Development of Integrated Algal Ponding Systems in the Treatment of Wine Distillery Wastewaters

THESIS

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Leendert Gideon Dekker

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Abstract

In South Africa, wastewater disposal in the wine and distilling industry is undergoing a profound transformation as a result of fundamental changes in regulations and license requirements. To deal with this problem conventional Waste Stabilisation Ponding systems have been used by the industry together with irrigation and evaporation disposal practises.

Although effective in the evaporation and containment disposal functions, these pond systems are generally not properly designed and/or managed, resulting in overloading and, at times, the generation of seriously offensive odour problems. Preliminary studies on the feasibility of utilising the Advanced Integrated Wastewater Ponding System as a core treatment technology in winery wastewater treatment were conducted. Results indicated that specific problems had to be addressed before successful ponding treatment could be achieved.

This research programme undertook an investigation of the performance of a demonstration ponding system treating household sewage, which formed the basis of the research due to limited experience reported on ponds treating wine industry wastewaters. Malfunctions identified were in correlation with the preliminary winery waste ponding survey, which included unstable fermentation pit functions and inadequate nutrient removal.

Retrofitting the fermentation pit with a nylon net across the rising water column resulted in improved retention of active anaerobic sludge, especially during periods of system start-up and/or organic overloading. An investigation into nutrient removal utilising algal biomass provided a valuable contribution towards development of an independent nutrient removal system. Harvested algal biomass was passively manipulated to release polysaccharides under anoxic conditions, with subsequent use as a carbon source by denitrifying organisms. Following denitrification, the still viable algal cells were introduced into a High Rate Algal Pond raceway for photosynthetically produced

alkalinity. This high pH environment resulted in induced calcium phosphate mineral formation and subsequent precipitation, as well as effective ammonia stripping from the water.

Based on the novel positive research outcomes a decision was made to proceed to the construction of a pilot-scale integrated ponding system treating wastewater from a wine lees factory. The system linked the Anaerobic Baffle Reactor, for pre-treatment, with the improved Advanced Integrated Wastewater Ponding System.

The potential of this system has shown that a Waste Stabilisation Ponding system can be engineered to treat wine industry wastewaters and thereby effectively reduce the organic and nutrient loads, by using low-cost retrofitted upgrading unit operations. Valuable algal biomass may also be recovered as a by-product of the treatment process.

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This work is dedicated to my wife, Wilna, for her moral support and encouragement throughout.

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List of Abbreviations

ABR	Anaerobic Baffle Reactor
AIWPS	Advanced Integrated Wastewater Ponding System
ASP	Algal Settling Pond
BFB	Biological Upflow Fluidised Bed
BOD	Biochemical Oxygen Demand
chl_a	$Chlorophyll_a$
COD	Chemical Oxygen Demand
CODs	Soluble Chemical Oxygen Demand
CODt	Total Chemical Oxygen Demand
CPS	Capsular Polysaccharides
DO	Dissolved Oxygen
DPB	Denitrifying Phosphorus Removing Bacteria
EAA	Essential Amino Acids
EBPR	Enhanced Biological Phosphate Removal
EC	Electrical Conductivity
EPS	Exocellular Polysaccharides
FP	Facultative Pond
GDW	Grahamstown Disposal Works
GLSS	Gas Liquid Solids Separator
HAP	Hydroxyapatite
HRAP	High Rate Algal Pond
HRT	Hydraulic Retention Time
I-HRAP	Independent High Rate Algal Pond
OLR	Organic Loading Rate
PAO	Phosphorus Accumulating Organisms
PAR	Photosynthetically Available Radiation
PFP	Primary Facultative Pond
SBR	Sequential Batch Reactor
SRP	Soluble Reactive Phosphorus

SS	Suspended Solids
TCP	Tricalcium Phosphate
TKN	Total Kjeldal Nitrogen
TPS	Total Polysaccharides
TVFA	Total Volatile Fatty Acids
UASB	Upflow Anaerobic Sludge Blanket
USA	United States of America
VFA	Volatile Fatty Acids
VSS	Volatile Suspended Solids
$\mathbf{V_{up}}$	Liquid Upflow Velocity
WSP	Waste Stabilisation Ponds

List of Publications

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

Dekker L. G., Hart O. O. and Rose P. D. (1999) UASB-type operation for improved performance of the Fermentation Pit in Advanced Facultative Ponds. IAWQ conference on Wastewater Stabilisation Ponding, Marrakech, Morocco.

Dekker L. G., Hart O. O. and Rose P. D. (2000) Combined Anaerobic-Algal Oxidation treatment of wine lees distillery effluent: the A_2O process. 2nd International Viticulture and Enology Congress, Cape Town, South Africa.

Clark S. J., Dekker L. G., Hart O. O. and Rose P. D. (2000) The High Rate Algal Pond as an independent unit operation for tertiary treatment: stress manipulation of carbon production for N and P removal. BioY2K, Grahamstown, South Africa.

Dekker L.G. and Rose P.D. (2002) Salinity, Sanitation and Sustainability: A study in Environmental Biotechnology and Integrated Wastewater Beneficiation in South Africa. Volume 3: Integrated Algal Ponding Systems and the Treatment of Domestic and Industrial Wastewaters. Part 5: Winery and Distillery Wastewaters. Water Research Commission, Pretoria.

Chapter One

The Wine Processing Industry and Waste Stabilisation Ponds

1. Introduction

1.1. Wine Making Process

The wine industry in South Africa comprises a number of closely related industrial operations engaged in the production and processing of grapes to a variety of alcoholic and non-alcoholic products. Harvesting of the grape crop is usually carried out from January to March every year and after delivery of the grapes at a winery, various operations are carried out which are illustrated in **Figure 1.1.** (Steffen Robertson and Kirsten, 1993). In 2000 approximately 100 980 hectares of land was under grape cultivation in South Africa, and producing around 1.1 million tons of grapes annually (SA Wine Industry Information & Systems, 2001).

Depending on a number of factors including regional differences, grape cultivar, equipment and processing methods, 1 ton of grapes yields 600 to 700 L of natural wine, and produces 100 to 120 kg of solid waste. The solid wastes from wineries, in the form of stalks, skins and pips, are usually returned to the land as soil conditioner. Water intake at a winery is used principally for washdown and cooling purposes. Depending on market forces, the total wine production is split half-and-half between "good wine" (for drinking) and "distilling wine" (to distillation for various purposes).

The unit operations used in spirit distillation are illustrated in **Figure 1.2.** Two methods of distillation are employed, namely column (continuous) distillation for the production of wine alcohol (96% absolute alcohol) and kettle (pot or batch) distillation for the production of brandy.

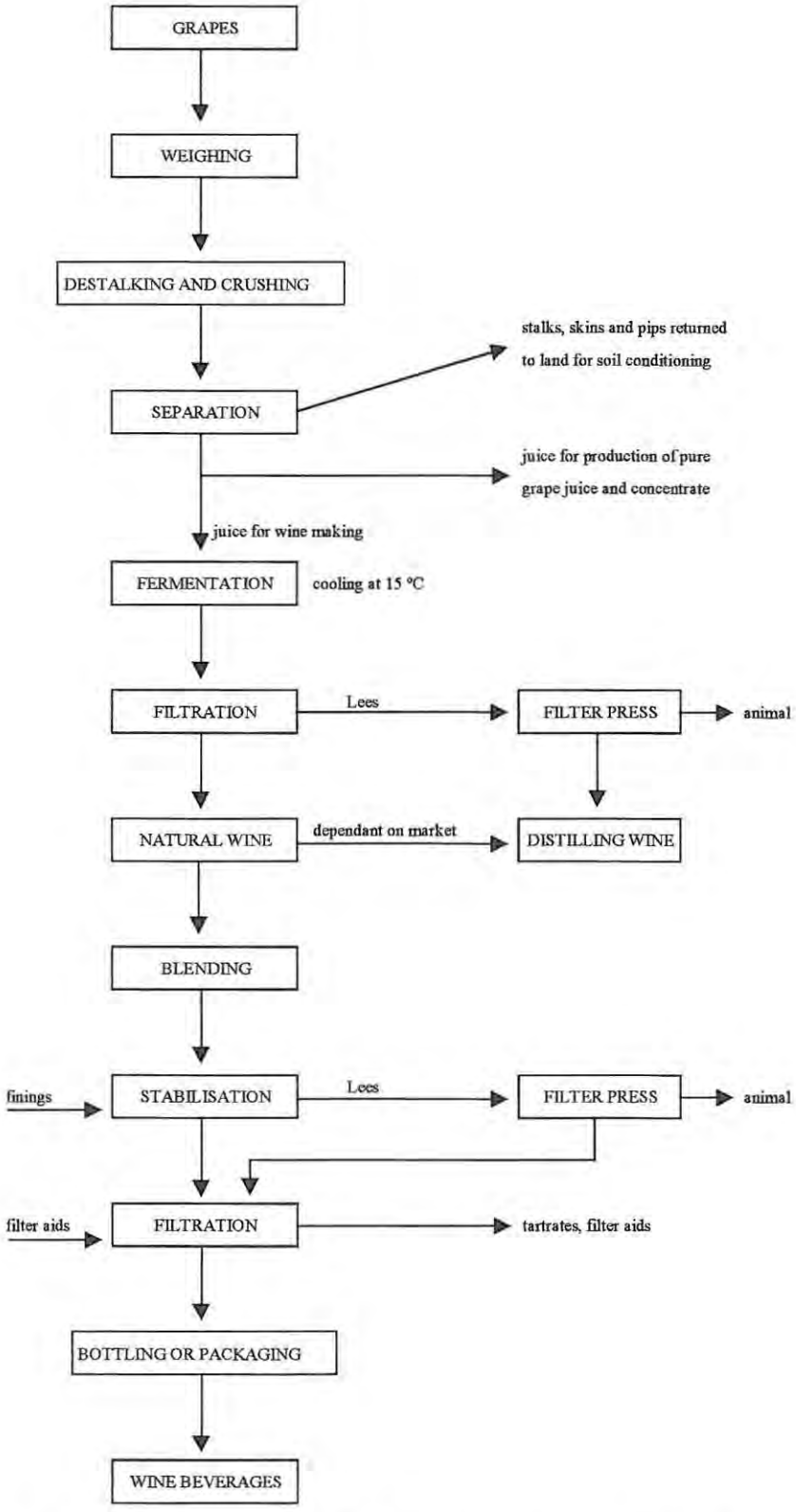


Figure 1.1. Schematic diagram of the unit operations applied in the winemaking process (Steffen Robertson and Kirsten, 1993).

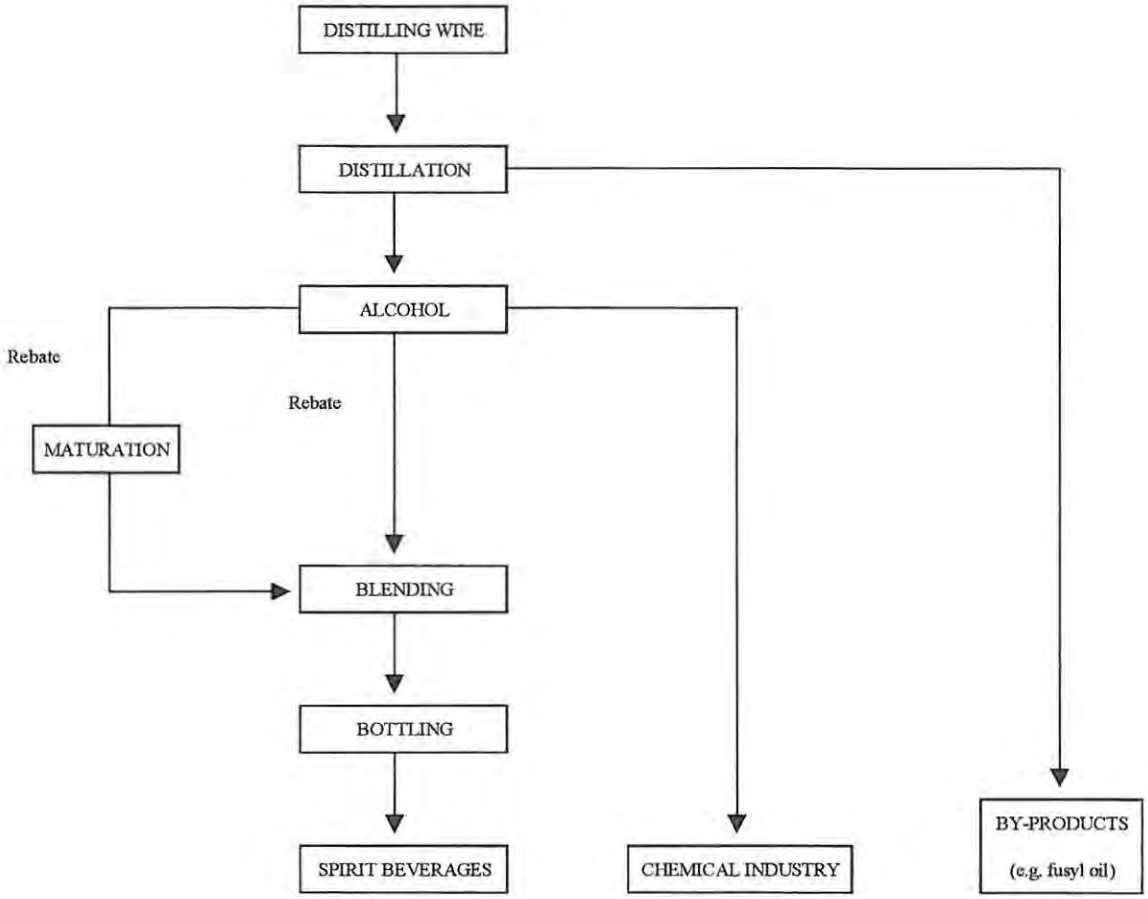


Figure 1.2. Schematic diagram of the unit operations applied in spirit distillation (Steffen Robertson and Kirsten, 1993).

1.2. The Environmental Problem

Winery and distillery operations produce both liquid and solid wastes. If the wastewater is not treated and allowed to accumulate, the decomposition of the organic material can lead to the production of seriously offensive odours. Due to the high COD (chemical oxygen demand) concentrations, seasonal variations, acidic characteristics and odour, wine wastes must be treated in some manner before being discharged or reused (Sheehan and Greenfield, 1980; Tofflemire, 1972). When wine wastewaters are released into the environment, oxygen levels are depleted in the receiving water environment, and besides

impacting on the natural biota, taste and odour generating compounds may be liberated requiring water purification treatment.

In South Africa, wastewater disposal in the wine and distilling industry is undergoing a profound transformation as a result of fundamental changes in regulations and license requirements. Established disposal procedures and criteria which have been used by the industry for decades are no longer compatible with current legislation, which is forcing the industry towards waste disposal in an environmentally safe and sustainable manner.

Historically, South African wineries and distilleries used to dispose of their effluents through direct river discharge. Today, South African effluent discharges must comply with either the General or Special Standards provided for by the National Water Act No. 36 of 1998. Therefore river discharge no longer presents a practical route for effluent disposal for any South African winery or distillery. The degree of on-site effluent treatment to be carried out is determined by whether the treated effluent is to be recycled or discharged to a municipal sewer, evaporation/stabilisation ponds or to land irrigation disposal.

A survey recently conducted by the researchers in the Western Cape Province, which is the prime wine producing area in South Africa, revealed that most wineries and distilleries were using either land irrigation practise, or waste stabilisation ponds to store and/or treat effluent. However, most of these systems were not properly designed and/or managed, resulting in overloading and, at times, the generation of seriously offensive odours. Additionally, land irrigation may also lead to salinisation of the soil, as well as possible groundwater contamination. Population growth around winery and distillery effluent producing areas, and changing land values, requires either more efficient operation of conventional ponds/irrigation areas or reinvestment in high cost physical-chemical and biological systems. Discharge of these wastewaters to nearby municipal sewage treatment facilities has often proved undesirable due to peak organic overloading being experienced, resulting in malfunctioning of these systems.

Therefore, a highly traditional and conservative industry is being required to change with regard to its environmental and waste management practices. Despite considerable research in the field, technology options remain constrained, and many of the fundamental problems remain largely unaddressed. These include high COD concentrations, nutrients in the form of organic nitrogen and phosphates, refractory organic compounds and relatively high salinity levels. This leads to renewed interest towards the development of an effective and sustainable treatment technology for the wine producing industry, with the emphasis being on technology which is compatible with the cost structures of the agro-industrial processing sector. Distillery and wine lees processing wastewaters can be regarded as the worst-case scenario of what may be encountered in wine industry effluents.

1.3. Wastewater Characteristics

1.3.1. Winery Effluent

One of the major problems in the operation of wineries is the disposing of large quantities of comparatively low-solids wastewater containing a medium to high content of chemical oxygen demand (COD). An average of 1.3 m^3 effluent is produced per ton of grapes processed in the winemaking industry. This represents about 70% of the water intake and results in a total of about 1 000 million litres wastewater produced annually by wineries in South Africa (Steffen Robertson and Kirsten, 1993). Winery effluents can vary widely in their hydraulic and pollution loads at different times in the year in relation to the working period (harvesting, fermentation, bottling), the type of wine (red, white, sparkling or special wine), and the methods employed for their production (Tofflemire, 1972; Fumi *et al.*, 1995). The raw winery effluent consists of all the typical substances of grapes and wine (organic acids, sugars, alcohol, etc.), including the residue from the winemaking process (yeast, bacteria, clarifying and fining agents, etc.), and cleaning and sterilising agents used for the treatment of tanks, equipment and winery rooms. The characteristics of typical winery wastewater are given in **Table 1.1.**

Seasonally there are two peaks in organic load, one relating to the maximum pressing activity at the winery and the other to re-filtering of the newly fermented wine.

1.3.2. Distillery Effluent

Wine distillery effluent is the residue left from the distillation of wine and contains residual organic acids, soluble protein, carbohydrates as well as various inorganic salts (Moosebrugger *et al.*, 1992). Distilleries produce effluent volumes of between 150 and 250 $m^3.d^{-1}$, which presents greater environmental problems than winery effluent due to the high organic pollution load, refractory organic compounds and salinity. Water is used primarily for washing and cooling purposes. The water intake for the spirit distillation industry was reported to be between 1.8 and 6.2 L of water per litre of ethanol produced, and generates a pollution load of between 95 and 145 kg COD per 100 L product. Distillery effluent COD concentration is very high at about 35 000 $mg.L^{-1}$, while the COD concentration in winery effluents is usually considerably lower (as low as 1 000 $mg.L^{-1}$). **Table 1.1.** reports the typical analysis of distillery effluent.

In any one winery or distillery there will be a statistical variation in waste flow characteristics. The magnitude of this variation will depend on the diversity of products manufactured, process operations contributing to wastes and whether the operations are batch or continuous processes. The nature of the effluent quality varies widely from winery to winery and distillery to distillery. This is mainly the result of production and housekeeping performance, the age of the facility, the technologies used in the process and operational habits.

Table 1.1. Typical analysis of wine distillery and winery wastewater (Ross, 1989; Torrijos and Moletta, 1997).

Parameter ($mg.L^{-1}$)	Distillery effluent	Winery effluent
pH	4.0	4.1
COD	35 000	4 600
Total dissolved solids	18 000	620
Kjeldahl-N	350	39
Ammonia-N	10	15
Nitrate-N	1	
Total P	150	12
PO_4-P	130	8
K	3 000	
Na	75	
Ca	410	
Mg	160	
Fe	15	
Cu	2	
Al	3	
Cl	160	
SO_4	150	
Co	0.6	
Ni	0.4	
Sr	0.1	
Mn	2.3	

1.3.3. Wine Lees Effluent

Wine lees is the proteinaceous material, containing yeast cells, potassium tartrate, grape skins and pips, that precipitate at the bottom of casks and tanks during wine fermentation. It is removed from the wine by filtration, after fermentation is completed, and is generally

regarded as a waste product by wine cellars. Wine lees can be used as part of the protein component of cattle feed (Steffen Robertson and Kirsten, 1993), or it can be processed to yield valuable products, such as tartrate (in the form of either calcium- or potassium bi-tartrate) as well as residual ethanol, which can be extracted by distillation (Boulton and Butzke, 1995). Wine lees effluent is the final effluent after the extraction of tartrate and ethanol.

The recovery of tartrate contributes to a reduction in the environmental impact of wine wastes. It can also reduce costs within the wine industry, as a percentage of the tartrate produced is returned to the wineries for use in the cold stabilisation of wine. Tartaric acid (produced from tartrate) is also consumed by the wine industry for the acidification of wine with a low natural acidity. In 2002 the world market price for tartrate was US$2/lb and US$3/lb for tartaric acid (Penkler 2002, pers comm.). Other uses for tartrate are in baking powder, sugar substitutes, plaster and play-dough while tartaric acid is used in candy and bakery goods because of its' relative microbial stability, and in the textile industry and for galvanising.

The processing and distillation of wine lees results in an effluent that has a high solids content (about 10%) which is mainly yeast biomass. The liquid fraction of distilled wine lees is considered to have characteristics similar to wine distillery effluent. Due to the high organic solids content the typical COD concentration is above 50 000 $mg.L^{-1}$, with TKN levels above 1 500 $mg.L^{-1}$.

1.4. Treatment of Wine Processing Effluent

Notwithstanding the general effluent volumes reported above, the extreme variability found in processing conditions in the wine industry calls for an individual investigation for each type of cellar or distillery effluent, and often entails the use of specific waste treatment processes. When considering a treatment option for a specific facility, the type of raw material used for fermentation and its resulting type of waste, the regulations for waste disposal as well as the energy profile of the facility must be taken into

consideration. These conditions vary and subsequently a broad variety of treatment solutions may be found, each with it's own drawbacks and advantages (Frostell, 1981).

Wastewater treatment for wineries and wine distilleries varies in detail but normally consists of some combination of the following steps:

1.4.1. Screening and sedimentation

Mechanical removal of stalks, skin, pips and suspended solids by screening or settling sumps is an important pre-treatment step prior to biological treatment or evaporation. Solids tend to block equipment and can cause unpleasant odours if allowed to decay. Simple screens or sieves and settling sumps with underflows are quite effective for this type of solids removal provided they are regularly cleaned. The purpose of sedimentation tanks is to control the upflow velocity of wastewater in a suitably designed tank, thus allowing heavier particulate matter to settle out. A corresponding decrease in COD of 15% has been reported (Squires and Cowan, 1986).

1.4.2. Biological treatment

Many methods have been reported for the biological treatment of winery wastewater where the focus has mainly been on aerobic or activated sludge treatment. Torrijos and Moletta (1997) showed that the sequencing batch reactor can be very effective in removing the organic and nutrient load from the effluent of small wineries, however, more than 50% of the operating costs went to sludge removal. In France an activated sludge plant treating municipal wastewater, has incorporated effluent from a number of wineries, and resulting in temporary overloading (Chudoba and Pujol, 1996). To overcome this problem a mineral material with low particle size was added, whose presence positively influenced the physical behaviour of the sludge, and allowing the nominal capacity of the plant to be surpassed without any oversizing modifications. The sludge production did however increase by 20 to 30%. Fumi *et al.* (1995) reported the use of several aerobic tanks in series which proved to be able to handle hydraulic and organic

shock loads, where the excess sludge generated was applied as a soil conditioner. The use of this type of sludge as soil amendment is worth considering, but care should be taken regarding the build-up of salinity, as noted by Saviozzi *et al.* (1994).

Anaerobic treatment with the upflow anaerobic sludge blanket (UASB) system is a well established technology for treatment of industrial effluents, such as wine distillery wastewater (Driessen *et al.* 1994). These systems are based on the development of granular sludge with a high settling velocity, and which is resistant to biomass washout due to organic and/or hydraulic overloading. This anaerobic process has proved to be very successful in high-rate COD removal systems, but capital costs and operating skills are perceived drawbacks. Also, residual organics and salinity remains in the treated effluent and secondary treatment is needed before final discharge.

Winery effluents have proved to be amenable to biological treatment in aerated pond systems. Aerated ponds have long detention times and require very little supervision or maintenance. While they are easily constructed, sufficient land area is required to accommodate the lagoons. Aerated pond systems for treating winery effluents have the advantage of storage and equalisation which prevent rapid changes in effluent characteristics (such as pH) from suppressing the optimum biological performance (Toffelmire, 1972). Aerated ponds are also not easily upset by shock loading, although the effluent load should remain within design limits. As with any biological system, extreme overloading and toxic materials should be avoided (Bowmer *et al.*, 1991).

Torrijos and Moletta (1998) evaluated the performance of a covered anaerobic pond system using suspended fishing nets for micro-organism attachment and found that the temperature was a significant factor in system design and performance. The system achieved 95% COD removal efficiency while treating winery wastewater. Anaerobic ponds provide the cheapest form of organic removal of any alternative primary treatment technology and should be used whenever they can be sited so as to prevent odour nuisance (Pescod, 1996). Recirculation and combining anaerobic pond systems with other treatment options might be beneficial in controlling odours.

1.4.3. Irrigation and evaporation

Irrigation is the most economical method of distillery effluent disposal in isolated areas, and where suitable land is available. An additional advantage is that grazing pasturage can be developed (Steffen Robertson and Kirsten, 1993). However, high concentrations of sodium and potassium were found in the irrigated areas, which could lead to the deflocculation of the clay particles in the soil (Ross *et al.*, 1981). Sandy soils drain well but care has to be taken with possible groundwater contamination.

Evaporation is also attractive because of the reduction in total effluent volume. However, the dried product does not have a high enough commercial value to justify the capital expenditures involved.

Land disposal has the advantages of requiring low capital investment and low maintenance costs as well as low manpower and maintenance requirements. The disadvantages are that if the unit is overloaded, or poorly operated, leaching and contamination of groundwater may occur. Land disposal also requires large areas of land (Anderson, 1995).

1.5. Waste Stabilisation Ponds

The ability of Waste Stabilisation Ponds (WSP) to absorb high organic loads has led to their widespread use in the treatment of domestic and industrial wastewaters in many industrialised countries (Kilani, 1992). One may define ponds as designed 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 pond system, correctly designed and managed to cultivate anaerobic and aerobic bacteria and green microalgae, can decompose waterborne organic wastes efficiently and achieve a high quality of treated effluent. Waste stabilisation ponds have been recognised to be one of the most efficient and cost-effective means of wastewater treatment in areas where land is relatively inexpensive and climatic conditions are favourable (Rose *et al.*, 1996).

WSP are operated in many different configurations from single mixed ponds to lined lagoons where the biological, physical and chemical processes are separated into a sequence of anaerobic, facultative and maturation ponds (Gomez de Sousa, 1987).

In spite of many decades of research on ponds, it is doubtful that anyone is able to state with any assurance of accuracy where and when the first WSP where introduced. Fishponds were used for flood control in Trebon, Czechoslovakia since the thirteenth century (Vuillot and Boutin, 1987). These produced carp which thrive in the algae laden waters. According to researchers these ponds were fertilised by human and animal waste, as well as stormwater runoff. Around 1919 in Munich, Germany, the first series of purpose-designed ponds were built along the edge of the Isar River to prevent sewage intrusion into water supplies. This long series of ponds was used to propagate fish, yielding more than 500 $kg.acre^{-1}.year^{-1}$.

Among the first well-documented descriptions of designed ponds in the USA are those of Caldwell (1946). According to Caldwell, clogged sewage percolation beds in Santa Rosa, California, were observed since 1935 to give remarkably good sewage treatment. Interest in ponds increased greatly and during the course of World War II many pond installations were constructed at military camps. Caldwell (1946) was the first to outline specific design criteria for ponds. It was suggested that a pond should not exceed five feet in depth so that wind would mix dissolved oxygen, produced by algae, to the bottom of the pond. This resulted in rapid growth of these systems, principally for the treatment of domestic sewage, but also for the treatment of industrial wastewaters (Boutin *et al.*, 1987; Middlebrooks, 1987). Marais and Shaw (1961) pioneered pond design criteria based on chemical engineering principles.

During the late 1960s disquieting information began to accumulate regarding shallow ponds (Barson and Ryckman, 1970). Some ponds that had initially performed satisfactorily began to discharge effluents which, with the algae remaining in them, at times had oxygen demand levels equal to or greater than their influent. Field research on ponds in California during the late 1950s and early 1960s revealed that those ponding

systems which had anaerobic zones in which methane fermentation could, and did, occur gave better overall performance than the shallower ponds. Due to wind mixing and bottom oxygenation, a shallow pond could not sustain methane fermentation and thus became acidic and odorous (Oswald *et al.*, 1963).

1.6. Integrated Advanced Pond Systems

It has long been known that one of the most efficient ways to dispose of waste organic matter is to convert it to methane and carbon dioxide. Scientific study of the production of methane from river mud and cattle intestines has been shown for at least 12 decades and with detailed investigation commencing in the early 1940s (Stephenson, 1949). The strict anaerobic requirement of the methane-producing bacteria was clearly illustrated. Early studies of oxidation ponds showed that significant amounts of methane are produced during warm periods, and that there is an inverse relationship between areal methane production and the distance from the pond inlet (Oswald *et al.*, 1963). Oswald's first efforts in improved design were to make ponds deeper, thereby increasing the probability of having a stable anaerobic bottom layer due to stratification (Oswald *et al.*, 1994). Deepening ponds did improve their performance, but the sludge layer tended to be cooler than desirable since the natural warmth of the sewage was dissipating quickly in the hypolimnion-like environment. These findings led his team to the idea of concentrating the settleable material into a smaller area that could be protected from intruding oxygen by submerged berms and could conserve the natural warmth of the entering sewage.

Early studies have also identified the important role of microalgae (both cyanobacteria and eucaryotic green algae) in the successful operation of WSP (Oswald *et al.*, 1957; de Pauw and Salomoni, 1991). It was shown that microalgae reduce the nutrient load through stripping and precipitation, produce oxygen for bacterial decomposition of organic matter, eliminate pathogenic bacteria, precipitate heavy metals and xenobiotic pollutants, and reduce offensive odours.

The significant developments in ponds with internally located fermentation pits, as 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 Advanced Integrated Wastewater Ponding System (AIWPS).

The AIWPS consists of a minimum of four ponds in series (Oswald, 1991). There are three main reasons for this. First, the several unit processes involved require distinct environments which sometimes cannot be overlapped. Secondly, use of four ponds in series, together with well arranged transfer structures, avoids all conceivable manners of short-circuiting of influent to effluent. Thirdly, in arid areas where annual evaporation generally exceeds annual precipitation, it is necessary to divide the ponding area into sectors to maintain sufficient depth to permit the essential biological and physical processes. The unit processes in the AIWPS do not differ greatly from those of conventional plants. They involve primary sedimentation, flotation, fermentation, aeration, secondary sedimentation, nutrient removal, storage and final disposal. The engineering strategies by which these unit processes are fostered in an AIWPS are described below.

1. The first of a four pond series is a Primary Facultative Pond (PFP). The advanced facultative pond has a unique design for incorporating three distinct microbial consortia: a deep anaerobic strata is overlain by a deep facultative strata which is overlain by an aerobic surface strata. One of the prerequisites for stable methane fermentation is the absence of dissolved oxygen (Oswald *et al.*, 1994). In the PFP sedimentation and methane fermentation occur in a specially designed submerged pit to avoid the intrusion of oxygenated water. After coarse screening, the influent raw sewage is discharged into the bottom of these internal digesters. A low upflow velocity, usually less than 2.5 $m.d^{-1}$, ensures the efficient sedimentation and fermentation of volatile settleable solids to methane and carbon dioxide. The low upflow velocity also ensures that most helminth (parasitic worms) ova remain in the pit (Oswald, 1991). Quiescent sedimentation in the fermentation pit is only the first

reaction. In the intensely anoxic environment of the pit acid forming and methane producing bacteria populate the surfaces of all sorts of solid particles originating from the wastewater. As gas is released from their surfaces, the solid particles become buoyant and tend to rise due to the attached gas bubbles. If the pit is sufficiently deep (5-6 metres) the gas bubbles expand as they rise and will usually break away from their attachment to the particles before they reach the aerobic surface waters of the pond. The bubbles then emerge and the particles with their adhering anoxic bacteria are free to settle again through the slowly rising bed of influent wastewater. In this way the entire raw wastewater flow is passed through a volume of intense anoxic activity where both soluble and insoluble organic matter is adsorbed and converted to carbon dioxide, methane and nitrogen gas. Furthermore, clogging is virtually impossible and maintenance minimal. To deal with floatables, PFP are designed with down-wind concrete "beaches" or scum ramps where floatable trash can be cast up by the wind to dry. These substances are largely inert, light in weight and low in odour. They can be collected periodically with a loader for burial or disposal as a solid waste. Since an overflowing PFP is constant in depth, bank erosion is best controlled with a paved water line. Depth in the PFP is regulated by the level of the invert of the outlet pipe, which should be located three or four feet below the surface to avoid transfer of floatables into the secondary pond.

2. The secondary pond of an AIWPS may be either a secondary facultative pond or a high rate algal pond (HRAP). The paddle wheel mixed HRAP is more advantageous since, although it is shallow, it requires a much shorter retention time and produces much more oxygen than a secondary facultative pond. HRAP are designed to promote the symbiosis between microalgae and aerobic bacteria, each utilising the major metabolic products of the other. Organic compounds are oxidised by aerobic bacteria utilising photosynthetic oxygen produced by microalgae which in turn utilise carbon dioxide and other nutrients released through bacterial oxidation (Ganapati, 1975). The cultivation of algae is uncontrolled in a conventional WSP, but in the AIWPS the growth of microalgae is optimised in the paddle wheel mixed HRAP. Algae in a HRAP has the tendency to raise the pH of the water to above 9, which will provide a

100% kill of *E. coli* and presumably most pathogenic bacteria within 24 hours (Parhad and Rao, 1974). On the other hand the short residence time of 3-5 days, continuous input of primary treated sewage and complete flow mixing tends to conceal its high rate of disinfection. Because the HRAP usually produces a surplus of dissolved oxygen (usually several times the applied organic load), some HRAP effluent is used to overlay the PFP with warm oxygen-rich water to absorb any odours of reduced substances emerging from the fermentation pit and to assure the presence of oxygen producing algae in the surface waters of the PFP.

3. The HRAP should be followed by an algal settling pond (ASP) or some other method for removing algal biomass. Natural sedimentation of algae in the overflow of a paddle wheel mixed HRAP is sufficient to remove 50 to 70% of the algae (Green *et al.*, 1995). If higher removal is required then mechanical harvesting is indicated. The settled algal sludge in the ASP should be periodically pumped out onto sand drying beds for drying and harvesting.

4. If wastewater from an AIWPS is to be discharged under conditions leading to possible human contact, storage for 10 to 20 days in a deep maturation pond will provide adequate die away of bacteria of human origin (Sarikaya and Saatchi, 1987). The maturation pond also aids in the removal of suspended algae not removed in the ASP (Oswald, 1991).

The demonstration AIWPS at the Environmental Biotechnology Field Station in Grahamstown, South Africa was designed by Prof. William Oswald and Dr. Baily Green (Environmental Engineering and Health Sciences Laboratory, University of California, Berkeley, California, USA). The plant (as shown in **Figures 1.3.** to **1.7.**) serves as both a demonstration plant and research facility. The design rationale used is according to that described by Oswald (1994), with the operation similar to the description given by Oswald *et al.* (1994). There are already two maturation ponds at the Grahamstown Disposal Works so that construction of another maturation pond as part of the pilot-scale AIWPS was not considered necessary.

The AIWPS concept therefore links an anaerobic digestion unit operation, usually located in the primary pond, with an HRAP raceway which, operated together and depending on loading regime, can provide reduction of organics, nitrogen and phosphate levels to surface water discharge standards. Separate optimisation of these two processes enables high rate performance of the advanced ponding system.

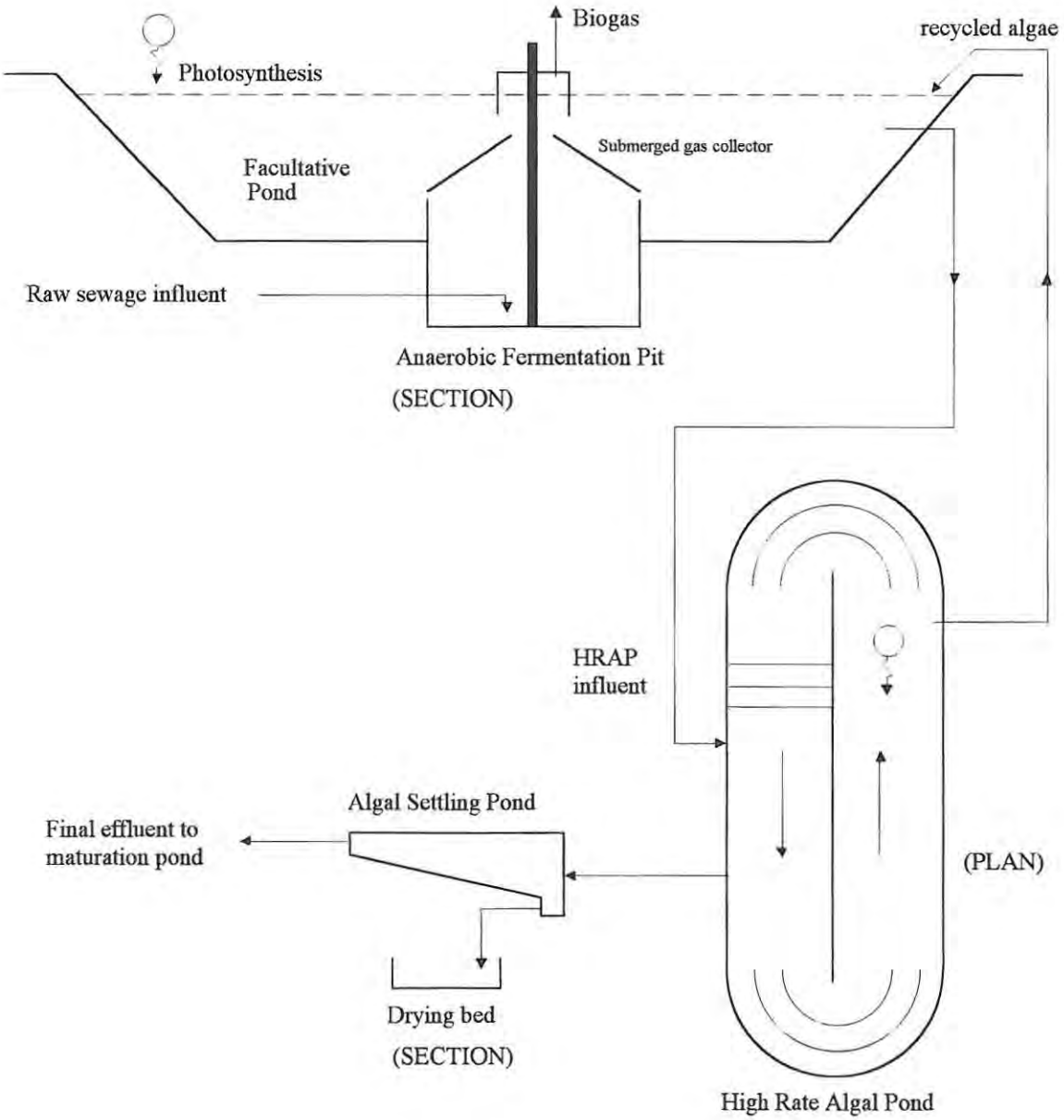


Figure 1.3. Schematic diagram showing the sub-units and sequence of treatment in the demonstration Advanced Integrated Wastewater Ponding System (AIWPS) in Grahamstown. The PFP and ASP are in section while the HRAP is shown in plan.

