The treatment of brewery effluent using an integrated high rate algal ponding system

A thesis

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Anneke Cilliers

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Abstract

The application of high rate algal ponds (HRAP) in the treatment of brewery effluent that met the South African Department of Water Affairs and Forestry's (DWAF) general limits for discharge into a natural water resource of 1998 were tested during a 10-month baseline phase, followed by an 11-month optimization phase. The objective of the baseline phase was to monitor the seasonal performance of HRAPs. The hydraulic retention time (HRT) fluctuated between 11.16 d and 12.00 d in HRAPs. The chemical oxygen demand (COD) increased from 130.12 ± 6.94 mg/L (post-AD), to 171.21 ± 7.99 mg/L (post-HRAP). The presence of algal cells and evaporation contributed towards an increase in post-HRAP COD. The ammonia (NH_4-N) concentration decreased from 46.59 ± 2.47 mg/L (post-AD), to 1.08 ± 0.12 mg/L (post-HRAP). The nitrite (NO_2-N) concentration remained below 1.00 mg/L in post-pilot plant AD, post-PFP and post-HRAP effluent. The phosphate (PO_4-P) concentration decreased from 29.81 ± 1.39 mg/L (post-AD) to 17.30 ± 1.16 mg/L PO_4-P. The objective of the optimization phase was to manipulate the HRT to achieve the maximum treatment rate that met the DWAF general limits for discharge into a natural water resource of 1998. Nitrogen (as NH_4-N, NO_3-N, NO_2-N) removal efficiency was used as an indicator of nutrient removal success. HRT was influenced by season. The optimal HRT for autumn was 4.30 d at a temperature of 20.53 °C in HRAP A2 (heated) and 18.96 °C in HRAP B2 (ambient). The optimal HRT for summer was 2.74 d at 29.90 °C in HRAP A2 (heated) and 26.36 °C in HRAP B2 (ambient). The COD decreased from 152.33 ± 4.85 mg/L (post-AD) to 95.00 ± 3.75 mg/L (post-HRAP A2), and to 100.82 ± 5.93 mg/L (post-HRAP B2). The incoming NH_4-N concentration decreased from 42.53 ± 1.38 mg/L (post-AD), to 1.70 ± 0.81 mg/L (post-HRAP). The nitrate (NO_3-N) concentration post-HRAP was 12 – 14 mg/L. The main methods for NH_4-N removal were probably NH_4-N volatilization through algal uptake. HRAPs were able to lower nitrogen and phosphorous concentrations to within the DWAF limits under normal operating conditions. It is recommended that HRAP treated brewery wastewater be used for irrigation after salt removal, or

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List of abbreviations

AD	Anaerobic digester
AS	Activated sludge
BOD	Biological oxygen demand
CH_4	Methane
COD	Chemical oxygen demand
CIP	Cleaning-in-place
DIC	Dissolved inorganic carbon
DO	Dissolved oxygen
DWAF	South African Department of Water and Environmental Affairs
EBRU	Institute of Environmental Biotechnology, Grahamstown, Rhodes University
EC	Electrical conductivity
H_2	Hydrogen gas
HRAP	High rate algal ponds
HRT	Hydraulic retention time
NGO	Non-governmental organization
NH_3	Ammonia gas
NH_4-N	Molecular unionized ammonia
N_2	Nitrogen gas
NO_2-N	Nitrite nitrogen
NO_3-N	Nitrate nitrogen
O_2	Oxygen gas
PFP	Primary facultative pond
PO_4-P	Orthophosphate
SAB Ltd.	South African Breweries Limited
SABSD 2010	South African Breweries sustainable development report 2010 (Reference)
SABWF 2010	South African Breweries water footprint report 2010 (Reference)
SABWFu 2010	South African Breweries water futures report 2010 (Reference)
WRC	Water Research Commission
WWF-UK	World Wildlife Fund United Kingdom

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I was part of a team of people that worked together to realize goals. I would like to express my humble wish that cooperation and resource sharing between people continues to implement clean and sustainable production technologies globally.

