridge University Press, Cambridge, UK.

Lichtenthaler, H.K. 1987. Chlorophylls and Carotenoids: Pigments of photosynthetic biomembranes. Methods in Enzymol. 148:350-371.

Livansky, K. and Doucha, J. 1996. CO_2 and O_2 gas exchange in outdoor thin-layer high density microalgal cultures. J. Appl. Phycol. 8(4/5):353-358.

Logie, M.M.R. 1995. Physiological signal transduction from the photosynthetic apparatus in the green alga *Dunaliella salina*. PhD Thesis, Rhodes University, Grahamstown, South Africa.

Losi, M.E., Amrhein, C., and Frankenberger, W.T. 1994. Environmental biochemistry of chromium. Rev. Environ. Contam. Toxicol. 136:91-121.

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

Macchi, G., Pagano, M., Pettine, M., Santori, M. and Tiravanti, G. 1991. A bench study on chromium recovery from tannery sludge. Wat. Res. 25(8):1019-1026.

Malik, K.A. 1983. A modified method for the cultivation of phototrophic bacteria. J. Microbiol. Methods. 1: 343-352.

Mara, D.D. (ed.) 1976. Sewage treatment in hot climates. John Wiley, London, UK.

Mara, D.D. and Marecos Do Monte, M.H. 1987. Waste stabilisation ponds. Wat. Sci. Technol. 19(12):1-401.

Mara, D.D., Pearson, H.W. and Silva, S.A. 1996. Waste stabilisation ponds: technology and applications. Wat. Sci. Tech. 33(7):1-262.

Marais, G.V. 1966. New factors in the design, operation and performance of waste stabilisation ponds. Bull. Wld. Hlth.. Org. 34:737-763.

Marais, G.V. and Shaw, V. 1961. Rational theory for design of waste stabilisation ponds in South Africa. Trans. South African Inst. Civ. Engr. 3(11):205.

Markl, H. 1980. Modelling of algal production systems. In: G. Shelef and C.J. Soeder (eds.), Algae biomass production and use. Elsevier, Amsterdam, Netherlands.

Marques, J.J. and d'Avila, J.S. 1995. An algorith optimisation to project and simulate aerobic and facultative stabilisation ponds. Proc. 3rd International Specialist Conference on Waste Stabilisation Ponds: Technology and Application, Brazil.

Materassi, R., Tredici, M. and Balloni, W. 1984. *Spirulina sp.* culture in sea-water. Appl. Micro. Biotech. 19:384-386.

Mathew, B., Sankaranarayanan, R., Nair, P., Varghese, C., Somanathan, T., Amma, B.P., Amma, N.S. and Nair, M.K. 1996. Evaluation of chemoprevention of oral cancer with *S. platensis.* Nutr. Cancer 24(2):197-202.

McGarry, M.G. 1970. Algal flocculation with aluminium sulphate and polyelectrolytes. J. Water Pollut. Control Fed. 42:191.

McGrath, J.E. and Harfoot, C.G. 1997. Reductive dehalogenation of halocarboxylic acids by the phototrophic genera *Rhodospirillum* and *Rhodopsuedomonas.* Appl. Environ. Microbiol. 63(8):3333-3335.

Menon, V.K.N. and Varma, A.K. 1982. Sulphate uptake in the cyanobacterium *S. platensis.* FEMS Microbiol. Letters.13:141-146.

Meiring, P.G., Drews, R.J., van Eck, H. and Stander, G.J. 1968. A guide to the use of pond systems in South Africa for the purification of raw and partially treated sewage. CSIR Special Reports, NIWR, Pretoria, South Africa.

Middlebrooks, E.J. 1987. Design equations for BOD removal in facultative ponds. Wat. Sci. Tech. 19(12):187-193.

Miller, L.P. 1950. Formation of metal sulphides through the activities of sulphate reducing bacteria. Contrib. Boyce Tompson Inst. 16:85-89.

Miller, A.G., Cheng, K.H. and Colman, B. 1971. The uptake and oxidation of glycolic acid by blue-green algae. J. Phycol. 7:97-100.

Miller, S., Abeliovich, A. and Belfort, G. 1977. Effects of high organic loading on mixed photosynthetic waste-water treatment. J. Water Pollut. Control Fed. March:436-440.

Mitchell, S.A. 1986. Experiences with the outdoor semi-continuous mass culture of *Brachionus calyciflorus* Pallas (Rotifera). Aquaculture 51:289-297.

Mitchell, S.A. and Richmond, A. 1988. Optimisation of a growth medium for *Spirulina* based on cattle waste. Biological Wastes 25:41-50.

Mohn, F.H. 1980. Experiences and strategies in the recovery of biomass from mass cultures of microalgae. In: G. Shelef and C.J. Soeder (eds.), Algae biomass production and use. p. 547-572. Elsevier, Amsterdam, Netherlands.

Mohn, F.H. 1988. Harvesting of micro-algal biomass. In: M.A. Borowitzka and L.J. Borowitzka (eds.), Micro-algal Biotechnology. p. 395-414. Cambridge University Press, Cambridge, UK.

Montanaro, F., Ceppi, M., Demers, P.A., Puntoni, R. and Bonassi, S. 1997. Mortality in a cohort of tannery workers. Occup. Environ. Med. 54(8):588-591.

Moutin,T., Gal, J.Y., El Halovani, H., Picot, B. and Bontoux, J. 1992. Decrease in phosphate concentration in a HROP by precipitation of calcium phosphate: theoretical and experimental results. Wat. Res. 26(1):1445-1450.

Naghavi, B. and Malone, R.F. 1986. Algae removed by fine sand/silt filtration. Wat. Res. 20:377-383.

Narasimha, D.L.R., Venkataraman, G.S., Duggal, S.K. and Eggum, B.O. 1982. Nutritional quality of the blue-green alga *S. platensis Geitler.* J. Sci. Food Agric. 33:456-460.

Natarajan, K.V. 1970. Toxicity of ammonia to marine diatoms. J. Water Pollut. Control Fed. 42(5):184-190.