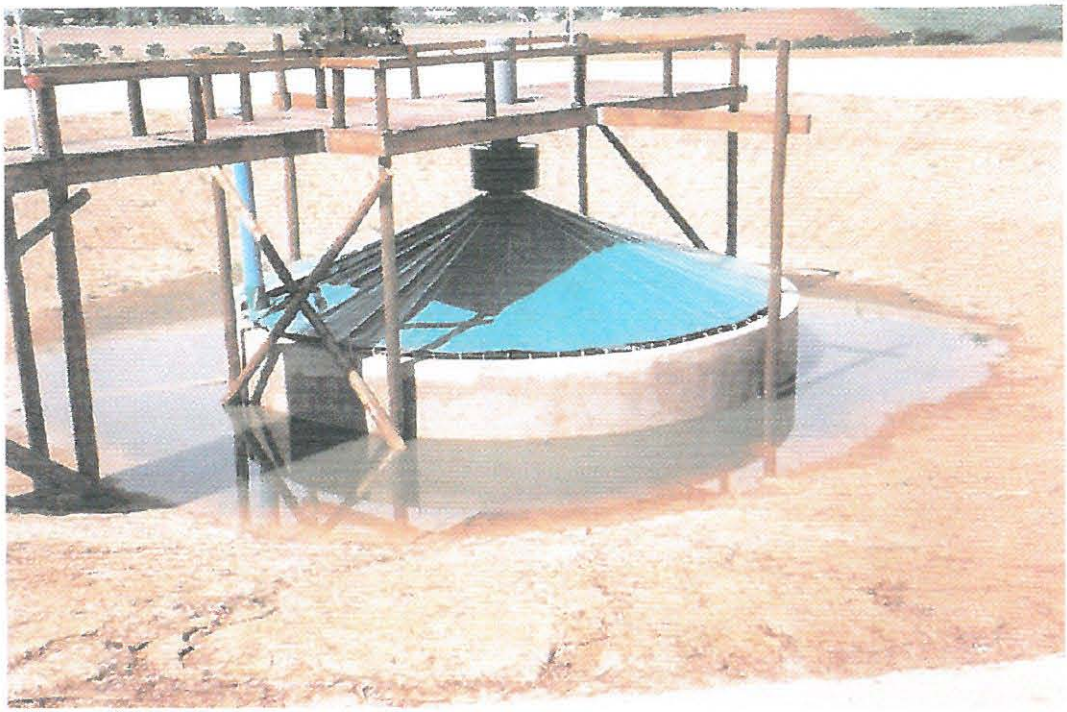


Figure 1.4. Fermentation pit inside the facultative pond after construction, Environmental Biotechnology Field Station, Grahamstown.

Figure 1.5. The primary facultative pond, Environmental Biotechnology Field Station, Grahamstown.

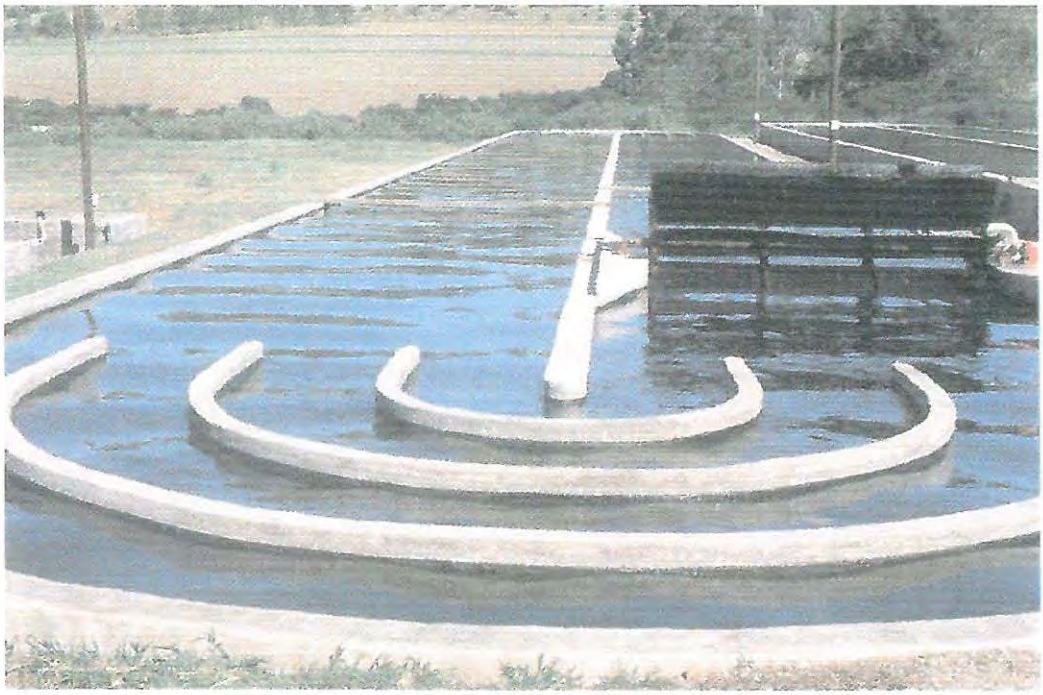


Figure 1.6. Paddle wheel mixed high rate algal pond, Environmental Biotechnology Field Station, Grahamstown.

Figure 1.7. Algal settling unit with sand drying bed, Environmental Biotechnology Field Station, Grahamstown.

1.7. Ponding Systems and Wine Industry Effluents

Wine industry effluents can be regarded as complex wastewaters, which conventionally require very specific treatment plant design criteria for implementation of specialised treatment systems, such as UASB and Activated Sludge technologies. These treatment systems are rather expensive, complex in design and require high levels of operator competency in their management. The ability to operate these systems during commissioning and shutdown also determines the successful establishment and sustainability, especially when treating seasonal effluents from the wine industry. The treatment of complex agricultural wastewaters, at low capital and operating costs, seems to be a favourable application for the AIWPS technology. The construction and operating costs for AIWPS are respectively 40% and 30% of conventional activated sludge systems treating domestic wastewater (Green *et al.*, 1995).

The potential for adding value to any wastewater treatment operation should also be factored into evaluation of technological options appropriate to resolve the wastewater problem. Dunn (1997) has shown that an integrated ponding system can be successfully engineered towards the treatment of tannery wastewater with valuable algal biomass being the beneficial product of the treatment process, thereby achieving a basis for long-term sustainability. The recovery of tartrate from wine lees already contributes to the reduction in the environmental impact of wine industry wastes. The investigation into ponding treatment of a "worst-case scenario" effluent produced by a wine lees/tartrate recovery operation, would be a good choice for an algal beneficiation operation. It should be noted that in the case of wine industry wastewaters this is not merely a case of trying to find another application for integrated ponding systems, but is driven by the unique complexity of wine wastes as well as the need for a positive cost benefit outcome.

The application of an integrated ponding process to the treatment of wine distillery effluents (including wine lees effluent) has not been previously reported and there is little reported on the ecology and performance of winery stabilisation ponds and approaches towards increasing their efficiency of operation. Although wine industry wastewaters

share some features with more common wastewaters, such as sewage effluents, there are also unique features that make the design and implementation of ponding systems rather tricky.

During the 1997 grape harvest season a laboratory-scale investigation was conducted by the researcher as a first proximate in the feasibility of developing an integrated ponding approach for the treatment of winery effluent. The following problems were experienced (results not shown):

1. The inability of the anaerobic fermentation pit to achieve acceptable organic removal rates, especially during periods of start-up and organic overloading.

2. Relatively high nutrient levels in the form of ammonia and phosphate were reason for concern when considering final discharge standards.

1.8. Research Objectives

Since the above problems would need to be addressed where distillery wastewaters were to be treated, and since little experience is available for the use of AIWPS in this application, a known system treating domestic wastewater was investigated initially as a model system. It was against this background that the research objectives for this study were formulated as follows:

1. Investigate AIWPS under known conditions treating a known domestic wastewater, although a low-strength example, identify and address critical features where problems are likely to be anticipated, including high COD loads to the Facultative Pond, and nutrient removal for high nitrogen and phosphate loads;

2. Investigate the optimisation of anaerobic and nutrient removal unit operations;

3. Based on the above findings to investigate application of AIWPS to high strength wine industry wastewaters (i.e. distillery and wine lees effluents);

4. To investigate algal biomass recovery as a potential value-adding operation, which would complement the value recovery framework of the wine lees processing operation, thereby dealing with the economic constraints of the wine processing industry.

Chapter Two

Operation and Performance of the Advanced Integrated Wastewater Ponding System

2.1. Introduction

There is a distinct lack of literature describing the performance of waste stabilisation ponds in the treatment of wine industry wastewaters and especially high-strength distillery effluents. This implies that there are no design criteria available for construction of AIWPS towards treatment of these wastewaters. The initial observation that high organic and nutrient loads seem to be problematic in AIWPS treatment of winery wastewater, was the first attempt to evaluate integrated ponding treatment of this complex wastewater. In order to proceed further with the potential development of the AIWPS technology for the treatment of wine industry effluents, a more comprehensive understanding of existing AIWPS components was identified as requiring further detailed investigations.

The physical, chemical and biological fundamentals underlining the function of AIWPS has already been discussed in Chapter 1. The domestic wastewater AIWPS, located in Grahamstown, South Africa, has become the focus of investigations conducted over a number of years by researchers in the Department of Biochemistry and Microbiology at Rhodes University, Grahamstown. This AIWPS serves as both a demonstration plant and research facility.

2.2. Research Objectives

Following the establishment of stable operating conditions the performance of the Grahamstown demonstration AIWPS would be monitored and the results together with the experiences and problems reported.

The performance of the following components would be of special interest:

1. The operation of the submerged fermentation pit inside the PFP with regard to anaerobic sludge retention and COD removal.

2. The potential of AIWPS to achieve effective nutrient removal, especially nitrogen and phosphorus.

2.3. Materials and Methods

2.3.1. Demonstration AIWPS

The demonstration AIWPS in Grahamstown has been sized to treat the sewage of 500 person equivalents (75 $m^3.d^{-1}$) and the design parameters used are summarised in **Table 2.3.1.** Two identical HRAP units are operated in parallel for research purposes.

Table 2.3.1. Design parameters of the AIWPS.

Parameter	Primary Facultative Pond	Fermentation Pit	High Rate Algal Pond	Algal Settling Pond	Drying Bed
HRT in days	20	3	4	0.5	_
Volume in m^3	1 500	225	150	19	_
Surface area in m^2	840	50	500	12.5	40
Depth in meter	3	4.5	0.3	1.5	_

* Note HRT refers to hydraulic retention time (calculated from volume / flow rate ratio).

2.3.2. Pond Sampling Procedures

Composite 24-hour samples were taken from the raw sewage influent using an automatic sampling device. The fermentation pit was fitted with multiple sampling points at 0.5,

1.5, 2.5, 3.5, 4.5 and 5.5 metres below the water surface down the centre of the pit, and samples were drawn using a vacuum pump. Sampling at each pond overflow monitored the performances of the PFP, HRAP and ASP.

2.3.3. Analysis of Pond Samples

The level of dissolved oxygen (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 pH of the wastewater was determined using a Cyberscan, 2500 pH meter, standardised at pH 4.0 and 7.0 with SAARCHEM standard buffer solutions.

Analysis of the following chemical parameters were performed using a Merck Spectroquant SQ 118: COD, ammonium as NH_3-N, nitrate as NO_3-N and orthophosphate as PO_4-P. Sample pre-treatment was done by filtering through Whatman GF/C microglass filters. The fractional analysis of phosphorus was done according to the method of Hieltjes and Lijklema (1980) which distinguished between organic-bound, calcium-bound and total phosphorus. Settleable solids (SS), volatile fatty acids (VFA) total Kjeldal nitrogen (TKN) and chlorophyll$_a$ (chl_a) were determined according to Standard Methods (A.P.H.A.,1980).

Biogas production in the fermentation pit inside the PFP was measured using an inverted funnel with an attached tube connecting the funnel to an aspirator bottle containing acidified water. Total biogas production was determined by monitoring acid solution displacement. Methane content in the biogas was determined by measuring carbon dioxide adsorption in an alkaline solution (Ross *et al.*, 1992).

2.4. Results and Discussion

2.4.1. Algal Integrated Wastewater Ponding System Operation

The performance of the system was closely monitored over a 74-week period (from July 1997 to December 1998) while operating under constant hydraulic retention, and the performance characteristics are summarised in **Table 2.4.1.**

Table 2.4.1. AIWPS performance characteristics over a 74 week period from July 1997 to December 1998. Standard deviations are in brackets.

Parameter		Influent sewage	PFP effluent	HRAP effluent	ASP effluent
pH		7.1 (0.4)	7.4 (0.4)	8.9 (0.9)	8.8 (0.8)
COD	$mg.L^{-1}$	969 (285)	197 (61)	338 (89)	101 (29)
TKN	$mgN.L^{-1}$	140	50	45	20
Ammonia	$mgN.L^{-1}$	31 (7)	29 (6)	3.7 (2.6)	4 (2.8)
$Chlorophyll_a$	$mg.L^{-1}$	_	0.21 (0.21)	3.21 (1.09)	0.33 (0.22)
Nitrate	$mgN.L^{-1}$	1.1 (0.8)	0.9 (0.7)	9.6 (3.9)	7.9 (3.9)
Phosphate	$mgP.L^{-1}$	7.1 (2.4)	7.4 (1.6)	4.5 (1.5)	4.9 (1.5)
Settleable solids	$ml.L^{-1}$	28	<1	4	0
Suspended solids	$mg.L^{-1}$	263 (48)	159 (34)	271 (68)	64 (27)
Total coliforms	per 100 ml	4.4×10^7	3.9×10^5	1.5×10^4	1.2×10^3

The screened sewage feed that passed into the fermentation pit showed substantial variation in COD load between 500 and 1 800 $mg.L^{-1}$ with an average of 970 $mg.L^{-1}$ (**Figure 2.4.1.**). This variation has been correlated with incidents of high rainfall during which storm-water entry into the Grahamstown sewerage system takes place with subsequent dilution. COD removal of 80% is achieved in the PFP, with a further 10% removal in the ASP after algal biomass settling. The function of the fermentation pit is mainly to receive, settle and digest the solids fraction present in the influent raw sewage.

Elevated total COD levels in the HRAP appear to exceed the load from the FP, which is almost entirely due to the production of algal biomass as shown by rising chl_a levels. Meiring and Oellermann (1995) report that the following equation can be used as a yardstick for determining the effect of suspended algae on pond COD levels:

$$100\ \mu g.L^{-1}\ \text{of}\ chl_a\ \text{give rise to}\ 5.6\ \text{mg}\ COD.L^{-1}$$

While chl_a levels (indicative of microalgal growth) varied quite widely it also coincided with high rainfall conditions.

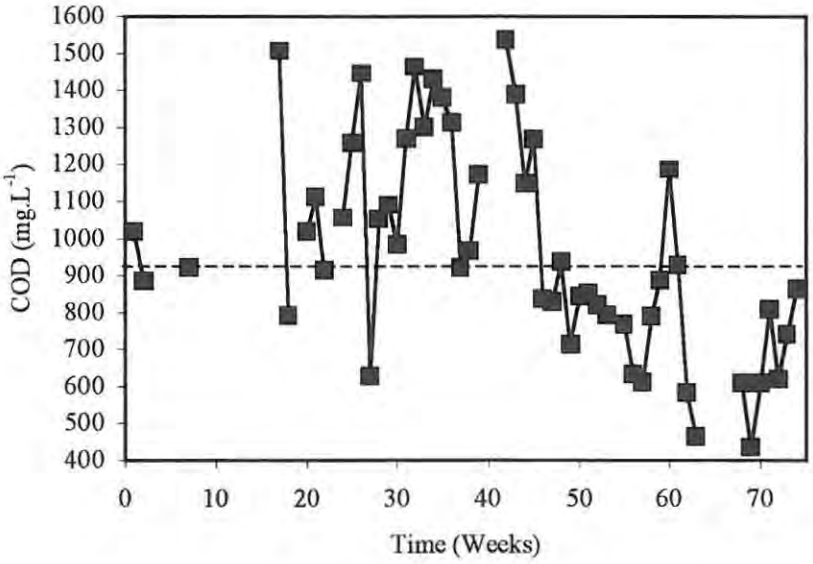


Figure 2.4.1. Variation in the facultative pond influent COD levels.

Figure 2.4.2. shows nitrogen removal across the AIWPS which averaged 86% removal for TKN, and 87% for ammonia, across the system. More than 60% of the total nitrogen is removed in the PFP, probably through the conversion of organic nitrogen (sewage suspended solids) to nitrogen gas. From a treatment standpoint, the removal of total nitrogen in facultative wastewater ponds is well documented and the process of nitrification and denitrification is a widely recognised phenomenon (Green *et al.* (1996). The work of Brockett (1976) and Green *et al.* (1996) indicates that from 30 to 50% of the total nitrogen entering an AIWPS is removed in the primary facultative pond (which is

confirmed in this study). This removal is effected by conversion of organic nitrogen and ammonium to nitrogen gas in the anoxic zones through a process known as heterotrophic nitrification. According to Verstraete and Alexander (1973) inorganic and organic nitrogen are metabolised by heterotrophic nitrifiers to produce nitrite and nitrate in the presence of undetectable traces of oxygen. The subsequent reduction of nitrate in the anoxic zones of the facultative pond would be expected.

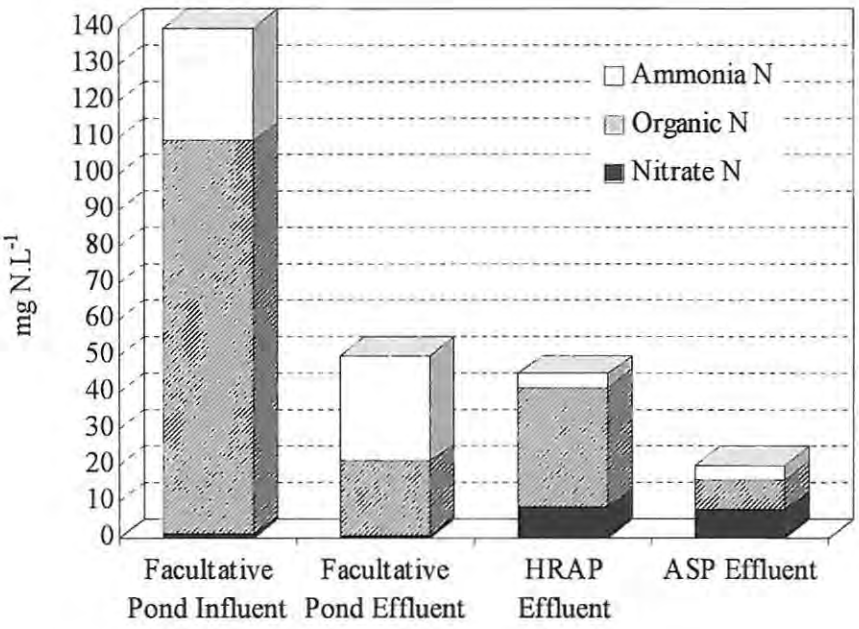


Figure 2.4.2. Nitrogen removal across the integrated ponding system.

The organic nitrogen fraction in the facultative pond effluent will consist of sewage suspended solids and suspended algae. About 87% of the inorganic nitrogen load to the HRAP was removed in this process unit through algal biomass uptake and bacterial nitrification. Ammonia stripping also occurred. Rapidly growing algae in the HRAP are generally high in nitrogen, and ammonium is taken up preferentially over nitrate and nitrite (Konig *et al.*, 1987). According to Green *et al.* (1996) the percentage of nitrogen and phosphorus in actively growing microalgae is usually about 9% and 1% respectively of the dry weight. During our study the average algal production rate was about 11 $g.m^{-2}.day^{-1}$ (dry weight) in the HRAP concerned. In the HRAP the levels of nitrate

increased from about 1 to 10 $mg.L^{-1}$ probably due to nitrification taking place under the aerobic conditions. A large percentage of the ammonium entering the HRAP was probably removed through stripping due to the high pH of about 9, and the provision of a large exchange surface area. Konig *et al.* (1987) pointed out that at pH 9 approximately 40% of ammoniacal nitrogen in the water is in the form of NH_3, which will be lost to the atmosphere if an exchange mechanism is provided. The removal of nitrogen in the demonstration AIWPS seemed to be achieved by five mechanisms. These were anoxic denitrification in the PFP, followed by algal uptake, ammonia stripping and nitrification in the HRAP, and finally algal cell removal in the ASP.

The results for phosphorus removal were less satisfactory and **Figure 2.4.3.** shows the fractionation of phosphorus across the system.

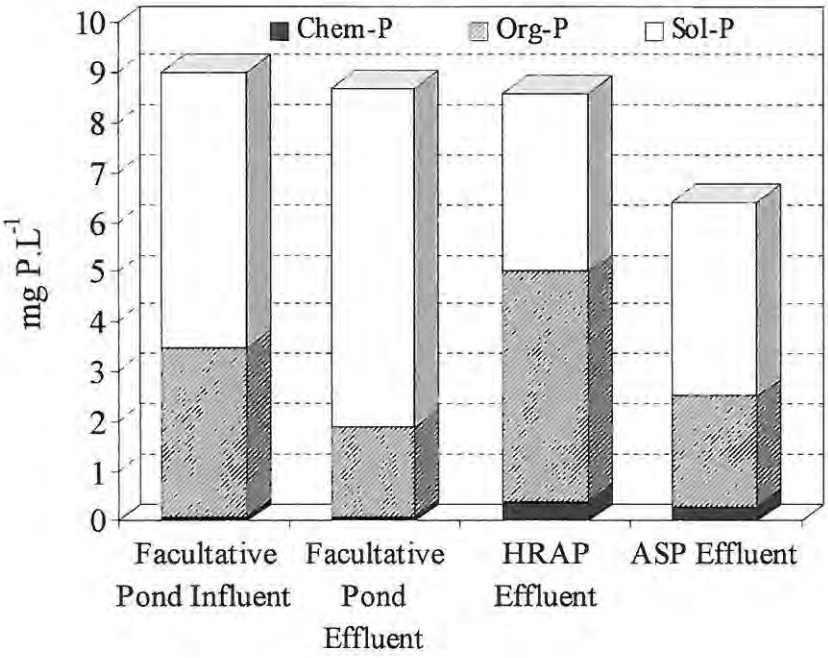


Figure 2.4.3. The fractionation of phosphorus across the AIWPS.

The increase in soluble phosphate levels in the PFP was expected due to the anaerobic digestion of waste organic solids, which tend to release nutrients in the form of

ammonium and phosphate. The overall phosphorus removal was only about 30% which was mainly attributed to algal uptake in the HRAP followed by biomass removal in the ASP. A relatively small fraction was removed through chemical precipitation. Moutin *et al.* (1992) reported the precipitation of calcium phosphate minerals in the presence of photosynthesising green algae where the algae played a central role in raising the pH and initiating the precipitation reaction. The presence of phosphorus accumulating organisms (PAO) is unlikely due to the operational design of the AIWPS, which does not meet the requirements for PAO cultivation according to Mino *et al.* (1998).

The degree of sewage purification achieved by the Grahamstown AIWPS does in general satisfy the design requirements of the system. However, in South Africa limited reliable water resources have necessitated the adoption of more stringent discharge standards, and today more effluent discharges must comply with the Special Discharge Limit as shown in **Table 2.4.2.** The relatively high levels of especially dissolved nitrogen and phosphorus would require further nutrient removal before final discharge.

Table 2.4.2. South African wastewater limit values applicable to discharge into a surface water course (South African National Water Act No. 36 of 1998).

All values in $mg.L^{-1}$	General limit	Special limit
COD	75	30
NH_4-N	3	2
NO_3-N	15	1.5
PO_4-P	10	1
Suspended solids	25	10

2.4.2. Anaerobic Fermentation Pit

During the construction phase the fermentation pit was fitted with a submerged gas collector shown in **Figure 1.4.**, which was fabricated from reinforced plastic liner. Gas emerging from the digester was focussed through the central opening between the collar

and the support column and was collected at the surface in a rigid circular PVC cap, also aligned by the support column. The advantage of biogas collection in a facultative pond system with a water column overlying the active digestion zone, is the provision for absorption of carbon dioxide, and thereby enriching the methane content of the biogas.

Towards the second half of 1997 large flocs of floating sludge became visible on the surface of the PFP which seemed to originate from the fermentation pit. Gas production was also visible above the submerged gas collector indicating the presence of anaerobic fermentation on top of the gas collector. Sludge sampled from the top of the gas collector outside the pit was analysed for biogas production – the activity test showed that 55 % of the produced biogas was in the form of methane, suggesting that active methanogenic sludge might have been washed out of the pit. It was further assumed that in order for sludge to wash out of the fermentation pit, it would have to be forced through the central opening between the submerged gas collector and the support column. The sludge probably re-settles on top of the gas collector from where it eventually slides down ending up outside the fermentation pit, and into the facultative compartment. In order to monitor whether this was actually taking place, a 4 litre PVC bucket was suspended next to the outside wall of the fermentation pit, below the top of the wall.

The fermentation pit was closely monitored over a period of 40 days and the results are reported in **Figure 2.4.4.** The results indicate that there was very little settleable solids in the pit, which correlated with the build-up of volatile fatty acids (on day 4 the VFA levels were 144 and 342 $mg.L^{-1}$ at 1.5 and 5.5 metres below the surface, respectively). On day 5 the pit was re-seeded with 8 m^3 of anaerobic digester sludge from the Grahamstown Disposal Works anaerobic digester. The pit was monitored daily before and after the re-seeding by sampling at 1.5 and 5.5 metres below the surface inside the pit, as well as at the pit overflow where cumulative 24-hour sampling was achieved using the suspended PVC bucket. The correlation between sludge washout and subsequent collection in the bucket is shown in **Figure 2.4.5.** The results show that the pit is not very effective in retaining the active anaerobic biomass due to washout through the opening of the gas collector resulting in build-up of the sludge on top of the gas collector outside the pit.

VFA level on day 40 was very much the same as on day 4. The fermentation pit was re-seeded twice afterwards and each time the results followed a similar trend.

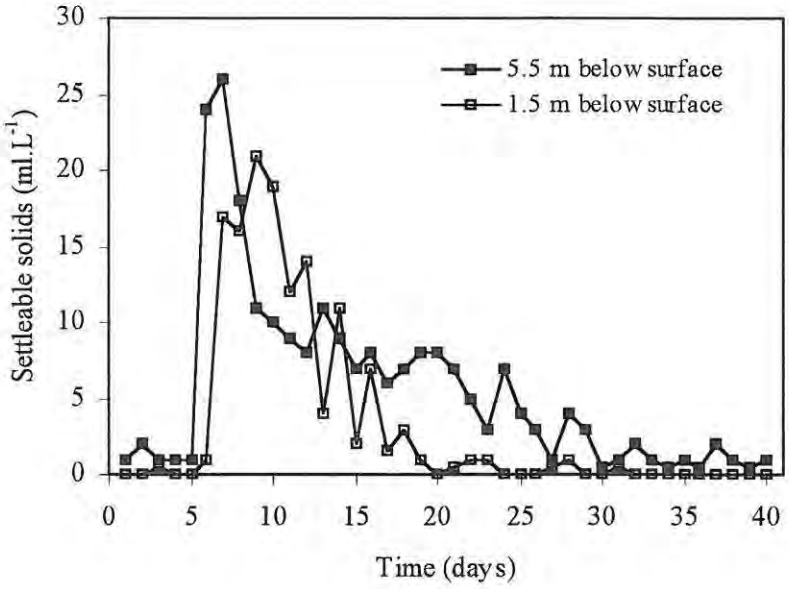


Figure 2.4.4. Anaerobic sludge washout from the fermentation pit after re-seeding on day 5 with active anaerobic sludge.

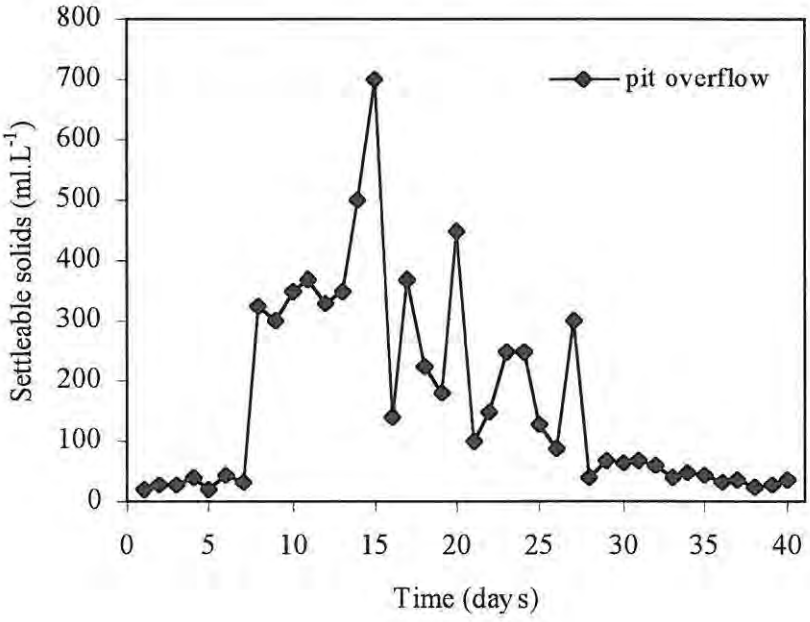


Figure 2.4.5. Collections of settleable solids in a PVC bucket outside the fermentation pit indicating anaerobic sludge washout.

During June 1998 the facultative pond was drained halfway in order to examine the submerged gas collector and, after exposure, a thick layer of sludge was visible on top of the gas collector (**Figure 2.4.6.**).

Figure 2.4.6. Active anaerobic sludge visible on top of the gas collector.

It was decided to remove the gas collection device to allow the re-settling of active sludge to the bottom of the pit. After a couple of weeks when the facultative pond has filled up again and started overflowing, the fermentation pit was re-seeded with conventional anaerobic sewage sludge. **Figure 2.4.7.** reports the results, indicating that the fermentation pit stabilised by retaining most of the anaerobic sludge, during which period very little settleable solids were collected in the PVC bucket outside the pit. VFA levels stabilised at 76 and 41 $mg.L^{-1}$ for 1.5 and 5.5 metres below the surface, respectively, which is an indication of the presence of active methanogenic bacteria. However, large sludge flocs did continue to surface, although less frequently than when the submerged gas collector was present.

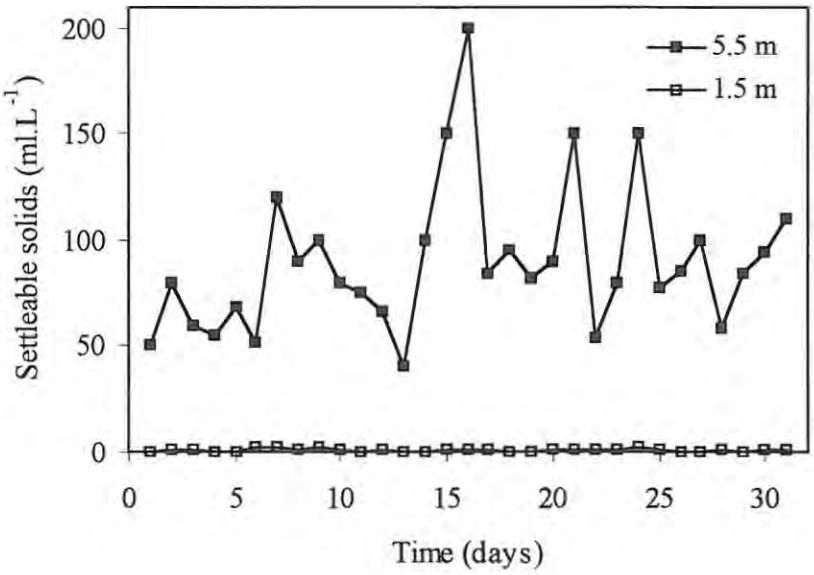


Figure 2.4.7. Retaining of anaerobic sludge in the fermentation pit after removal of the submerged gas collector.

2.4.3. Facultative Pond

Recirculation of HRAP medium to the surface of the facultative pond is designed to introduce algae-rich warm oxygenated water and provide a trap for odours released by the subsurface anaerobic layers. In practice the large algal flocs present in the HRAP medium (2-3 mm in diameter) rapidly settle to the bottom of the facultative pond together with the predominant algae *Scenedesmus, Micractinium* and *Actinastrum spp.* The algal species composition able to survive and establish themselves in the upper layer of the facultative pond are quite different, with the motile algae *Chlamydomonas* and *Euglena spp.* predominating. Recirculation from the HRAP at a rate of 10% for 8 hours per day was quite reliable in controlling odour release, but proved relatively less effective where HRAP performance was affected in colder weather. In these circumstances populations of purple photosynthetic bacteria predominated in the PFP surface layers. These 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 (Almasi and Pescod, 1995). The accumulation of a floating white film, which is characteristic of elemental sulphur, revealed their presence.

It was decided to stop the recirculation of HRAP medium to the facultative pond after the extent of settleable algal biomass build-up became evident around the HRAP recycle influent, and near the edge of the PFP. Eventually algal biomass will be degraded anaerobically and release nutrients resulting in a degree of short-circuiting. Instead, surface waters of the PFP was recirculated (refer to **Figure 2.4.8.**) and thereby aiding in maintaining an evenly distributed mixed algal population. Odour control was as reliable as before with the introduction of HRAP overflow once every 4 to 6 months for re-seeding to maintain the surface algal population.

2.4.4. Nutrient Release in Algal Settling Pond

The results shown in **Table 2.4.1.** indicate that levels of ammonia and phosphate in the ASP overflow slightly exceeded the HRAP, while showing a slight decrease in nitrate concentration. A depth profile was conducted in the ASP and the results are reported in **Table 2.4.3.** which indicate possible fermentative nutrient release at the bottom of the settling pond. It has been reported previously that green microalgae can remain in an ASP for weeks, even months, before releasing any nutrients (Green *et al.*, 1996). The ASP profile suggests, however, that fermentative products are possibly available as a carbon source for biological denitrification, with released ammonia and phosphate as the by-products. Regular cleaning of the ASP on a weekly basis improved the quality of the settler overflow with almost no release of nutrients detected.

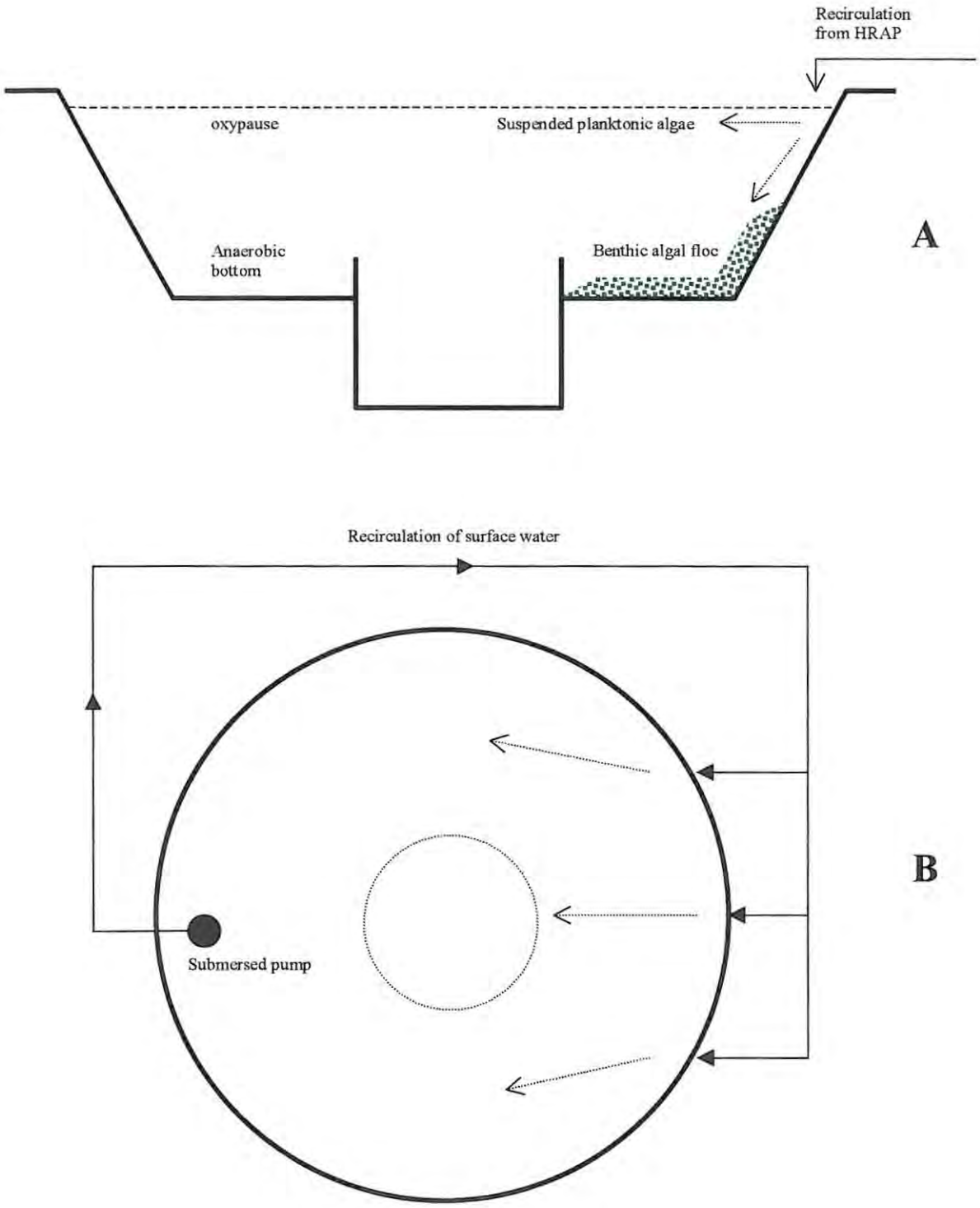


Figure 2.4.8. The original system operation included recirculation of HRAP medium to the surface of the PFP, which resulted in unwanted algal biomass settling (**A**). Improved system operation included recirculation of PFP surface water in order to maintain a planktonic algal population (**B**).

Table 2.4.3. Depth profile of the algal settling pond after collecting biomass for 2 weeks.

Parameter	Surface sample	Middle sample	Bottom sample
pH	8.7	7.6	5.7
PO_4 (mg $P.l^{-1}$)	3.9	10.1	27
NH_3 (mg $N.l^{-1}$)	3.0	10.2	18.3
NO_3 (mg $N.l^{-1}$)	5.3	0.4	0.1

2.5. Conclusion

The AIWPS in Grahamstown, South Africa, proved to be efficient in receiving and treating raw sewage to the extent that 90% of COD, 86% of fixed nitrogen and 87% of ammonia were removed over the study period. Total coliform removal was at least 99%, which is impressive for a biological process. This disinfection effect is attributed mainly to a combination of UV and high alkalinity production in the HRAP, which is a well-known mechanism for inactivating pathogenic organisms.

The following areas were the cause of concern regarding the potential of AIWPS as a long-term treatment system:

1. The biogas collection system, which was added above the fermentation pit in order to evaluate the potential of methane collection, did not adequately serve the purpose. Due to the washout of active anaerobic sludge over time an adequate sludge blanket could not be maintained in the pit. This substantially reduced its performance. An open pit design, alternatively, did retain most of the anaerobic sludge but the extend of exposure of the rising methanogenic sludge to dissolved oxygen in the surface layers was also cause for concern, as the toxicity of oxygen to methane producing organisms is well known. It is evident that if biogas is to be collected, a different and more efficient gas collection system needs to be devised. Despite the variable performance of the fermentation pit, the facultative pond and subsequent units

demonstrated the highly effective buffering capacity of the system throughout the course of the study, notwithstanding the incidents of high and low COD load. Follow-up studies on improving the design of the fermentation pit are described in Chapter 3 of this report.

2. Phosphorus removal was only 30% of the influent value, which resulted in the ASP overflow containing phosphate levels of more than 4 $mgP.L^{-1}$. As indicated in **Figure 2.4.3.** the removal that took place was probably due to the uptake by, and subsequent removal of, the microalgal biomass.

3. The combined ammonia and nitrate levels were also constantly higher than the standard discharge limit, which together with the presence of phosphate would not be allowed for final discharge to a receiving natural water body, as this could lead to eutrophication.

It was apparent that the above problems would have to be addressed before applying the AIWPS technology in wine industry effluent treatment, and these issues relate to anaerobic sludge retention in the fermentation pit and improved nutrient removal in the final effluent.

Chapter Three

Design Evaluation for Improved Performance of the Fermentation Pit inside an Advanced Facultative Pond

3.1. Introduction

The stabilisation of household sewage by means of anaerobic digestion was first described in 1881, and by 1914 the process had come into extensive use at municipal sewage treatment works (McCarthy, 1981). One of the most important assets of the process is that considerable purification of wastewater is achieved without the formation of excessive biomass (Weber *et al.*, 1984). Biogas, consisting mainly of methane and carbon dioxide, is the most important by-product of the anaerobic digestion process. Biochemical energy, locked-up in the waste stream, is thus converted and conserved by the anaerobic process.