Chapter one

Introduction to Project Eden

Recycling of waste has become a global imperative which is being written into policy, legislation and corporate governance (Ridout & Pfister 2010). Water management practices need to become more sustainable in order to secure enough water to support the growing world population, to underpin economic growth, as well as to meet environmental needs (SABWF 2010). Societies have largely failed to value and govern their freshwater resources adequately. This has led to water shortages and pollution in many locations (Ridout & Pfister 2010, SABWF 2010). Climate change is expected to intensify the variability and predictability of rainfall patterns. Water in the 21st century is expected to become a scarce and highly contested resource, similar to what oil was in the 20th century (Barlow & Clarke 2002).

There has been a growing awareness in the private sector of the importance of water for the well-being of society, economic growth and environmental health (Ridout & Pfister 2010, SABWF 2010). Business forums such as the United Nations Global Compact's CEO Water Mandate, the World Economic Forum and the Water Stewardship Forum have emerged as platforms to host these important debates (SABSD 2010). Organisations such as the Water Footprint Network and the Alliance for Water Stewardship have been established to help measure and set standards around water use globally (SABWF 2010). Companies are increasingly focusing on their water footprint to assess their risk and to assist with their strategic planning. Corporate water strategies, water footprint and "offset approaches" are still in their infancy, although the attention that businesses are giving to proper water management is steadily accelerating (Ridout & Pfister 2010, SABWF 2010).

The current research project formed part of "Project Eden", which was implemented by Rhodes University to promote SABMiller's global sustainability objectives and to reduce the costs associated with brewery effluent disposal at South African Breweries Limited (SAB Ltd.) iBhayi Brewery in Port Elizabeth, South Africa. In a joint development agreement with Rhodes University, a 10-month "proof-of-concept" phase (May 2009 – February 2010) was implemented, followed by an 11-month "optimization phase" (March 2010 – January 2011) to test the application of high rate algal ponds (HRAP) and a constructed wetland in the treatment of brewery effluent that met the Department of Water Affairs and Forestry's (DWAF) general limits for discharge into a natural water resource of 1998 (Table 1, Appendix 1). The treated effluent was subsequently used in aquaculture and hydroponic lettuce production. Additional support was secured by the Water Research Commission

for the optimization phase and the beneficiation of recycled water and algae. This thesis reports on the efficiency of HRAP systems in the treatment of brewery effluent in order to realize SABMiller's global sustainability objectives (Box 1).

Box 1 – SAB Miller Sustainability Objectives Addressed by Project Eden

- **Making beer with less water**
- **Improved use of energy**
- **Reduced carbon footprint**
- **Water reuse and recycling**
- **Working towards zero waste operations**
- **Benefiting communities**
- **Transparency in progress reporting**

1.1 SABMiller's approach to water management

SABMiller is one of the world's largest brewers, with brewing interests and distribution agreements across six continents and 34 countries. By its nature, brewing is a water-intensive process (Braeken *et al.* 2004, Brito *et al.* 2007). SAB Ltd. faced particular challenges due to the widespread nature of its activities (SABWF 2010). An approximate 2.6 billion L beer and 3.3 billion L soft drinks were distributed annually from seven breweries and seven soft drink bottling plants and 41 sales and distribution centres (SABWF 2010). SAB Ltd. Head of Sustainable Development, Mr Andre Fourie, said: "*As a leading socially responsible corporate, it is imperative that we take responsibility for the impact of our operations on the environment and the communities in which we operate*".

SABMiller has recognized the importance of water conservation and has developed a strategy to make more beer, using less water (SABSD 2010). SABMiller is reducing its water footprint by employing new processes, and by changing behaviour to reduce water consumption in plants. Furthermore, there is a focus on recycling wastewater for re-use in non-brewing activities (SABSD 2010). SABMiller's target was to reduce its operational water use by 25 % by 2015 (SABSD 2010). The goal is to reduce the company's consumption from an industrial average of 5.00 L water for 1.00 L beer, to 3.50 L water for 1.00 L beer (SABSD 2010). Project Eden tested new water recycling technologies that could potentially enable SABMiller to realize its water strategy objective with regard to its *reduce*, *re-use*, *recycle* and *redistribute* sustainable water management principles (SABSD 2010, SABWF 2010, SABWFu 2010).