Neytzell-de Wilde, F.G., Orbin, A., Solymosi, A.M. and Simpson, A. 1992. The treatment of industrial effluents with high salinity and organic content. Water Research Commision Report No. 123/1/87. Pretoria, South Africa.

Nielson, A.H. and Larsson, T. 1980. The utilisation of organic nitrogen for growth of algae : physiological aspects. Physiologia Planatarum 48:542-553.

O'Brien , D.J., Stenske, G.E. and Komanowsky, M. 1986. Ammonia removal from deliming effluent by stripping-reabsorption. J. Am. Leather Chem. Assoc. 81:125-136.

OECD. 1996. Wider application and diffusion of bioremediation technologies. The Amsterdam '95 Workshop. OECD, Paris, France.

Oliviera, L. and Huynh, H. 1989. Ultrastructure and cytochemistry of *Dunaliella tertiolecta* (Butcher) and *Pavlova lutheri* (Droop) Green grown on three different sources of nitrogen. New Phytol. 113:481-490.

Oliviera, L. and Huynh, H. 1990. Phototrophic growth of micro-algae with allantoic acid or hypoxanthine serving as a nitrogen source: Implications for purine-N utilisation. Canad. J. Fish Aquat. Sci. 47:351-356.

Oremland, R.S., Hollibaugh, J.T., Maest, A.S., Presser, T.S., Miller, L.G. and Culberston, C.W. 1989. Selenate reduction to elemental selenium by anaerobic bacteria in sediments and culture: Biogeochemical significance of a novel, sulphate independent respiration. Appl. Eniron. Micro. 55(9):2333-2343.

Oren, A. 1992. Bacterial activities in the Dead sea; 1980-1991 a survival at the upper limits of salinity. Int. J. Salt Lake Res. 1:7-20.

Oron, G. and Shelef, G. 1980. An optimization model for high rate algae ponds. In: G. Shelef and C.J. Soeder (eds.), Algae biomass production and use. p. 497-504. Elsevier, Amsterdam, Netherlands.

Oswald, W.J. 1963. The HROP in waste disposal. Dev. Ind. Microbiol. 76:3:112-119.

Oswald, W.J. 1980. Algal production-problems, achievements and potential. In: G. Shelef and C.J. Soeder (eds.), Algae biomass production and use. p. 1-8. Elsevier, Amsterdam, Netherlands.

Oswald, W.J. 1991. Waste treatment by Pond Systems, Engineering Aspects. Proc. IAWPRC Conference on Appropriate Waste Management Technologies, Perth, Australia.

Oswald, W.J. 1995. Ponds in the Twenty-first Century. Wat. Sci. Tech. 31(12):1-8.

Oswald, W.J. 1988a. Micro-algae and waste-water treatment. In: M.A. Borowitzka and L.J. Borowitzka (eds.), Micro-algal Biotechnology. p. 305-328. Cambridge University Press, Cambridge, UK.

Oswald, W.J. 1988b. Large-scale algal systems (engineering aspects). In: M.A. Borowitzka and L.J. Borowitzka (eds.), Micro-algal Biotechnology. p. 357-394. Cambridge University Press, Cambridge, UK.

Oswald, W.J., and Golueke, C.G. 1960. Biological transformations from solar energy. Appl. Environ. Microbiol. 2:233-262.

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

Oswald, W.J., Green, F.B. and Lundquist, T.J. 1994. Performance of methane fermentation pits in advanced integrated wastewater ponds. Wat. Sci. Tech. 30(12):287-295.

Paerl, H.W. 1996. Microscale physiological and ecological studies of aquatic cyanobacteria:macroscale implications. Microsc. Rec. Tech. 33(1):47-72.

Paerl, H.W., Tucker, J. and Bland, P.T. 1983. Carotenoid enhancement and its role in maintaining blue-green algal (*Mycrocystis aeruginosa*) surface blooms. Limnol. Oceanogr. 28:847.

Palmer, C.M. 1969. A composite rating of algae tolerating organic loading. J. Phycol. 5:78.

Pano, A. and Middlebrooks, E.J. 1982. Ammonia nitrogen removal in facultative waste-water stabilisation ponds. J. Water Pollut. Control Fed. 54:344-351.

Pearson, H.W. 1996. Expanding the horizons of pond technology and application in an environmentally concious world. Wat. Sci. Tech. 33(7):1-9.

Pearson, H.W., Mara, D.D. and Bartone, C.R. 1987. Guidelines for minimum evaluation of performance of full-scale waste stabilisation pond systems. Wat. Sci. Tech. 21(9):1067-1075.

Pelroy, R.A. and Bassham, J.A. 1972. Photosynthetic and Dark carbon metabolism in unicellular blue green algae. Arch. Mikrobiol. 86:25-38.

Pescod, M.B. 1996. The role and limitations of anaerobic pond systems. Wat. Sci. Tech. 33(7):11-22.

Pettine, M., Millero, F.J. and Passino, R. 1994. Reduction of chromium (IV) with H_2S in NaCl medium. Mar. Chem. 46(4):335.

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

Phillips, L.G., Cowan, A.K., Rose, P.D. and Logie, M.R.R. 1995. Operation of the Xanthophyll Cycle in non-stressed cells of *Dunaliella salina* Teod. in response to diurnal changes in incident irradiation: a correlation with intracellular β-carotene content. J. Plant Physiol. 146:547-553.

Pohland, A.E., Dowell Jr., V.R. and Richard, J.L. 1990. Microbial toxins in foods and feeds - cellular and molecular modes of action. Proceedings of a Symposium on Cellular and Molecular Mode of Action of Selected Microbial Toxins in Foods and Feeds, Maryland, Plenum Press, New York, USA.

Post, F.J. 1981. Microbiology of the Great Salt Lake north arm. Hydrobiologia 81:59-69.

Pruel, H.C. and Wagner, R.A. 1987. Waste stabilization pond prediction model. Wat. Sci. Tech. 19(12):205-211.