Anaerobic digestion has only much more recently been applied to the industrial level. During the 1950's the process was understood well enough to allow the development of full-scale anaerobic digesters treating industrial effluents (Ross *et al.*, 1989). The process has since been tried and tested on many types of industrial effluents, both in the laboratory and at full-scale. Since the definitions of digester types are sometimes rather vague and overlapping, it is difficult to accurately categorise each digester design. However, in terms of their biomass retention mechanisms, all digesters can be broadly categorised into one of only four types (Strydom *et al.*, 2001). These are attached biofilm systems, systems where gravitational sludge settling takes place, systems where solids and liquids are separated by some mechanical means such as membranes and finally completely mixed systems with no mechanism for biomass retention. Among the high-rate anaerobic reactors developed and successfully applied in recent years the upflow anaerobic sludge blanket (UASB) reactor has become one of the most popular designs for the biological treatment of effluents (Lettinga *et al.*, 1997).

The anaerobic pond, or facultative pond with submerged fermentation pit, are the simplest types of anaerobic digesters. Ponds are far easier to construct than vertical digester types, with the only drawback being the relatively large land area required. During the 1980s interest in renewable energy technologies hastened the development of large-scale biogas collection devices. Several animal waste ponds were retrofitted with floating plastic covers to collect biogas (Oswald and Swanson, 1981; Safley and Westerman, 1992). Given their exposure to the elements, surface covers are vulnerable to high winds, heavy rains, UV degradation and vandalism. Oswald *et al.* (1994) reports the development of submerged gas collectors for fermentation pit digesters, which avoided the difficulties experienced with surface gas collectors.

The inefficient retention of anaerobic sludge due to the inadequate design of the submerged gas collector in the AIWPS demonstration plant at Grahamstown has been discussed in Chapter 2 of this report. This initiated a study into the possible re-design of the fermentation pit inside the facultative pond, which would combine gas collection with improved solids retention. The obvious advantage of applying some of the UASB characteristics to the in-pond fermentation pit would be to improve the retaining of active methanogenic sludge in a low-cost construction, resulting in improved performance.

Limited data is available on the performance of pond-type anaerobic digesters treating wine industry wastewaters. In view of the availability of a reference AIWPS facility receiving sewage, it was considered appropriate to simultaneously compare the anaerobic digestibility of both winery and sewage effluents. It has been mentioned in Chapter 1 that the fermentation pit (inside the facultative pond) experienced some difficulty in handling high organic loads when receiving winery wastewater. Against this background it would seem appropriate to introduce an alternative anaerobic pre-treatment step when treating higher strength wine wastewater, before entering the fermentation pit of an AIWPS. Therefore, evaluating the fermentation pit performance with winery wastewater, and not high-strength wine lees wastewater, seemed more appropriate at this stage.

3.2. Research Objectives

To evaluate reactor designs for improved sludge retention based on the improved performance of the fermentation pit inside the facultative pond, where the treatment of household sewage and winery wastewater are simultaneously compared.

3.3. Materials and Methods

3.3.1. Anaerobic Reactor Designs

The laboratory-scale anaerobic reactors used in this study were made from PET plastic and are shown in **Figures 3.3.1. & 3.3.2.** Each digester (10 cm wide and 39 cm high) had an operational pit volume of 3 litres and was surrounded by a small 2 litre facultative pond. The reactors were all operated in duplicate to make the simultaneous treatment and comparison of sewage and synthetic winery wastewater possible.

Reactor A simulated the open fermentation pit found in conventional AIWPS where no gas collection device is present. The facultative pond was not to scale with the fermentation pit because the study focussed on the pit performance alone and the outside pond's function was merely to collect washout anaerobic sludge.

Reactor B incorporated a gas/liquid/solid separator (GLSS) present in the upper zone of the digester which simulated the conventional UASB-type design according to Van Haandel and Lettinga (1994).

Reactor C contained a nylon net, of 10 mm aperture size, across the rising column of the fermentation pit, which divided it in two chambers. The inserted net was evaluated for its function in retaining large sludge particles.

Reactor D combined both the UASB-type GLSS and the nylon net insertion.

Each reactor was seeded with 600 ml of anaerobic sludge obtained from a conventional anaerobic digester treating primary sewage sludge, which had a concentration of 83.4 g $SS.L^{-1}$ and 28.6 g $VSS.L^{-1}$, and a methanogenic activity of 0.24 g CH_4-$COD.g^{-1}$ $VSS.d^{-1}$ at 25 °C. The anaerobic sludge was pre-treated by passing it through a 3 mm sieve.

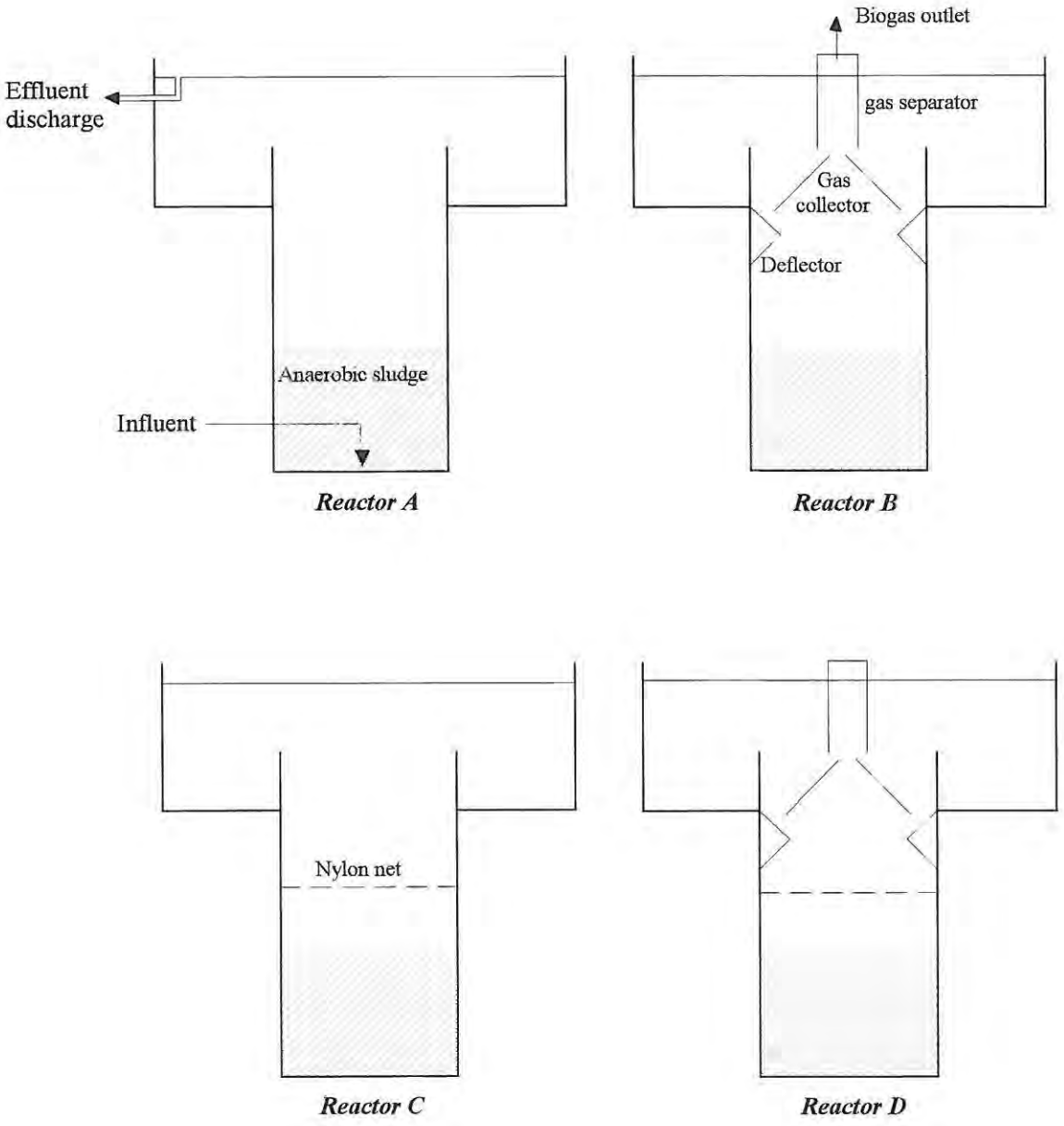


Figure 3.3.1. Anaerobic digester designs evaluation for improving the fermentation pit performance.

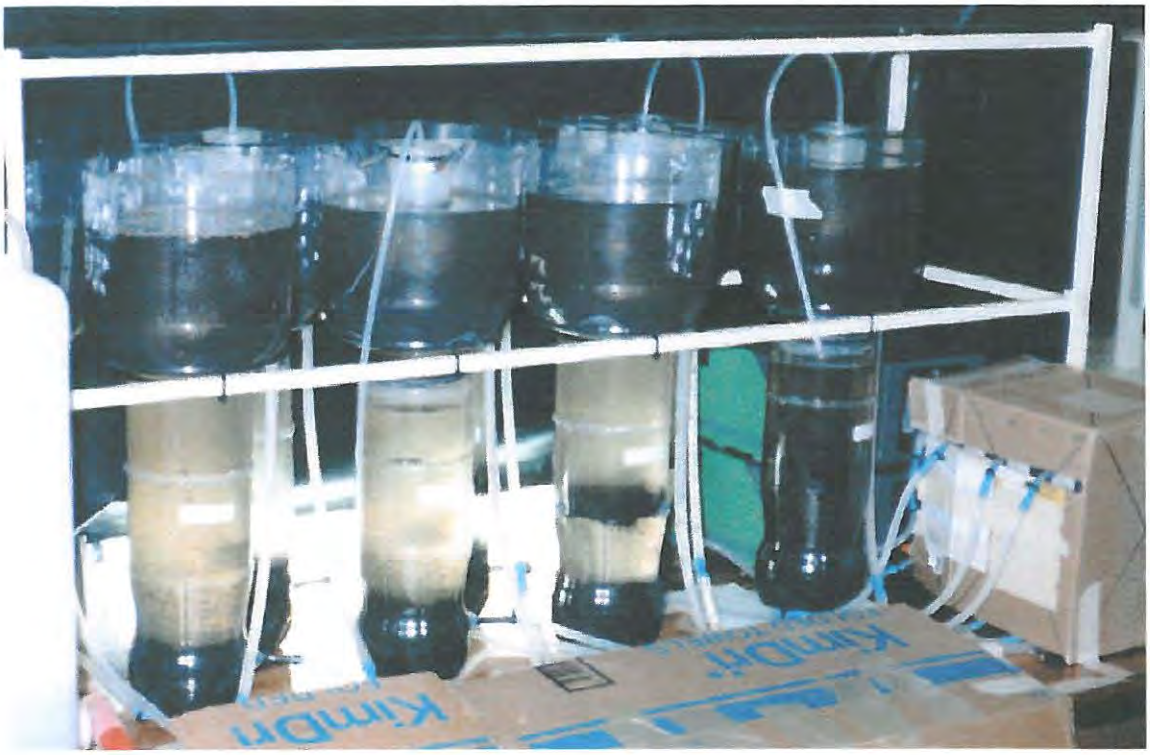


Figure 3.3.2. Photograph of the laboratory-scale reactors used in this study.

3.3.2 Sewage Reactors Start-up

Sewage wastewater was collected from a main sewer at the Grahamstown Disposal Works. The sewage was passed through a 2 mm sieve before analyses and used as feed to the anaerobic sewage reactors. The sewage feedstock was diluted with tapwater, when necessary, to give a final total COD of about 700 $mg.L^{-1}$, and renewed daily. **Table 3.3.2.** shows the sewage characteristics over the study period of 140 days. The screened sewage was fed to the reactors by means of a peristaltic pump (Watson Marlow 101), starting with a hydraulic residence time of 17 hours inside each fermentation pit. Room temperature was controlled at 25 ± 2 °C.

Table 3.3.2. A Typical average analysis of the Grahamstown domestic wastewater used in the study, with standard deviations (note that three replicates were used).

Parameter		Average	SD
pH		7.3	0.5
CODt	$mg.L^{-1}$	708	32
CODs	$mg.L^{-1}$	239	41
SS	$mg.L^{-1}$	263	89
Settleable solids	$ml.L^{-1}$	5	3
Alkalinity	mg $CaCO_3.L^{-1}$	312	63
Ammonia	mg $N.L^{-1}$	28	6
Phosphate	mg $P.L^{-1}$	7.5	3.2

3.3.3 Winery Wastewater Reactors Start-up

The composition of the synthetic winery wastewater was red wine that has been diluted with tapwater to the required COD concentration. The pH was then adjusted to 7.2 with sodium bicarbonate and the characteristics of the synthetic substrate together with the average composition from a local winery effluent are given in **Table 3.3.3.** The synthetic substrate was also fed to the reactors with a peristaltic pump (Watson Marlow 101), starting with a hydraulic residence time of 17 hours inside each fermentation pit. The feed was replaced daily and all experiments were carried out at 25 ± 2 °C.

Table 3.3.3. Comparison of winery effluent with the synthetic substrate used in the study.

Parameter		Winery wastewater	Synthetic wastewater
pH		4.3	7.2
COD	$mg.L^{-1}$	2 900	2 000
TKN	$mg.L^{-1}$	48	11
Ammonia	mg $N.L^{-1}$	1	1.6
Nitrate	mg $N.L^{-1}$	23	6
Phosphate	mg $P.L^{-1}$	6.3	10
VFA	$mg.L^{-1}$	–	992
Alkalinity	mg $CaCO_3.L^{-1}$	–	1 000

3.3.4 Sampling Procedures and Analysis

In order to monitor each reactor's performance, samples were drawn with a syringe below the outside pond surface at the inside of the fermentation pit wall. Sample pre-treatment was done by filtering through Whatman GF/C microglass filters.

Analysis of all parameters was undertaken as previously described in Chapter 2.

Total volatile fatty acids (TVFA) were determined according to the titration method of Ripley *et al.* (1986). Biogas production and composition were determined by acid and alkaline solution displacement as well as by gas chromatography using a thermal conductivity detector. Biogas production in the reactor A and C type designs was measured using a specially designed inverted funnel with an attached tube connecting the funnel to the surface, while monitoring acid solution displacement.

Methanogenic activity assays were carried out in 100-ml serum bottles as described previously (Soto *et al.*, 1993). A stock solution of VFA, previously neutralised with NaOH, was used as substrate feed. Concentration in the assay medium was 3.8 $gCOD.L^{-1}$ (2.0 $g.L^{-1}$ acetic acid, 0.5 $g.L^{-1}$ propionic acid and 0.5 $g.L^{-1}$ of *n*-butyric acid). The seed sludge concentration used in anaerobic assays was in the range of 1 to 3 g $VSS.L^{-1}$. Methane production was measured by the displacement of alkaline solution at 25 °C.

3.4. Results and Discussion

3.4.1. Sewage Fed Reactors

The volume of sludge that accumulated over time in each facultative pond of the sewage fed reactors gives a clear indication of each reactor's ability to retain active anaerobic biomass in the form of sludge. **Figure 3.4.1.** shows the sludge accumulation in the facultative ponds of each reactor during the first 22 days after start-up. During the first week after seeding the reactors, the fermentative gas production was very high resulting

in sludge piston formation, where the dense sludge bed caused gas entrapment at the bottom of each digester. When the buoyancy of the entrapped gas was high enough there was an incidental flotation of sludge resulting in subsequent biomass washout.

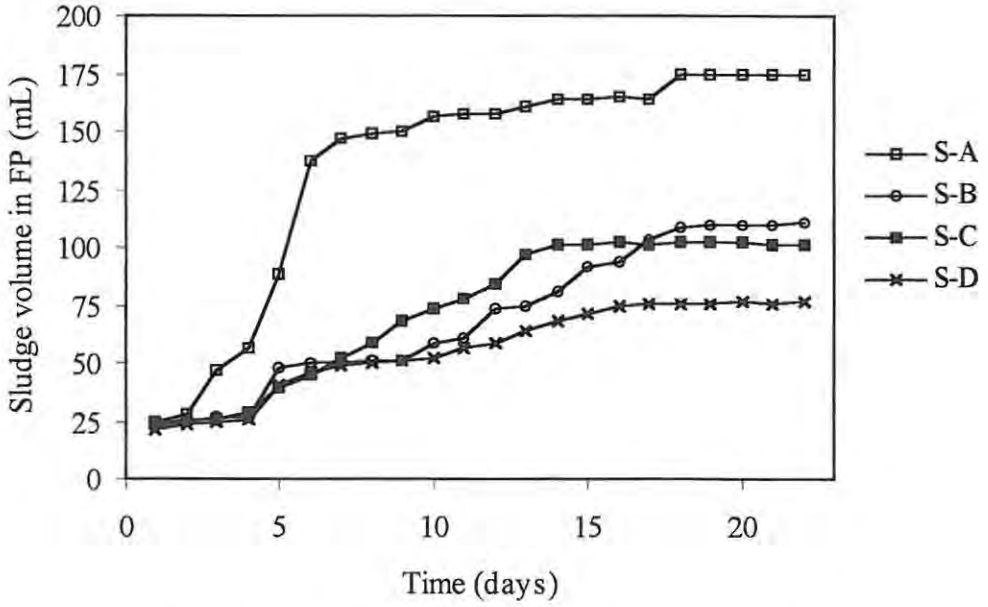


Figure 3.4.1. Overflow sludge accumulation in the facultative ponds of the sewage fed reactors, indicating the degree of sludge loss from each fermentation pit (see **Figure 3.3.1.** for design and configuration of reactors).

In reactor S-A the sludge washout was high due to the open pit design, which did not prevent the carryover of sludge to the surrounding pond. In reactor S-B the opening of the submerged gas collector blocked, but was eventually unblocked by rising gas bubbles after a few days. The nylon net in reactor S-C was very successful in preventing large sludge fractions from reaching the surface whenever an incidental sludge flotation occurred. After each flotation the net seemed to block because the sludge fractions were bigger than the actual openings in the net, but rising gas bubbles helped in unblocking the net after a few hours.

In reactor S-D sludge retention was optimal due to the flocculation and re-settling of small particles. It was observed that, as in the case of reactor S-C, the net created a barrier

which effectively separated large solid fractions from the entrapped gas bubbles. This resulted in the return of the large particle fraction to the sludge bed. The small solids fraction, however, passed through the net into the upper compartment of the pit where the bubbles had now aggregated into large units. While the upper compartment did not provide a truly turbulence-free environment, smaller particles were not entrained with the larger gas bubbles and a component also returned and settled back into the lower compartment. The gas/liquid/solid separator (GLSS) present in the upper zone of the digester seemed to operate according to the original design. Owing to the inclined walls of the phase separator (gas collector) the area for the liquid flow increased as the liquid approached the water surface, so that the upflow velocity of the liquid decreased when it flowed towards the top of the fermentation pit wall. Due to the decreased liquid velocity, small sludge flocs drawn into the settling zone could flocculate and/or settle out. At some stage the weight of the accumulated sludge aggregating on the phase separator exceeded the frictional force that kept it on the inclined surface and slid back towards the digester bottom to become, once again, part of the sludge bed that digested the influent organic matter.

A week after start-up, reactor S-A had lost about 65% more sludge than the other reactors, but seemed to stabilise after 3 weeks. During the first week all reactors, except S-A, performed similarly in retaining the very active anaerobic sludge, indicating that all the structural interfaces inside each pit contributed towards absorbing the volatile sludge bed. Sludge piston formation occurred frequently (about every 2 hours) during this period, but seemed to become less frequent after day 8 (about once or twice a day). Reactor S-C, containing the nylon net, became stable after only two weeks of operation compared to S-B which, although having lost less sludge at the time, was still experiencing solids washout at a fairly steady rate. The improved performance of reactor S-C can be attributed to the fact that a dense sludge layer of about 2 cm thick developed around the nylon net (visible in **Figure 3.3.2.**) which acted as a "filter" by trapping the rising small sludge flocs. This second sludge layer seemed to prevent the steady rise of small gas bubbles by trapping the bubbles only to subsequently release large gas bubbles at a less frequent rate.

A serious drawback of the GLSS in reactor S-B was that the gas production inside the gas collector gave rise to liquid flows both beneath and above it (through the opening between the gas separator and the inclined side). These convective currents therefore made settling less efficient.

Reactor S-D showed the best performance from all the configurations and here too the net played a very important role in stabilising the sludge bed, where a second sludge layer also developed. The net contributed in preventing the opening of the gas collector from being blocked with sludge during reactor start-up. On day 22, after all reactors stabilised, reactor S-D proved to retain 56% more sludge than the open pit design. The UASB-design of reactor S-B also retained 37% more sludge than reactor S-A, but what is interesting is that by just inserting a low-cost nylon net into the fermentation pit, resulted in 42% more anaerobic sludge being retained.

The performance of each anaerobic sewage-fed reactor relating to the COD concentration in the fermentation pit overflow is shown in **Figure 3.4.2.** There was a clear correlation between initial loss of active anaerobic sludge from the anaerobic pits and digester performance after stabilisation has been reached. Reactor S-A proved to be very unstable and every incident of high overflow COD was due to sludge washout, as a clear correlation between COD and suspended solids concentration was found during start-up (**Figure 3.4.3.**). Reactor S-D performed the opposite of S-A where the standard deviation of overflow COD was only 20 $mg.L^{-1}$, compared to 50 $mg.L^{-1}$ for reactor S-A. **Table 3.4.1.** shows the performance of each digester on day 96, with the HRT still at 17 hours. For each reactor there was a correlation between the %COD removal achieved and the methane content in the biogas produced. Reactor S-A had a relatively high volatile fatty acid concentration which probably related to the loss of fairly large numbers of methanogenic bacteria (especially during start-up). From day 97 the HRT in all the reactors was shortened from 17 to 10 hours by increasing the feed rate to each fermentation pit. The graph of reactor S-A seemed to follow a slight upwards trend for the last 30 days, while that of reactor S-D was downwards over the same period (**Figure 3.4.2.**), which indicated the inability of reactor S-A in handling higher organic loading

compared to the improved stability of reactor S-D. Reactors S-B and S-C maintained their efficiency in COD removal even with the shortened retention time. The performance of each digester is shown in **Table 3.4.2.** on day 137.

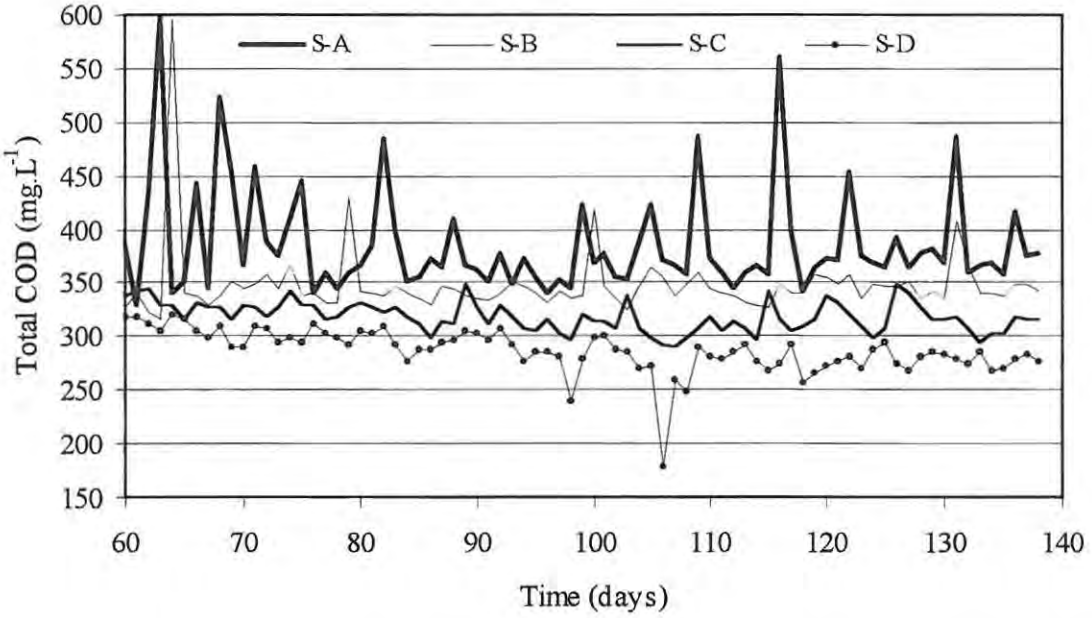


Figure 3.4.2. Performance of each sewage digester relating to the concentration of total COD in the pit overflow stream.

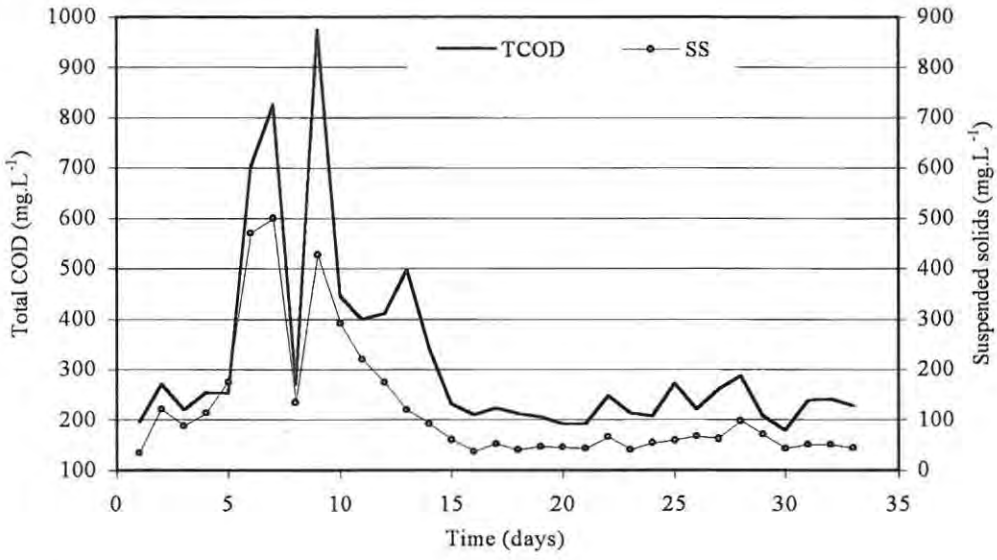


Figure 3.4.3. Comparison between COD and SS levels in S-A overflow during start-up.

Table 3.4.1. Performance of the sewage reactors on day 96. Each reactor operated at HRT = 17 hours, OLR = 1.0 kgCOD.m^{-3}.d^{-1}, V_{up} = 0.55 m.d^{-1}.

Reactor	pH	%$COD_{removal}$	Alkalinity mg $CaCO_3$.L^{-1}	VFA mg.L^{-1}	Biogas L.d^{-1}	%Methane
S-A	7.2	52	460	201	0.265	51
S-B	7.5	53	570	118	0.270	54
S-C	7.5	55	560	114	0.281	56
S-D	7.4	60	570	92	0.306	58

Table 3.4.2. Performance of the sewage reactors on day 137. Each reactor operated at HRT = 10 hours, OLR = 1.7 kgCOD.m^{-3}.d^{-1}, V_{up} = 0.94 m.d^{-1}.

Reactor	pH	%$COD_{removal}$	Alkalinity mg $CaCO_3$.L^{-1}	VFA mg.L^{-1}	Biogas L.d^{-1}	%Methane
S-A	7.3	46	420	208	0.399	48
S-B	7.4	51	490	121	0.442	51
S-C	7.5	55	540	98	0.477	55
S-D	7.5	61	550	76	0.529	59

3.4.2. Winery Wastewater Reactors

The behaviour of the sludge beds in the winery wastewater reactors during the first few weeks of start-up were similar to that observed in the sewage fed reactors, where sludge piston formation occurred. However, in the winery wastewater reactors the anaerobic sludge gradually changed its character by becoming less dense and with smaller flocs observed. The sludge mass appeared to reduce in volume, not from sludge washout, but rather through sludge digestion which resulted in the development of a sludge with a good settling character. As can be seen in **Figure 3.4.4.** the reactors stabilised after about 2 weeks with no more sludge piston formation or biogas build-up observed.

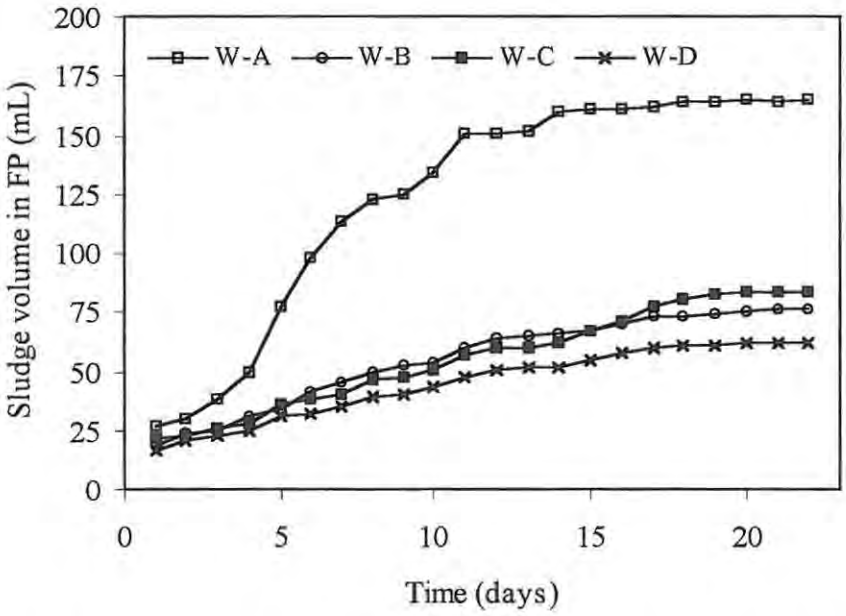


Figure 3.4.4. Overflow sludge accumulation in the facultative ponds of the winery effluent fed reactors, indicating the degree of sludge loss from each fermentation pit (see **Figure 3.3.1.** for design and configuration of reactors).

Reactor W-A showed substantial washout of sludge in the beginning although it retained about 6% more biomass than reactor S-A. This could be attributed in part to the fact that the synthetic winery wastewater contained no suspended solids compared to the sewage feed, and therefore once the sludge bed stabilised there was no continuous build-up of suspended material in the sludge bed.

The nylon nets in both reactors W-C and W-D did contribute to the formation of sludge layers in and on them (similar to S-C and S-D), but when the sludge started to change character these layers disappeared. In this case the nets seemed to be more functional during the first 2 weeks, by trapping the sludge beds and to a large extend preventing it from leaving the fermentation pits. Due to the early disappearance of the second sludge layer in reactor W-C, small sludge flocs rising from the bottom were not prevented from passing through the net and were subsequently washed out. This is probably the reason why from day 16 reactor W-C began to perform slightly worse than W-B. Reactor W-D gave the best performance by retaining 62% more sludge than reactor W-A. It is

interesting to note that reactor W-D retained 65% more sludge than reactor S-A, thereby showing an even better performance than reactor S-D.

Figure 3.4.5. shows the performance of the winery wastewater reactors after the sludge beds has reasonably stabilised. The graph of reactor W-A performance follows an upward trend emphasising the instability of the open fermentation pit design. The gradient is, however, considerably higher than that of S-A indicating that this design might not be very effective in treating a low solid effluent like winery wastewater. In the case of sewage treatment, the settleable solids present in the influent are considered to be beneficial by trapping small suspended anaerobic flocs, thereby preventing large numbers of active methanogens from leaving the anaerobic reactor.

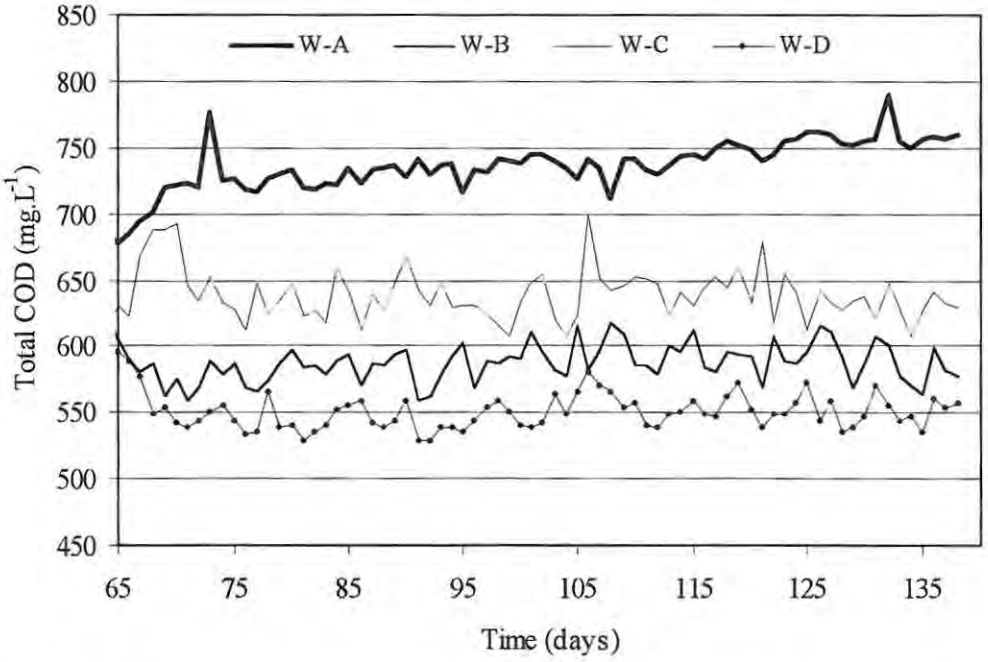


Figure 3.4.5. Performance of the winery wastewater reactors relating to the levels of CODt in each pit overflow stream.

Table 3.4.3. shows the performance of winery wastewater reactors digesters on day 97 when the HRT was still at 17 hours, before shortening it to 10 hours. The difference between reactors W-B, W-C and W-D is not as dramatic as their sewage fed counterparts,

but here too, reactor W-A is showing the least methane content in the biogas which correlated with the high VFA concentration. **Table 3.4.4.** shows the performance characteristics on day 137 where the stability of all the reactors, except W-A, is evident.

Table 3.4.3. Performance of the winery reactors on day 97. Each reactor operated at HRT = 10 hours, OLR = 2.8 kgCOD.m^{-3}.d^{-1}, V_{up} = 0.55 m.d^{-1}.

Reactor	pH	%$COD_{removal}$	Alkalinity mg $CaCO_3$.L^{-1}	VFA mg.L^{-1}	Biogas L.d^{-1}	%Methane
W-A	6.8	63	840	939	0.900	49
W-B	6.9	71	845	713	1.014	52
W-C	7.0	69	840	795	0.985	52
W-D	7.0	72	860	660	1.029	55

Table 3.4.4. Performance of the winery reactors on day 137. Each reactor operated at HRT = 10 hours, OLR = 4.8 kgCOD.m^{-3}.d^{-1}, V_{up} = 0.94 m.d^{-1}.

Reactor	pH	%$COD_{removal}$	Alkalinity mg $CaCO_3$.L^{-1}	VFA mg.L^{-1}	Biogas L.d^{-1}	%Methane
W-A	6.9	62	830	940	1.441	48
W-B	7.0	71	845	730	1.718	53
W-C	6.9	68	835	789	1.624	51
W-D	7.1	72	865	671	1.762	55

3.5. Conclusion

The performance of the facultative pond in AIWPS design may be limited to a degree by the loss of biomass carried out of the fermentation pit during process operation. The evaluation of a modified laboratory-scale fermentation pit that combined the GLSS of a UASB and the insertion of a nylon net across the rising column, proved to be effective interventions for enhancing biomass retention in these systems. The nylon net appeared to be most effective during reactor start-up (or organic overload) by preventing large

flocs of gas entrained sludge from leaving the bottom sludge bed. In treating sewage wastewater (which contains high suspended solids) a second sludge layer developed on the net over time, which prevented smaller particles from rising to the surface.

During winery wastewater treatment the nylon net was mainly functional during reactor start-up and did not develop a second sludge layer due to the nature of the anaerobic sludge. The insertion of such a net of appropriate aperture size in an open fermentation pit could well be a low-cost method for improving the anaerobic system's robustness relating to improved sludge retention during reactor start-up and/or incidences of organic overloading. The net as a novel device in the operation of the FP could therefore also play an important role during the treatment of higher strength wastewaters, such as wine distillery effluent, due to sludge retention in the presence of the increased organic load.

Chapter Four

Tertiary Treatment in the High Rate Algal Pond

4.1. Introduction

The relative high levels of phosphate present in winery and distillery wastewater has already been identified in Chapter 1. The performance of the AIWPS described in Chapter 2 revealed that, among others, inadequate phosphate removal was cause for concern when treating household sewage. The final treated effluent does therefore not comply with the relevant discharge standard regulations. Phosphate levels are at least 15 times higher in wine distillery wastewaters, compared to household sewage (refer to **Tables 1.1. & 3.3.2.**). It is foreseen that inadequate phosphate removal will therefore be even more significant when treating distillery wastewater in a conventional AIWPS.

Eutrophication of freshwaters, particularly due to excessive phosphorus concentrations is an increasing problem, with more than 30% of rivers and lakes in the USA already affected (Hecky and Kilham, 1988). The reduction of inorganic phosphate concentration in wastewater before final discharge is therefore an inevitable preoccupation for modern society, and today still exists (Arnz *et al.*, 2001).

Biological phosphate removal from wastewater can be achieved in two ways: stoichiometric coupling to microbial growth or enhanced storage in the biomass as polyphosphate (poly-P). The latter was formerly called "luxury uptake" (Levin and Shapiro, 1965) and is the key mechanism in the enhanced biological phosphate removal (EBPR) process. The EBPR process is primarily characterised by circulation of activated sludge through anaerobic and aerobic phases, coupled with the introduction of influent wastewater into the anaerobic phase (Barnard, 1975). By this anaerobic-aerobic configuration, micro-organisms which accumulate poly-P, and thus have a high phosphorus content, are selected and grow to dominance in the process. High phosphate removal efficiency can be achieved by withdrawing the excess sludge with high

phosphorus content. Predominance of the polyphosphate accumulating organisms (PAO) can be explained as follows: if an anaerobic phase is introduced in which activated sludge is mixed with the influent wastewater, micro-organisms capable of anaerobically taking up organic carbon sources from the influent are favoured. PAO can do this because they are able to hydrolyse stored poly-P in order to supply energy for the anaerobic uptake of carbon sources. Thus, in the anaerobic phase, PAO take up organic carbon and store it in the form of polyhydroxyalkanoates accompanied by the degradation of poly-P and consequent release of orthophosphate. In the subsequent aerobic phase, PAO grow aerobically and take up orthophosphate to recover the poly-P level by using the stored polyhydroxyalkanoates as the carbon and energy source (Van Loosdrecht *et al.*, 1997). After the aerobic phase a settling phase is introduced where the sludge is divided into return sludge and waste sludge (removal of phosphate in biomass). Since pure cultures that possess complete characteristics of PAO have not been isolated yet, the biochemical mechanism can not be definitely described (Mino *et al.*, 1998). The EBPR process is well established in practice and many full-scale plants are in operation world-wide (Arnz *et al.*, 2001).

Removal of phosphorus from wastewater by chemical addition has found widespread application and the most commonly used chemicals are lime, alum and ferric chloride and they may be added at any suitable point in a treatment plant. The principle of chemical phosphorus removal is to transfer the dissolved orthophosphates into particulate form by producing chemical precipitates of low solubility, which are then commonly removed by solids separation processes such as sedimentation, flotation or filtration (Maurer and Boller, 1999). The quantity of chemical required is determined by the concentration of phosphorus species in the wastewater and the degree of purification required. A number of other factors such as pH, alkalinity, ratio of metal salt to phosphorus, intensity of mixing and the presence of other interfering substances will also affect the actual quantity of chemical required (Thomas *et al.*, 1996). The transfer of dissolved phosphorus species (present as H_3PO_4, $H_2PO_4^-$, HPO_4^{2-}, PO_4^{3-} depending on pH) into particulate form includes three mechanisms: (1) chemical precipitation of metal-hydroxo-complexes of low solubility; (2) selective adsorption of dissolved phosphorus species onto freshly

precipitated metal-hydroxo-complex surfaces; and (3) flocculation and co-precipitation of finely dispersed colloidal matter. The latter mechanism is independent of the phosphorus speciation in the water but depends mainly on size and surface chemical properties of the phosphorus containing colloids. These mechanisms are not independent of each other but take place simultaneously when precipitation chemicals are added to the wastewater.