SABMiller is affiliated with the Water Footprint Network. The Water Footprint Network is a body that promotes the transition towards sustainable fair and efficient use of freshwater resources worldwide, by advancing the "water footprint" concept, by increasing awareness of the "water footprint" concept in communities, and by encouraging forms of water governance that reduce the negative ecological and social impacts of the water footprints of communities, countries and businesses (SABWF 2010). The water footprint concept implies water management that not only focuses on internal processes, but also considers supply chains that companies source from and the communities and ecosystems that supply these (SABWF 2010). In 2009, SABMiller and the World Wildlife Fund – United Kingdom published a report: "*Water Footprinting: Identifying and addressing water risks in the value chain*" (SABWF). The report focused on the entire value chain for SABMiller's beers in South Africa and the Czech Republic. Results for South Africa indicated that the total water footprint for 1.00L beer was 155.00 L water. This figure was significantly more than the figure for beer production in the Czech Republic, which was 45.00 L water for 1.00 L beer. In both cases more than 90 % of the water footprint's origin stemmed from the cultivation of crops, both local and imported (virtual water movement). The international trade in agricultural commodities implies that a trade in virtual water is occurring between countries (Chapagain & Hoekstra 2008). Virtual water is the total volume water that is required to produce a commodity. The main risks that were identified in the report included 1) water scarcity; 2) competition for water; 3) declining water quality; and 4) the social dimension of water and interactions with business . Water footprinting can be useful from a business perspective by helping to identify the scale of water-use in water scarce areas, as well as the potential business risks that can arise (SABWF 2010). The real value of a water footprint is that it can assist businesses to make better operational decisions concerning facility management, supply chain management and stakeholder engagement with the goals of reducing risk and improving environmental sustainability performance (SABWF 2010). The report emphasised that collaboration between local government, business and non-governmental organizations was the only way to solve local water problems.

1.2 South African water management legislation

The operations at SAB Ltd. iBhayi brewery were subject to South African legislation. A brief description of the governmental regulations for effluent disposal, the cost implications of handling effluent at SAB Ltd. iBhayi brewery, and the importance of reducing the chemical oxygen demand (COD) with HRAP and wetland effluent treatment technology follows, in order to put the objectives of this research project into perspective.

South African water resources are owned and governed by the national DWAF. The work of the department is informed by the following key legislative policy and regulatory frameworks (DWAF annual report 2009/10):

1. The National Water Act, 1998 (Act No. 36 of 1998). The objective of this act is to ensure that South Africa's water resources are protected, used, managed and controlled in a sustainable and equitable manner, for the benefit of all persons.

2. The Water Services Act, 1997 (Act No. 108 of 1997). The objective of this act is to provide for the right of access to basic water supply and basic sanitation by setting national norms and standards.

3. The Water Research Act, 1971 (Act No. 34 of 1971). The purpose of this act is to provide for the promotion of research in connection with water affairs and, for that purpose, to establish the Water Research Commission and Water Research Fund (DWAF annual report 2009/10).

The governmental wastewater discharge limits for the release of industrial effluent into natural water resources that were used as a benchmark in this study are currently being revised. In the interim those published in the National Gazette No. 26187 (26 March 2004) – *Revision of the general authorizations in terms of Section 39 of the National Water Act, 1998: The DWAF general limits for discharge into a natural water resource*, were used (Table 1, Appendix 1). One of the objectives of this research project was to test if it would be possible to treat brewery effluent with HRAP technology to produce an effluent that met these standards.

Another objective of Project Eden was to test the potential to reduce SABMiller's costs of purchasing water and the cost of municipal effluent treatment, by recycling effluent. The resultant effluent could potentially be used on-site for cleaning or irrigation and subsequently reduce the cost of purchasing water for these purposes. The tariff that applied for purchasing water from the municipality was R7.72/kL in 2010, and could fluctuate depending on the water availability in the area. SAB Ltd. iBhayi brewery's water consumption for 2009 was 743 240 kL/year, of which 380 425 kL/y was discharged to municipal sewage works. Fifty one per cent of the brewery's total water consumption was discharged as effluent (Mabuza, *pers. comm.,* engineering manager, iBhayi brewery, Port Elizabeth, SAB Ltd., SABMiller, November 2010). This means that the cost of purchasing water during 2010 was approximately R 5 737 812.80. The cost of treating effluent sent to the sewage works varied between R0.87/kL and R1.19/kL in 2010 (Mabuza, *pers. comm.,* engineering manager, iBhayi brewery, Port Elizabeth, SAB Ltd., SABMiller, November 2010). Using the R 0.87 rate, it would mean the the brewery spent around R330 969 for municipal wastewater treatment. If alternative water treatment technology could recycle effluent into drinking water, it

could save iBhayi brewery 51.2 % of its costs of purchasing water from the municipality by using recycled effluent in its production processes, as well as eliminating the cost of municipal sewage treatment. This would amount up to R2 937 760.15 per year, using 2010 figures.