Pushparaj, B., Pelosi, E., Tredici, M.R., Pinzani, E. and Materassi, R. 1997. An integrated system for outdoor production of microalgae and cyanobacteria. J. Appl. Phycol. 9(2):113-119.

Quereshi, M.A. and Ali, R.A. 1996. *Spirulina platensis* exposure enhances macrophage phagocytic function in cats. Immunolpharmacol. Immunotoxicol. 18(3):465-476.

Quereshi, M.A, Garlich, J.D. and Kidd, M.T. 1996. Dietry *Spirulina platensis* enhances humoral and cell-mediated immune functions in chickens. Immunopharmacol. Immunotoxicol. 18(3):465-476.

Ramus, J. 1985. Light. In: M.M. Littler and D.S. Littler (eds.), Handbook of Phycological Methods, Ecological Field Methods: Macroalgae. p33-52. Cambridge University Press, Cambridge, UK.

Raven, J.A. 1988. Limits to growth. In: M.A. Borowitzka and L.J. Borowitzka (eds.), Micro-algal Biotechnology. Cambridge University Press, Cambridge, UK.

Richmond, A. 1981. *Spirulina* production - A final report on six years of experimentation to develop the biotechnology for the commercial production of algae. The unit of applied Hydrobiology, The Jacob Bluastein Institute for Desert Research, Ben-Gurion University, Sede Boqer, Israel.

Richmond, A. 1986a. Microalgae of economic potential. In: A. Richmond (ed.), CRC Handbook of Microalgal Mass culture. p. 199-244. CRC Press Inc., Boca Raton, USA.

Richmond, A. 1986b. Future prospects. In: A. Richmond (ed.), CRC Handbook of Microalgal Mass culture. p. 485-487. CRC Press Inc., Boca Raton, USA.

Richmond, A. 1986c. Outdoor mass cultures of microalgae. In: A. Richmond (ed.), CRC Handbook of Microalgal Mass culture. p. 285-330. CRC Press Inc., Boca Raton, USA.

Richmond, A. 1987. The challenge confronting industrial micro-agriculture: high photosynthetic efficiency in large-scale reactors. Hydrobiol. 151/152:117-121.

Richmond, A. 1988. *Spirulina*. In: M.A. Borowitzka and L.J. Borowitzka (eds.), Micro-algal Biotechnology. p. 85-121. Cambridge University Press, Cambridge, UK.

Richmond, A. 1996. Efficient utilisation of high irradiance for production of photoautotrophic cell mass: a survey. J. Appl. Phycol. 8:381-387.

Richmond. A. and Becker, E.W. 1986. Technical aspects of mass cultivation - A general outline. In: A. Richmond (ed.), CRC Handbook of Microalgal Mass culture. p. 245-263. CRC Press Inc., Boca Raton, USA.

Richmond, A. and Preiss, K. 1980. The biotechnology of algaculture. Interdisciplinary Sci. Rev. 5(1): 60-70.

Richmond, A. and Vonshak, A. 1978. *Spirulina sp.*, culture in Israel. Archiv fur Hydrobiologie, Beihefte Ergebnisse der Limnologie 11:274-280.

Richmond, A., Karg, S. and Boussiba, S. 1982. Effects of Bicarbonate and Carbon Dioxide on the Competition between *Chlorella vulgaris* and *Spirulina platensis*. Plant Cell Physiol. 23(8):1411-1417.

Richmond, A., Vonshak, A. and Arad, S. 1980. Environmental limitation in outdoor production of algal biomass. In: G. Shelef and C.J. Soeder (eds.), Algae biomass production and use. p. 65-72. Elsevier, Amsterdam, Netherlands.

Richmond, A., Boussiba, S., Vonshak, A. and Kopel, R. 1993. A new tubular reactor for mass production of microalgae outdoors. J. Appl. Phycol. 5:327-332.

Rippka, R. 1972. Photoheterotrophy and chemoheterotrophy among unicellular blue-green algae. Arch. Mikrobiol. 87:93-98.

Rippka, R., Deruelles, J., Waterbury, T.B., Herdman, M. and Stanier, R.Y. 1979. Generic assignments, strain histories and properties of pure cultures of cyanobacteria. J. Gen. Microbiol. 111:1-61.

Rodgers, P.W. and DePinto, J.V. 1981. Algae-bacteria interaction in a light-dark cycle. J. Fresh. Wat. Ecol. 1(1):71-80.

Rodrigues, A.M. and Oliviera, J.F.S. 1987a. HROP treatment of waste-waters and protein production: chemical composition of biomass produced from swine wastes. Wat. Sci. Tech. 19(12):243-248.

Rodrigues, A.M. and Oliviera, J.F.S. 1987b. Treatment of waste-waters from the tomato concentrate industry in HRAP. Wat. Sci Tech. 19(12):43-49.

Rodriguez-Valera. 1992. Biotechnological potential of halobacteria. Biochem. Soc. Symp. 58:135-147.

Rose, P.D. 1991. Algal Biotechnology and the benefication of saline effluent wastes. PhD Thesis, Rhodes University, Grahamstown, South Africa.

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 HROP treatment of saline organic effluents with the recovery of products of value. Wat. Sci. Tech. 25(10):319-327.

Rose, P.D., Maart, B.A., Dunn, K.M., Rowswell, R.A. and Britz, P. 1995. Ponding presents potential. Leather. 197(4643):83-90.

Rose, P.D., Maart, B.A., Dunn, K.M., Rowswell, R.A. and Britz, P. 1996. High Rate algal oxidation ponding for the treatment of tannery effluents. Wat. Sci. Tech. 33(7):219-227.

Ross, E., Puapong, D.P., Cepeda, F.P. and Patterson, P.H. 1994. Comparison of freeze-dried extruded *Spirulina platensis* as yolk pigmentation agents. Poult. Sci. 73(8):1282-1289.

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

Rowswell, R.A. and Rose, P.D. 1990. Report on an investigation of environmental problems at King Western Leathers, Wellington, South Africa. LIRI Internal Report No. 1497, October 1990.