Biologically mediated chemical precipitation of phosphorus in EBPR plants has previously been reported (Fuhs and Chen, 1975; Arvin, 1983; Mino *et al.*, 1985; Appeldoorn *et al.*, 1992). The relative amount of phosphorus precipitated was found to be as high as 80% and the influence of high pH and high concentrations of calcium was pointed out. The chemical precipitation of phosphorus in conventional biological nutrient removal plants may be mediated in at least two ways: First the elevated P-concentrations created by anaerobic phosphate release from PAO can initiate and accelerate calcium phosphate precipitation. Secondly, biological denitrification in fixed biofilms and possibly also in bacterial flocs can lead to phosphate precipitation due to the elevated pH-conditions inside the biofilms (Arvin and Kristensen, 1979). In both cases the precipitation conditions must be generally favourable, i.e. the calcium concentration should be reasonably high, roughly above 50 $mg.L^{-1}$, and the concentration of precipitation inhibitors low; magnesium, pyrophosphates and bicarbonate (alkalinity). It is also essential that the pH is relatively high, preferably above 7.5. Struvite, or magnesium ammonium phosphate hexahydrate, is a mineral that often precipitates from wastewater during anaerobic biological treatment of hog wastes (Maqueda *et al.*, 1994), poultry wastes (Manninen *et al.*, 1989), wine distillery effluents (Lowenthal *et al.*, 1998), and biosolids from biological phosphorus removal processes (Fujimoto *et al.*, 1991).

The concentration of orthophosphate may depend on the content of calcium in calcium rich waters (Hepher, 1958; Golterman and Meyer, 1985) including lime-treated wastewaters (Banister *et al.*, 1998). It therefore appears that the equilibrium between the orthophosphate and the solid phase determines the concentration of phosphate in solution. Although the relationship between phosphate and calcium is unquestionable, different authors disagree on the composition of the solid phase (Arvin and Kristensen, 1979;

Golterman and Meyer, 1985; House, 1990); it seems that apatite, $Ca_5(PO_4)_3OH$, amorphous tricalcium phosphate, $Ca_3(PO_4)_2$, brushite, $CaHPO_4 \cdot 2H_2O$, as well as octacalcium phosphate, $Ca_4H(PO_4)_3$, may determine the equilibrium phosphate concentration.

Hartley *et al.* (1997) reported the co-precipitation of phosphate with calcite in the presence of photosynthesising green algae where the algae played a central role in raising the pH and initiating the precipitation reaction. In a solution containing calcium bicarbonate the rising pH, due to algal uptake of dissolved CO_2, resulted in the precipitation of calcite and during this process orthophosphate is incorporated in the calcite crystals. The precipitation of calcium phosphate minerals in a high rate algal pond was reported by Moutin *et al.* (1992), although phosphorus removal was only 40% with more than 4 $mg.L^{-1}$ dissolved phosphorus remaining in the treated wastewater. In literature two possible reaction mechanisms for calcium phosphate precipitation are proposed. Firstly, the co-precipitation of inorganic phosphorus with calcium carbonate (calcite) which is a phenomenon that occurs naturally in hardwater lakes. According to Hartley *at al.* (1997) calcite starts precipitating from a solution containing calcium bicarbonate at pH of >8.8, which causes the production of carbon dioxide:

$$Ca^{2+} + 2(HCO_3^-) = CaCO_3\,(solid) + H_2O + CO_2$$

In abiotic experiments there was a decrease in pH during the precipitation reaction which was due to CO_2 release, however, in the presence of algae the process of photosynthesis constantly utilised CO_2 produced during calcite precipitation. It is believed that phosphate first absorbs to the calcite crystal surface and then a fraction of this becomes incorporated into the crystal at active kink sites during crystal growth (House and Donaldson, 1986; House, 1990).

Secondly, the removal of calcium and phosphate from slightly alkaline wastewater can be approximately represented as the precipitation of calcium hydroxyapatite (HAP):

$$5Ca^{2+} + 3PO_4^{3-} + OH^- = Ca_5(PO_4)_3OH \text{ (solid)}$$

Although HAP is the thermodynamically stable state, the phosphate concentration is determined by the solubility of the amorphous tricalcium phosphate [$Ca_3(PO_4)_2$], which was confirmed by the calculation of the theoretical predicted solubility as well as various experiments by Moutin *et al.* (1992).

The development of the algal turf scrubber for phosphorus removal from secondary treated wastewater resulted in effective removal of phosphate through combined biological and chemical precipitation induced by a microalgal colony growing on an inclined flow way (Craggs *et al.*, 1996).

Against the above literature background it was hypothesised that the HRAP can be engineered as a free-standing tertiary unit operation for the removal of phosphates from conventionally treated sewage wastewaters.

4.2. Research Objectives

To evaluate if phosphate removal in the HRAP can be optimised to investigate its use as a free-standing tertiary treatment operation, with simultaneous removal of residual ammonium nitrogen through stripping.

4.3. Materials and Methods

4.3.1. Pilot Plant

The independent high rate algal pond (I-HRAP) used for piloting was a 500 m^2 and 30 cm deep pond, which could treat final effluent from either the conventional Grahamstown Disposal Works sewage treatment plant (GDW-final) or from the AIWPS (AIWPS-final) as described in Chapter 2. The hydraulic residence time in I-HRAP was kept at four days with an influent of 37.5 $m^3.d^{-1}$.

4.3.2. Laboratory Experiments

The culture apparatus used for the algal-mediated experiments consisted of a 1-litre cylindrical glass vessel with two ports (**Figure 4.3.1.**). A pH electrode connected to a Cyberscan 2500 pH meter was inserted through one of the ports and the remaining port was used for sampling or the addition of algal culture. The culture apparatus was surrounded by a wooden shield with 6 fluorescent tubes, giving a total photosynthetical available radiation (PAR) of 158 $\mu mol.m^{-2}.s^{-1}$, as energy source for photosynthesis. A magnetic stirrer and follower stirred the solution. Precipitation of calcium phosphate was investigated using fresh algal biomass from I-HRAP and washing the algae with distilled water. Each batch precipitation experiment was repeated at least three times, temperature 25°C, and chlorophyll-a concentrations in the glass vessel were similar to that of the I-HRAP medium.

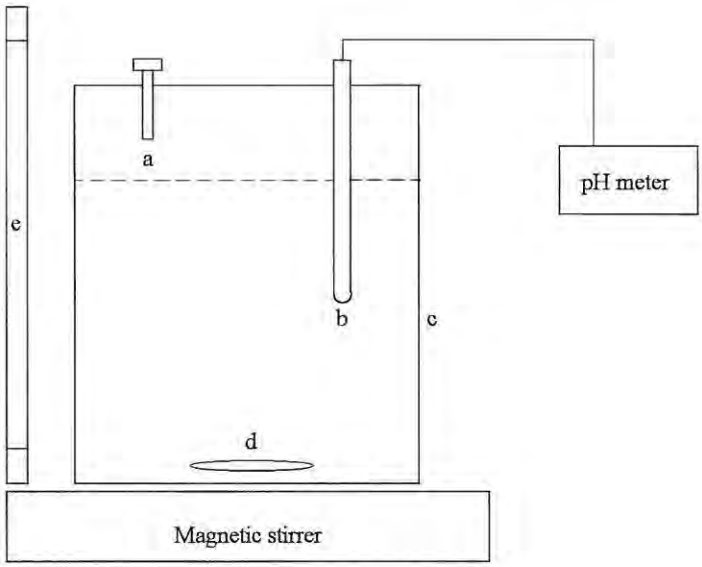


Figure 4.3.1. Culture apparatus used for algal mediated experiments with the following components: (a) small sampling port; (b) pH electrode; (c) 1-litre cylindrical glass vessel; (d) magnetic follower; (e) fluorescent tube x 6.

4.3.3. Analytical Methods

Analysis of all parameters was undertaken as previously described in Chapter 2.

4.4. Results and Discussion

4.4.1. Evaluation of I-HRAP for phosphate and ammonia removal

Observations on the continuous operation of the 500 m^2 I-HRAP receiving GDW-final as influent are shown in **Figure 4.4.1.** The removal of soluble phosphate was about 90% resulting in effective levels of less than 1 $mgP.L^{-1}$ being achieved in the algal pond. **Table 4.4.1.** summarises the I-HRAP performance, where the loss of phosphate by mineralisation with calcium is in accordance with that of the flask studies. Effective ammonia removal of 75% was probably due to stripping into the atmosphere due to the high pH and aerobic environment. When the water pH is very high the majority of the ammoniacal nitrogen is in the form of NH_3 which will be lost to the atmosphere at a high rate when a large exchange surface area is provided (Konig *et al.*, 1987). Although the I-ASP overflow had an average ammonia concentration of 1.12 $mgN.L^{-1}$ the total dissolved nitrogen level was more than 16 $mgN.L^{-1}$, which was due to the relatively high nitrate level present in the I-HRAP influent.

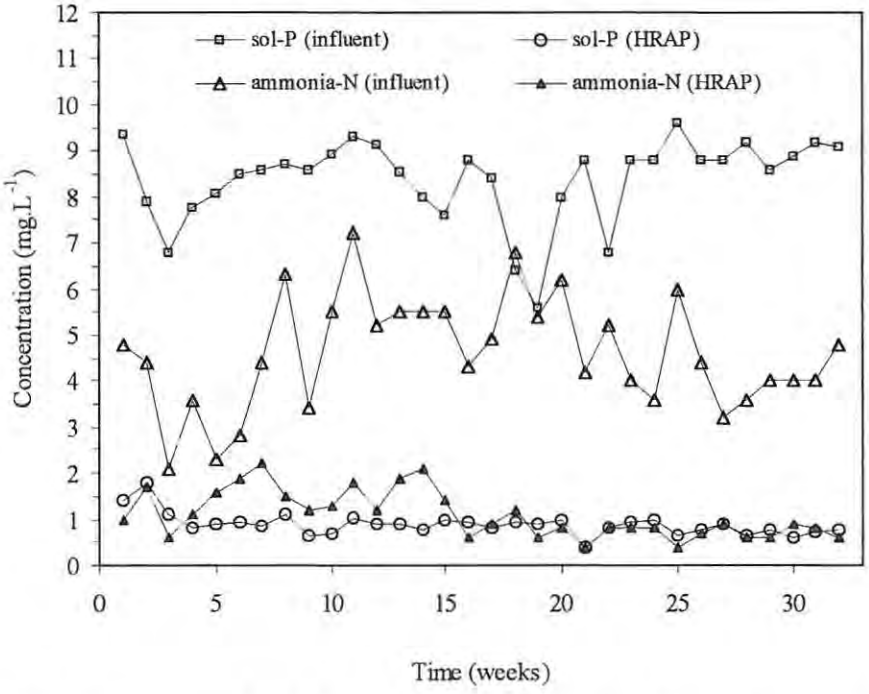


Figure 4.4.1. Treatment of GDW-final in I-HRAP over a 32-week period, indicating the effective removal of phosphate and ammonia.

Table 4.4.1. Performance of I-HRAP during treatment of GDW-final.

Parameter		Influent		I-HRAP		I-ASP *	
pH		7.2	(0.2)	10.5	(0.6)	10.4	(0.7)
sol-P	$mgP.L^{-1}$	8.39	(0.91)	0.88	(0.25)	0.92	(0.24)
NH_3	$mgN.L^{-1}$	4.6	(1.22)	1.09	(0.51)	1.12	(0.52)
NO_3	$mgN.L^{-1}$	14.7	(1.92)	15	(1.7)	15.1	(1.6)
Calcium	$mg.L^{-1}$	29	(2)	17	(1)	17	(1)
SCOD	$mg.L^{-1}$	60	(16)	54	(14)	55	(15)
TCOD	$mg.L^{-1}$	88	(17)	335	(21)	59	(32)
$Chlorophyll_a$	$\mu g.L^{-1}$	–	–	478	(120)	48	(29)

*ASP overflow results shown for period week 23-32.
Note standard deviation in brackets.

During this 32-week period different procedures for harvesting of settled algal biomass from the I-ASP were performed and the release of phosphate in the I-ASP into the final discharge is evident in **Figure 4.4.2.**

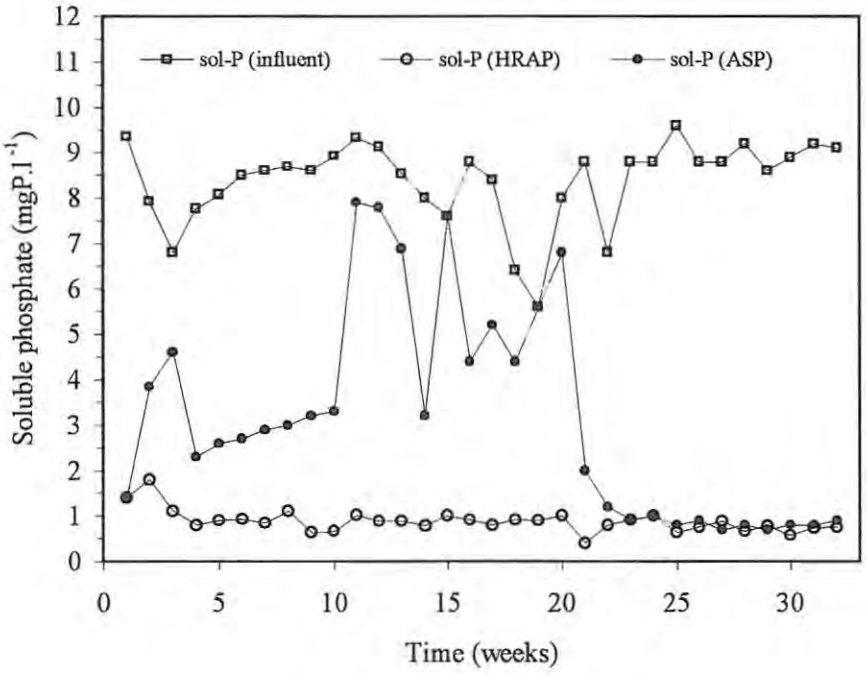


Figure 4.4.2. Release of phosphate from I-ASP during different biomass harvesting procedures.

Week 1 to 10:

During this period the I-ASP was cleaned weekly by withdrawing the slurry from the bottom without first decanting the supernatant. This procedure seemed to result in a degree of mixing between the bottom phase (where P-release took place) and the phosphate free I-ASP overflow.

Week 11 to 20:

From week 11 daily recycling of algal biomass from the I-ASP was initiated in an attempt to increase the chlorophyll concentration in the I-HRAP for possible enhanced phosphate removal. During this period algal biomass remained in the system with no harvesting taking place. This operating procedure did not have a measurable effect on the phosphate level inside the I-HRAP, however, it resulted in a higher degree of P-contamination of the I-ASP overflow. The slurry pump probably did not remove all the biomass during each cycle and again mixing between the bottom and surface took place. Flask studies (reported below) showed that under the current four day hydraulic retention time in the I-HRAP the algal biomass concentration was not the limiting factor for phosphate removal, but rather the low calcium level. The addition of ± 5 $mgCa.L^{-1}$ in the form of calcium chloride resulted in further calcium phosphate precipitation to achieve a level of less than 0.5 $mgP.L^{-1}$.

Week 21 to 32:

Daily biomass recycling stopped and the method for harvesting returned to the standard operating procedure, where the I-ASP is cleaned properly on a weekly basis while preventing phosphorus contamination of the final discharge. The standard procedure for settled biomass removal is to first decant the supernatant liquid down to the concentrate and then remove the concentrate with a slurry pump.

I-HRAP was also evaluated for its performance over a 24-hour period during week 24 and the results are shown in **Figure 4.4.3.**, during which time the influent characteristics were very similar to the average values shown in **Table 4.4.1.** Note that for the time of the year, of which this study was undertaken, sunrise is at about 06:00 and sunset at

19:00. In **Figure 4.4.3.(a)** the graphs for dissolved oxygen (DO), temperature and pH follow the same profile, where DO and pH appears to be directly linked to algal photosynthetic activity.

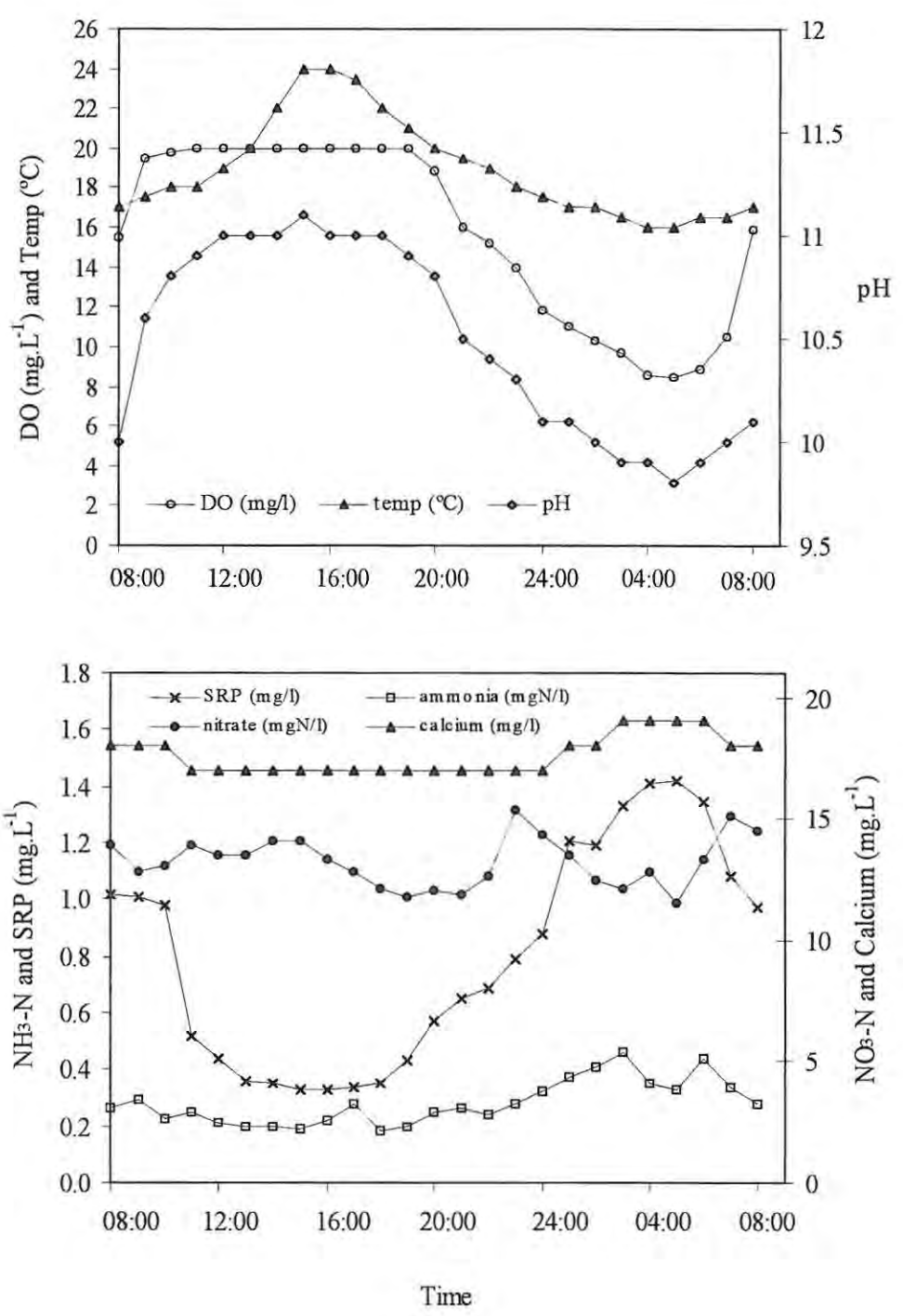


Figure 4.4.3. The I-HRAP performance monitored over a 24-hour period.

In the idealised photosynthetic equation

$$6CO_2 + 12H_2O \xrightarrow[\text{Algae}]{\text{light}} 6(CH_2O) + 6O_2 + 6H_2O$$

CH_2O is regarded as the organic matter fixed in algal material and O_2 on the right side of the equation has been shown to come entirely from the water on the left hand side (Rabinovitch and Govindjee, 1969). The algae are primarily dependant on dissolved carbon dioxide to fill their photosynthetic carbon requirements. In waste treatment the major source of inorganic carbon for algal growth is the organic carbon in the waste, which must first be released as CO_2 by bacteria decomposing the waste. Another source is the bicarbonate ion, HCO_3^-, from which most algae have a mechanism for extracting CO_2. Another source is CO_2 that can be absorbed from the air and at a pH of 8 the rate of absorption is about 10^{-11} $mol.atm^{-1}.cm^{-2}.s^{-1}$. But at a pH of 10 the rate is 100 times as great (Oswald, 1994). Given the low COD (consisting mainly of slowly biodegradable organics) in the I-HRAP influent, it is believed that the source of CO_2 is from atmospheric absorption. Most algae have an inorganic carbon assimilation mechanism involving the enzyme, carbonic anhydrase. The HCO_3^- is converted to carbon dioxide, which is internalised and incorporated into the photosynthetic pathway, and hydroxide ions remain in the medium. The hydrogen ions generated are removed from the solution, it is believed, by accumulation into the algal cells. The increase in pH of the I-HRAP medium during the day is therefore mainly due to CO_2 uptake including the generation hydroxyl ions.

During night-time respiration occurs which is basically the reverse of photosynthesis, where dissolved oxygen is absorbed and carbon dioxide released by the algal cells and thereby causing lowering of the pH. From **Figure 4.4.3.(b)** it is evident that soluble phosphorus removal from the I-HRAP medium is highest when the algal photosynthetic activity is at its maximum during daytime, and the opposite for night-time. Again, the correlation between calcium and phosphate levels reflects the laboratory results. Ammonia stripping was very effective at these high pH levels, but the change in nitrate concentration was minimal under these conditions, where the I-HRAP medium never reached anoxic conditions for denitrification to occur.

4.4.2. Calcium phosphate precipitation

The removal of phosphate from GDW-final in the presence of green algae was evaluated first. The induction period before the onset of precipitation lasted for more than 2 hours, during which time there was a steady rise in pH from the starting pH of 7.8 to 9.4 (**Figure 4.4.4.**). This rise in pH was due to the microalgal photosynthetic activity, as it did not occur in the absence of algae or when the algae were maintained in darkness. The correlation between phosphate and calcium levels is clear with no change in the phosphate concentration observed prior to precipitation.

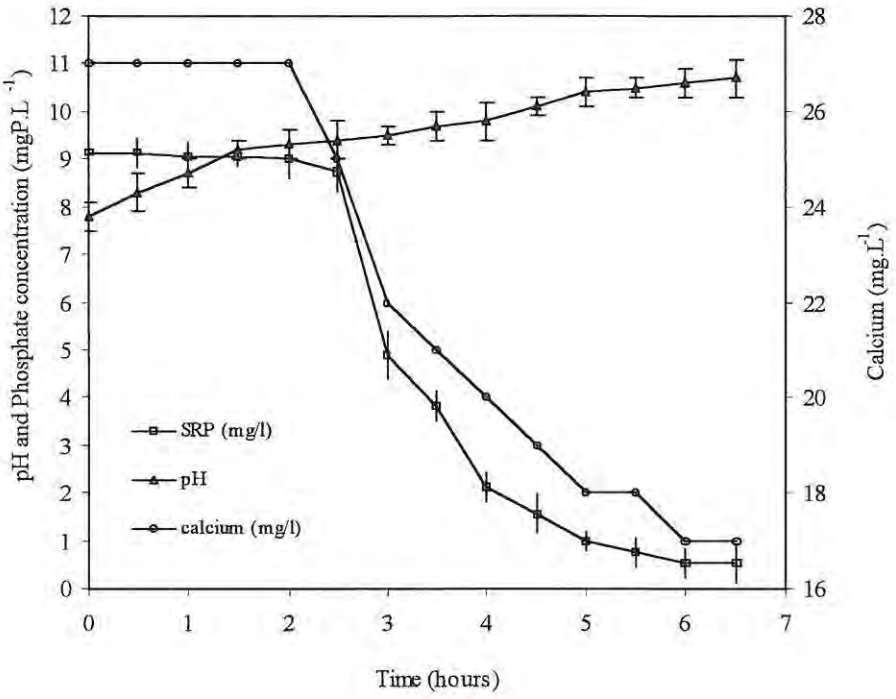


Figure 4.4.4. Precipitation of calcium phosphate minerals from GDW-final in the presence of photosynthesising algae.

It appears as if the mechanism for calcium-bound removal is regulated not only by the pH and various other ionic species, but more importantly by the ratio of calcium to dissolved phosphorus (Ca/P). In the case of calcite co-precipitation the Ca/P molar ratios before the onset of precipitation was in excess of 525, where the phosphate concentration (< 0.3 $mgP.L^{-1}$) was low enough not to inhibit calcite crystal formation. According to Hartley *et*

al. (1997) soluble reactive phosphorus concentrations near 0.33 $mgP.L^{-1}$ caused inhibition of calcite precipitation in abiotic conditions. In the case of tricalcium phosphate (TCP) formation the Ca/P molar ratios before the onset of precipitation was not more than 20, which ruled out the formation of calcite due to the high phosphate inhibition factor. Precipitation of magnesium phosphate and especially struvite ($MgNH_4PO_4$) was not considered in this study because the ratio of Mg/Ca is about 0.2 in GDW-final, which is expected to rather favour calcium phosphate phase precipitation (Abbona *et al.*, 1986). Also the Ca/P for GDW-final is 3.5 which suggests that the formation of HAP and/or TCP is the most probable mechanism involved in phosphate removal from this type of wastewater.

Precipitation of ionic crystals from solution is notoriously difficult to describe rigorously. However, many such reactions follow a general pattern where a period of very slow precipitation or reactant removal is followed first by rapid removal and then by further slow removal as the reactant concentration approaches an equilibrium value (Ferguson *et al.*, 1973). The same general pattern is followed in **Figure 4.4.4.** where the graphs of calcium and soluble reactive phosphorus seems to indicate that a precipitation mechanism is indeed involved. During the precipitation reaction, the culture medium cleared as the algal cells settled to the bottom of the flask. This was demonstrated by a rapid decrease in suspended chlorophyll-a concentration before and after precipitation (from 413 to 126 $\mu g.L^{-1}$). The algal cells self-flocculated in a manner similar to that described by Koschel *et al.* (1983), in studies of algal assemblages in Lake Breiter Lucin. It is likely that precipitation occurs on or in close vicinity of the algal cells where the highest pH gradients are expected (Hartley *et al.*, 1996). **Table 4.4.2.** shows the fractionation of phosphorus in a mixed sample from the same experimental apparatus, which indicates that the majority of phosphorus is indeed in the chemical precipitate form.

Table 4.4.2. The fractionation of phosphorus after calcium phosphate mineralisation.

Parameter	Phosphorus ($mgP.L^{-1}$)	Percentage
Soluble reactive phosphorus	0.62	5.5
Iron-bound phophates	0.02	0.2
Calcium-bound phosphates	9.81	87.3
Organic phosphates	0.79	7
Total phosphorus	11.24	-

Iron-bound phosphate levels were very low which would be expected as sewage contains relatively low concentrations of iron. The organic phosphate fraction is probably incorporated with the algal biomass present in the culture medium. A small fraction still remains in the soluble form (5.5% of total P), which was expected given the presence of slowly biodegradable organic compounds (CODs 50-60 $mg.L^{-1}$). Organic compounds such as humic, fulvic and tannic acids are known inhibitors of calcium hydroxyapatite precipitation (Inskeep and Silvertooth, 1988). When the medium was acidified with dilute hydrochloric acid the release of phosphate coincided with a rise in calcium concentration (**Figure 4.4.5.**) similar to what it was before the precipitation experiment, thereby emphasising the pH-dependence of the reaction mechanism.

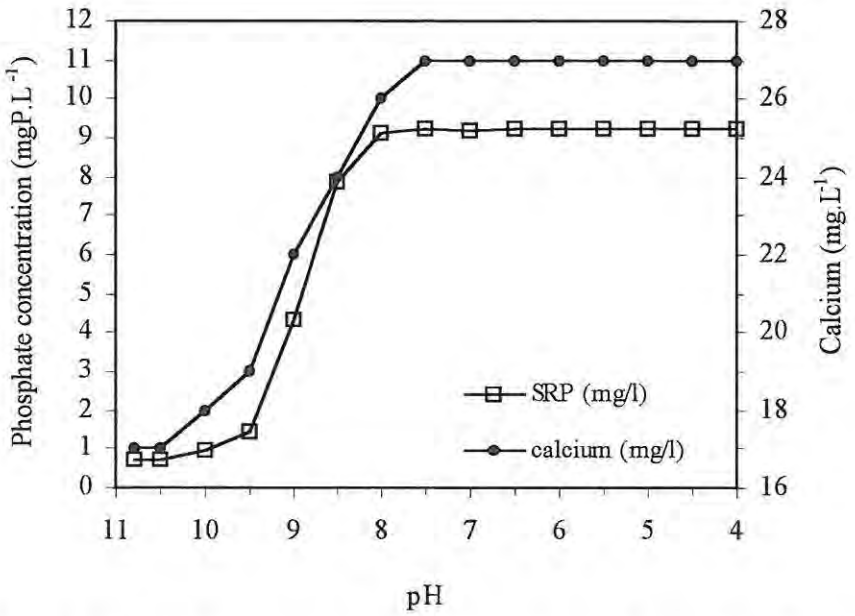


Figure 4.4.5. The release of chemically bound phosphate during the addition of acid, showing the relationship between pH and soluble reactive phosphorus concentration.

4.5. Conclusion

This research has demonstrated that it is possible to achieve average phosphate residuals of below 1 $mgP.L^{-1}$ by calcium phosphate precipitation at a pH of above 10. The high rate algal pond can be used as a free-standing unit operation where, by photosynthetic activity, the algae play a central role by maintaining a high pH and thereby not only induce calcium phosphate precipitation, but also aid in the removal of ammonia through stripping. In the case where calcium concentration is the limiting factor dosing with small amounts of lime and/or calcium chloride at the HRAP influent might be required.

During harvesting of the settled algal biomass in the following algal settling pond, either for drying or recycling purposes, care should be taken in applying the correct harvesting procedure. This would prevent the dissolution of calcium phosphate in the low pH environment of the anoxic concentrated algal sludge, generated by CO_2 release from sludge respiration.

The process offers considerable hope for savings in the chemical and capital costs associated with phosphate removal because of the possibility of utilising passive algal photosynthesis in a low operational cost HRAP. This process could potentially be applied for treatment of final discharge waters emanating from a sewage treatment works, or indeed other treatment systems, such as for wine industry wastewater. Denitrification is not achieved in the HRAP and will be discussed in the following chapter.

Chapter Five

Denitrification using Algal Biomass as the Carbon Source

5.1. Introduction

5.1.1. Background

Nitrate contamination of groundwater has been documented world-wide (Delwiche and Bryan, 1976; Brezonik, 1977; Focht and Verstraete, 1977). Because ingestion of high levels of nitrate may cause negative effects on human health, such as infant methemoglobinemia (blue baby syndrome), efficient and economical removal processes are needed. Three methods show some potential for full-scale application: ion exchange, reverse osmosis and biological denitrification (Mateju *et al.*, 1992). Ion exchange is limited by two problems. The first is that a resin of high selectivity for nitrates over ions that are commonly present in water would be required. The second problem involves providing an adequate resin regenerant, such that regenerant disposal does not itself become a problem. The problem with reverse osmosis is that the membranes used generally do not exhibit high selectivity for nitrates, as the removal of multivalent ions is preferred. One of the most promising and versatile approaches being studied is biological denitrification. This process has been used for years in wastewater treatment (Clayton *et al.*, 1991; Borregaard, 1997; Semon *et al.*, 1997; Bailey *et al.*, 1998). Biological denitrification is highly selective for nitrate removal with very high efficiency, often reaching nearly 100%. The potential bacterial contamination of treated water is the main disadvantage and subsequent treatment and disinfection are required.

5.1.2. Microbiology

Denitrification is the reduction of nitrate to nitrogen gas and this process occurs in four steps (Brezonik, 1977):

$$NO_3^- \longrightarrow NO_2^- \longrightarrow NO \longrightarrow N_2O \longrightarrow N_2$$

nitrate nitrite nitric oxide nitrous oxide nitrogen gas

Each step is catalysed by an enzyme reductase system. Denitrification requires an electron donor which can be organic material or reduced compounds such as sulphide or hydrogen. Heterotrophic denitrifying bacteria require an organic carbon source for respiration and growth. A variety of organic compounds has been used, such as methanol (Borregaard, 1997) and formate (Soares *et al.*, 1991) as well as different industrial wastes including fusel oil (Klapwijk *et al.*, 1981) and primary sewage sludge (Moser-Engeler *et al.*, 1998). Numerous species of facultative denitrifying bacteria, including *Pseudomonas*, *Micrococcus*, *Achromobacter* and *Bacillus* are capable of converting nitrate to nitrogen gas. Nitrate replaces oxygen in the respiratory process of the organisms capable of denitrifying under anoxic conditions. Autotrophic denitrifying bacteria utilise hydrogen or sulphur as electron donors, and carbon dioxide or bicarbonate is used as a carbon source for microbial cell synthesis (Anderson and Levine, 1986; Flere and Zhang, 1998). Generally, autotrophic denitrifiers grow slowly and denitrification rates are lower, whereas contamination of denitrified water with organic materials requires extensive post-treatment in the heterotrophic processes. Most full-scale applications make use of heterotrophic processes due to their efficiency, high specific denitrification rate and operational simplicity.

5.1.3. Biological denitrification processes

Biological upflow fluidised bed (BFB) technology has been used since the 1970s for industrial waste treatment and more recently for denitrification of contaminated groundwater and municipal waste (Sutton and Mishra, 1990). The BFB technology has the potential to remove large quantities of nitrogen with relatively small space requirements, which is of particular importance in densely populated cities with limited space for expansion. Semon *et al.* (1997) described the results of a BFB with sand media that was operated at a flow rate of 1 296 $m^3.d^{-1}$, and an average loading rate of 1 843 $kgNO_3.m^3.d^{-1}$, treating final effluent from a conventional activated sludge treatment

facility. Approximately 3 mg methanol per mg of influent nitrate was used as external carbon source. Denitrification in the reactor was very rapid resulting in average effluent nitrate levels of 0.4 $mg.L^{-1}$ (influent 7.7 $mg.L^{-1}$) with reaction times of less than 5 minutes. Borregaard (1997) reported another high rate process where a combination of nitrification-denitrification was achieved in a fixed-film system utilising methanol as carbon source. The Biostyr unit has polystyrene granules which offer a high specific surface area and are therefore very compact. This process removed at least 70% of total nitrogen in order to comply with Danish final discharge standards.

The sequential batch reactor (SBR) can be modified to provide secondary, advanced secondary treatment, nitrification, denitrification and biological nutrient removal. An SBR treatment cycle consists of timed sequences which typically includes the following steps: fill, react, settle, decant, idle (Arora *et al.*, 1985). When biological nutrient removal is desired, the steps in the cycle are adjusted to provide anoxic or anaerobic periods within the standard cycles. Surampalli *et al.* (1997) reported complete nutrient removal using a SBR that followed a specific sequence: Aerated fill (COD removal, nitrification, phosphorus uptake), react (COD removal, nitrification, phosphorus uptake), settle (waste P-containing sludge), idle (denitrification, growth of P-removing bacteria). Biological dephosphatation by activated sludge under denitrifying conditions has been optimised for a full-scale activated sludge treatment plant, where denitrifying phosphorus removing bacteria (DPB) were cultivated in an anaerobic-anoxic SBR (Kuba *et al.*, 1997). A problem with the conventional system is the competition for COD between phosphorus and nitrate removing organisms, since organic substances in municipal wastewater are often limiting. After determining the culture conditions for DPB it was clearly shown that 50% of the phosphorus removal occurs via denitrifying activities, resulting in less competition for organic substrate.

Semi-passive treatment of wastewater to achieve denitrification in the form of subsurface flow constructed wetlands has also been reported, where hydroperiod manipulation and vegetation presence/absence in two-stage treatment systems were applied (Kemp and George, 1997).

5.1.4. Extracellular polymeric substances for carbon source

Changes in the composition of extracellular polymeric substances in activated sludge during anaerobic storage have been reported due to microbial degradation (Nielsen *et al.*, 1996). The results showed that a fast decrease in total sludge protein and carbohydrate took place within 3 days of anaerobic storage as a result of degradation processes, which accounted for approximately 20% of the organic fraction. Stress production of extracellular polymeric substances by microalgae and cyanobacteria is known to respond to changes in several external factors, such as nitrogen concentration, irradiance or temperature (Arad *et al.*, 1992; Moreno *et al.*, 1998) where carbohydrate and protein are found to be the major components (Flaibani *et al.*, 1989).

In the previous chapter algae were shown to play a central role in biologically pH-induced precipitation of calcium phosphate. This chapter now links the production and release of algal extracellular polymeric substances with nitrate removal under anoxic conditions with the utilisation of these substances as carbon source, followed by algal mediated phosphorus removal.

5.2. Research objectives

The evaluation of harvested algal biomass from I-HRAP treating sewage waters (with subsequent application to wine industry wastewaters) for:

1. Stress induced release of extracellular polymeric substances and the utilisation thereof as carbon source for biological denitrification, and

2. Subsequent removal of the released phosphate and ammonia under photosynthetic conditions.

5.3. Materials and Methods

5.3.1 Laboratory experiments

Batch denitrification using algal biomass was conducted in a covered 2-litre flask at 25°C while mixing with a magnetic follower (**Figure 5.3.1.**). Fresh algal biomass was harvested from I-HRAP and concentrated to give a final total COD value of about 2000 $mg.L^{-1}$. Nitrate was supplemented in the form of KNO_3 to give a final concentration of 20 $mgN.L^{-1}$. Samples were taken every 12 hours and analysed for nutrient levels and polysaccharides (with the addition of KNO_3 when NO_3-N<1$mg.L^{-1}$).

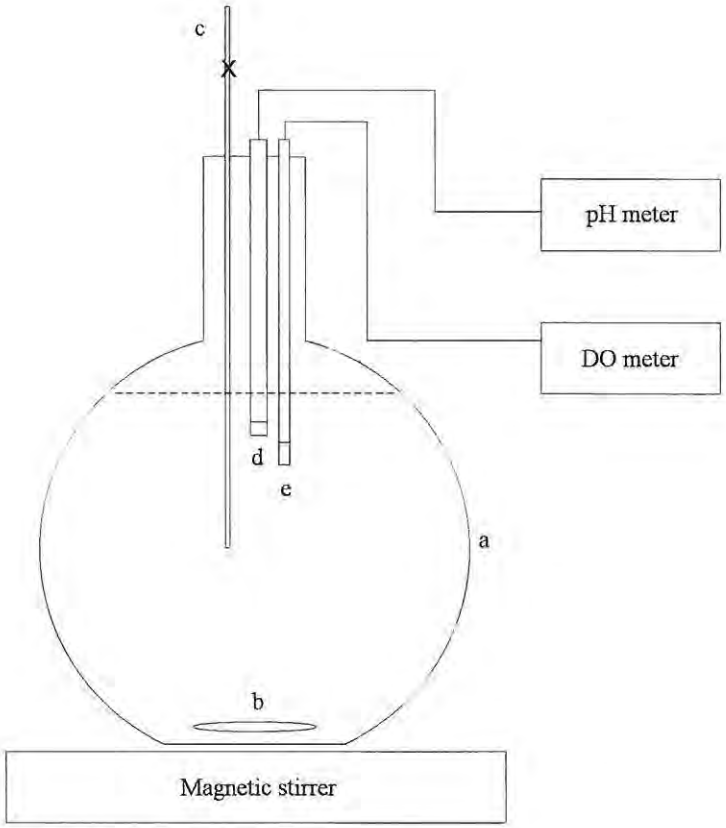


Figure 5.3.1. Experimental apparatus used for batch denitrification. (a) Foil covered glass flask (b) magnetic follower (c) sampling port (d) pH-electrode (e) DO-electrode.