HRAP and wetland technology could also assist in reducing the cost of penalties imposed by the municipality for the treatment of effluent. The municipality's pricing mechanism to treat effluent was based on an effluent's COD and pH (Mabuza, *pers. comm.,* engineering manager, iBhayi brewery, Port Elizabeth, SAB Ltd., SABMiller, November 2010). Municipal effluent treatment required effluent to have a COD of less than 500 mg/L and a pH above 6.00. If the COD and pH in effluent exceeded these limits, penalties were calculated for which the brewery was subsequently charged (1 July 2010 – 30 June 2011). HRAP treatment technology could therefore potentially assist to prevent effluent from exceeding municipal COD and pH limits, and assist with reducing the associated cost of penalties (Mabuza, *pers. comm.,* engineering manager, iBhayi brewery, Port Elizabeth, SAB Ltd., SABMiller, November 2010).

To sum it up, it was important to test whether HRAP systems could produce an effluent that met the DWAF general limits for discharge into a natural water resource (Table 1, Appendix 1). Treated effluent could either be re-used onsite at the brewery, or be used to recharge the groundwater supply. This could lead to reduced company cost of sending effluent to municipal sewage works and/or the cost of purchasing water from the municipality.

1.3 The brewing process

In order to understand the research approach it is necessary to present a summary of the brewing process in order to shed light on the chemical composition of brewery effluent and the subsequent treatment methods that have been used to remove macro-pollutants from effluent (Section 1.3.1).

Beer is obtained through the alcoholic fermentation by selected yeasts of the genus *Saccharomyces* of wort prepared from malt cereals (mainly barley), amylaceous /sugar-based raw materials to which hop flowers are added, and water (Figure 1.3.a, Brito *et al.* 2007). Water is used during the brewing process itself, but the majority of water is used as rinsing water during cleaning-in-place (CIP) process (Table 1.3.a, Braeken *et al.* 2004)