Ruffier, P.J., Boyle, W.C. and Kleinschmidt, J. 1981. Short-term acute bioassay to evaluate ammonia toxicity and effluent standards. J. Wat. Pollut. Control Fed. 53:367-377.

Russel, A.E., Tandt, H. and Kohl, R. 1996. Liricure powder biocide composition for Hide and Skin preservation. J. Soc. Leather Technol. Chem. 81:137-142.

Ryther, J.H., Goldman, J.C., Gifford, C.E., Huguenin, J.E., Wing, A.S., Clarner, J.P.,Williams, L.D. and Lapointe, B.E. 1975. Physical models of integrated waste recycling: marine polyculture systems. Aquaculture 5:163.

Sandbank, E. and Hepher, P.H. 1980. Microalgae grown in wastewater as an ingredient in the diet of warm water fish. In: G. Shelef and C.J. Soeder (eds.), Algae biomass production and use. Elsevier, Amsterdam, Netherlands.

Sandbank, E., and Shelef, G. 1987. Harvesting of algae from high-rate ponds by flocculation-flotation. Wat. Sci. Tech. 19(12):257-263.

Santillan, C. 1982. Mass production of *Spirulina.* Experientia 38:40-43.

Saxena, P.N., Ahmad, M.R., Shyam, R. and Amla, D.V. 1983. Cultivation of *Spirulina* in sewage for poultry feed. Experientia 39:1077-1083.

Sbrana, I., Caretto, S. and Battaglia, A. 1991. Chromosomal aberration analysis of workers in tannery industries. Mutat. Res. 260(4):331-336.

Shah, N. and Syfrett, P.J. 1984. The uptake of guanine and hypoxanthine by marine micro-algae. Mar. Biol. Assoc. UK.

Shanz, F., Allen, E.D. and Gorham, P.R. 1979. Bioassay of seasonal ability of water from a eutrophic Alberta lake to promote selective growth of strains of *Anabaena flos-aquae* and other blue-green algae. Can. J. Bot. 57:2443-2451.

Sharp, J.H. 1977. Excretion of organic matter by marine phytoplankton: do healthy cells do it ? Limnol. Oceanogr. 22:381-399.

Shelef, G. 1982. HROP algae ponds for waste-water treatment and protein production. Wat. Sci. Tech. 14:439-452.

Shelef, G. and Azov, Y. 1987. High-rate oxidation ponds: the Israeli experience. Wat. Sci. Tech. 19(12):249-255.

Shelef, G., Azov, Y., Moraine, R. and Oron, G. 1980. Algal mass production as an integral part of watsewater and reclamation system. In: G. Shelef and C.J. Soeder (eds.), Algae biomass production and use. p. 163-190. Elsevier, Amsterdam, Netherlands.

Shelef, G, and Soeder, C.J. (eds.) 1980. Algal biomass production and use. Elsevier, Amsterdam, Netherlands.

Sherwood, J.E., Stagnitti, F., Kokkinn, M.J. and Williams, W.D. 1992. A standard table for predicting equilibrium dissolved oxygen concentrations in salt lakes dominated by sodium chloride. Int. J. Salt Lake Res. 1:1-6.

Shimamatsu, H. 1987. A pond for edible *Spirulina* production and its hydraulic studies. Hydrobiologia 151/152:83-89.

Shipin, O.V., Dunn, K.M., Shipin, V.Y. and Rose, P.D. 1994. Saline anaerobic digestion in advanced algal high rate oxidation ponding for the treatment of organics in saline effluents. Presented at the 7th International Symposium on Anaerobic digestion, Cape Town, South Africa.

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

Silva, S.A., Mara, D.D. and de Oliveira, R. 1987. The performance of a series of five deep waste stabilisation ponds in northeast Brazil. Wat. Sci. Tech. 19(12):61-64.

Skinner, B.J. 1969. Earth Resources. Prentice Hall, New Jersey, USA.

SLTC. 1996. Proc. Society of Leather Technologists and Chemists Annual Conference (South Africa section).

Smith, A.J., London, J. and Stanier, R.Y. 1967. Biochemical basis of obligate autotrophy in blue-green algae and thiobacilli. J. Bact. 94(4):972-983.

Soares, J., Silva, S.A., de Oliviera, R., Aranjo, A.L., Mara, D.D. and Pearson, H.W. 1995. Ammonia and TKN removal in a pilotscale WSP complex in north east Brazil. 3rd. IAWQ International Specialist Conference Workshop; Waste Stabilisation ponds: Technology and Applications. Brazil.

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

Stanley, J.T., Bryant, M.P., Pfennig, N. and Holt, J.G. 1989. Bergy's Manual of Systematic Bacteriology, Vol.3. Williams and Wilkins, London, UK.

Stander, J.V.R. 1987. Fighting South Africa's salinity problem. SA Water Bulletin 13:10-13.

Stanier, R.Y. 1977. The position of Cyanobacteria in the world of phototrophs. In: J.R. Rosowski and B.C. Parker (eds.), Selected Papers in Phycology. Phycological Society of America Inc., USA.

Stanier, R.Y. and Cohen-Brazire, G. 1977. Phototrophic protokaryotes: The cyanobacteria. Ann. Rev. Microbiol. 31:225-274.

Stanier, R.Y., Sistrom, W.R., Hansen, T.A., Whitton, B.A., Castenholz, R.W., Pfennig, N., Gorlenko, V.N., Kondratieva, E.N., Eimhjellen, K.E., Whittenbury, R., Gherna, R.L. and Truper, H.G. 1978. Proposal to place the Nomenclature of the Cyanobacteria (Blue-green algae) Under the rules of the International Code of Nomenclature of Bacteria. In: J.R. Rosowski and B.C. Parker (eds.), Selected Papers in Phycology. p. 51-52. Phycological Society of America Inc., USA.

Storey, R.D. and Wagner, F.W. 1986. Plant proteases: A need for uniformity. Phytochemistry 25:2701-2709.