Continuous denitrification was conducted in a 7.4 litre covered upflow glass column with a height of 1.17 m and internal diameter of 9.0 cm (**Figure 5.3.2.**). The reactor was seeded with 5 litres concentrated algal biomass (COD 37 500 $mg.l^{-1}$, chlorophyll-a 53.8 $mg.L^{-1}$) obtained from I-HRAP and the feed consisted of settled I-HRAP medium with KNO_3 supplementation to a final concentration of 20 $mgNO_3\text{-}N.L^{-1}$. The NO_x-N mass balance was determined by setting up another smaller column (volume 540 mL) that operated in parallel to the 7.4 L column under the same conditions. Gas produced in the reactor was collected in a gas meter filled with dilute hydrochloric acid solution. Both systems were operated in a temperature controlled room at 25 ± 2 °C.

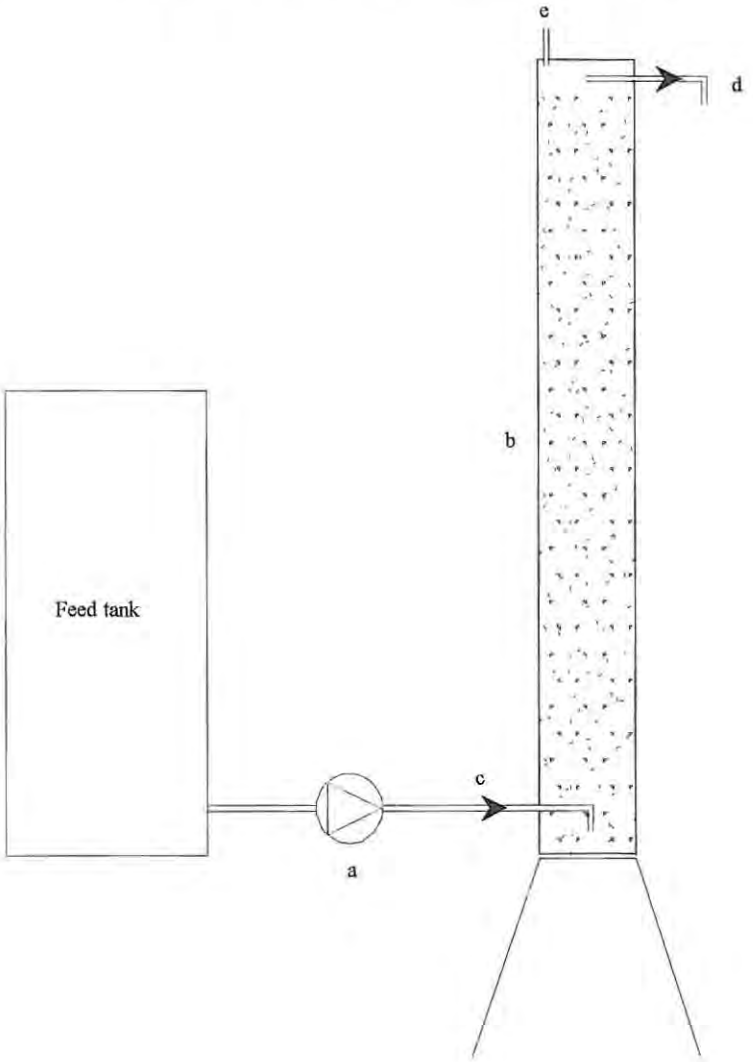


Figure 5.3.2. Upflow anoxic column with settled algal biomass. (a) Peristaltic pump (b) covered cylindrical glass column (c) influent (d) overflow (e) gas vent.

5.3.2. Analytical methods

Analysis of all parameters was undertaken as previously described in Chapter 2.

Biogas production was measured by acid solution displacement and the gas content was determined by gas chromatography using a thermal conductivity detector. Total polysaccharides (TPS) in the cultures and exocellular polysaccharides (EPS) in the supernatants, resulting from culture centrifugation at 5 000 g for 15 min, were determined by the phenol-sulphuric method (Dubois *et al.*, 1956). Capsular polysaccharides (CPS) was determined similar to EPS, but after stirring the sample in distilled water at 50°C for 30 min (Vincenzini *et al.*, 1990). The Lowry method (Lowry *et al.*, 1951) was applied for protein determination.

5.4. Results and Discussion

5.4.1. Nutrient release in algal settling ponds

A depth profile in the ASP at the AIWPS demonstration plant in Grahamstown was undertaken in order to monitor nutrient release and nitrate removal at the bottom of these ponds. The results are shown in **Table 5.4.1.** where the "middle samples" were taken just above each algal sludge bed after each collected biomass over a period of one week.

Table 5.4.1. Depth profile in each algal settling pond showing the correlation between phosphate and calcium levels. Note that ASP follows HRAP, and I-ASP follows I-HRAP.

		ASP			I-ASP		
Parameter		Surface	Middle	Bottom	Surface	Middle	Bottom
pH		8.7	7.6	5.7	10.4	8.9	8.3
sol-P	$mgP.L^{-1}$	3.9	10.1	58	0.91	1.21	9.73
Calcium	$mg.L^{-1}$	26	27	27	18	18	32
NH_3	$mgN.L^{-1}$	3.04	10.2	18.3	0.54	1.77	4.98
NO_3	$mgN.L^{-1}$	5.3	0.4	0.1	14.3	10.7	1.2

Note that HRAP has been treating the overflow from the facultative pond and I-HRAP received GDW-final for phosphate removal. For I-ASP there was a correlation between the phosphate and calcium concentrations, but this time the calcium phosphate precipitate seemed to be re-solubilising due to a decrease in pH, which is in agreement with results from Chapter 4. Fermentative phosphorus release is unlikely because green microalgae are known to degrade very slow under anaerobic conditions. The P-release by PAO is also unlikely due to the operation of I-HRAP, which did not meet the requirements for PAO cultivation according to Mino *et al.*, 1998. Even more nutrients were release from ASP than I-ASP, possibly due to a higher sludge volume accumulated. There is no evidence to suggest that the released phosphate was from chemical origin as there was no simultaneous increase in calcium concentration. It is quite possible that PAO were present in large numbers, for the operation of the AIWPS is such that it does include recycling within the facultative pond as well as from HRAP to the facultative pond. Under these circumstances PAO are likely to accumulate and release phosphate in an anoxic environment when there is organic substrate available in the form of fermentative products. The fact that nitrate removal and ammonification is taking place in the algal sludge beds suggests that fermentative products are indeed available for denitrification and/or biological phosphorus release. When performing harvesting and drying of the algal biomass care should be taken to collect the drainage from the drying bed, which may be returned to I-HRAP for removing released NH_3 and PO_4.

5.4.2. Nitrate removal using algal biomass as reagent

I-HRAP can be considered as a reactor in which photosynthetic carbon production is maximised. Influent COD concentrations were low (< 90 $mgCOD.L^{-1}$) and consisted mainly of recalcitrant organic compounds (after primary and secondary wastewater treatment). It is known that carbon dioxide will dissolve in high alkalinity water, which in turn will be assimilated by the algal biomass present. When an excess of carbon flux occurs in the response to a deviation from optimal growth conditions, green microalgae often will accumulate internally stored carbon in the form of starch (Preiss and Romeo, 1989; De Philippis *et al.*, 1992). Under stress conditions (for example, in conditions of

nitrogen starvation) EPS release could be stimulated, which might serve as carbon source for denitrification organisms. Nitrate removal in the bottom of both ASP and I-ASP indicate that easily biodegradable carbon is available to denitrifying organisms. When algal biomass (containing also cyanobacteria) was harvested from I-HRAP and submitted to anoxic dark conditions, polysaccharide production and subsequent release was stimulated. **Figure 5.4.2.(a)** indicates the correlation between total COD and denitrification. The differentiation into four phases with respect to nitrate removal are described below:

Day 0 to 2:

Although the system turned anoxic within 2 hours (dissolved oxygen from 14.5 to < 0.2 $mg.L^{-1}$) it is generally assumed that the nitrate and nitrogen oxides are repressed by O_2. When this gas is removed, the reductase enzymes are depressed within a period of 40 minutes to 3 hours (Payne *et al.*, 1971; Baumann *et al.*, 1996) Also, the rate of nitrate reduction is possibly to a degree dependant on the rate of EPS hydrolysis into more easily accessible products. During the first 24 hours polysaccharide production seemed to be higher than its rate of consumption (**Figure 5.4.2.(b)**). It was assumed that fermentative organisms are responsible for the breakdown of released complex carbohydrate into more easily accessible carbon, which in turn is utilised by the denitrifying organisms. The algal biomass from the I-HRAP appears to contain high enough numbers of facultative denitrifying organisms, so that culturing or an additional seed was not required.

Day 2 to 4:

The rate of nitrate reduction increased, but as soon as NO_3-N decreased to less than 5 $mg.L^{-1}$ the rate decreased as nitrate became the limiting factor. Between days 3 and 4 the rate of polysaccharide production appeared to equal its consumption.

Day 4 to 8:

The rate of nitrate removal slowed down, possibly due to a decrease in TPS production rate, and therefore a smaller COD:NO_3-N became limiting. Note the rise in

polysaccharide levels between days 5 and 6, indicated a continued release when nitrate again became the limiting substrate.

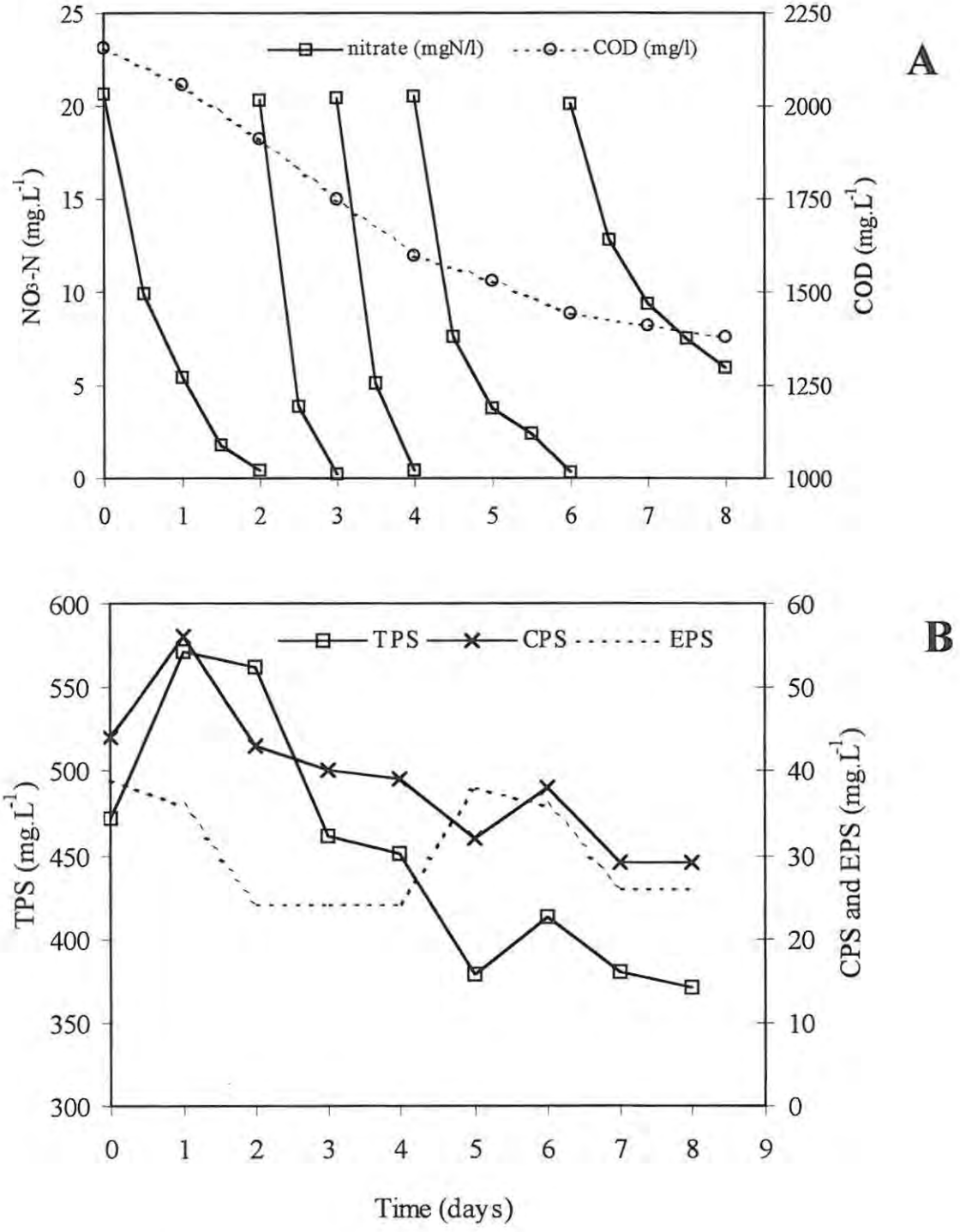


Figure 5.4.2. Batch denitrification using algal biomass as reagent.

Towards the end of the experiment the algal biomass seemed to reach its limit with respect to converting internally stored carbon into polysaccharide. In this experiment 776 mg COD (36% of total COD) was consumed while eliminating 94 mg NO_3-N, giving an average COD:NO_3-N removal ratio of 8.26:1 (mg/mg) over the 9 day period. The calculated stoichiometric value of TPS, which is expressed in glucose equivalents, gave a COD:TPS ratio of 1.07:1. Therefore the COD:NO_3-N ratio for the first 6 hours is 8.0:1, and indicates that 90% of the COD consumed was from polysaccharide origin. When the nitrate level reached ± 5 mgN.L^{-1} after 24 hours there was an increase in the TPS level. Not enough data is available yet to conclude whether the polysaccharide production rate is related to low nitrate concentration alone.

It was calculated that 121 mgCOD.L^{-1} of I-HRAP-medium will be available for removing 15 mgNO_3-N.L^{-1} and according to the COD:NO_3-N ratio of 8.26:1, approximately 98% nitrate could theoretically be removed by utilising the produced and harvested algal biomass in an appropriate denitrifying reactor design.

In the batch experiments the release of phosphate and ammonia were also observed (**Figure 5.4.3.**). During polymeric substrate fermentation ammonification is taking place due to the breakdown of protein that forms part of the microalgal EPS. The ammonia concentration reaches 20 mgN.L^{-1} within 3 days, but stays constant for the remaining 5 days. This is possibly due to ammonia stripping taking place because of the high pH medium (pH>9.5).

Phosphate release (from 0.6 to 2.2 mgP.L^{-1}) correlates well with dissolved calcium release (from 24 to 26 mgCa.L^{-1}) which is due to the change in pH (from 10.0 to 9.6). This has been verified with the adjustment of the pH of a I-HRAP sample with hydrochloric acid solution from pH 10.5 to pH 7, resulting in the release of both calcium and phosphate (refer to **Figure 4.4.2.**). There is not much indication of phosphate release by PAO due to organic substrate uptake, either because the PAO numbers are very low or the denitrifying organisms are more competitive regarding easily accessible carbon uptake.

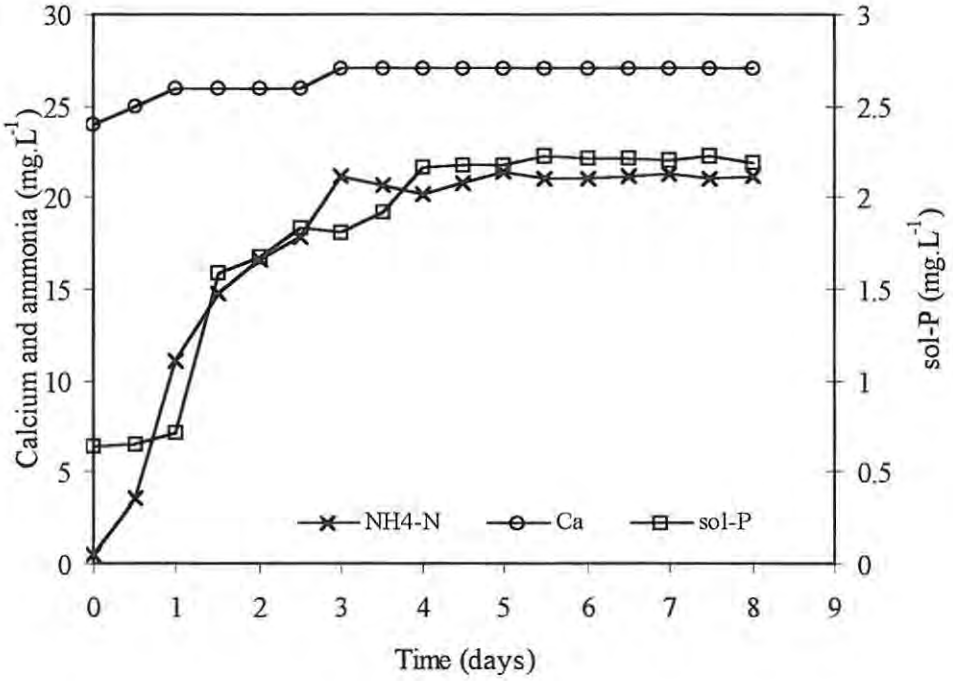


Figure 5.4.3. The release of phosphate and ammonia during denitrification.

5.4.3. Continuous denitrification in an upflow column

The 7.4 L upflow column was operated UASB-style for 45 days and the results can be seen in **Figure 5.4.4.**

Day 1 to 18:

Denitrification started immediately with the HRT set at 1.03 days. The feed rate was increased from day 4 to give a retention time of 0.6 days. During the first 5 days the fermentative gas production was high, resulting in sludge piston formation, where the dense algal sludge bed caused considerable gas entrapment at the bottom of the reactor. When the buoyancy of the accumulated gas was high enough, a sporadic flotation of sludge occurred resulting in subsequent biomass washout. The retention time was adjusted to 0.5 days (from day 8) and the sludge bed stabilised with no more serious loss in algal biomass observed. Nitrate removal efficiency of 99% was achieved.

Nutrient release was high during this period, and there was again a clear correlation between phosphate and calcium levels. The anoxic fermentation process was probably responsible for the initial fall in pH and thereby causing the calcium phosphate precipitate to re-solubilise.

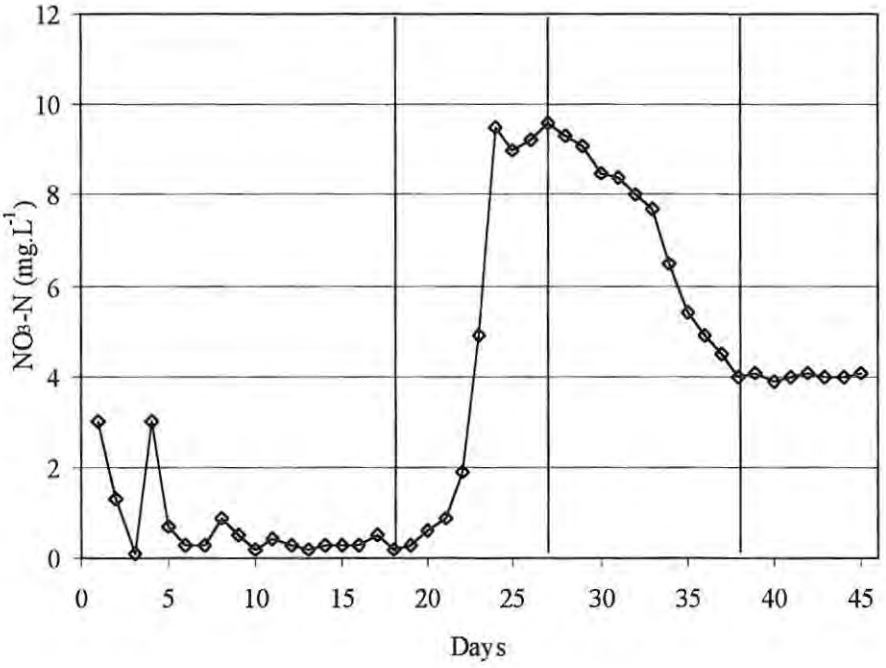


Figure 5.4.4. Performance of the denitrifying upflow column using settled algal biomass as reagent.

Day 19 to 27:

As the rate of stress induced release of algal polysaccharides declined, the rate of nitrate reduction decreased from 99 to 55%.

Day 28 to 38:

From day 27 fresh algal biomass was included in the feed, by changing to I-HRAP medium for feed collection. The total COD in the feed was 335 $mg.L^{-1}$ and according to the batch experiment more or less 36% of the COD should be available for

denitrification, thereby removing 14.6 $mgN.L^{-1}$. Immediately nitrate reduction improved with the addition of fresh algae and the performance increased gradually from 52 to 80% of nitrate removed. It was expected that only 73% nitrate removal with a sludge age of 9 days would be observed, but because of the large original inoculum still present 80% removal was achieved.

Day 39 to 45:

The column had stabilised possibly due to equilibrium being established between polysaccharide release from algal cell leakage or lysis, and polysaccharide consumption by fermentative organisms. Examination of the column effluent with a light microscope revealed that the majority of the suspended algae were indeed stressed, but still viable (**Figure 5.4.5**). It is important that the algal cells are not digested under these conditions for the release of intracellular components will be undesirable for tertiary wastewater treatment. This reactor achieved an eliminated loading rate of 32 $mgNO_x\text{-}N.L^{-1}$ reactor volume per day. According to these results a denitrification unit of 18 m^3 could be operated parallel to I-HRAP for treating GDW-final (which contains 15 $mgNO_3\text{-}N.L^{-1}$). The relatively low ammonia (< 2 $mgN.L^{-1}$) and phosphate (< 3 $mgP.L^{-1}$) levels together with the pH (> 9.5) in the reactor effluent should not result in a significant additional load to a scale HRAP, if operated in parallel to the denitrification unit.

5.4.4. Denitrification mass balance

The results from the steady state 540-mL denitrifying column are presented in **Table 5.4.2.** The biogas analysis shows that only nitrogen is present and no carbon dioxide or methane were detected. Carbon dioxide will remain in solution as the denitrified medium has an alkaline pH above 9.5. The experimental study showed that this reactor removed 56.6 $mgNO_x\text{-}N.d^{-1}$ of which 88% was recoverable in nitrogen gas, which indicate that anoxic denitrification is mainly responsible for nitrate removal.

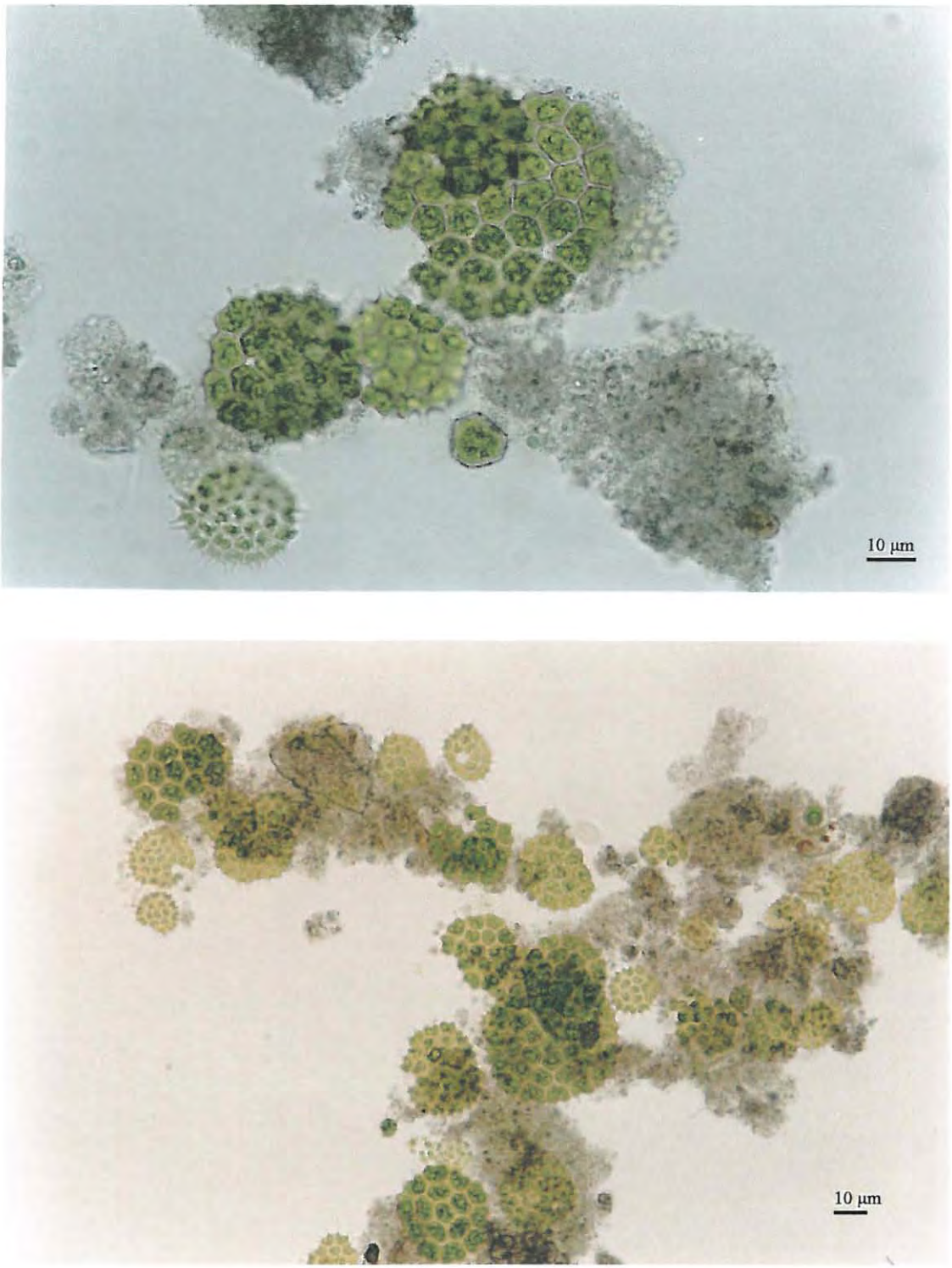


Figure 5.4.5. Algal biomass present in the inlet (A) and overflow (B) of the denitrifying column.

Table 5.4.2. NO_x-N mass balance from the 540 ml denitrifying column.

Hydraulic load	$L.d^{-1}$	3.7
Influent NO_3	$mgN.L^{-1}$	20
Influent NO_2	$mgN.L^{-1}$	3.2
Effluent NO_3	$mgN.L^{-1}$	0.8
Effluent NO_2	$mgN.L^{-1}$	2.3
NO_x removed	$mgN.d^{-1}$	56.6
N_2 gas collected	$mgN.d^{-1}$	50

5.4.5. Photosynthetic treatment of denitrified liquor

Effluent from the 7.4 L anoxic column, which contained viable algal biomass, was collected and exposed to light with the algae concentration similar to that found in I-HRAP. The precipitation of released phosphate with calcium was induced by high pH and ammonia removal was probably due to stripping (**Table 5.4.3.**). Ammonia stripping should be enhanced if the denitrified effluent is introduced back to a medium where the pH is already above 10, i.e. into I-HRAP. This experiment demonstrates that operating a phosphate precipitation reactor (such as I-HRAP) linked to a denitrification unit could result in an effective nutrient removal system.

Table 5.4.3. Removal of phosphate and ammonia from the denitrified medium in the presence of photosynthesising algae.

Time (hours)	pH	sol-P ($mgP.L^{-1}$)	Ca ($mg.L^{-1}$)	NO_3 ($mgN.L^{-1}$)	NH_3 ($mgN.L^{-1}$)
0	9.4	4.02	23	0.3	6.21
6	10.6	0.43	15	0.3	2.09

5.5. Conclusion

The concentration of soluble phosphate in I-HRAP treating either AIWPS-final or GDW-final was determined by the solubility of calcium phosphate minerals (possibly amorphous tricalcium phosphate [$Ca_3(PO_4)_2$] according to Moutin *et al.*, 1992). The photosynthetic activity by the algae clearly plays the central role in calcium phosphate precipitation by maintaining a high pH environment.

Harvested algal biomass from I-HRAP can be used as a reagent for denitrification by releasing polymeric substances, which probably serves as a carbon source for the biological denitrification process. The polysaccharides released were possibly due to stress conditions, for example low nutrient levels and/or the anaerobic dark environment. Results so far indicate that released phosphate and ammonia from the denitrification process can be removed after exposure to photosynthetic conditions.

Not enough data is available yet to put forward optimised design parameters, but a system design has been proposed for combined nutrient removal (**Figure 5.5.1.**): The use of the HRAP as a free-standing operation for tertiary treatment of wastewater, where calcium phosphate precipitation is induced by high pH in the presence of photosynthesising green algae. Ammonia stripping will be achieved simultaneously. Algal rich water gravitationally overflows into an ASP from which a percentage of the settled algal biomass will be recycled to the denitrification unit in front of the HRAP, where microalgae are passively manipulated into stress induced release of polysaccharides. The denitrification unit will receive the nutrient containing water (i.e. AIWPS-final or GDW-final) where the released polysaccharides are then utilised as carbon source for denitrification. The denitrified medium then enters the HRAP for removal of released phosphates (through calcium phosphate precipitation) and ammonia (alkaline stripping). During the anoxic phase the carbon dioxide produced immediately dissolved in the alkaline medium and would therefore be available in the form of bicarbonate for photosynthetic uptake. Suspended algae in the anoxic overflow should still be viable for regeneration and floc formation in the raceway.

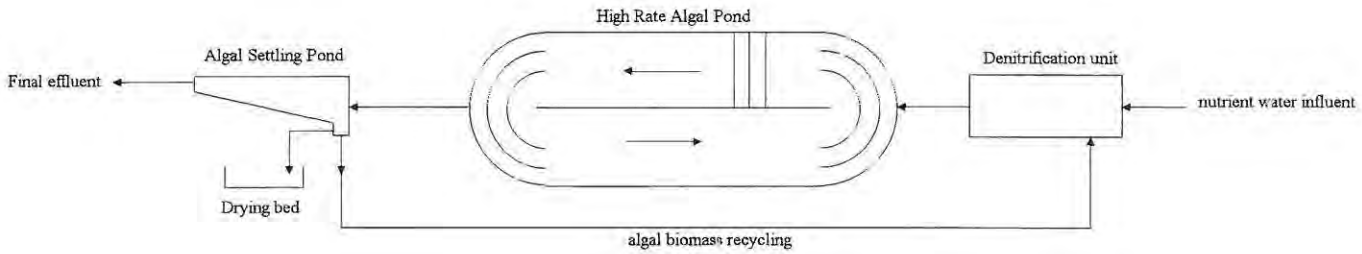


Figure 5.5.1. Proposed process design for combined N and P removal.

This novel system configuration is currently under investigation and future research will be aimed at understanding the mechanisms involved with stress induced release of algal polysaccharides, as well as optimising reactor design for the subsequent denitrification.

The engineering of an I-HRAP combined with denitrification using the algal biomass is indeed regarded as one of the most important and novel products to emerge from this study. The removal of N and P may be achieved in a low-maintenance, low-cost operation, appropriate to applications across a range of wastewater treatment needs, including wine industry wastewater. This proposed process for combined N and P removal was subsequently demonstrated on pilot-scale during the treatment of wine lees effluent.

Chapter Six

Combined Anaerobic and Algal Integrated Ponding treatment of Wine Lees Distillery Wastewater

6.1. Introduction

The domestic sewage treatment AIWPS survey detailed in Chapter 2 showed that a number of problems had to be addressed before applying this technology towards treatment of wine industry wastewaters. Follow-up studies then revealed novel improvements on the original concept and thereby making a fundamental contribution to the AIWPS specifically. The preceding studies were preparatory to the conceptualisation, development and piloting of the integrated ponding treatment of wine industry wastewater. The integrated ponding treatment of wine lees distillery wastewater was then chosen as a model for such a study, because it provides a worst-case scenario of what may be encountered in this industry. In addition to high COD loads, wine lees effluents contain a range of components including high levels of ammonia and protein nitrogen, phosphates and sulphates.

Due to the high COD concentration anticipated with this type of wastewater (in excess of 30 000 $mgCOD.L^{-1}$) it was decided to introduce anaerobic pre-treatment in order to remove 60-70% of the total COD (the easily biodegradable organic fraction) before entering an AIWPS. The anaerobic baffle reactor (ABR) was chosen due to several advantages over well-established systems such as the upflow anaerobic sludge blanket (UASB) and the anaerobic filter. These include: better resilience to hydraulic and organic shock loadings, longer biomass retention times, lower sludge yields, and the ability to partially separate between the various phases of anaerobic catabolism (Barber and Stuckey, 1999). It also combines the advantages of a repeated series of UASB chambers allowing the reactor to separate acidogenesis and methanogenesis longitudinally down the reactor, therefore behaving like a two-phase system without the associated control

problems and high costs (Weiland and Rozzi, 1991). The ABR may be constructed relatively inexpensively as lined and covered trenches in the ground and do not require highly skilled management.

Our research was carried out over a period of two consecutive seasons (1999 and 2000) to verify the efficiency of a combined anaerobic and integrated ponding treatment system and to assess its flexibility, simplicity and the running and maintenance costs on pilot scale.

6.2. Research Objectives

Evaluation of a pilot plant in treating wine lees distillery effluent with emphasis on the following components:

1. Anaerobic pre-treatment with the anaerobic baffle reactor in order to reduce the COD concentration of the wastewater to below 10 000 $mg.L^{-1}$, prior to entering an AIWPS.

2. Evaluation of the fermentation pit inside the facultative pond for improved sludge retention after insertion of an appropriate nylon net across the rising water column;

3. Effective nutrient removal using algal biomass in a denitrification process followed by ammonia stripping and phosphate precipitation in a high rate algal pond unit;

4. Downstream beneficiation options play an important role in the cost-benefit analysis relating to both the treatment technology applied as well as the final route for treated water disposal. Beneficiation options identified included yeast and algal biomass recovery for evaluation in specialist animal feed production;

5. To derive input values, cost and design detail for the construction of a full-scale system treating wine lees effluent.

6.3. Materials and Methods

6.3.1. Pilot plant

The pilot plant design details and site layout are presented in **Table 6.3.1.** and **Figures 6.3.1.** to **6.3.3.** The plant was constructed by Grahamstown Engineering CC and commissioned on site in Worcester (Western Cape, South Africa) next to Brenn-O-Kem (Pty) Ltd, a wine lees processing plant, which recovers by-products in the form of cream-of-tartar and ethanol from spent wine lees. The factory produces around 200 $m^3.d^{-1}$ of wine lees effluent during the processing season (annually from February to October). The tartrate by-product recovery plant is operated adjacent to and receiving waste streams from two distilleries. The disposal of wastewater is currently a joint endeavour by the three operations. They generate 167 megalitres of effluent per year during their season, which is transported via a 4.5 km pipeline, to a land disposal facility, owned by the local municipality, where it is currently discharged. The typical analysis of both distillery and wine lees effluents are shown in **Table 6.3.2.**

Table 6.3.1. Pilot plant design criteria.

Parameter	Anaerobic Reactor 1	Anaerobic Reactor 2	Fermentation Pit	Facultative Pond	HRAP 1	HRAP 2
Volume (m^3)	27	10	3	20	3	3
Surface area (m^2)	15	5	1.1	20	20	20
Influent ($m^3.d^{-1}$)	4.8	2.9	1	1	1	1
HRT (days)	5.6	3.5	3	20	3	3

In addition to the construction of a mobile site laboratory the pilot plant included the following unit operations:

1. Centrifugation of wastewater to achieve maximum settleable solids removal in the form of yeast biomass;

2. A 45 m^3 buffer tank to provide a constant supply of clear effluent to the pilot pant;

3. Heat exchanger utilising hot distillery effluent to maintain an increased operating temperature during the winter months;

4. Anaerobic Baffle Reactor (ABR). In the current application the ABR was divided into two separate reactors ABR1 (27 m^3) and ABR2 (10 m^3) for the purpose of optimisation. The ABR combines the advantages of a repeated series of upflow sludge blanket chambers, which includes internal recycle loops. The aim was to achieve rapid removal of the incoming COD (from 30 000 - 40 000 to between 5 000 and 10 000 $mg.L^{-1}$) before entering the facultative pond (FP).

5. Integrated Algal Ponding System. This included a facultative pond (fermentation pit volume 3 m^3 and outside pond volume 20 m^3) and two High Rate Algal Pond (HRAP) raceways (each 20 m^2) with algal biomass recovery settlers. The HRAP units were operated in series in order to implement the nitrate removal system described in Chapter 5, and using algal biomass as a denitrifying reagent in the first algal settler. This was followed by biologically induced calcium phosphate mineral precipitation in the second HRAP (refer to **Figure 6.3.4.**). Note the design of the fermentation pit which is similar to the proposed design described in Chapter 3.

Each following process component was designed to be smaller than the previous one to ensure that there was enough partially treated effluent available in order to optimise each component separately.

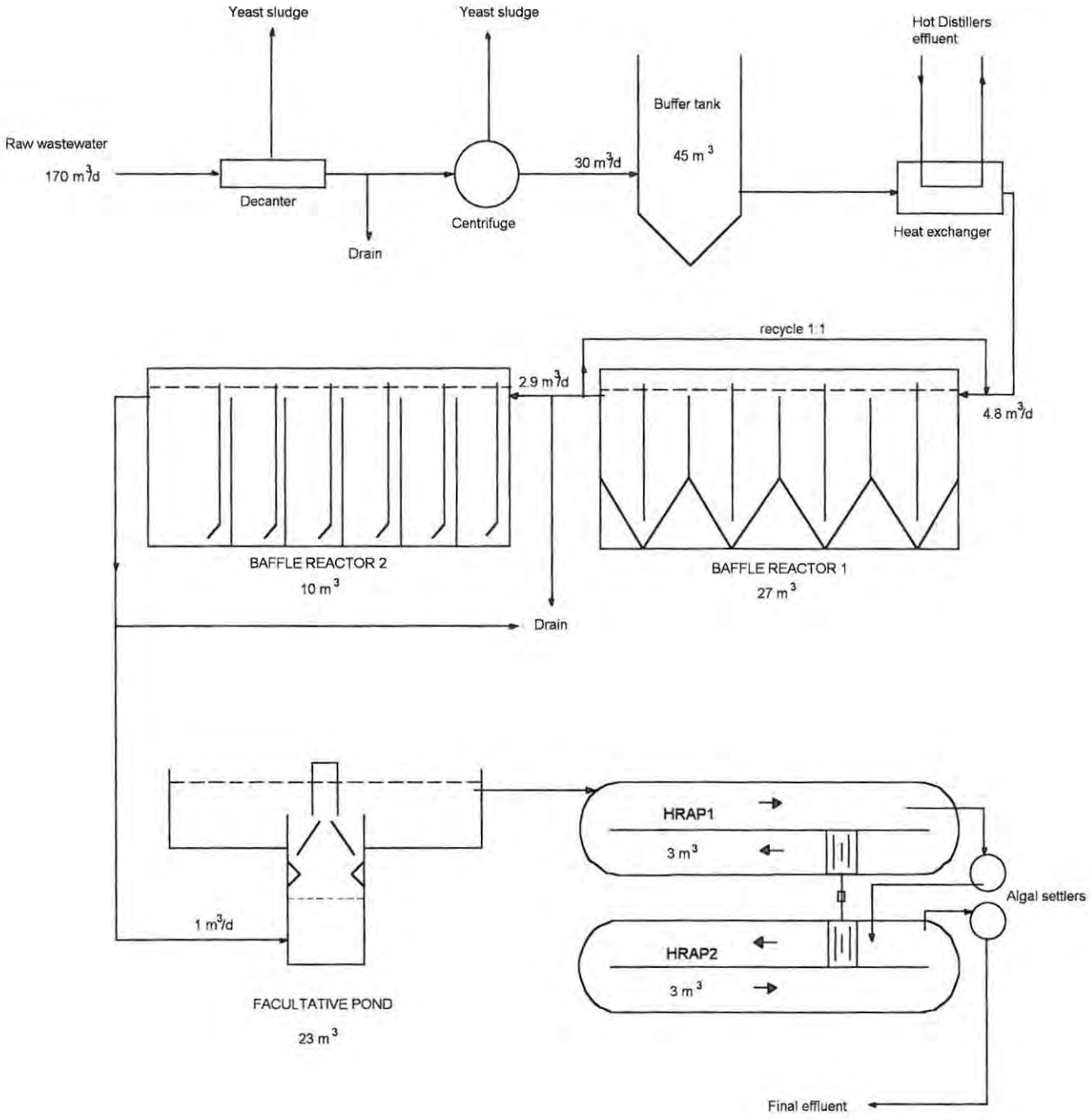


Figure 6.3.1. Conceptual diagram of the pilot plant constructed for the treatment of wine lees and distillery wastewaters.