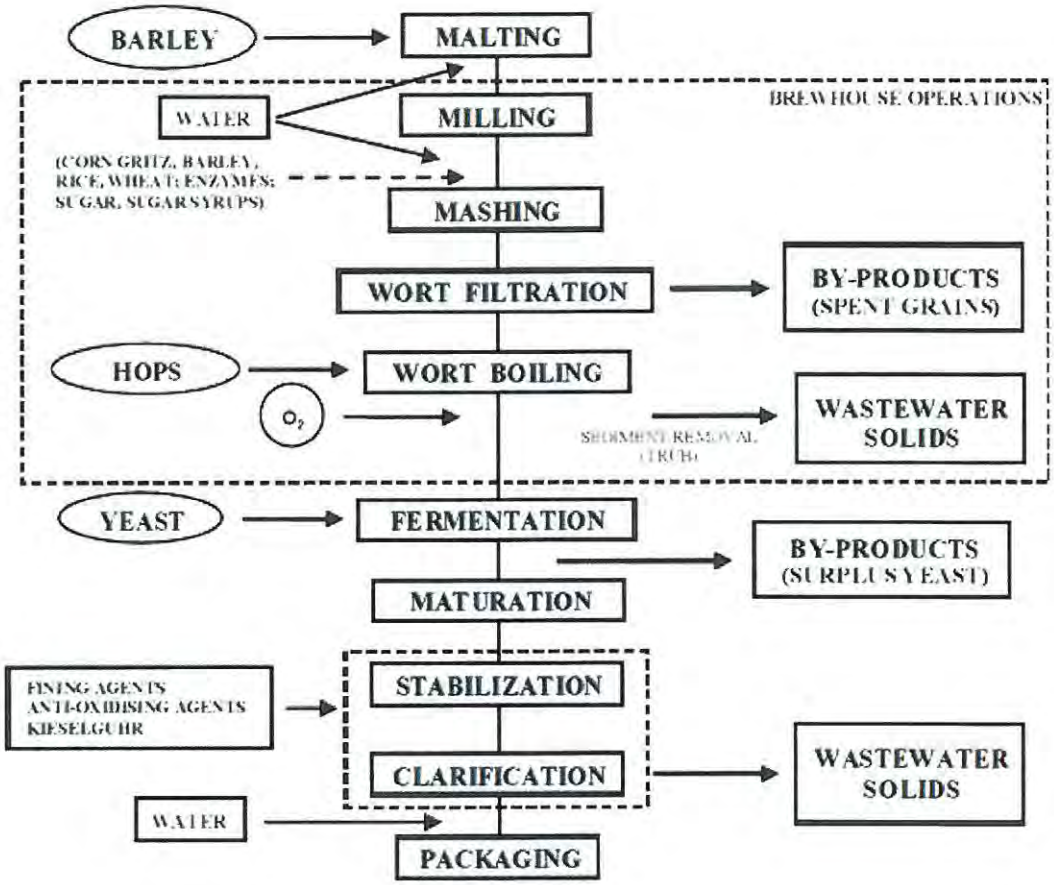

Figure 1.3.a: A schematic representation of the brewing process (Brito *et al.* 2007).

Table 1.3.a: The chemical oxygen demand (COD), sodium (Na^+) and chloride (Cl^-) concentration, electrical conductivity (EC) and pH of selected brewery wastewater streams (table adapted from Braeken *et al.* 2004).

Selected wastewater stream	COD (mg/L)	Na^+ (mg/L)	Cl^- (mg/L)	EC (µS/cm)	pH
Biologically treated water	72.00	256.00	53.00	2080.00	8.80
Bottle rinsing water	592.00	287.00	140.00	3730.00	11.90
Rinsing water bright beer reservoir	2632.00	295.00	39.00	1296.00	7.10
Rinsing water brewing room	102.00	231.00	26.00	2020.00	11.30

Brewery effluent contains macromolecules originating from wort, beer and CIP processes. These include carbohydrates such as maltose, dextrose, lactose, proteins and amino acids, hop compounds, vitamins and minerals, alcohol, yeast, yeast derived fermentation products, sodium hydroxide, nitric and phosphoric acid, sequestering agents, chelating agents, wetting agents, Kieselguhr, silica hydrogel, small amounts of spent grain, calcium sulphate and lactic acid, and water at 85 °C (Viljoen, *pers. comm.*, brewing master, SAB Ltd. iBhayi brewery, SABMiller, April 2010).

Wastewater characteristics can differ, and the amount of wastewater produced depends on the amount of water that is used in production and CIP processes (Table 1.3.b). SAB Ltd. iBhayi brewery discharged approximately 380 425 kL/month, or an average of 1041 kL/d in 2010 (Mabuza, M. *pers. comm*, engineering manager, SAB Ltd. iBhayi brewery, Port Elizabeth, SABMiller, November 2010).

Brewery effluent is thus primarily a relatively dilute organic effluent, which is either disposed of directly to sewage works, or pre-treated to lower the COD before sending it to the municipal sewage works.

Table 1.3.b: General brewery wastewater characteristics (Rao *et al.* 2007, Simate *et al.* 2011).

Parameter	Value
pH	3 - 12
Temperature (°C)	18 - 40
Chemical oxygen demand (mg/L)	2000 - 6000
Biological oxygen demand (mg/L)	1200 - 3600
COD:BOD ratio	1.67
Volatile fatty acids (mg/L)	1000 - 2500
Total Kjeldahl nitrogen (mg/L)	25 - 80
Phosphate (mg/L)	10 - 50
Total solids (mg/L)	5100 - 8750
Total suspended solids (mg/L)	2901 - 3000
Total dissolved solids (mg/L)	2020 - 5940

1.3.1 Wastewater treatment in the brewing industry

Proper wastewater treatment and recycling can benefit companies by reducing the cost for fresh water as well as the cost of treating wastewater. Wastewater reuse in the brewing industry for the production of beer is not very popular due to public perception and possible product quality deterioration problems (Simate *et al.* 2011). Nonetheless, when the present project was initiated, a percentage of the wastewater effluent at SAB Ltd.'s iBhayi brewery was recycled for irrigation purposes. It will become increasingly necessary for brewery effluent water to be recycled due to the relatively intensive water-use that is associated with the industry, and the fact that water shortage has become a serious global and environmental problem (Ridout & Pfister 2010, Simate *et al.* 2011). The treatment of brewery wastewater is costly and complex due to the need to meet governmental regulations and corporate social responsibility. Simate *et al.* (2011) reviewed all of the treatment methods used in the brewing industry except for HRAP systems, and provided a comprehensive discussion about their advantages and disadvantages (Table 1.3.1.a). They compiled a table with different wastewater processes treatment processes, their COD removal efficiency and whether the

reclaimed water was suitable for primary process water or secondary non-process water. The table was compiled from various studies with different experimental designs. The study provided a useful comparative benchmark to evaluate HRAP systems as a brewery effluent treatment method in the light of the treatment technologies currently in use (Table 1.3.1.b).