Tadesse, I. 1993. Tanning Industries in Ethiopia - Water pollution impact and possible interventions, Tampere University of Technology, Tampere, Finland.

Talinli, I. 1994. Pretreatment of tannery wastewaters. Wat. Sci. Technol. 29(9):175-178.

Tamiya, H. 1957. Mass culture of algae. Ann. Rev. Plant Physiol. 8:309-334.

Tel-Or, E., Boussiba, S. and Richmond, A.E. 1980. Products and chemicals from *Spirulina platensis*. In: G. Shelef and C.J. Soeder (eds.), Algae biomass production and use. p. 611-618. Elsevier, Amsterdam, Netherlands.

Tipnis, H.P. and Pratt, P. 1960. Protein and lipid content of *Chlorella vulgaris* in relation to light. Nature 188:1031-1032.

Thorstensen, T. 1984. Practical Leather Technology. Krieger Inc., Florida, USA.

Tomaselli, L., Torzillo, G., Giovanetti, L., Pushparaj, B., Bocci, F., Tredici, M., Papuzzo, T., Balloni, B. and Materassi, R. 1987. Recent research on *Spirulina* in Italy. Hydrobiologia 151/152:79-82.

Torres, J.J., Soler, A., Saez, J. and Ortuno, J.F. 1997. Hydraulic performance of a deep wastewater stabilisation pond. Wat. Res. 31(4):679-688.

Trilli, A. 1986. Scale-up of fermentations. In: A.L. Demain and N.A. Solomon (eds.), Industrial Microbiology and Biotechnology. p. 277-307. .American Society for Microbiology, USA.

Tsotsos, D. 1986. Tanneries: A short survey of the methods applied for waste water treatment. Wat. Sci. Tech. 18:69-76.

van Baalen, C., Hoare, D.S. and Brandt, E. 1971. Heterotrophic growth of blue-green algae in dim light. J. Bact. 105(3):685-689.

van Eykelenberg, C. 1979. The ultrastructure of *Spirulina platensis* in relation to temperature and light intensity. Antonie van Leeuwenhoek 45:369-390.

van Eykelenberg, C. 1980. Ecophysiological studies on *Spirulina platensis*: Effect of temperature, light intensity and nitrate concetration on growth and ultrastructure. Antonie van Leeuwenhoek 46:113-127.

van Haandel, A.C. and Catunda, P.F.C. 1995. Improved performance and increased applicability of waste stabilisation ponds by pretreatment in a UASB reactor. 3rd IAWQ International Specialist Conference and Workshop; Waste Stabilisation Ponds: Technology and Applications, Brazil.

Venkataraman, L.V., Nigam, B.P. and Ramanathan, P.K. 1980. Rural oriented fresh water cultivation and production of algae in India. In: G. Shelef and C.J. Soeder (eds.), Algae biomass production and use. p. 81-96. Elsevier, Amsterdam, Netherlands.

Venkataraman, L.V., Somasekaran, T. and Becker, E.W. 1994. Replacement value of blue-green alga (*Spirulina platensis*) for fishmeal and a vitamin- mineral premix for broiler chicks. Br. Poult. Sci. 35(3):373-381.

Vonshak, A., Abeliovich, A., Boussiba, S., Arad, S. and Richmond, A. 1982. Production of *Spirulina* biomass: effects of experimental factors and population density. Biomass 2:175-185.

Vonshak, A. and Guy, R. 1992. Photoadaptation, photoinhibition and productivity in the blue-green alga, *S. platensis* grown outdoors. Plant, Cell and Environment. 15:613-616.

Vonshak, A., Kancharaksa, N., Bunnag, B. and Tanticharoen, M. 1996. Role of light and photosynthesis on the acclimation process of the cyanobacterium *Spirulina platensis* to salinity stress. J. Appl. Phycol. 8(2):119-124.

von Sperling, M. 1995. Design of facultative ponds based on the uncertainty analysis. Wat. Sci. Tech. 31(12):41-47.

Vuillot, M. and Boutin, C. 1987. Waste stabilisation ponds in Europe: A state review. Wat. Sci. Tech. 31(12):1-6.

Walsby, A.E. and Klemer, A.R. 1974. The role of gas vacoules in the microstratification of a population of *Oscillatoria agardhii* var. *isothrix* in Denning Lake, Minnesota. Arch. Hydrobiol. 74:375.

Walsh, A.R. and O'Halloran, J. 1996. Chromium speciation in tannery effluent - II. Speciation in the effluent and in receiving estuary. Wat. Res. 30(10): 2401-2412.

Whang, J.S. 1982. Soluble-Sulphide precipitation for Heavy Metal Removal from Wastewaters. Environmental Progress 1(2):110-113.

Wheeler, P. A. and Kirchman, D.L. 1986. Utilization of inorganic and organic nitrogen by bacteria in marine systems. Limnol. Oceanogr. 31:998-1009.

Wood, T. 1987. Interpretation of laboratory-scale waste stabilisation pond studies. Wat. Sci. Tech. 19(12):195-203.

World Leather. 1992. Tanning and the Environment. 5:1-58.

World Leather. 1993. Tanning and the Environment. 6(4):1-56.

World Leather. 1994. Tanning and the Environment. 7(2):1-83.

World Leather. 1996. Tanning and the Environment. 9(7):1-76.

Winters, D. 1986a. Thoughts on choice of tannery effluent treatment systems (Part 1). Leather June:20-28.

Winters 1986b. Thoughts on choice of tannery effluent treatment systems (Part 2). Leather September:164-168.

WRC. 1996. Technical Report. Water Research Commission, Pretoria, South Africa.