Figure 6.3.2. Photograph of the Rhodes University pilot plant at Brenn-O-Kem, Worcester, showing the Buffer Tank (left rear), the two Anaerobic Baffle Reactors (centre rear & right), the Facultative Pond (centre left) and the High Rate Algal Ponds (front right), and the site laboratory (left).

Figure 6.3.3. Photograph of the Integrated Algal Ponding System showing the Facultative Pond (right) and the two High Rate Algal Ponds (left).

Table 6.3.2. Typical analysis of the wine distillery and wine lees effluents produced at Worcester.

Parameter	Distillery Effluent (after lime neutralisation)	Wine Lees Effluent
pH	6.7	5.8
Conductivity ($mS.m^{-1}$)	880	2 670
TKN ($mg.L^{-1}$)	234	1 662
Ammonia-N ($mg.L^{-1}$)	260	376
Nitrate-N ($mg.L^{-1}$)	2	44
Phosphate-P ($mg.L^{-1}$)	43	318
CODt ($mg.L^{-1}$)	21 600	56 800
CODs ($mg.L^{-1}$)	19 040	46 600
Calcium ($mg.L^{-1}$)	960	903
Potassium ($mg.L^{-1}$)	680	6 200
Magnesium ($mg.L^{-1}$)	44	113
Sodium ($mg.L^{-1}$)	601	582
Sulphate ($mg.L^{-1}$)	120	320
Chloride ($mg.L^{-1}$)	36	142

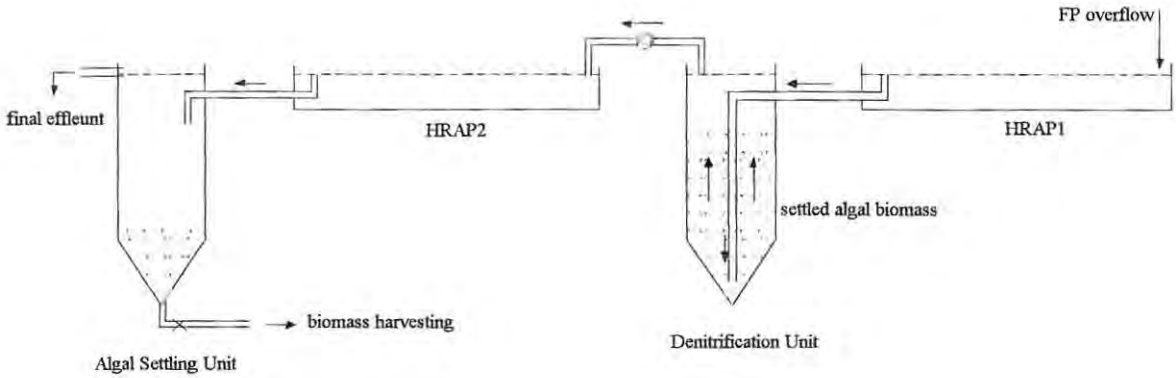


Figure 6.3.4. Configuration of the ponding system which was operated in order to achieve denitrification in the first algal settling unit (denitrification unit) followed by calcium phosphate precipitation and ammonia stripping in HRAP2.

6.3.2. Analytical Methods

Analysis of all parameters as previously described in Chapter 2. Soluble COD fraction (CODs) was determined after sample pre-treatment by filtering through Whatman GF/C microglass filters. Algal biomass production was determined according to the method described by Oswald (1994). Yeast biomass was recovered from the wine lees effluent and spray dried. Algal biomass was harvested from the HRAP units and sundried on site at Worcester. Yeast and algal biomass feed composition analysis and feed evaluation studies was undertaken by the Department of Ichthyology at Rhodes University, Grahamstown.

6.4. Results and Discussion

6.4.1. Process performance for the 1999-operating season

The operational milestones associated with the piloting exercise are summarised in **Table 6.4.1.** and the operation and of the individual unit operations are discussed below.

Table 6.4.1. Operational milestones covering the piloting process between May and December 1999.

Week 1	May '99	Pilot plant construction	*Week 24*	29-Sep	Plant audit 1
5	June '99	Inoculation & start-up of all systems	*26*	8-Oct	Introduce Westphalia centrifuge
			27	13-Oct	Plant audit 2
12	12-Jul	Acclimatise microbial populations			
			28	22-Oct	Ammonia toxicity inhibits ABR
17	12-Aug	Start feeding ABR1 continuously			
			29	26-Oct	Feed Distillers effluent
19	28-Aug	Start feeding ABR2 continuously			
			30	2-Nov	Plant audit 3
22	17-Sep	FP begin overflowing			
			34	3-Dec	Plant audit 4
24	27-Sep	HRAP culture developed		4-Dec	Plant shutdown

6.4.1.1. Centrifugation

The wine lees effluent had a high solids content (~ 10% TS) mainly in the form of yeast biomass. The effluent volume was divided between three decanters, and with the addition of flocculent 5% of the total solids was removed. A Westphalia centrifuge was also introduced in order to achieve as high a removal of the remaining yeast cells as possible, which resulted in a total COD reduction of 35%.

Apart from the potential beneficiation value associated with the effective recovery of the yeast biomass, the pilot study also showed that solids separation is important for the efficient operation of the ABR process. Excess build-up of yeast cells in the ABR may be related to an ammonia toxicity effect. Both points were investigated in some detail and will be discussed later in this chapter.

6.4.1.2. Anaerobic Baffle Reactors

ABR1 was seeded with 13 m^3 anaerobic sludge obtained from a digester treating similar wastewater, and 6 m^3 anaerobic sewage sludge (from a conventional sewage anaerobic digester). This reactor was then filled with 3 m^3 raw wastewater and 5 m^3 tapwater. During the first four weeks the anaerobic biomass was acclimatised by adding effluent at 5% reactor volume twice a week with continuous recirculation at 0.5 $m^3.h^{-1}$. The pH of the reactor was monitored regularly and adjusted with sodium hydroxide solution addition when the pH decreased to below 7. From 12 August 1999 the standard operating procedure for ABR1 was followed as reported in **Table 6.4.2.**

Table 6.4.2. Standard operating procedure for ABR1.

Influent	HRT	Overflow recycle	*Sludge recycle
4.8 $m^3.d^{-1}$	5.6 days	1:1	30 $min.d^{-1}$ at 1 $m^3.h^{-1}$

*Sludge recycle from the bottom of the last compartment.

ABR2 was seeded with 5 m^3 anaerobic sewage sludge. Start-up was similar to that of ABR1 with the influent being changed from raw effluent to ABR1 overflow. This reactor stabilised at a feed rate of 2.9 $m^3.d^{-1}$ ABR1 overflow (HRT of 3.4 days) with no recirculation. The overall COD removal achieved in the combined anaerobic treatment was between 70 and 80% with an influent COD range of 20 000 - 60 000 $mg.L^{-1}$ for the wine lees effluent and between 20 000 - 30 000 $mg.L^{-1}$ for the distillers effluent. The ABR performance trend over the 1999 study period is reported in **Figure 6.4.2.(a)** and **Figure 6.4.2.(b)** reports the same results adjusted for hydraulic retention time in the system.

A result of particular significance which emerged was the substantial reduction in salinity in the ABR while treating wine lees effluent. An electrical conductivity (EC) removal of 25% was observed (from 2 850 $mS.m^{-1}$ to 2 280 $mS.m^{-1}$) and follow-up investigation of these observations was undertaken during the 2000 season.

6.4.1.3. Facultative Pond

The anaerobic pit in the facultative pond was seeded with 1 m^3 anaerobic sewage sludge and the pond was filled with 15% ABR1 effluent resulting in a starting COD load of 2 130 $mgCOD.L^{-1}$ and conductivity of 385 $mS.m^{-1}$. The pond was inoculated with 40 litres of algal pond water obtained from waste stabilisation ponds treating tannery wastewater. Algal innocula ware also collected at various winery effluent ponds near Worcester. On 8 September 1999 continuous feeding of the facultative pond with ABR2 effluent commenced at a rate of 1 $m^3.d^{-1}$. The feed COD to the FP ranged between 3 500 - 16 000 $mg.L^{-1}$ and averaging 4 000 - 6 000 $mg.L^{-1}$ during periods of stable operation. Combined COD removal performance across the algal ponding system is recorded in **Figure 6.4.3.**

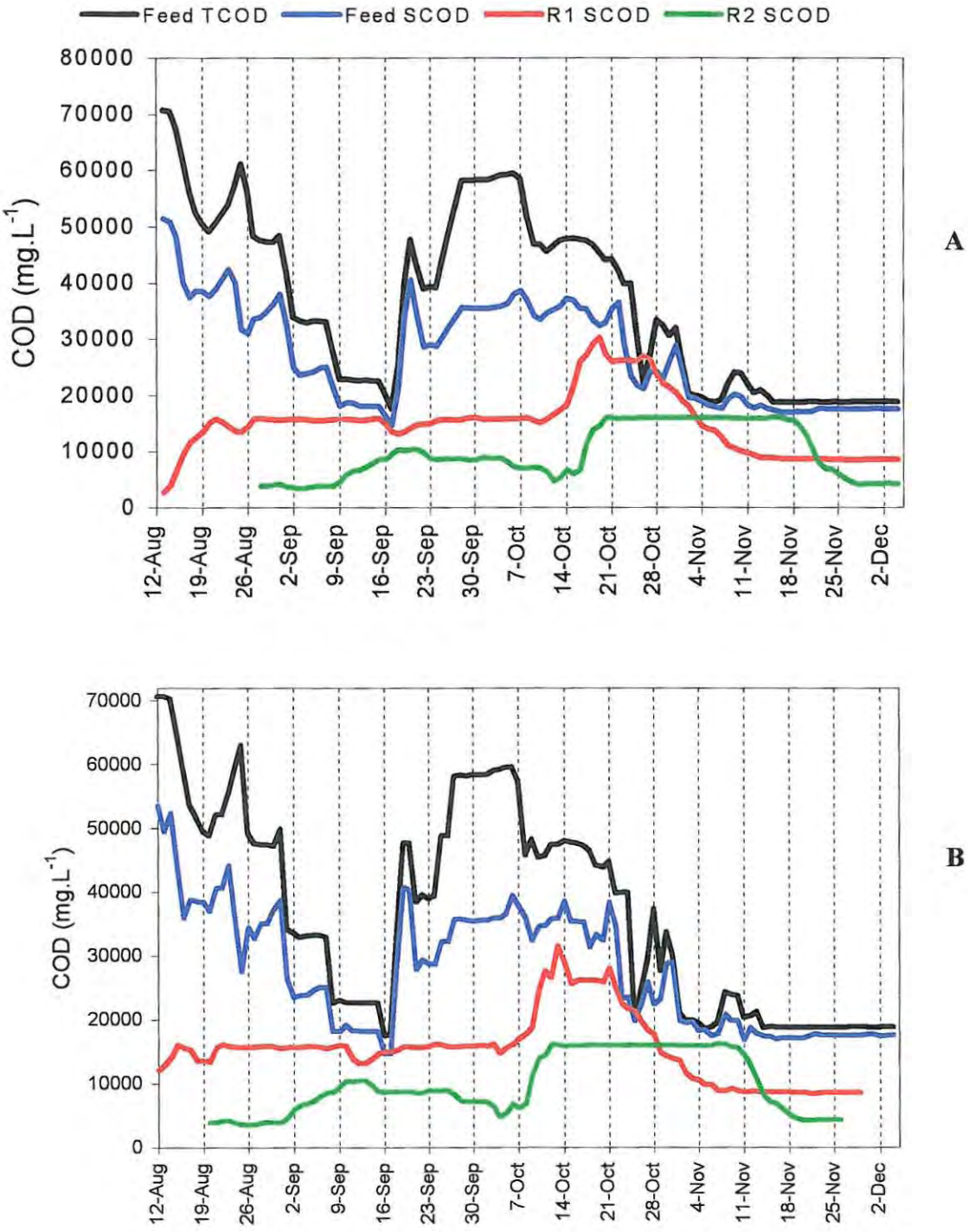


Figure 6.4.2. The COD removal performance trend for ABR1 and ABR2 covering the study period from 12 August 1999. (**A** reflects full treatment history for inlet stream on a particular day; **B** has been adjusted to remove time differences in HRT between the unit operations).

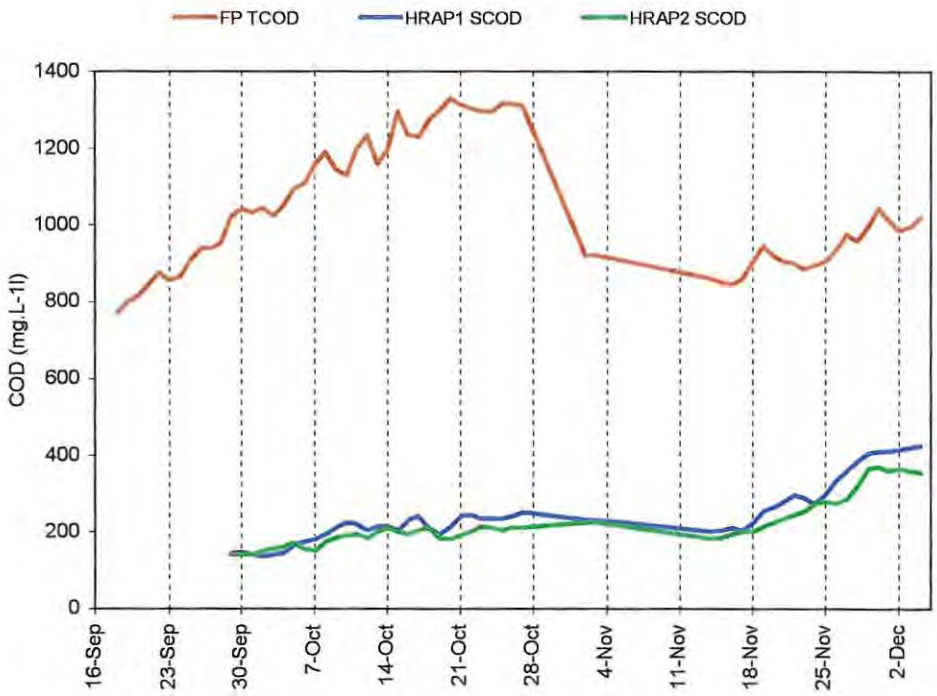


Figure 6.4.3. COD removal performance in the Integrated Algal Ponding System for the period 16 September to 4 December 1999.

The design of the anaerobic fermentation pit inside the facultative pond can be seen in **Figure 6.3.1.**, which is in accordance with the improved design evaluated in Chapter 3. The improved design was evaluated for its performance relating to anaerobic sludge retention and the results are reported in **Table 6.4.3.** The nylon net was effective in retaining active sludge for there is a clear difference in settleable solids below and above the net, indicating the possible presence of a sludge layer which formed around the net interface (which was a phenomenon observed with the laboratory reactors). Although soluble COD reduction achieved in the pit was only 34%, it was to be expected given the anaerobic baffle reactor pre-treatment which probably removed most of the easily biodegradable organic fraction. The pit overflow contained very little settleable solids, showing the retention of small sludge particles by the gas-liquid-separator which provided a quiescent zone for flocculation and re-settling of these small particles which were not retained by the net.

Table 6.4.3. Performance of the fermentation pit 8 weeks after start-up.

Parameter	pH	Settleable solids ($ml.L^{-1}$)	SCOD ($mg.L^{-1}$)
influent	7.6	0.6	8 940
bottom	7.7	520	6 250
0.1 m below net	7.8	95	5 870
0.1 m above net	7.8	2	5 880
overflow	7.8	0.5	5 860

6.4.1.4. High Rate Algal Ponds

Both HRAP1 and HRAP2 were filled with defined Zarouk's growth medium (Zarrouk, 1966) in order to try and stimulate the growth of *Spirulina*, but later it became evident that the natural selection process did not favour *Spirulina* growth. Instead, a unicellular algal species dominated in the facultative pond (provisionally identified as *Chlorellae spp.*) with subsequent inoculation of the HRAP units as the FP overflowed directly into the shallow algal ponds. HRAP1 received the total overflow volume of the FP and after algal biomass settling, HRAP1 overflowed to HRAP2. The COD removal performance is recorded in **Figure 6.4.3.** and the algal production for peak summer conditions was 29 $g.m^2.d^{-1}$.

During the last 4 weeks before plant shutdown the HRAP cascade was operated in such a way as to facilitate novel nitrogen and phosphorus removal, whereby algal biomass was used as reagent for denitrification followed by pH induced ammonia stripping and calcium phosphate precipitation. The algal settling unit following HRAP1 was converted into a denitrification unit by (a) extending the inlet of the settler to the bottom and (b) terminating weekly algal biomass harvesting. From **Figure 6.3.4.** it is evident that the first algal settler has therefore been converted to operate in an upflow mode, allowing the larger microalgal flocs to settle while passing the water through the algal sludge blanket before leaving the denitrification unit at the top overflow. The biomass was allowed to build up in the denitrification unit and thereby eventually leaving the unit at the overflow

as small suspended flocs into HRAP2. **Table 6.4.4.** summarises the performance of this type of nutrient removal operation and there seems to be good correlation between these results and the results discussed in chapters 4 and 5.

Table 6.4.4. Performance of the ponding cascade regarding N and P removal over a 4 week period (values in $mg.L^{-1}$, except for pH units).

	pH		SCOD		Calcium		PO_4-P		NO_3-N		NH_3-N	
$HRAP_1$ influent	8.1	(0.3)	933	(66)	34	(7)	11.1	(1.2)	8.4	(1.5)	253.9	(10.6)
$HRAP_1$ overflow	8.9	(0.5)	313	(84)	32	(3)	8.5	(0.9)	8.8	(1.4)	44	(5.3)
Den. Unit overflow	8.7	(0.5)	324	(88)	33	(2)	12.6	(2.0)	0.6	(0.2)	46.7	(3.3)
$HRAP_2$ overflow	9.9	(0.7)	274	(69)	8	(3)	0.8	(0.2)	0.7	(0.1)	4.5	(0.9)
ASP_2 overflow	9.9	(0.8)	269	(71)	8	(3)	0.8	(0.3)	0.8	(0.1)	4.4	(0.9)

Nitrate removal in the denitrification unit was 93% which is very efficient considering the short hydraulic retention time of 5.3 hours and nitrate eliminating load of 37.3 $mgNO_3$-$N.L^{-1}$ reactor volume per day, which compares very well with the performance of the laboratory denitrifying upflow column described in chapter 5. The performance was slightly better than that of the laboratory upflow column, probably due to the higher concentration of algal cells present in the influent to the denitrifying unit.

Phosphate removal was 93% which correlated with the associated decrease in calcium levels. Ammonia removal was 98%.

6.4.1.5. Ammonia Toxicity

During the course of the piloting programme rising levels of ammonia were noted in the ABRs. This was probably related to inefficient removal of yeast biomass solids during start-up, in the earlier stages of plant operation, and before the introduction of the Westphalia centrifuge step. Also slug loads of yeast biomass reached the first reactor on a number of different occasions during the period in which operating procedures were being established. This resulted in solids retention in the sludge compartments, followed

by cell degradation with protein and ammonia release. The ammonia load to the system was probably exacerbated somewhat by the processing of old batches of wine lees towards the end of the season, although the ammonia increase in the wine lees effluent was substantially below the toxic threshold.

From day 61 ammonia toxicity to the anaerobic digestion process was observed which impacted severely on the COD removal efficiency of the system. (see **Figure 6.4.2.(a)** from 7 October). Detailed recording of ammonia levels in the various stages of the system over this period are reported in **Figure 6.4.4.** and indicates a possible toxic threshold to the system at ammonia concentrations around 700 $mg.L^{-1}$.

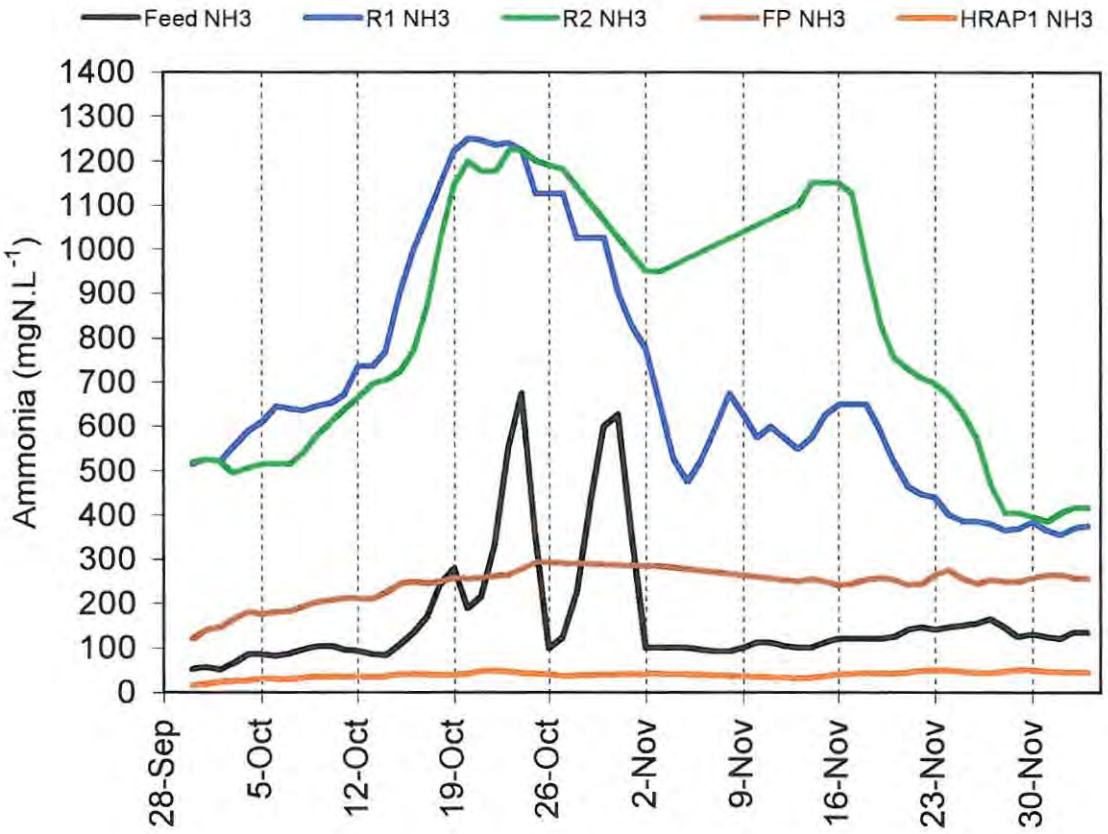


Figure 6.4.4. Ammonia concentrations recorded in the pilot plant over the period 28 September to 4 December 1999.

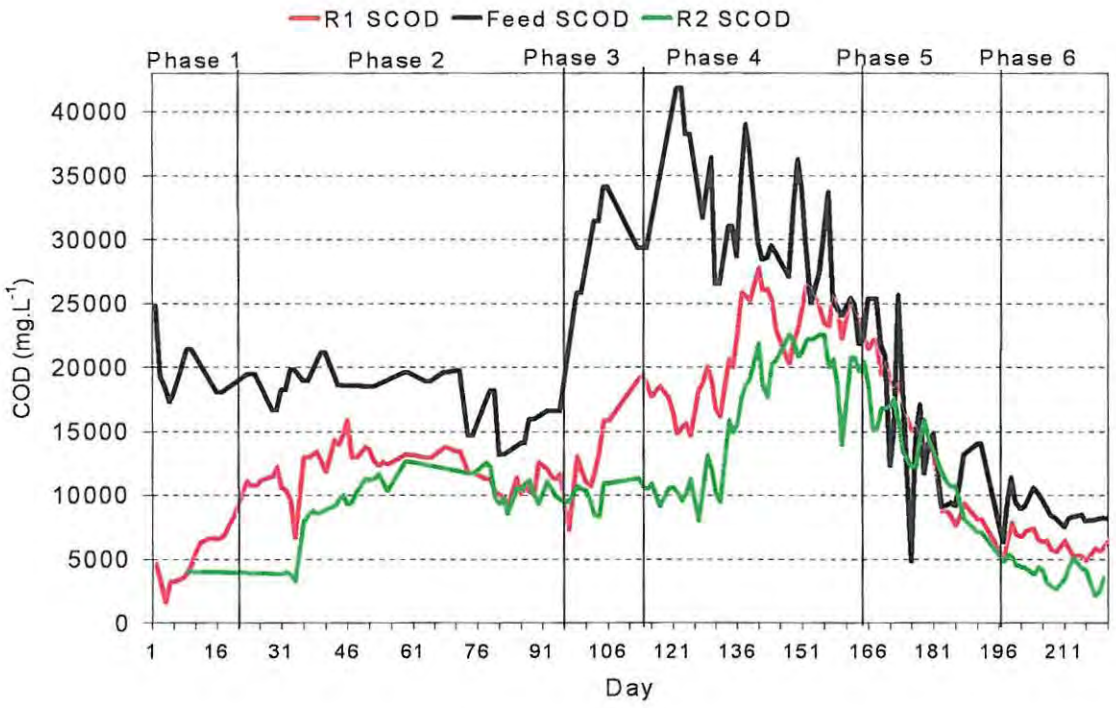
From day 76 the feed was changed to neutralised distillery effluent where both ammonia and COD levels were substantially lower than in the wine lees effluent. With the reduction in ammonia load a resumption of anaerobic processes was observed within 4-5 days with full operational performance re-established in ABR1 after about 15 days. By this time 42% CODs removal was achieved in ABR1 compared to an approximate 50% removal in wine lees effluent measured shortly before the toxicity incident. The anaerobic biocatalyst, which had taken many months to reach full operational performance, showed no lasting deleterious effects indicating a certain robustness, and the operation of typical inhibition/relief of inhibition response by the ABR system.

6.4.2. Process performance for the 2000-operating season

Given the relatively short period of four months of operational data acquired for the 1999 season it was decided to continue pilot plant operations for the 2000 production season.

The pilot plant was shut down from early December 1999 until start-up in April 2000. During this period, the supernatant was retained in ABR1 and ABR2 and the FP remained full and contained algae. The HRAP had been kept operational as far as algal growth was concerned, by the continued use of the paddles and addition of tapwater. Nutrients were also added in the form of urea and mono-ammonium-phosphate.

The pilot plant was started up on 10 April 2000, which will be regarded as day 1 of the 2000 operating period. For the first 93 days of operation the pilot plant was treating neutralised distillery effluent, and with the resumption of centrifugation of final effluent at the wine lees plant was run on wine lees effluent from day 94 to 220. The 2000 operating period has been reported in six phases according to the type of effluent that was treated and the monitoring of ammonia toxicity response. Performance for the 2000 season is reported in **Figures 6.4.5** to **6.4.9.**, where each phase is characterised by:



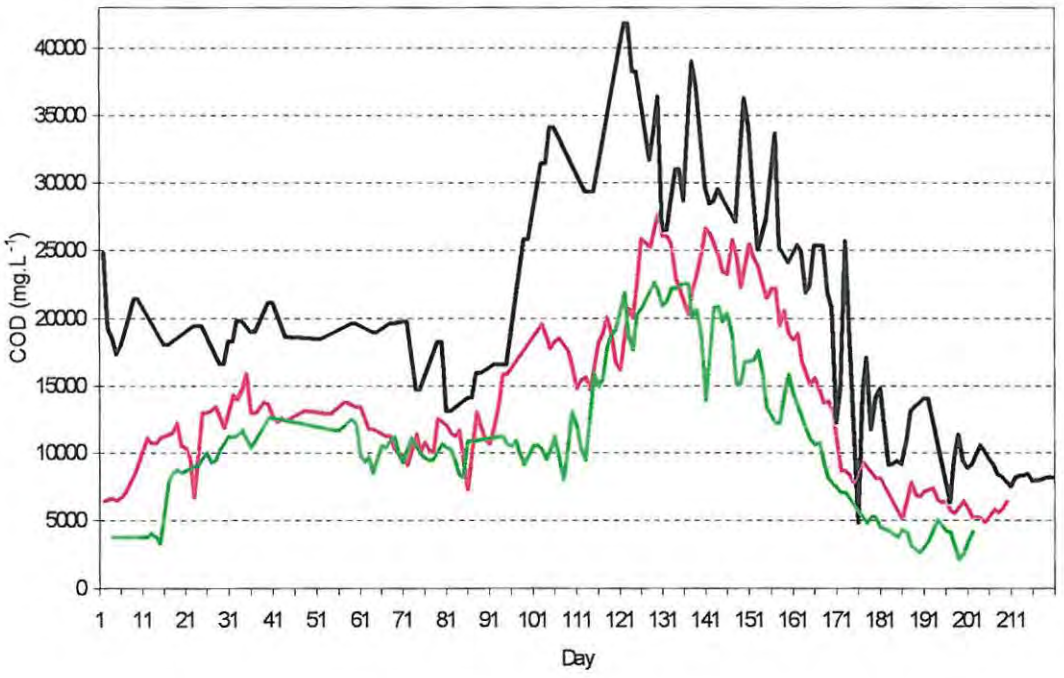


Figure 6.4.5. The COD removal performance trend for ABR1 and ABR2 over the 2000 study period. (**A** reflects full treatment history for inlet stream on a particular day; **B** has been adjusted to remove time differences in HRT between the unit operations).

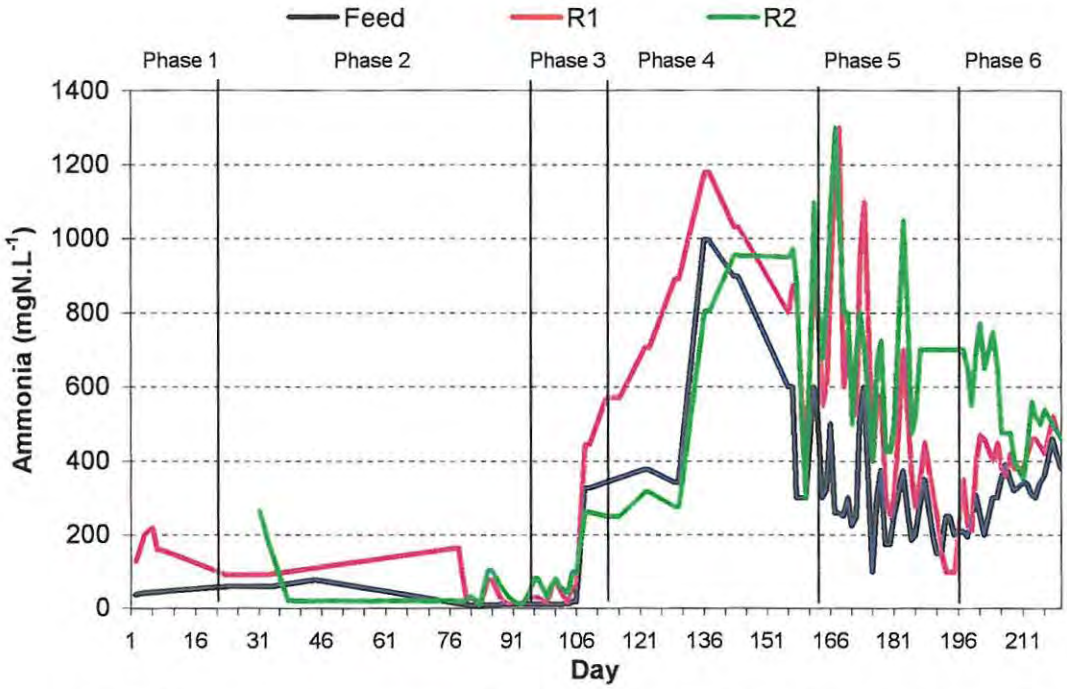


Figure 6.4.6. Ammonia concentrations for the feed and ABR, indicating the toxicity experienced during Phase 4.

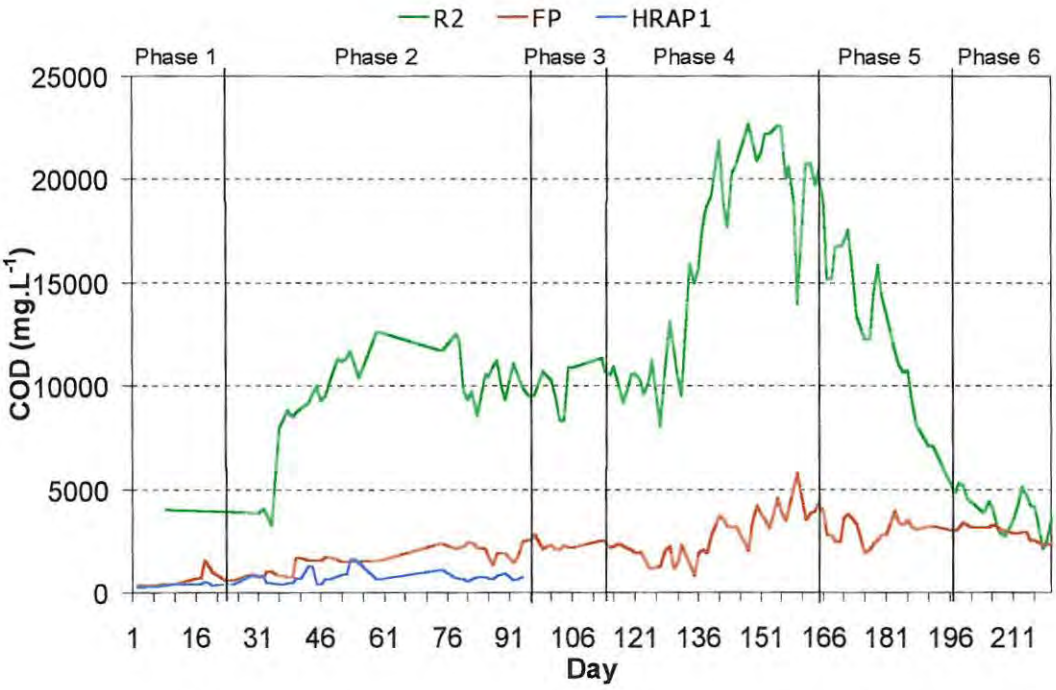


Figure 6.4.7. COD removal performance in the ponding system. Note that the HRAP units received FP overflow only during Phases 1&2, after which algal production was separately optimised for maximum biomass production.

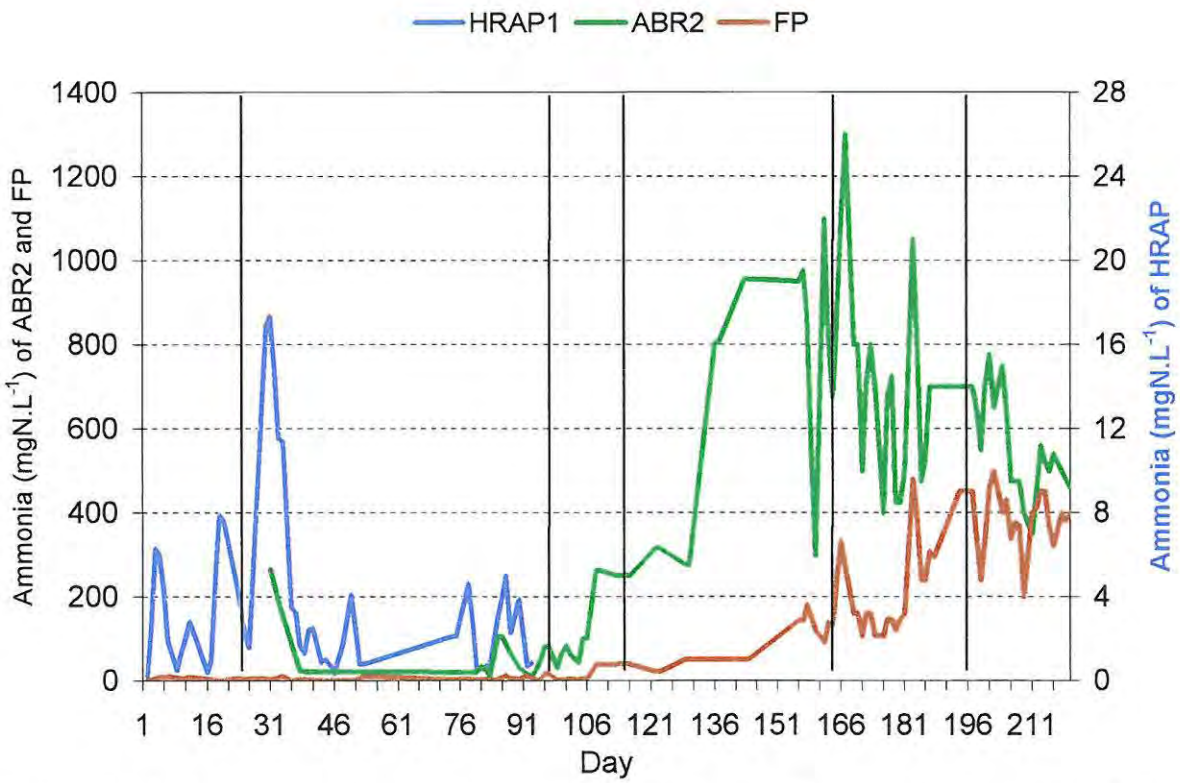


Figure 6.4.8. Ammonia concentrations reported for the ponding system showing ABR2 feed to the FP and FP feed to HRAP1.

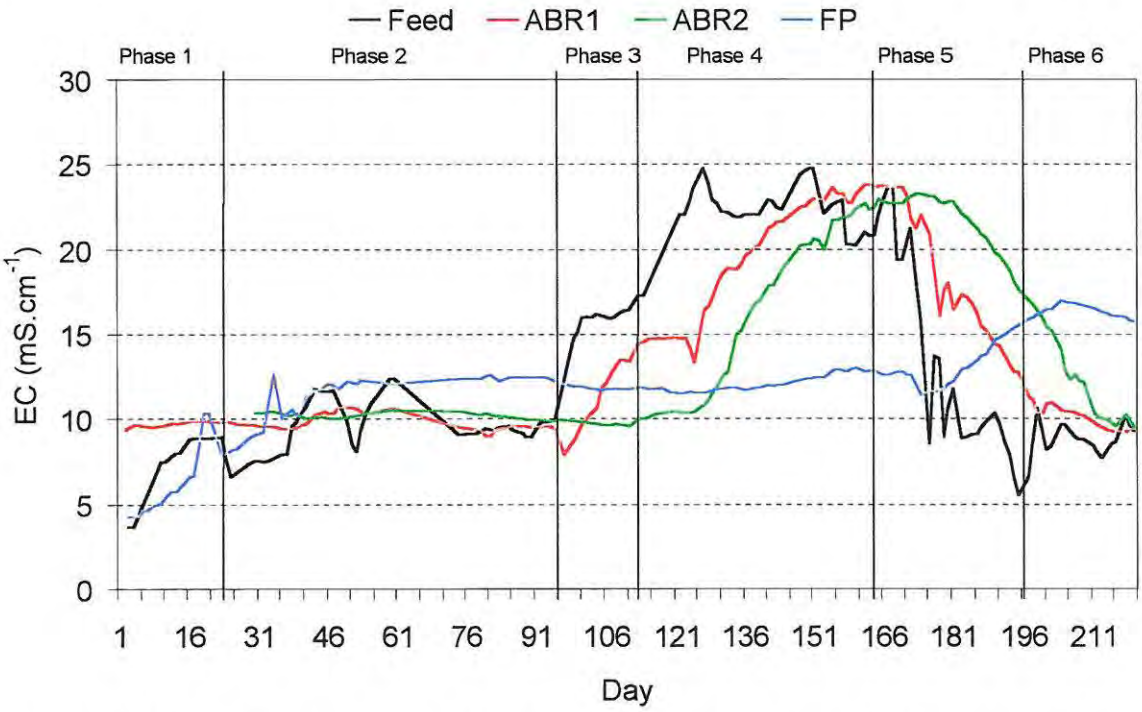


Figure 6.4.9. Conductivity profile for the pilot plant. Note that the FP received a high conductivity feed (1 700 $mS.m^{-1}$) during Phase 1.