Table 1.3.1.a: Wastewater treatment operations (physical, chemical and biological) used in the brewery industry (Simate *et al.* 2011).

Operation	Method
Physical unit operations	Screening
	Comminution
	Flow equalization
	Sedimentation
	Flotation
	Granular-medium filtration
Chemical unit operations	Chemical precipitation
	Adsorption
	Disinfection
	Chlorination
	Other chemical applications
Biological unit operations	Activated sludge processes
	Aerated lagoons
	Trickling filters
	Rotating biological contactors
	Pond stabilization
	Anaerobic digestion
	Biological nutrient removal
	HRAP
	Constructed wetland

Table 1.3.1.b: A summary of brewery wastewater treatment processes for re-use (Simate *et al.* 2011).

Process	Initial COD (mg/L)	Final COD (mg/L)	COD reduction (%)	Potential use	
				Primary process water	Secondary non-process water
Quenched plasma	1018	18	98	No	No
Upflow anaerobic sludge blanket	1947-3079	Not given	73-91	No	No
Aerobic reactor	Not given	Not given	90-98	No	No
Combined bioreactor	Not given	Not given	98	No	No
Membrane bioreactor	500-1000	40	96	No	No
Electrochemical methods	2470	64	97	No	No
Microbial fuel cells	1710	105	94	No	No
Nanofiltration	3692	143	96	No	No
Reverse osmosis	850	0	100	Yes	Yes

The treatment system that was used at iBhayi brewery consisted of a combination of some of the technologies described above. The full volume effluent was screened through a drum filter that removed solid wastes such as stones, plastics, glass, paper and labels from the wastestream, after which it was sent to an anaerobic digester (AD). The onsite effluent treatment plant consisted of an AD, an activated sludge (AS) digester, a clarifier, sand filters, activated carbon filters, micro-filtration, reverse osmosis and a chlorine dosing facility (Figure 1.3.1.a). Approximately 1041 m^3 effluent was treated in the AD per day in 2010/2011, of which 670 m^3 (64 %) was treated in the AS system. The remaining 500 m^3 (35 %) was sent to the municipal sewer. Post-AS treatment, 170 m^3 (16 % of the original volume effluent) was recycled through filtration, and was used onsite (Mabuza, *pers. comm*, engineering manager, SAB Ltd. iBhayi brewery, Port Elizabeth, SABMiller, November 2010).

Conventional brewery effluent treatment technologies are well developed and can convert effluent into potable water. Sustainable effluent treatment technologies, on the other hand, are less developed. One of the disadvantages of the conventional technologies is that it relies on fossil fuels to operate. It is unable to sustain itself without fossil fuel electricity, and therefore contributes to greenhouse gas emissions (Craggs *et al.* 2011). Conventional treatment systems are also relatively expensive, and can rely on the availability of chemicals or very fine membranes (Simate *et al.* 2011). The disposal of sludge is not sustainable. Sludge contains valuable nutrients that could be re-used. The continued disposal of sludge will eventually lead to the deterioration of the environment and the loss of ecosystem resilience (Oswald 2003). More sustainable treatment methods are those that are able to function properly with fewer manpower requirements and chemical additives, and those that produce energy (Craggs *et al.* 2011). HRAP systems are potentially able to recycle nutrients, produce biofuel, fix carbon and cut on emissions. They cost less to construct and operate, and require relatively little manpower (Craggs *et al.* 2011). For these reasons, SABMiller and Rhodes University decided to test HRAP technology in the treatment of brewery effluent.