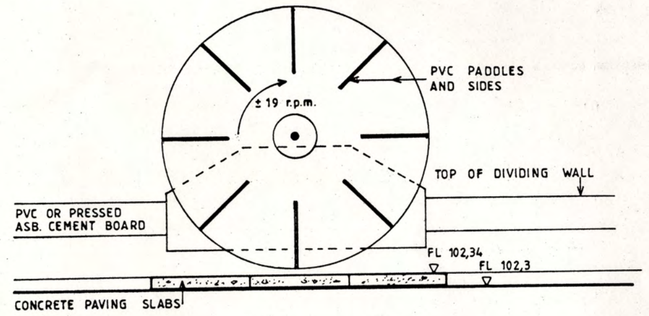
Yang, H.N., Lee, E.H. and Kim, H.M. 1997. *Spirulina platensis* inhibits anaphylactic reaction. Life Sci. 61(13):1237-1244.

Zarrouk, C. 1966. Cited by Vonshak (1986) In: A. Richmond (ed.), CRC Handbook of Microalgal Mass culture. CRC Press Inc., Boca Raton, USA.

Zitelli, G.C., Tomasello, V., Pinzani, L.M. and Tredici, M.R. 1996. Outdoor cultivation of *Athrospira platensis* during autumn and winter in temperate climates. J. Appl. Phycol. 8(4/5):293-301.

Zviagintseva, I.S., Gerasimenko, L.M., Kostrikina, N.A., Bulygina, E.S. and Zavarzin, G.A. 1995. Interaction of halobacteria and cyanobacteria in a halophilic cyanobacterial community. Mikrobiologia 64(2)252-258.

LIRI TECHNOLOGIES	TITLE	SCALE	DRAWN
ENVIRONMENTAL DIVISION P O BOX 185 GRAHAMSTOWN 6140 Rep. South Africa	PROPOSED SITING & CONSTRUCTION OF HIGH RATE OXIDATION PONDS FOR ALGAL PROJECT TRIALS	AS SHOWN	RAR LDH DATE JAN 1993



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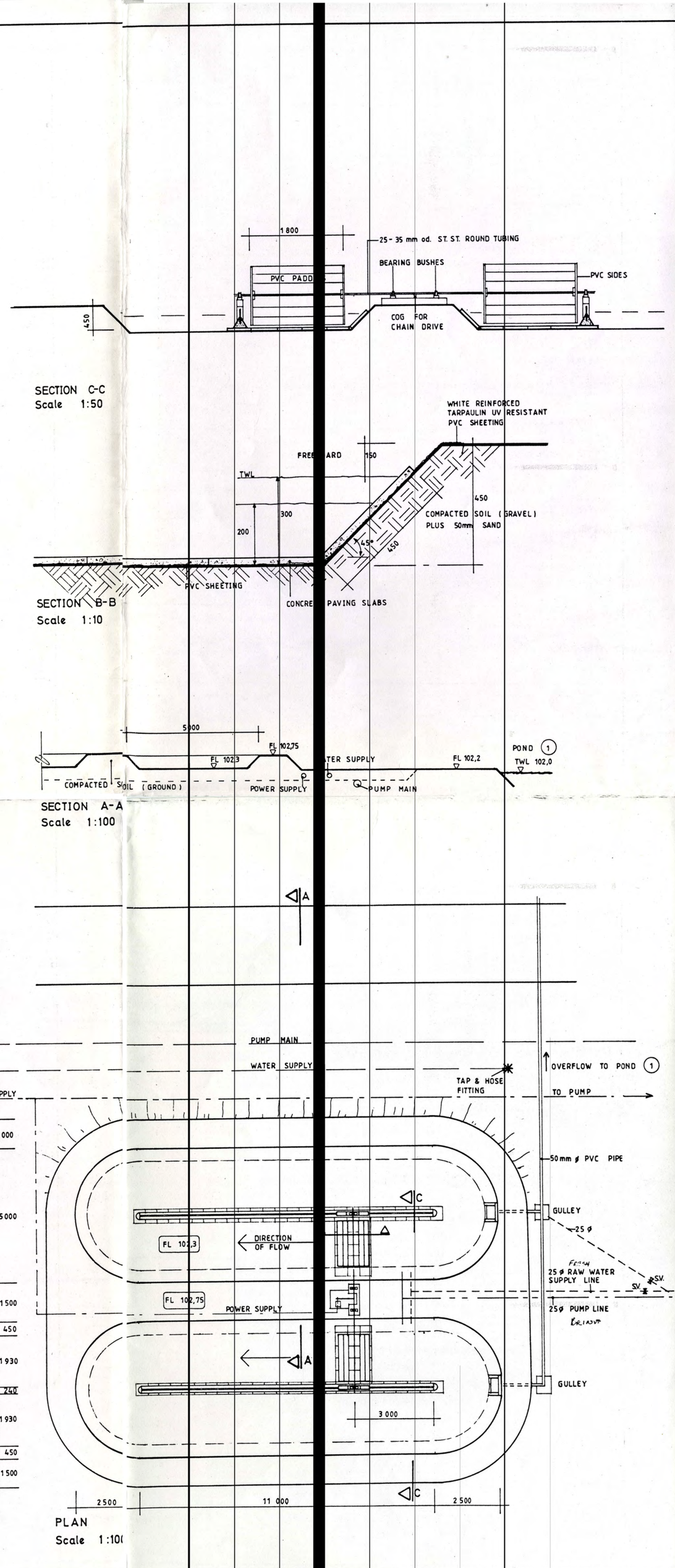

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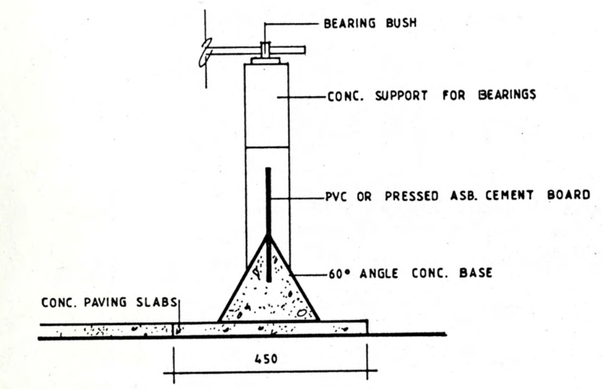
SECTION B-B
Scale 1:10