Phase 1: Start-up period using Distillery Effluent

Phase 2: Treating neutralised Distillery Effluent

Phase 3: Start-up with Wine Lees Effluent

Phase 4: Treating Wine Lees Effluent & Ammonia Toxicity period

Phase 5: Diluting wine lees feed 1:1 with water & Relief from Ammonia Toxicity

Phase 6: Increased hydraulic loading rates

6.4.2.1. Phase 1: Day 1-23 (Start-up period using Distillery Effluent)

Phase 1 consisted of the 23 days of the start-up period, using neutralised distillery effluent as feed. Prior to start up of the ABR, flask studies were performed to establish the start up criteria for ABR1 and ABR2. It was determined that ABR1 and ABR2 contained 2% and 7% anaerobic sludge respectively. The distillery effluent was neutralised by adding lime as it was pumped into the buffer tank. The FP was started up using partially digested effluent from another wine lees treatment plant. **Table 6.4.5.** details the start-up procedure that was followed during the 2000 season.

6.4.2.2. Phase 2: Day 24-93 (Treating neutralised Distillery Effluent)

For the period from day 24 to 93 the pilot plant treated distillery effluent at a fairly steady rate. For the duration of this phase the feed rate was maintained at $2.5m^3.d^{-1}$, which gave a HRT in ABR1 of 13.5 days. Due to the build up of volatile fatty acids in the reactor, the retention time in ABR1 could not be reduced and the feed and recirculation ratio of 1:1 remained the same throughout this phase. The flow rate, HRT, organic loading rate (OLR) and volume displacements for each component are reported in **Tables 6.4.6.** and **6.4.7.**

During Phase 2 a 45% reduction in COD was achieved in the ABR and a further 70% in the FP, with a total COD reduction of 97% in the HRAP final effluent. Throughout this period there was a fairly stable and consistent reduction in the ABR units and the ABR2 overflow had an average COD of around $10\ 000mg.L^{-1}$, which correlated well with the

Table 6.4.5. Start-up procedure for pilot plant during 2000 season.

Day 1	Each valley of R1 was filled up separately with neutralised distillery effluent,
	Start feeding FP with Wolseley anaerobic effluent
Day 2	Contents of each valley of R1 mixed and allowed to settle
Day 3	Recirculation of R1 was started at a rate of $1m^3$/day
	Each baffle of R2 was filled with neutralised distillery effluent
Day 4	Increase R1 recirculation rate to $2m^3$/day
	Start feeding neutralised distillery effluent at $2m^3$/day
Day 8	R1 feed rate increased to $2.5m^3$/day
Day 14	Stop feeding R1 due to build up of acidity in reactor
Day 15	Re-seed R1 with anaerobic sludge from R2
	Start feeding at a rate of $1m^3$/day
Day 16	Increase R1 feed rate to $2m^3$/day
Day 17	Increase R1 feed rate to $2.5m^3$/day
Day 19	Start feeding R1 overflow to R2 at a rate of $0.83m^3$/day
Day 20	Start feeding FP R2 overflow

Table 6.4.6. Feed flow rates, HRT and OLR for the Phase 2 operations.

Day		24-30			30-93		
	Vol (m^3)	Feed Rate (m^3/day)	HRT (days)	OLR (kg COD/m^3 reactor/ day	Feed Rate (m^3/day)	HRT (days)	OLR (kg COD/m^3 reactor/ day
ABR1	27	2.5	10.8	1.67	2.5	10.8	1.65
ABR2	10	0.83	8.3	0.91	1.2	8.3	1.44
FP	23	1	23	0.17	1	23	0.42
HRAP 1	3	1	3	0.23	1	3	0.56
HRAP 2	3	1	3	0.20	1	3	0.24

Table 6.4.7. Volume displacements for each component during Phase 2 operations.

	ABR1	ABR2	FP	HRAP 1	HRAP 2
Volume (m^3)	27	10	23	3	3
No of Volume displacements	6.4	8.3	2.8	21	21

Table 6.4.8. Phosphate concentrations and percentage reduction during Phase 2.

	Feed	R1	R2	FP	HRAP 1	HRAP 2
Average PO_4(mgP.L^{-1})	**36**	**11.6**	**7.8**	**2.9**	**1.3**	**0.9**
% reduction		**67.7**	**78.3**	**92.1**	**95**	**97.4**

1999 results. While the COD removal in the FP averaged 70% it had not yet stabilised at the time of the switchover to wine lees treatment.

The total reduction of ammonia while treating distillery effluent was 96%, which also correlated well with the 1999 results where 97% ammonia removal was reported. The majority of ammonia reduction occurred in the ponding system, which reduced the ammonia in the ABR overflow by around 95%. This was due to two reasons. Firstly ammonia volatilisation is accelerated in the high pH environment of the FP and HRAP. At a pH of >9 most of the ammoniacal nitrogen present is in the NH_3 form. The second reason is the ready uptake of ammonia by the algal biomass.

The percentage of phosphate reduction while treating distillery effluent, was 97 - 98% across the system during Phase 2, with the majority (80%) occurring in the ABR units and the remainder in the ponding system (see **Table 6.4.8.**). The possibility of struvite and calcium phosphate crystallisation was investigated and will be discussed later in this chapter.

The EC of the feed averaged at 935 $mS.m^{-1}$, and remained relatively constant at 981 $mS.m^{-1}$ in ABR1 and 1 019 $mS.m^{-1}$ in ABR2. The FP EC increased initially due to the high EC of the Wolseley influent that was used for the start-up during Phase 1.

6.4.2.3. Phase 3: Day 94-113 (Start-up with Wine Lees Effluent)

For the start-up phase with wine lees effluent, the buffer tank was filled with centrifuged effluent and feeding commenced on day 94 at a rate of $2.5m^3.d^{-1}$ to ABR1. Difficulties with the availability of centrifuged effluent were experienced during the first few days of start up, which accounts for the fairly long duration of the start-up period. During the 1999 operating period it was established that the centrifugation of the wine lees effluent was necessary to remove the maximum amount of settleable solids (mainly yeast cells) for the efficient operation of the ABR. The removal of the solids also resulted in a 35% reduction in the total COD of the raw wine lees effluent. The centrifuged effluent was

monitored on a daily basis to ensure the maximum removal of solids to less than 1% settleable solids in the raw effluent was achieved, before it was pumped into the $45m^3$ buffer tank.

At this stage the HRAP units were withdrawn from use as the final polishing step, and were operated for maximum algae production for the feed study. It is also at about this time that a bloom of purple phototrophic bacteria occurred in the FP, possibly due to the high organic load. The bloom did not appear to have any negative effects on treatment and performed the same odour reducing function achieved by the algal cap.

The flow rates, HRT, OLR and volume displacements during Phase 3 are reported in **Tables 6.4.9.** and **6.4.10.**

The COD of wine lees effluent is much higher than that of distillery effluent, and averaged at 30 000 mg/l. During this phase a 70% reduction in COD in the ABR was recorded and a total of 92% reduction in the FP overflow. It is important to bear in mind that due to extended retention times in ABR2 and FP the 92% COD reduction measured during Phase 3 refers to the treatment of distillery effluent.

Initially the ammonia in the feed was relatively low, but as the season progressed and older batches of wine lees were processed, ammonia levels increased substantially. The ammonia levels in ABR1 and ABR2 also increased.

The phosphate levels of the wine lees effluent were considerably higher than distillery effluent, averaging $200mg.L^{-1}$. The removal efficiency in ABR1 and ABR2 was good during this period, at 92 and 96% respectively and results are reported in **Table 6.4.11.**

Table 6.4.9. Feed flow rates, HRT and OLR for the Phase 3 operations.

DAY		94-113		
	Volume (m^3)	**Feed Rate (m^3/day)**	**HRT (days)**	**OLR (kg COD/ m^3 reactor/ day**
ABR1	27	2.5	10.8	2.79
ABR2	10	1.2	8.33	1.49
FP	23	1	23	0.43

Table 6.4.10. Volume displacements for each component during Phase 3 operations.

	ABR1	**ABR2**	**FP**
Volume (m^3)	27	10	23
No of Volume displacements	1.9	2.4	0.4

Table 6.4.11. Phosphate removal during Phase 3 operations.

	Wine lees effluent	**ABR1**	**ABR2**	**FP**
Average PO_4 ($mgP.L^{-1}$)	229.3	17.5	8.6	4.2
% reduction		92.4	96.3	98.2

Table 6.4.12. Feed flow rates, HRT and OLR for the Phase 4 operations.

DAY		114-166		
	Volume (m^3)	**Feed Rate (m^3/day)**	**HRT (days)**	**OLR (kg COD/ m^3 reactor/ day**
ABR1	27	2.5	10.8	2.82
ABR2	10	1.2	8.33	2.59
FP	23	1	23	0.7

Table 6.4.13. Volume displacements for each component during Phase 4 operations.

	ABR1	**ABR2**	**FP**
Volume (m^3)	27	10	23
No of Volume displacements	4.8	6.2	2.2

The EC of wine lees effluent was higher than that of distillery effluent and as Phase 3 progressed an increase in the EC of ABR1 was evident (see **Figure 6.4.9.**).

6.4.2.4. Phase 4: Day 114-166 (Ammonia Toxicity)

Given the potential for ammonia toxicity to affect the process during the latter part of the 1999 season, the careful assessment of system performance under high ammonia loading was identified as an important objective for the 2000 study.

The switchover from distillery to wine lees effluent which commenced with Phase 3 was accompanied by a rapid rise in both feed COD and ammonia levels. The Phase 4 component of the study commenced at the point where ammonia concentrations exceeded 600 $mg.L^{-1}$, which flask studies had shown was near the toxic threshold of 700 $mg.L^{-1}$. When the ammonia concentration rose above 800 $mg.L^{-1}$, around day 130, COD levels started to rise indicating a reduction in the overall COD removal efficiency in the system from around 50% to only 20% of influent COD.

The reduced COD removal performance in ABR1 in particular was associated with the formation of a fluffy floating sludge which was partially lost to the system by reducing the bottom sludge bed and also via the reactor overflow. On day 141 recirculation of settled sludge from the end to the beginning of ABR1, commenced at a rate of 1 $m^3.d^{-1}$. This step was associated with a partial improvement in the overall performance of the system.

The feed flow rates, HRT, OLR and volume displacements during this phase are reported in **Tables 6.4.12.** and **6.4.13.**

Flask studies concluded that during anaerobic digestion of wine lees effluent the ammonia concentration had to be maintained at below 700 $mgN.L^{-1}$ to avoid toxicity induced inhibition of methanogenesis.

Koster and Koomen (1998) and Van Velsen (1979) reported the inhibitory effect of high ammonia-nitrogen levels on the methanogenic activity in anaerobic systems. During the scientific investigation of pellet formation in UASB systems it was found that an increase in NH_3 species concentration had an inhibitory effect on sludge production and more specifically on the hydrogenotrophic methanogens, at near neutral pH levels. They also found that by increasing the ammonium concentration in the influent, the system only showed a temporary loss of overall COD removal and reverted quickly once the ammonia concentration was reduced.

6.4.2.5. Phase 5: Day 167-195 (Dilution of Raw Wine Lees Effluent)

This phase is marked by the decision to dilute the raw wine lees effluent feed to a ratio of 1:1 with fresh water in order to reduce the dissolved protein concentration of the feed (TKN < 600 $mg.L^{-1}$) and therefore reduce the ammonia concentration to less than 400 $mg.L^{-1}$. It took 2.5 volume displacements before the ammonia levels were reduced to below toxicity levels before ABR1 recovered its biological activity.

The feed flow rates, HRT, OLR and volume displacements during this phase are reported in **Tables 6.4.14.** to **6.4.16.**

The 1:1 dilution ratio in ABR1 influent produced an average feed COD of 15 000 $mg.L^{-1}$. COD reduction in the ABR averaged 30% given the ammonia toxicity experienced during the first half of this phase. The lowered reduction efficiency of 72 % in the FP was due to this unit only entering the ammonia toxicity phase some time later while treating almost double the designed organic load. The sludge in the pit of the FP will also have been inhibited by the high influent ammonia levels.

The appearance of the sludge changed, it became less "fluffy" and assumed a much higher settleability characteristic. A reduced percentage of settleable solids was found in the overflow of ABR1 as it emerged from the toxicity phase (see **Figure 6.4.10.**). This is

Table 6.4.14. Feed flow rates, HRT and OLR for ABR1 in the Phase 5 operations.

ABR1				
Day	**Vol (m^3)**	**Feed Rate (m^3/day)**	**HRT (days)**	**OLR (kg COD/m^3 reactor/ day**
167-171	27	2.5	10.8	1.43
172-177	27	3	9	2.02
178-184	27	3.5	7.7	1.47
185-195	27	4	6.8	1.68

Table 6.4.15. Feed flow rates, HRT and OLR for ABR2 in the Phase 5 operations.

DAY		167-195		
	Volume (m^3)	**Feed Rate (m^3/day)**	**HRT (days)**	**OLR (kg COD/m^3 reactor/ day**
ABR2	10	1.2	8.3	1.71
FP	23	1	23	0.58

Table 6.4.16. Volume displacements for each component during Phase 5 operations.

	ABR1	**ABR2**	**FP**
Volume (m^3)	27	10	23
No of Volume displacements	3.9	3.6	1.3

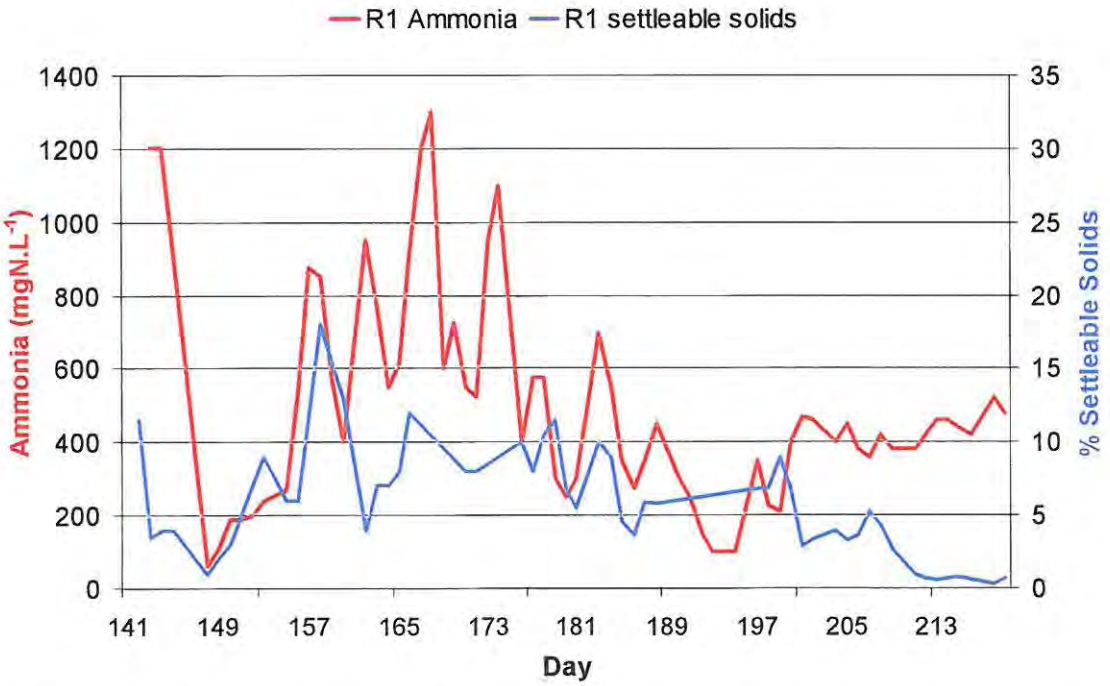


Figure 6.4.10. Ammonia levels in ABR1 and the settleable solids present in overflow.

probably due to the relief of inhibition of the methanogens and improved bacterial floc formation.

6.4.2.6. Phase 6: Day 196-220 (Increased hydraulic loading of dilute feed)

Where a relative stable feed regime had been established by the end of Phase 5 the HLR was increased to evaluate improved throughput of the system on the dilute wine lees effluent feed. The system responded well to the increase in organic loading rates and reduced HRT. The final hydraulic loading rate to ABR1 was 6 $m^3.d^{-1}$ and to ABR2 was 2.2 $m^3.d^{-1}$. The overflow from both reactors was much clearer and contained very little sludge in the form of settleable solids. Any sludge that it did contain had a noticeably higher settleability than during the ammonia toxicity phase. The purple phototrophic bacteria in the FP were still present at this stage.

The feed flow rates, HRT, OLR and volume displacements during this phase are reported in **Tables 6.4.17.** to **6.4.19.**

A stable COD removal efficiency of 55% in the ABR was reached, and remained fairly constant with the increasing feed rate and reduced HRT. In the FP the COD removal was low at only 66%, but his was due to the FP still experiencing residual ammonia toxicity and COD overloading during the early part of Phase 6. The ammonia concentration in the feed averaged around 300 $mgN.L^{-1}$ and in ABR1 between 300 and 500 $mgN.L^{-1}$.

The EC of the feed and ABR overflows decreased and stabilised at about the same level towards the end of this phase. There was an initial increase in the FP EC, but subsequently started to decrease and would probably have continued decreasing if feeding had continued. On day 220 the pilot plant was shut down due to the ending of the wine lees processing season in early December 2000.

Table 6.4.17. Feed flow rates, HRT and OLR for ABR1 in the Phase 6 operations.

ABR1 Day	Vol (m^3)	Feed Rate (m^3/day)	HRT (days)	OLR (kg COD/m^3 reactor/ day
196-197	27	4	6.8	0.94
198-203	27	4.5	6	1.69
204-207	27	5	5.4	1.78
208-220	27	6	4.5	1.82

Table 6.4.18. Feed flow rates, HRT and OLR for ABR2 in the Phase 6 operations.

DAY		196-197			204-220		
	Volume (m^3)	Feed Rate (m^3/day)	HRT (days)	OLR (kg COD/m^3 reactor/ day	Feed Rate (m^3/day)	HRT (days)	OLR (kg COD/m^3 reactor/ day
ABR2	10	1.2	8.3	0.75	2.2	4.5	1.33
FP	23	1	23	0.21	1	23	0.27

Table 6.4.19. Volume displacements for each component during Phase 6 operations.

	ABR1	ABR2	FP
Volume (m^3)	27	10	23
No of Volume displacements	4.9	4.7	1.1

6.4.3. Salinity removal

Results from the 1999 season had shown a conductivity reduction of 20% in the ABR and FP in treating wine lees effluent, while no substantial reduction was observed when treating distillery effluent. The treatment system did not reach steady-state for wine lees effluent treatment during the 2000 season and therefore the 1999 results with respect to salinity reduction could not be confirmed.

The formation of struvite has been documented during the anaerobic treatment of distillery type effluents (Loewenthal *et al.*, 1998). Struvite precipitation occurs at a molar ratio of 1:1:1 of P:N:Mg at the optimum conditions of a pH above 8 and low CO_2 partial pressure (pCO_2). The concentrations of the elements concerned has to be high enough in

order to induce struvite formation, due to the presence of inhibiting substances (primarily dissolved organic compounds and suspended solids).

Flask studies confirmed a correlation between phosphate precipitation and conductivity reduction. Anaerobic digestion of wine lees effluent gave the best salinity reduction results, with precipitation occurring only when the COD levels were below 10000 $mg.L^{-1}$. Supplementation with phosphate, ammonium and lime did not have any significant effect on EC levels. It was concluded that the concentration of phosphate is not high enough in the distillery effluent in question for perceptible struvite precipitation to occur.

After pilot plant shutdown the precipitates inside the ABR units were sampled and analysed - results shown in **Table 6.4.20.** The precipitate in ABR1 contained more calcium and potassium indicating that calcium-phosphate had co-precipitated with potassium. The yellowish precipitate was observed to be more evident near the water-gas interface and towards the final compartment of ABR1. The relatively clear crystals that formed in ABR2 could have been mainly struvite in combination with some calcium-phosphate precipitate. X-ray diffraction analysis would reveal the true identity of these crystals, but was not within the scope of the study. The density of the struvite crystals increased towards the baffle overflows, possibly due to the reduced pCO_2 near the water-gas interface.

Table 6.4.20. Composition of the precipitates sampled in the ABR units.

Parameter	ABR1 precipitate	ABR2 precipitate
N	0,7%	2,7%
P	6,4%	5,2%
Mg	4,3%	4,3%
K	3,9%	0,3%
Ca	30%	24%
Ash	67%	50%

Flask studies concluded that not more than a 25% EC reduction, as calcium-phosphate and struvite formation, could be expected during biological treatment of this wastewater.

6.4.3. Algal biomass production

Over the five month shutdown period (Dec 1999 - April 2000) the algal ponding system became deficient in macro-nutrients, nitrogen and phosphorus, and supplementation with urea and mono-ammonium-phosphate was required to initiate algal biomass production on start-up. Notwithstanding the highly variable operating regime experienced by the HRAP the algal biomass yield averaged 15 $g.m^{-2}.d^{-1}$ for the year. Biomass yields are reported in **Figure 6.4.11.**

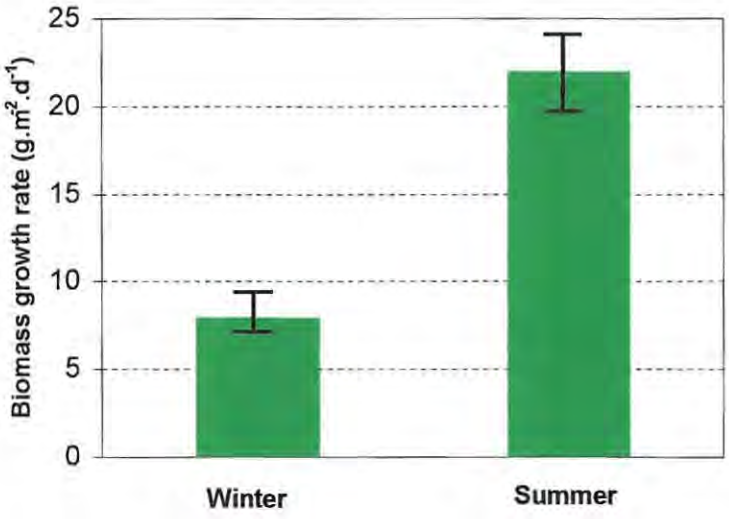


Figure 6.4.11. Average algal biomass production for the 2000 season.

Algal production during winter might be increased by heating the HRAP units utilising hot distillery effluent, but experimentation with the pilot HRAP showed that care has to be taken with the design of the heat exchanger in order to avoid a high temperature gradient in the raceway.

Chlorella-type unicellular algae appeared as the dominating species present in the HRAP and algal flocs were harvested in the algal settling units which followed the HRAP. The study showed that on average 50% of the biomass produced in the HRAP was in a settleable form, and the remaining suspended algae would have to be removed with the

aid of a flocculent. Oven-dried biomass was analysed (**Table 6.4.21.**) and contained 5,5% nitrogen which would indicate a total protein content of 34%.

Table 6.4.21. Analysis of oven-dried algal biomass harvested from the pilot HRAP.

Parameter	Percentage (%)
Nitrogen	5,5
Phosphorus	1,9
Potassium	5,8
Calcium	2,2
Magnesium	1,1
Ash	35,8

6.4.4. Biomass nutritional quality evaluation

A role for the beneficiation of by-products had been identified at the outset as an important objective of the pilot plant project. Algal biomass was harvested from the HRAP units, concentrated and sundried. Wine yeast biomass was recovered from the wine lees effluent and spray dried. Chemical analysis was undertaken on both samples and preliminary biological nutritional evaluation was undertaken only on the yeast biomass by the Department of Ichthyology at Rhodes University, Grahamstown. The results of the chemical analysis and comparison with other feed ingredients are shown in **Tables 6.4.22.** to **6.4.24.** for the wine yeast and algal biomass respectively.

The level of protein in wine yeast is lower than typical bulk proteins used in the animal feed industry such as fishmeal (71%) and soya oil cake (36.5%). Its protein level is similar to ingredients such as sunflower oil cake (26%) and peanut oil cake (29%) which are widely used feed ingredients. The wine yeast possesses a very well balanced essential amino acid profile with high levels of lysine and histidine. Note the similarity with the EAA profiles of fishmeal and soya **(Table 6.4.23.)**. The high level of lysine is a very positive attribute as lysine is usually the first limiting amino acid in compound animal feeds. The high level of ash (23%) indicates that the wine yeast is possibly rich in essential minerals.

Table 6.4.22. Amino acid analysis of spray dried wine yeast. The sample contained 25.66% protein and 1.87% ammonia.

Amino acid	% sample	Amino acid	% sample
Aspartic	2.62	Isoleucine	1.51
Threonine	1.27	Leucine	2.09
Serine	1.20	Tyrosine	0.75
Glutamic	3.05	Phenylalanine	1.33
Proline	1.44	Histidine	0.69
Glycine	1.53	Lysine	1.88
Alanine	1.90	Arginine	1.23
Valine	1.70	Methionine	0.4

Table 6.4.23. Comparison of the essential amino acid profile (expressed as % of protein), protein content and price of common feed ingredients with wine yeast.

Amino acid	Wine yeast	Fishmeal	Soya oil cake, solvent extr.	Sunflower oil cake – mech. extr.	Torula yeast
Arginine	4.8	5.12	6.76	10.1	4.6
Histidine	7.33	2.88	2.38	3.19	2.6
Isoleucine	5.93	3.85	4.54	4.99	6.2
Leucine	8.15	7.13	7.3	8.06	7.5
Lysine	7.33	7.77	5.98	4.34	8.2
Meth + Cys	1.56 (meth only)	4.63	2.82	5.15	2.4
Threonine	4.95	4.42	3.7	4.61	5.6
Phen+Tyr	2.07	7.04	7.68	9.09	8.6
Trytophan	N/a	1.15	1.42	3.46	0.8
Valine	6.63	4.75	4.5	6.14	6.2
Protein %	25.7	71	36.5	26	50
Price Rands	?	4.40	2.30	1.5	10

Table 6.4.24. EAA profile of sundried algal biomass containing 26.33% protein and 2.5% ammonia.

Aspartate	2.77	Isoleucine	1.31
Threonine	1.3	Leucine	2.32
Serine	1.03	Tyrosine	0.83
Glutamate	3.05	Phenylalanine	1.56
Proline	1.17	Histidine	0.38
Glycine	1.7	Lysine	1.12
Alanine	1.93	Arginine	1.4
Valine	1.73		

While damage to algal cell protein is a well-known effect of sundrying methods, alternative options were not available in the given time. Other studies on spray dried algal biomass would indicate predictable protein recovery around twice that recorded for the sundried sample (i.e. 50% protein). Nevertheless, the amino acid composition is particularly impressive, and while results for cysteine and tryptophan are also still outstanding, the results indicate real potential for following up biological screening trials for this feed source.

6.5. Conclusion

The pilot plant performance studies for the 1999 and 2000 seasons, over which the investigation was undertaken, showed broadly comparable results for both distillery and wine lees effluent treatment. Although longer operating periods were possible during the 2000 season, lending considerable more confidence to the results achieved, the outcomes for the process were broadly confirmed by the second year of pilot plant operation.

With respect to distillery effluent treatment, which was fed at the end of the 1999 season and the beginning of the 2000 season, steady state operating conditions were achieved early in the operation of the pilot plant and maintained throughout. The system performed well without serious perturbation and produced a consistent effluent quality at an OLR around 2.5 $m^3.d^{-1}$. Extrapolating results for the two seasons indicates anticipated treatment performance for distillery effluent across all units of the integrated ponding system with the following removal efficiencies: COD 97%, ammonia 96%, and phosphate 97%. No effective salinity reduction was observed with the treatment of distillery effluent, and with this exception a quality of effluent would be produced close to compliance with surface water discharge standards. The quality of the final stream would be amenable to further optimisation by HRAP polishing operations which were, however, not undertaken in this study.

Treatment of wine lees effluent gave a less clear-cut picture than distillery effluent, mainly due to the occurrence of ammonia toxicity in the system when fed partially

degraded late season wine lees. While ammonia levels present in the feed to the pilot plant contribute to the problem, digestion of other effluent constituents, probably mainly proteinaceous components, also gives rise to the ammonia levels rising to the 700 $mg.L^{-1}$ toxic threshold for the anaerobic digestion system. Notwithstanding ammonia toxicity incidents occurring during both seasons, it was demonstrated that the problem may be effectively overcome with the introduction of effluent and sludge recycling operations. It was shown with the plant operated using dilute feed at higher hydraulic loading rates it was possible to re-establish both equilibrium in the system and a final effluent treatment efficiency comparable to treated distillery effluent. During conditions where low levels of ammonia prevail OLRs double that of distillery effluent may be conveniently managed.

The ammonia toxicity incidents illustrated a number of important points. The ABR was able to function for some time at or near the upper limits of the ammonia toxic threshold for anaerobic systems. Also that the recovery of the system followed very closely on the relief of inhibition thus demonstrating a satisfactory robustness to the process. While the system also handled the fluctuations in ammonia levels present in the range of wine lees effluents treated during the first part of the piloting operation, sudden increases in ammonia may impact severely where the final levels in the ABR are close to the toxic threshold. It had been noted at the outset that a high level of yeast cell removal would be required to prevent such an eventuality. It is evident that this was not achieved for the first few months of operation, and that additionally a few slug loads of yeast sludge did inadvertently enter the system during this period. It was shown that the problem might be overcome with appropriate design. Nutritional evaluation studies indicate that yeast biomass removal may be accomplished at negative cost given the beneficiation potential demonstrated for yeast biomass recovery.

Taking the above into account the overall process may be anticipated to achieve a CODS removal of 95-98%, with around 80% of this removal achieved in the ABR operation. While the final COD of < 300 $mg.L^{-1}$ remains higher than the surface water discharge standard, it seems likely that a substantially improved polishing performance is achievable with the optimisation of system performance. Both ammonia and nitrate levels

were reduced by 97% and 80% respectively, and phosphate by at least 97%. When operating the HRAP cascade in such a manner as to facilitate denitrification, followed by pH induced ammonia stripping and calcium phosphate precipitation, a final effluent of discharge standard may be produced.

The improved design of the anaerobic fermentation pit inside the facultative pond, with the introduction of the nylon net, proved to be effective in retaining the active anaerobic sludge in accordance with the design evaluation described in Chapter 3.

A result of particular significance, which emerged from the 1999 season piloting study, was the substantial reduction in EC achieved in the anaerobic units during the processing of wine lees effluent. This is probably accounted for by the precipitation of potassium and magnesium together with calcium salts in the ABR and FP units. A combination of flask studies and observations of EC removal in the pilot plant showed that a 20-25% reduction could be expected for wine lees effluent treatment from a starting level of above 2 000 $mS.m^{-1}$. Very little EC reduction was observed during the treatment of distillery effluent.

The scale-up development of the results discussed up to this point in the study is detailed in the following Chapter 7 of this report.

Chapter Seven

Development and Scale-up design of the Anaerobic-Algal Oxidation Process for treating Wine Lees Distillery Wastewater

7.1. Introduction

A principal objective of the project, following demonstration of process feasibility in the pilot studies, was to develop design criteria on which a process scale-up initiative could be evaluated. Following completion of the piloting operations in December 2000 an approach to the development of design and cost estimates was undertaken by the researcher in collaboration with MBB Consulting Engineers, who specialise in civil construction design. Brenn-O-Kem (Pty) Ltd decided to proceed with a scale-up design evaluation for treating only wine lees wastewater.

7.2. Research Objectives

Following the successful demonstration, both in the laboratory and pilot-scale investigations, of the feasibility of the Anaerobic-Algal Oxidation Process, the conceptual design, engineering design and cost estimates of a scale-up treatment system would be undertaken.

7.3. Materials and Methods

Methods used are as previously described.

7.4. Results and Discussion

The following reasoning was followed in developing the conceptual design of the Anaerobic-Algal Oxidation Process.

7.4.1. Pre-treatment

The current pre-treatment practise of centrifugation being implemented at the wine lees factory proved to be satisfactory. Apart from the potential beneficiation value associated with the effective recovery of the yeast biomass, the pilot study also showed that solids separation is important for the efficient operation of the ABR process. Yeast biomass removal results in a 35% COD reduction, as well as reducing the ammonia toxicity effect in the primary anaerobic treatment phase.

7.4.2. Anaerobic Baffle Reactor

Monitoring at the Brenn-O-Kem factory showed that processing of wine lees generated only 130 m^3 of wastewater per day during the 2000-operating season. Due to the simplicity in design of the pilot plant facility, it was decided to apply linear scale-up design criteria for the design and construction of the full-scale treatment system.

The results obtained from Chapter 6 indicated that the ABR system was indeed capable of successfully treating the dilute wine lees wastewater by reducing the organic load by 55% at a volumetric loading rate of 11% per day. Dilution of the wastewater with 1 part freshwater proved to be sufficient in avoiding ammonia toxicity inside the ABR. Partially treated wastewater from the facultative pond overflow would be recycled for dilution purposes for the scale-up ABR, due to the relatively low concentration of ammonia expected in the surface water of the FP. Another important factor is that the alkaline FP water will aid in neutralising the influent acidic stream prior to treatment in the ABR.

The scale-up ABR should have a total volume of 2 400 m^3 in order to maintain a HRT of 9 days. The downward velocity at each baffle will be 11 $m.d^{-1}$ and the upward velocity 5.4 $m.d^{-1}$. Provision will be made for an internal recycling ratio of approximately 1:1.

A schematic diagram of the design layout for the scale-up system is shown in **Figure 7.4.1.**

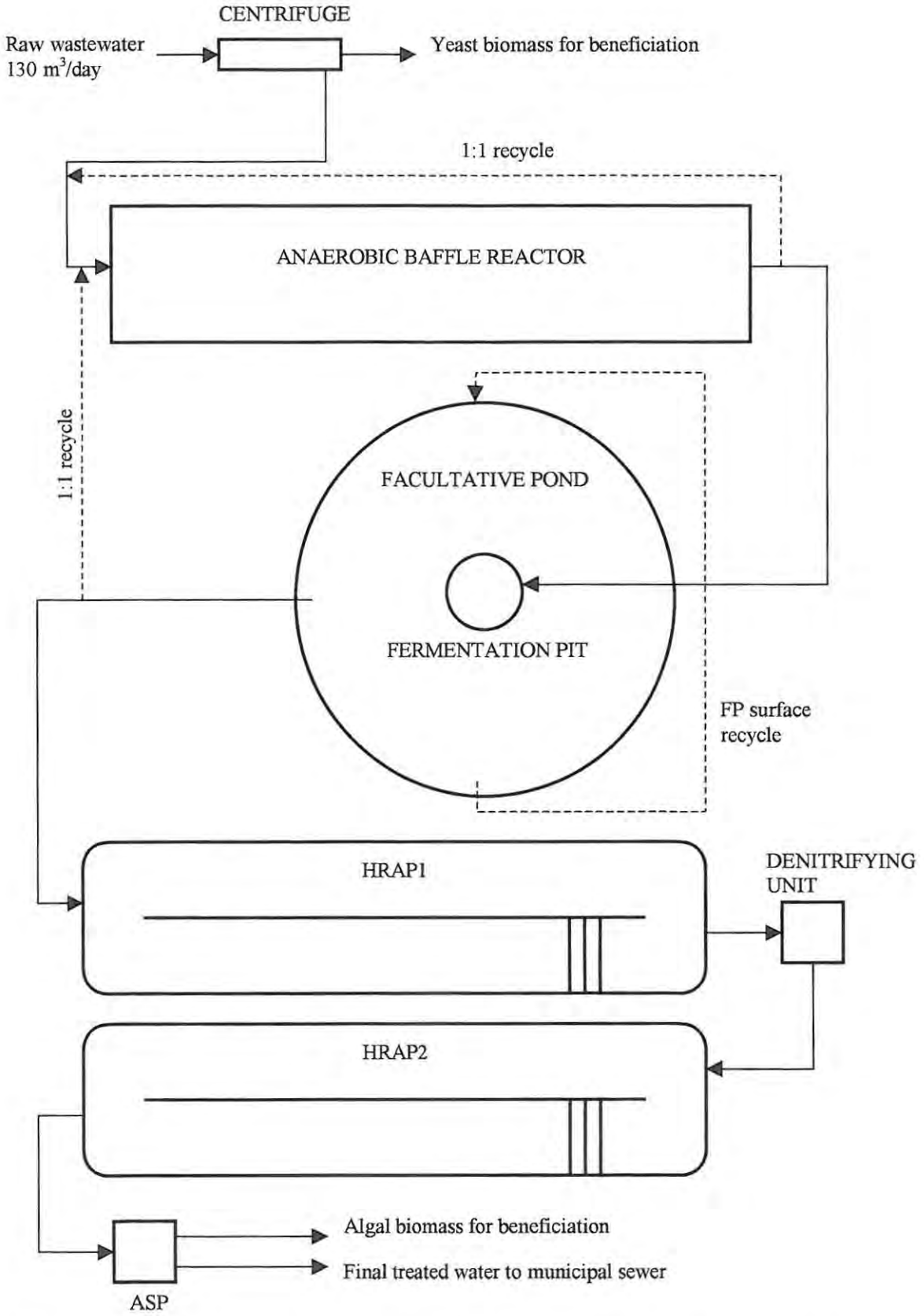


Figure 7.4.1. Conceptual diagram of the scale-up Anaerobic/Algal Oxidation Process for the treatment of wine lees wastewater at Brenn-O-Kem.

7.4.3. Facultative Pond

The fermentation pit inside the FP removed only 34% of the influent COD load (refer to **Table 6.4.3.**) However, during the pilot plant studies the fermentation pit functioned well in retaining active anaerobic sludge present in the overflow from the ABR. The inserted nylon net played an important role in sludge retention and would therefore also be a useful retrofit for the scale-up unit. A hydraulic retention of 1 day would be sufficient for the anaerobic pit, due to the efficient primary anaerobic treatment expected in the ABR. Sludge accumulating inside the fermentation pit could then be recycled to the ABR when required.

Although the pilot FP was operated with a 20-day retention period, it was decided to increase the retention to 30 days for enhanced stabilisation of the partially treated wastewater. The FP surface water will be internally recycled for enhanced planktonic algal growth, according to the design described in **Figure 2.4.8.**

7.4.4. High Rate Algal Pond

The HRAP conceptual design and operation is similar to the description in Chapter 6, where the HRAP is constructed into two separate units for enhanced algal biomass production in the first unit, followed by nutrient removal in the second unit. The scale-up HRAPs will have a surface area of 2 000 m^3 each. The volumetric loading rate to each will be 22% per day.

7.4.5. Algal Settling Pond

Each ASP will provide a hydraulic retention of approximately 10 hours. The first ASP can easily be converted into a denitrification unit, while the second ASP be used for removal of settleable algal biomass from the final treated water.

The Brenn-O-Kem wine lees factory is situated within a municipal area, which allows for discharge of the treated wastewater into the municipal sewer system for final blending of the relatively high saline water. This practise will be acceptable to the local municipality given the fact that the problematic compounds, in the form of organic and nutrient loads, have been removed from the wastewater prior to blending with sewage.

7.4.6. Cost for construction of the scale-up treatment plant

The cost for construction of the Anaerobic/Algal Oxidation Process is R5 million (South African Rand value in 2001) with a plant footprint of 1,5 hectare. Appendix I contains the MBB engineering design including a breakdown of the unit costs.

7.5. Conclusion

The AIWPS in an adapted form, which was originally designed by Oswald for the treatment of sewage wastewaters, could offer an appropriate solution to the wastewater problems experienced by Brenn-O-Kem.

The ABR offers efficient COD removal in order to not only reduce the pollution load to the ponding system, but also reduce the land area requirement for a conventional AIWPS treating this type of high strength wastewater.

The operation and maintenance costs for the proposed system is believed to be much less than conventional treatment plants, which is the result from a number of factors unique to the system. The specialisation level required of personnel is not as great as that needed to maintain high technology treatment systems. The transfer and disposal of day-to-day sludge is also eliminated, which aids in the minimisation of mechanical equipment and electrical energy requirements. The decreased energy requirement for aeration is another important factor which lowers the cost of operation and maintenance. It has been argued that AIWPS operate at a fraction of the cost of conventional wastewater treatment

systems, without factoring into the calculation the value of the biomass produced during treatment (Fallowfield *et al.*, 1992).

The potential commercial value of the yeast and algal biomass, which is recoverable from the wine lees wastewater application in a feed-grade form, is an added bonus to this industry.