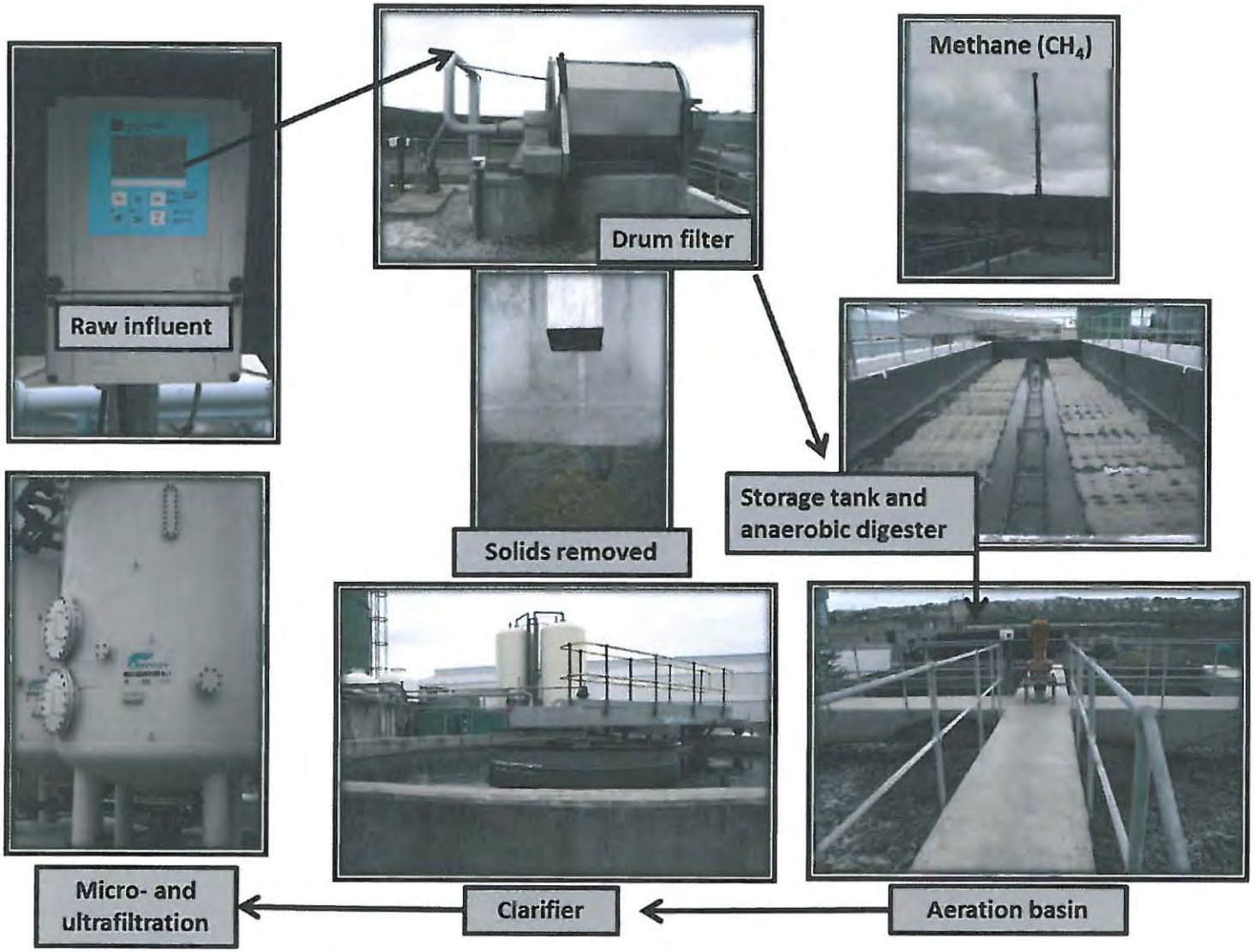

Figure 1.3.1.a: Brewery effluent treatment technology at iBhayi brewery.

1.4 High rate algal ponds

1.4.1 An introduction to the applications of high rate algal ponds

High rate algal ponds (HRAP) are used in the treatment of wastewater, as well as in the cultivation of commercial algal species in the nutraceuticals sector. The design was originally developed by William James Oswald in 1968 at the University of California, Berkeley, and has since been applied in South Africa, the United States, France, the Netherlands, Spain, Malaysia, Israel, Morocco and New Zealand to remove nitrogen and phosphorus from wastewater (Green *et al.* 1996, Idelovitch & Michail 1981, Gaigher *et al.* 1985, Phang 1990, El Hamouari *et al.* 1994, Pagand *et al.* 2000, Craggs *et al.* 2004, Kabede-Westhead *et al.* 2006, De Godos *et al.* 2009). HRAPs were first tested in the treatment of brewery effluent at the Hamilton Brewery in Bloemfontein in 1985 as part of an integrated system (Gaigher *et al.* 1985). Since then no work on the treatment of brewery effluent with HRAP technology has been published.