SECTION A-A
Scale 1:100



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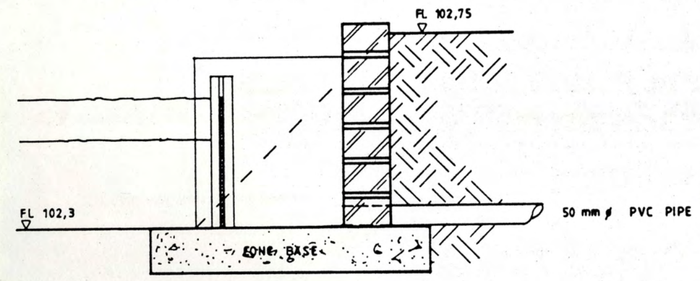

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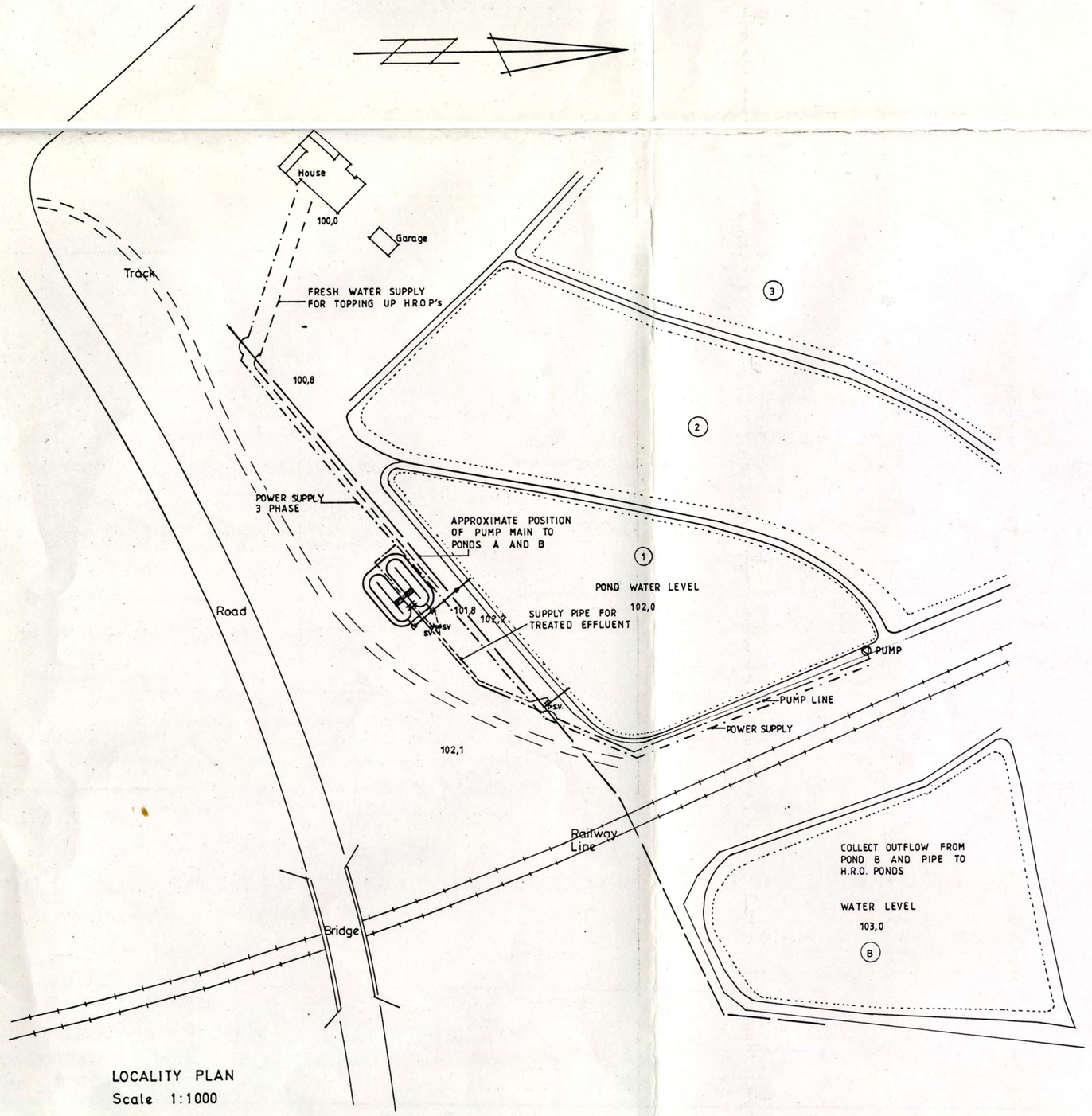
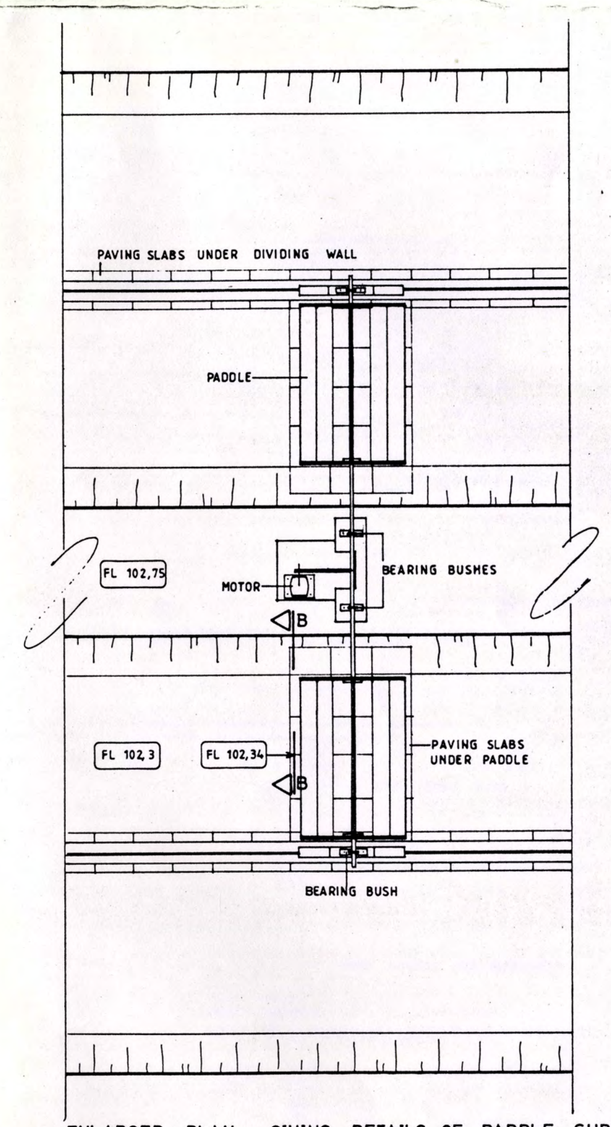