Chapter Eight

Conclusion

Ponds are by far the most economical reactors available for liquid waste management and for efficient capture of solar energy (Oswald, 1995). A pond system, correctly designed and managed to cultivate anaerobic and aerobic bacteria and green microalgae, can decompose waterborne organic wastes efficiently and synthesise valuable algal biomass from the products of decomposition. Few studies of wine industry WSP have been reported and limited guidelines are available on operation and management of winery wastes in ponds. A survey conducted by the researchers in the Western Cape Province, which is the prime wine producing area in South Africa, revealed that many wineries and distilleries were using waste stabilisation ponds to store or treat effluent. However, most of these systems were not properly designed and/or managed, resulting in overloading and, at times, the generation of seriously offensive odours. Population growth around winery and distillery effluent producing areas, and changing land values, requires either more efficient operation of conventional ponds or reinvestment in high cost physical-chemical and biological systems.

Preliminary studies on the feasibility of treating winery wastewater utilising AIWPS technology were conducted. The results indicated problems with regard to active sludge retention by the in-pond anaerobic digester, as well as inadequate nutrient removal rates achieved in the secondary and tertiary ponding treatment sequence. In this study a demonstration AIWPS treating household sewage has been used as the basis for the investigation towards understanding the associated problems of malfunction.

The investigation has shown that a number of factors influence the retention of anaerobic sludge within the submerged fermentation pit inside the PFP. These include the inappropriate positioning of the gas collection device, as well as sludge floatation during the open pit design. The insertion of an appropriate aperture nylon net across the rising water column proved to be an effective low-cost retrofit. During system start-up and/or

incidences of organic overloading, fermentation gas production is very high, resulting in gas entrapment in the dense bottom sludge bed. When the buoyancy of the entrapped gas is high enough there is an incidental flotation of sludge resulting in sludge piston formation. The net prevents these large sludge fractions from washout and effective separation of gas bubbles and solids is created at the net interface, with sludge particles returning to the bottom sludge bed.

The development of the HRAP application as a potential stand-alone unit operation for the removal of phosphate and ammonia from treated wastewaters was also investigated. The research has demonstrated that photosynthetic activity, through microalgal activity, play a central role by maintaining a high pH and thereby not only induce calcium phosphate precipitation, but also aid in the removal of ammonia through stripping. Phosphate removal to below 1 $mgP.L^{-1}$ has been sustained over prolonged periods of operation. This independent HRAP is followed by algal biomass removal through settling, together with excess calcium phosphate solids.

During investigation into the ASP component of the sewage AIWPS it was revealed that denitrification occurred inside the settled algal biomass column. Research into this phenomenon indicated that active algal biomass from the HRAP can be used as a reagent for denitrification. The algal biomass releases polymeric substances which probably serve as carbon source for denitrifying micro-organisms. Nitrate removal levels from 20 to 1 $mgN.L^{-1}$ have been achieved in the algal denitrifying reactor. Under these anoxic conditions the algal cells are not degraded and proved to be active after subsequent light exposure. The viable algal biomass could therefore be introduced back into any HRAP unit to complete alkalisation for pH-induced phosphate and ammonia removal. The I-HRAP can therefore be considered as a reactor in which photosynthetic carbon production is maximised by converting dissolved carbon dioxide into algal biomass.

The preceding studies were therefore preparatory to the conceptualisation, development and piloting of the integrated ponding treatment of wine industry wastewater. The integrated ponding treatment of wine lees distillery wastewater was chosen as a model for

such a study. In addition to high COD loads, wine lees effluents contain a range of components including high levels of ammonia and protein nitrogen, phosphates and sulphates, providing a worst-case scenario of what may be encountered in wine industry effluents. Due to the additional high organic load of this effluent an ABR was introduced as pre-treatment prior to the receiving AIWPS. The overall process achieved a COD removal of 95 - 98 %, with around 80% of this removal achieved in the ABR operation. Ammonia toxicity was experienced by the anaerobic units of the process during periods when treating wine lees effluent batches with high nitrogen levels. However, the recovery of the system followed closely on the relief of inhibition which demonstrated a certain robustness to the process.

The improved design for the fermentation pit proved to be successful during treatment of wine industry wastewater. The nylon net played an important role especially during start-up and periods of ammonia toxicity.

Both nitrogen and phosphorus were effectively reduced by the algal ponding components. When operating the HRAP cascade in such a manner as to present algal biomass development for subsequent use in a denitrifying unit, followed by ammonia stripping and phosphate precipitation, a good quality final effluent may be produced. Another important advantage of the HRAP is that the high alkalinity final water may be recycled for upstream control and neutralisation of the influent acidity.

The recovery and evaluation of algal and yeast biomass was shown to offer opportunities for by-product beneficiation. Analysis and results of the fish feeding trials undertaken, allow a preliminary conclusion that winery effluent grown algae has no demonstrated toxicological constraints.

The successful evaluation of the improved AIWPS for treatment of wine industry effluents has provided insights into the managing of malfunctioning winery WSP, and in this way the investigations undertaken here have provided verification of the research objectives.

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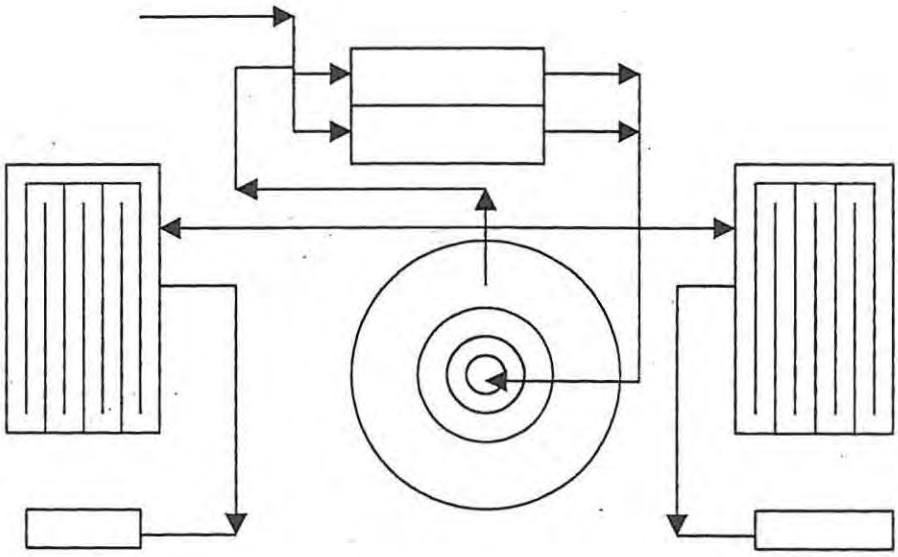
CONTENTS

RHODES UNIVERSITY

INTERGRATED BIOPROCESS TREATMENT PLANT



PRELIMINARY DESIGN AND COST ESTIMATE
FEBRUARY 2001

CONSULTING ENGINEERS:

MBB Consulting Engineers Inc
PO Box 509
GRAHAMSTOWN
6140

TEL: (046) 622 7223
FAX: (046) 622 9761

CLIENT:

Professor P Rose
Dept. of Microbiology & Biochemistry
Rhodes University
GRAHAMSTOWN
6140

TEL: (046) 6038630

February 2001

INTEGRATED BIOPROCESS TREATMENT PLANT

1. INTRODUCTION

On January 19, 2001 representatives of **MBB Consulting Engineers Inc, (MBB)** met with Mr Len Dekker and Professor Peter Rose from Rhodes University. Professor Rose and Mr Dekker briefed MBB on the proposed Algal Integrated Ponding System (A.I.P. System) developed by Rhodes to clean effluent stemming from wine farms. This A.I.P System consists of four parts: An anaerobic baffle reactor, a fermentation pit and facultative pond, a high rate algal pond and an algal settling pond.

2. BRIEF

MBB were required to undertake a preliminary infrastructure design and establish an estimate of cost for the proposed works. The specifications given for the preliminary design were as follows:

2.1 Anaerobic Baffle Reactor

- Total volume: 2400 m^3
- Depth: 3 m
- Surface area: 800 m^2
- Flowrate: 400 m^3/day
- It needs to be lined and covered to prevent gasses escaping
- There is to be a downward velocity of 11 m/day and an upward velocity of a maximum of 5.4 m/day.
- The effluent is to be recirculated by means of a recirculation pump (10 m^3/hour).

2.2 Facultative Pond

- Total volume: 8000 m^3
- Depth: 4 m
- Flowrate: 400 m^3 /day
- Attached is an internal settling pit (Fermentation Pit), 200 m^3 and 2 m deep with a sludge evacuation pump.
- Surface recirculation pump (10 m^3 /hour)
- Subsurface recirculation pump (10 m^3 /hour) to the Baffle tank.

2.3 High Rate Algal Pond

- Depth: 30 cm
- Two 2000 m^2 units.

2.4 Algal Settling Pond

- Two 50 m^3 units.

3. PRELIMINARY DESIGN

The preliminary design details of the respective items which make up the Integrated Bioprocess Treatment Plant are as follows:

3.1 General Layout Plan of Process Plant - Annexure A

3.2 Anaerobic Baffle Reactor - Refer to Annexure B.

3.3 Fermentation pit and Facultative pond- Refer to Annexure C.

3.4 High Rate Algal Pond (x2) – Refer to Annexure D.

3.5 Algal Settling Ponds (x2) – Refer to Annexure E.

4. COST ESTIMATE

A cost estimate was undertaken for the proposed works with the following assumptions in mind:

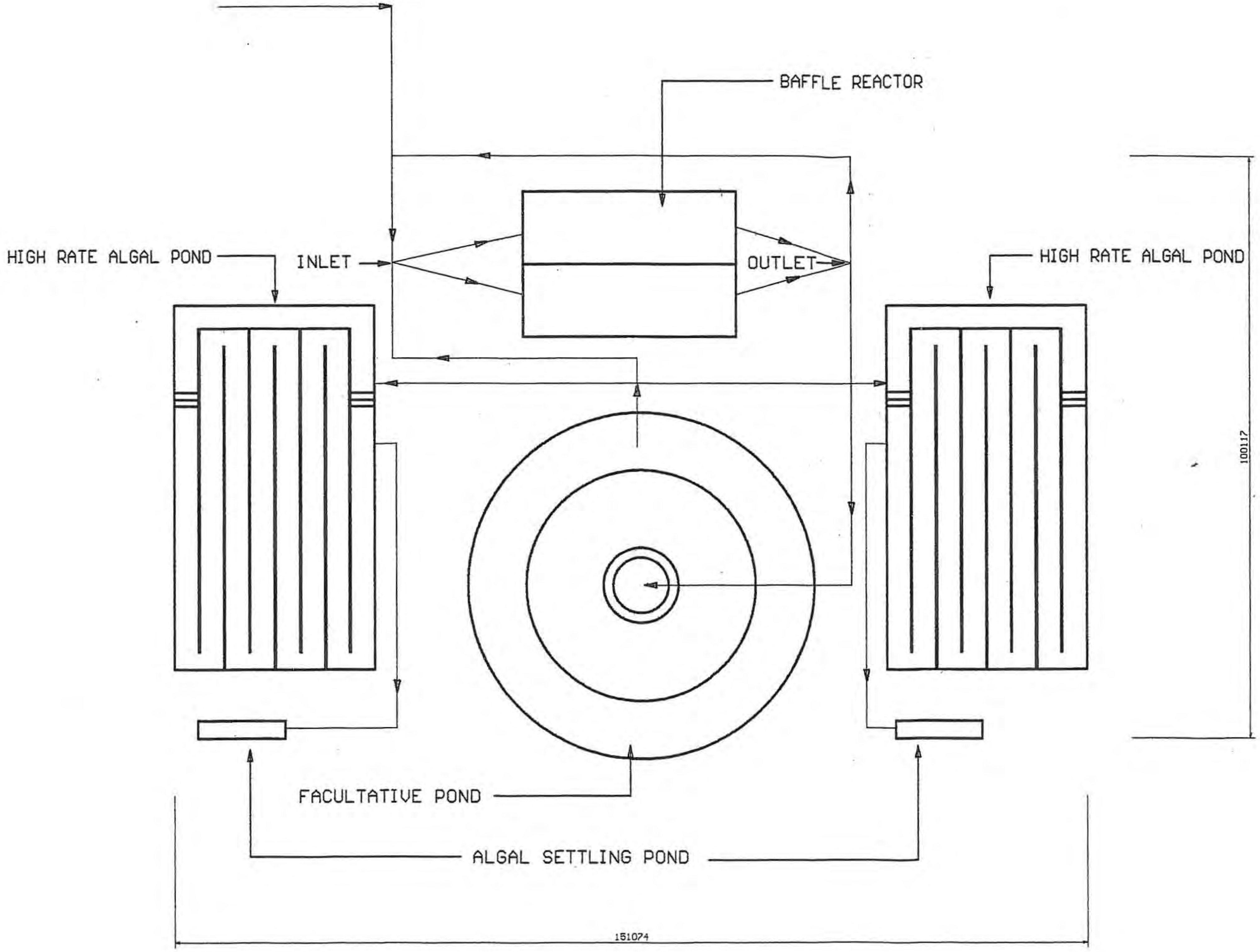
- A level terrain which is geotechnically suitable for development.
- Costs are based on local construction industry rates.

Item No.	Item				Cost
	Preliminary & General (15%)				R450,000.00
1	Anaerobic Baffle Reactor *				R1,350,660.00
2	Fermentation Pit and Facultative pond *				R369,550.00
3	High Rate Algal Pond (x2) *				R756,310.00
4	Algal Settling Ponds (x2) *				R84,250.00
5	Pumps *				R225,000.00
6	100mm uPVC Pipeline *				R81,500.00
	Contingencies (+-15%)				R450,000.00
	Consulting Fees and Recoverable Costs				R550,000.00
	Subtotal				R4,317,270.00
	14% VAT				R604,418.00
	Total				**R4,922,000.00**

* Refer to Annexure F for a breakdown of the unit costs used in determining the costs of respective items that make up the Integrated Bioprocess Treatment Plant.

ANNEXURE A

GENERAL LAYOUT PLAN
OF
PROCESS PLANT



NOTE :
AREA COVERED 15125 m^2

Wys. Rev.	Datum Date	Ruit Grid	Beskrywing Description	G.V. O.A.

WYSIGINGS/REVISIONS

Kontroleer tekeningmates voor werkuitvoering. Geskaalde mates is ongeldig.
Check drawing measurements before commencing work. Scaled measurements are not valid.

KANTOOR/OFFICE:	✉	☎	FAX
STELLENBOSCH	3011	8871028	8838514
PRETORIA	35560	488332	488381
GRAHAMSTOWN	609	27223	29781
NELSPRUIT	498	25213	52115
ROBERTSON	440	61003	3538
PIETERMARITZBURG	2831	453630	427728

REG NO 77/04353/21

MBB RAADGEWENDE INGENIEURS INGELYF
CONSULTING ENGINEERS INCORPORATED

Namens/For: MBB RAADGEWENDE INGENIEURS INGELYF
MBB CONSULTING ENGINEERS INCORPORATED

Pr Ing / Pr Eng .... Datum/Date ....

RHODES BIOCHEMISTRY

INTERGRATED BIOPROCESS TREATMENT PLANT

GENERAL LAYOUT

Geteken/Drawn	HS/IB	Opgemeet/Surveyed	
Datum/Date	JANUARY 2001	Ontwerp/Designed	HS/IB
Skaal/Scale	1 : 400	Nagesien/Checked	PJE
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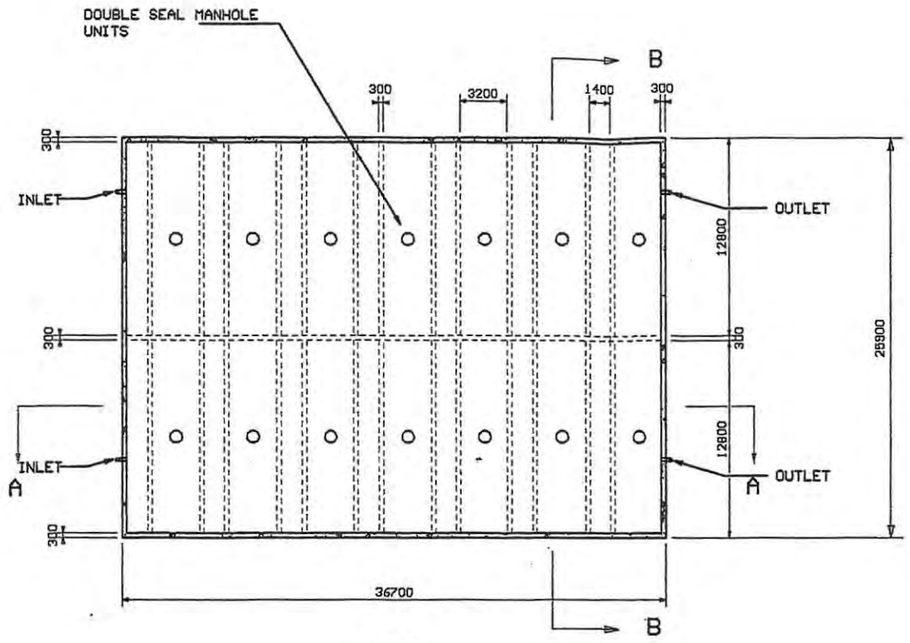
ANNEXURE B

ANAEROBIC BAFFLE REACTOR



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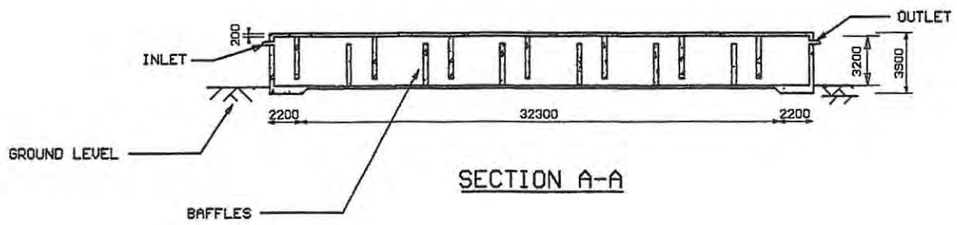

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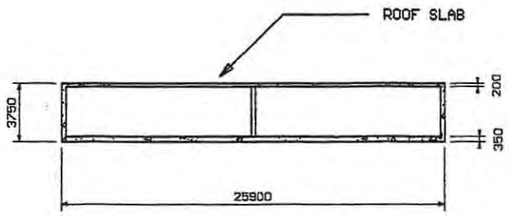

SECTION B-B

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WYSIGINGS/REVISIONS

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STELLENBOSCH	3011	8871025	8838514
PRETORIA	35660	468332	468391
GRAHAMSTOWN	509	27223	28761
NELSPRUIT	488	28213	52115
ROBERTSON	440	61005	2536
PIETERMARITZBURG	2821	453530	427728

REG. NO. 77/04351/21

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CONSULTING ENGINEERS INCORPORATED

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MBB CONSULTING ENGINEERS INCORPORATED

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RHODES BIOCHEMISTRY

INTERGRATED BIOPROCESS TREATMENT PLANT

ANAEROBIC BAFFLE REACTOR

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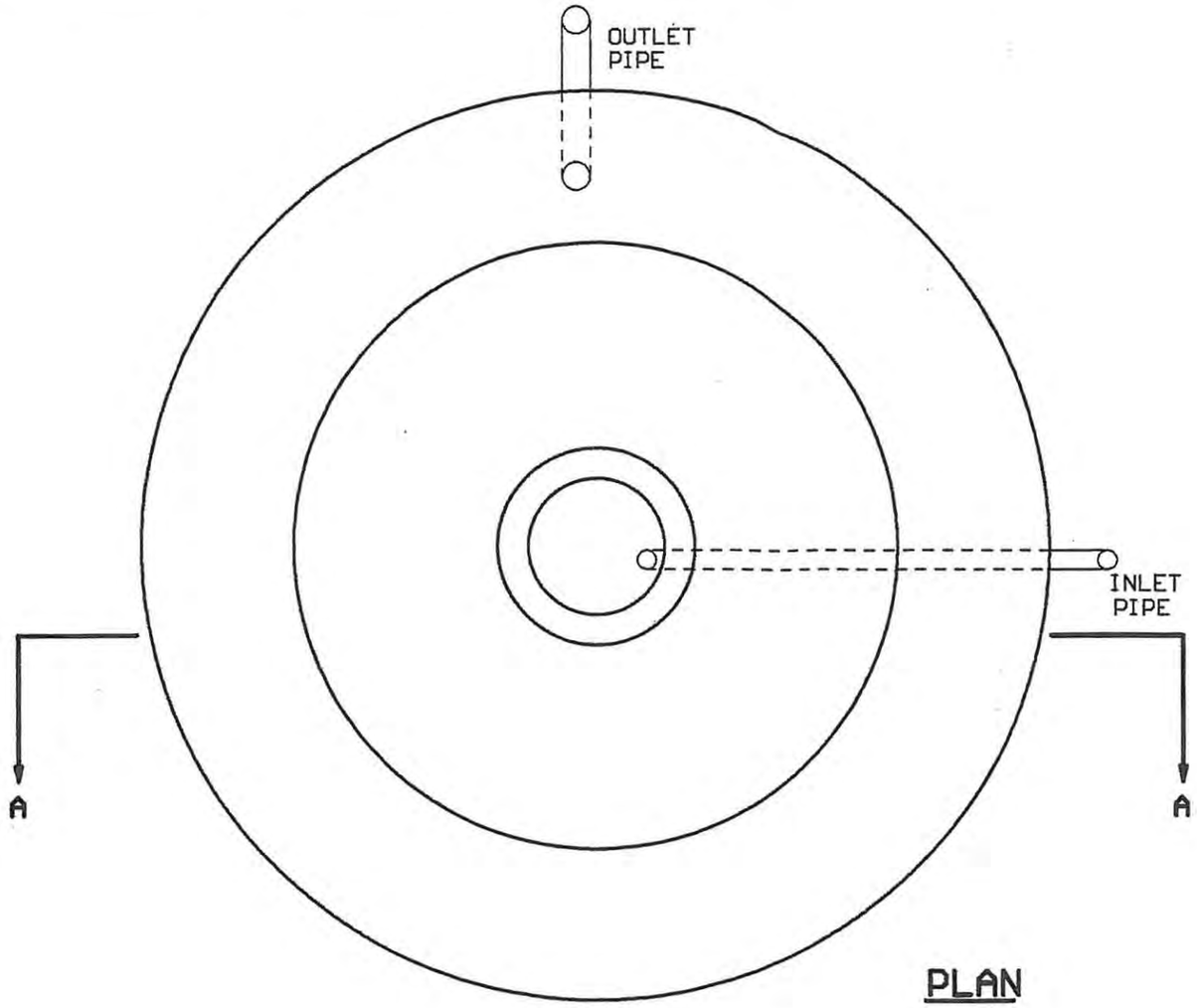
Skaal/Scale	1 : 200	Nagesien/Checked	AJE

TEKENINGNOMMER / DRAWING NUMBER: KODE/CODE G | PROJEKNOMMER/PROJECT NUMBER 1930 | TEKENINGKODE/DRAWING CODE 101 | UITGAWE NO./REVISION NO. R00

ANNEXURE C

FERMENTATION PIT
AND
FACULTATIVE POND



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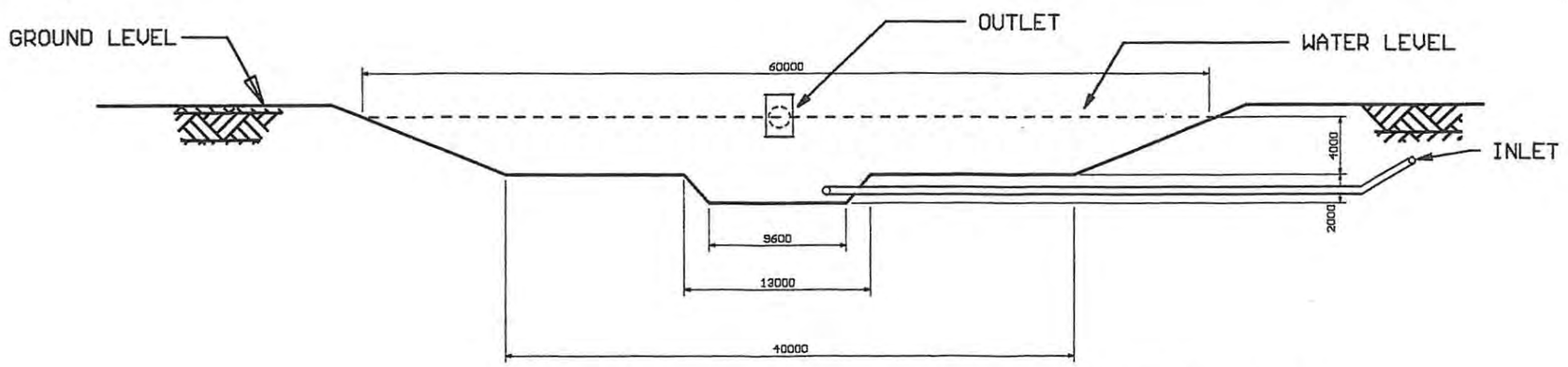

SECTION A-A

Wys. Rev.	Datum Date	Ruit Grid	Beskrywing Description	G.V. Q.A.

WYSIGINGS/REVISIONS

KANTOOR/OFFICE: | TEL | FAX
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PRETORIA | 38660 | 466332 | 466351
GRAHAMSTOWN | 509 | 27223 | 28761
KOLSPRUIT | 498 | 28213 | 52115
ROBERTSON | 440 | 61008 | 2538
PIETERMARITZBURG | 2821 | 453530 | 427728
REG.NO. 77/04253/21

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RHODES BIOCHEMISTRY

INTERGRATED BIOPROCESS TREATMENT PLANT

FACULTATIVE POND/FERMENTATION PIT

Getoken/Draun	IBAB	Opgemeet/Surveyed	
Datum/Date	31/01/01	Ontwerp/Designed	IBAB
Skaal/Scale	1:200	Nagesien/Checked	PJE

TEKENINGNOMMER / DRAWING NUMBER: F CODE | PROJECTNOMMER / PROJECT NUMBER | TEKENINGNOMMER / DRAWING CODE | WYSIGING NO. / REVISION NO.
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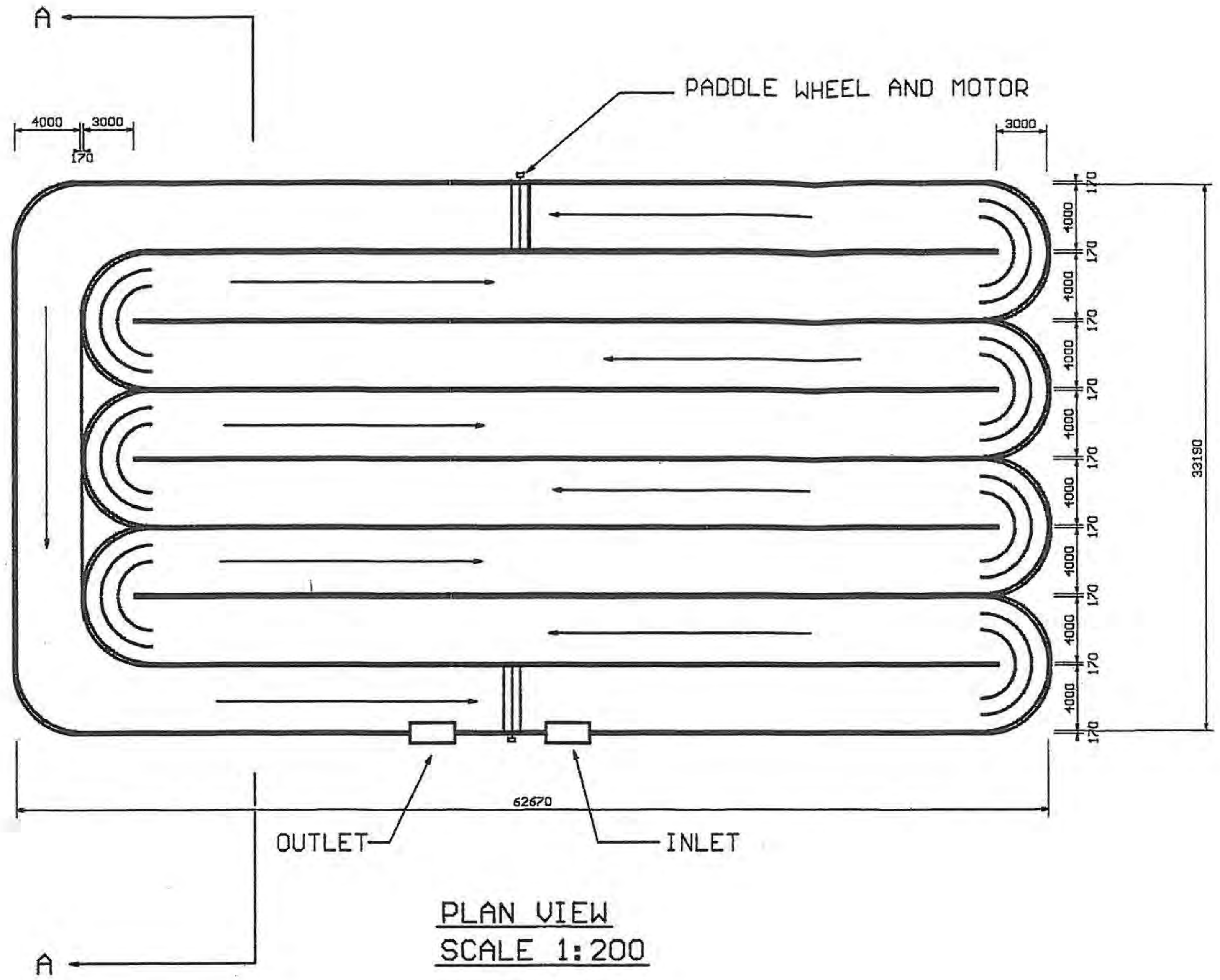
ANNEXURE D

HIGH RATE ALGAL POND



PLAN VIEW
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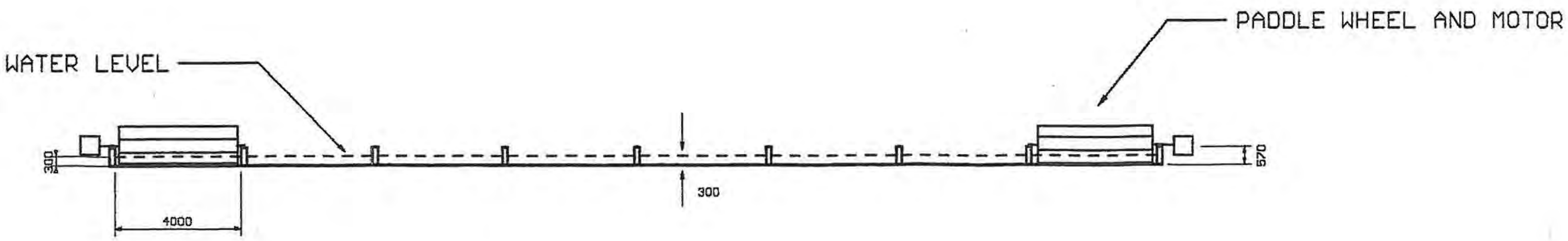

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Wys. Rev.	Datum Date	Ruit Grid	Beskrywing Description	G.V. Q.A.

WYSIGINGS/REVISIONS

KANTOOR/OFFICE:	✉	☎	FAX
STELLENBOSCH	3011	8871028	8838514
PRETORIA	35880	465332	488391
GRAHAMSTOWN	509	27223	29761
NELSPRUIT	498	28213	52115
ROBERTSON	440	61005	2538
PIETERMARITZBURG	2821	453530	427728

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CONSULTING ENGINEERS INCORPORATED

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MBB CONSULTING ENGINEERS INCORPORATED

Pr Ing / Pr Eng Datum/Date

RHODES BIOCHEMESTRY

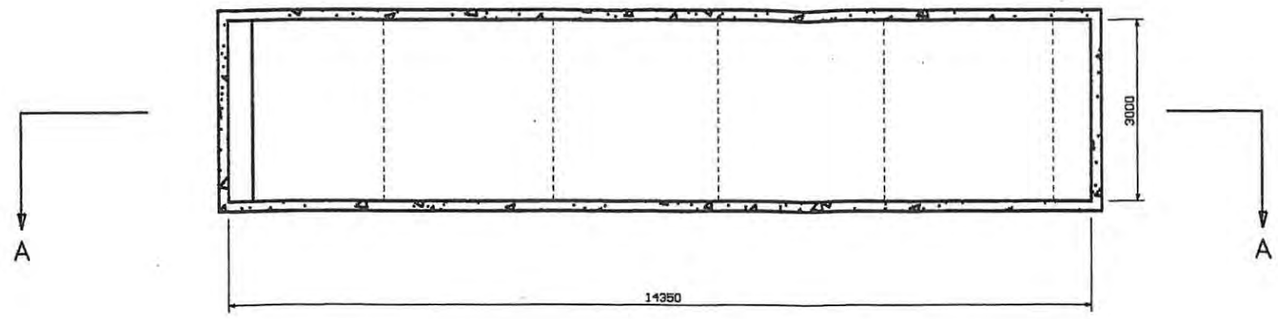
INTERGRATED BIOPROCESS TREATMENT PLANT

HIGH RATE ALGAL POND

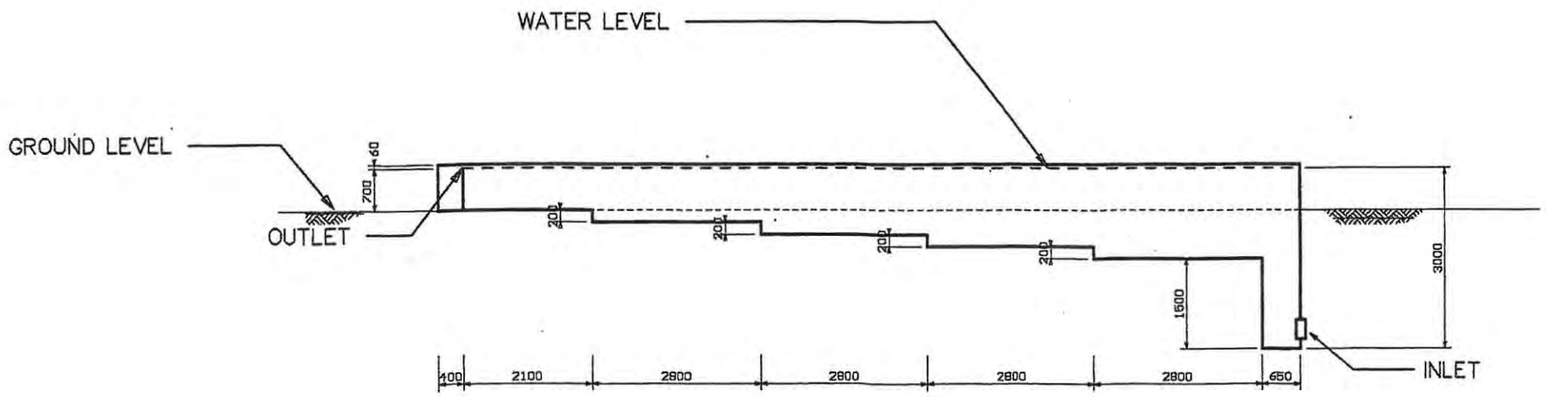
Geteken/Drawn	IB/HS	Opgemeet/Surveyed	
Datum/Date	31/01/01	Ontwerp/Designed	IB/HS
Skaal/Scale	AS SHOWN	Nagesien/Checked	PJE
TEKENINGNOMMER DRAWING NUMBER	G1930 1 03		R00

ANNEXURE E

ALGAL SETTLING POND



PLAN



SECTION A–A

Wys. Rev.	Datum Date	Ruit Grid	Beskrywing Description	G.V. Q.A.

WYSIGINGS/REVISIONS

KANTOOR/OFFICE:	TEL	FAX
STELLENBOSCH	3011	8571028	8838514
PRETORIA	35880	468332	465391
GRAHAMSTOWN	809	27233	29761
HELSPRUIT	468	28213	53115
ROBERTSON	440	81005	2536
PIETERMARITZBURG	2821	453930	427728

REG. NO. 77/04383/21

MBB RAADGEWENDE INGENIEURS INGELYF
CONSULTING ENGINEERS INCORPORATED

Namens/For: MBB RAADGEWENDE INGENIEURS INGELYF
MBB CONSULTING ENGINEERS INCORPORATED

Pr Ing / Pr Eng ..... Datum/Date .....

RHODES BIOCHEMISTRY

INTERGRATED BIOPROCESS TREATMENT PLANT

ALGAL SETTLING TANK

Geteken/Drawn	IB/HS	Opgemeet/Surveyed	
Datum/Date	31/01/01	Ontwerp/Designed	IB/HS
Skaal/Scale	1:100	Nagesien/Checked	PJE

TEKENINGNOMMER DRAWING NUMBER	F KODE F CODE	PROJEKNOMMER PROJECT NUMBER	TEKENINGKODE DRAWING CODE		WYSIGING NR. REVISION NR.
	G	1930	104		R00

ANNEXURE F

COST ESTIMATE

m No.	Description	Unit	Quantity	Rate (R)	Cost (R)
	Anaerobic Baffle Reactor Drawing G1930/101 - Annexure B				
	Foundation Preparation	m2	470	40	18,800.00
	Concrete for walls and baffles (40 Mpa)	m3	400	675	270,000.00
	Concrete for roof slab (40 Mpa)	m3	180	675	121,500.00
	Concrete for floor (40MPa)	m3	160	675	108,000.00
	Reinforcing steel (Y12)	kg	88440	4	353,760.00
.1	Formwork and finish				
.2	Smooth: Roof slab soffit	m2	960	110	105,600.00
	Smooth: Walls	m2	3200	110	352,000.00
	Double Seal man hole covers	sum	14	1500	21,000.00
	Sub total of Item 1				1,350,660.00
	Fermentation Pit & Facultative Pond Drawing G1930/102 - Annexure C				
	Excavations	m3	8000	15	120,000.00
	Cement stabilized base (75mm)	m2	3100	11	32,550.00
	Plastic Lining (fitted)	m2	3100	70	217,000.00
	Sub total of Item 2				369,550.00
	High Rate Algal Ponds (x2) Drawing G1930/103 - Annexure D				
	Concrete Building Blocks	sum	9030	7	63,210.00
	Plastering	m2	1510	30	45,300.00
	Plastic Lining (fitted)	m2	6550	70	458,500.00
	Supply and Installation of paddle wheel complete with motors and electrical connection	sum	4	28000	112,000.00
	Foundation Excavations	m3	100	12	1,200.00
	Foundation Concrete (20MPa)	m3	100	530	53,000.00
	Cement stabilized base (75mm)	m2	2100	11	23,100.00
	Sub total of Item 3				756,310.00
	Algal Settling Ponds (x2) Drawing G1930/104 - Annexure E				
	Excavations	m3	300	12	3,600.00
	75mm Concrete Blinding (15MPa)	m3	25	490	12,250.00
	150mm Reinforced Concrete (30MPa)	m3	50	620	31,000.00
	Reinforcing Mesh	m2	400	11	4,400.00
	Formwork and finish	m2	300	110	33,000.00
	Sub total of Item 4				84,250.00
	Subtotal carried forward				2,560,770.00

n No.	Description	Unit	Quantity	Rate (R)	Cost (R)
	Subtotal brought forward				**2,560,770.00**
	Pumps				
	Supply and installation of recirculation pumps including electrical connections	sum	3	50000	150,000.00
	Supply and installation of sludge pump including electrical connections	sum	1	75000	75,000.00
	Subtotal of item 5				225,000.00
	110mm uPVC Pipeline				
	Under Ground	m	1000	28	28,000.00
	Above Ground	m	300	15	4,500.00
	Valves & Fittings	sum			30,000.00
	Pipe trench excavations: 0 to 1m	m	1000	19	19,000.00
	Subtotal of item 6				81,500.00

No.	Summary Of Costs				
	Preliminary & General (15%)				450,000.00
1	Anaerobic Baffle Reactor				1,350,660.00
2	Fermentation Pit and Facultative pond				369,550.00
3	High Rate Algal Pond (x2)				756,310.00
4	Algal Settling Ponds (x2)				84,250.00
5	Pumps				225,000.00
6	100mm uPVC Pipeline				81,500.00
	Contingencies (+-15%)				450,000.00
	Consulting Fees and Recoverable Costs				550,000.00
	Subtotal				4,317,270.00
	14% VAT				604,418.00
	Total				**4,922,000.00**