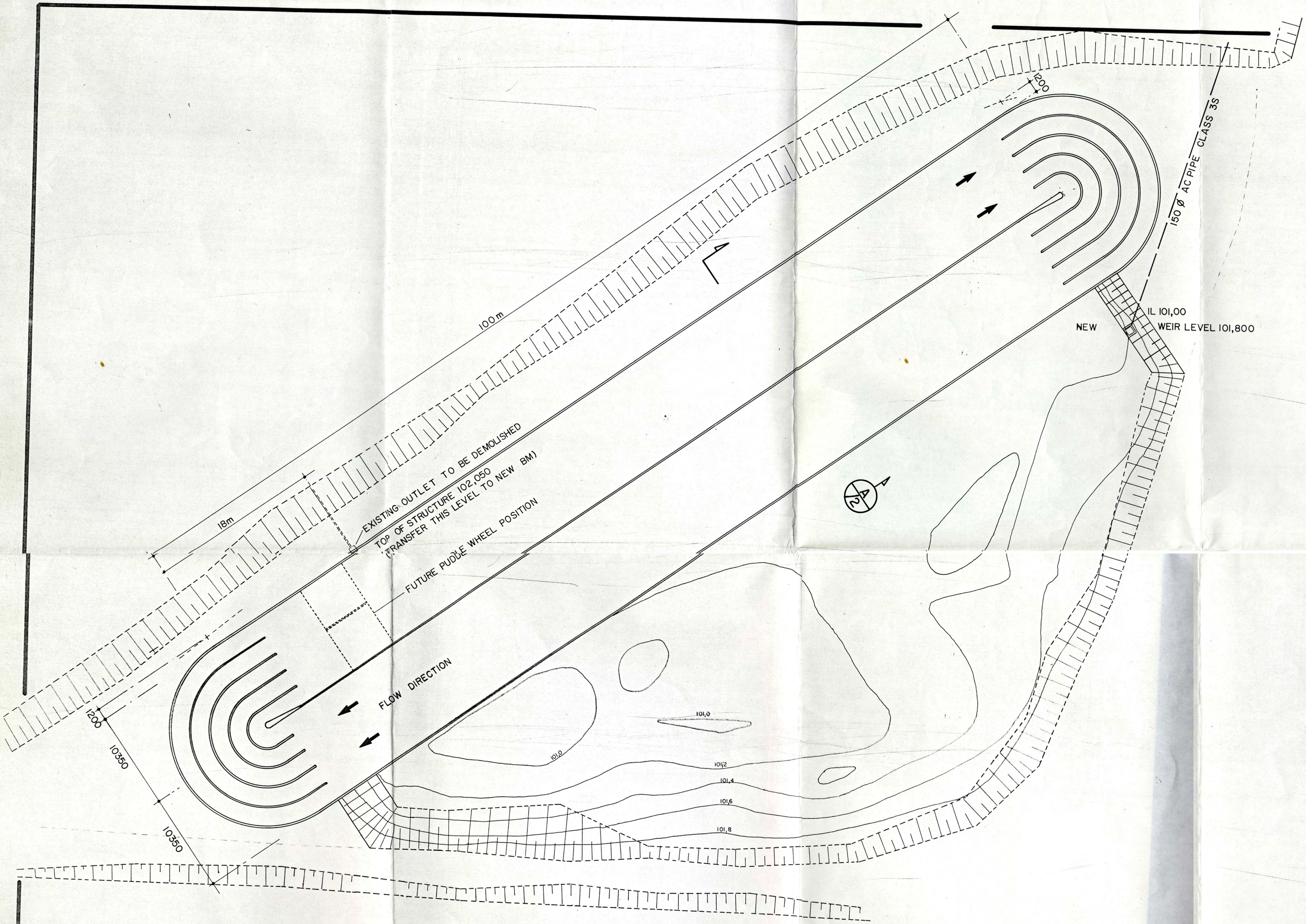
LOCALITY PLAN
Scale 1:1000

PLAN
Scale 1:10



ENLARGED PLAN - GIVING DETAILS OF PADDLE SUPPORTS
Scale 1:50

A1/003



LAYOUT

SCALE: 1:200

NOTE

ALL RELEVANT CLAUSES OF SABS 1200 SHALL APPLY TO THIS CONTRACT

THE CONTRACTOR SHALL READ THE DRAWINGS TOGETHER WITH THE PROJECT SPECIFICATIONS

Wysigings /Revisions

Nr No	Datum Date	Beskrywing Description

en Medewerkers BK
Chris Erasmus
and Associates cc
Raadgewende Ingenieurs en Tegnoloë
Consulting Engineers and Technologists

Diens / Service
WESTERN TANNING Co Ltd

THE CONSTRUCTION OF HIGH RATE PONDS AND APPURTANANT WORKS

Titel / Title
LAYOUT OF HIGH RATE PONDS

Skaal / Scale AS SHOWN	Ontwerper Designer	:C P Erasmus :
	Geteken Drawn	:C P E :
Datum: Date :	Nagesien Checked	: :

Tekening Nr / Drawing No.

9408/1

Wysiging Revision