A HRAP train generally consists of two ponds in series, with the first pond overflowing into the second pond, each in the shape of a typical D-ended raceway. The channels are separated down the middle and the water is mixed by paddle wheels. An incoming stream provides the nutrients that promote algal growth. This is usually wastewater derived from a number of possible industrial processes (e.g. brewery effluent, sugarcane effluent, steel plant effluent or domestic sewage). Algae grow in the circulated water, and algal productivity is dependent on light, temperature and nutrient concentration (Fogg 1991, Knud-Hansen 1998).

Industries and countries in which the applications of HRAPs in industrial effluent treatment have been tested include the treatment of agro-industrial and agricultural effluent in Malaysia. Nutrients were recycled in HRAP systems and they could be useful if integrated in rural fish-rearing systems (Phang 1990, Azov & Shelef 1982). HRAPs have been used in the tertiary level treatment of 2000 m^3/d domestic sewage in four HRAPs on 1.25 ha with CO_2-addition in Christchurch, New Zealand (Craggs *et al.* 2010). The algae produced in HRAPs were a source for biodiesel, but also focussed on the application of algae as animal feeds, fertilizers and a means to offset greenhouse gas emissions. The treatment of swine manure effluent with HRAP technology was tested in Spain and the United States (Kabede-Westhead *et al.* 2006, De Godos *et al.* 2009). The hydraulic retention time (HRT) is the measure of the average length of time that a soluble compound remains in a reactor. A HRT of 10 d yielded COD removal efficiencies of 76 % under optimum environmental conditions (De Godos *et al.* 2009). Phosphate removal efficiency of 10 % was probably due to the absence of pH-mediated phosphate precipitation (De Godos *et al.* 2009). Moderate temperature and irradiation conditions combined with short HRTs supported higher algal productivity than found during winter (De Godos

et al. 2009). The treatment of diary-farm effluent with HRAPs was tested in New Zealand (Craggs *et al.* 2004). HRAP effluent quality was considerably better than that of conventional two-stage oxidation ponds with regard to ammonia, phosphate, and *Eschericea coli* (*E. coli*) concentration (Craggs *et al.* 2004). The treatment of domestic sewage for the removal of faecal coliforms, nematodes and pathogens (*Salmonella* sp.) with HRAP technology was tested in Rabat, Morocco and was and were successful in the complete removal of *Salmonella* sp. and nematodes (El Hamouari *et al.* 1994). HRAP design parameters played a role in the removal of faecal coliforms (El Hamouari *et al.* 1994). HRAPs can play a complementary role in generating alkalinity and facilitating the bioadsorptive removal of metals in acidic and metal mining effluent (Rose *et al.* 1998). HRAPs can be used as a carbon source in the generation and precipitation of metal sulphides from pilot stage to commercial implementation (Rose *et al.* 1998). The treatment of marine effluent with HRAPs from a circulating fish rearing system was tested in France (Pagand *et al.* 2000). Results of this study indicated 59 % dissolved nitrogen removal and 56 % phosphorous removal, and commented on the seasonal variation in space that was needed to treat effluent (Pagand *et al.* 2000). The use of HRAP technology in the treatment of palm oil mill effluent was tested in Malaysia (Phang & Kim-Chong 1988). One of the main findings of this study was that light was the limiting factor, and that the algal species composition changed at different HRTs, *Chlorella* species being dominant at a long HRT. The application of HRAPs in the treatment of sago starch effluent was tested in Malaysia (Phang *et al.* 2000). This study reported increased productivity at shorter HRT and almost 100 % removal efficiency for COD, nitrogen and phosphate (Phang *et al.* 2000). The treatment of brewery effluent in an integrated system that consisted of bacteria, algae, fish and macrophytes has been tested in South Africa (Gaigher *et al.* 1985). The COD was reduced from 4000 mg/L where effluent entered bacterial ponds, to 100 mg/L in the macrophyte pond.

HRAPs could play an important role in energy self-sufficiency and present a superior way of treating effluent with regard to economics as well as its environmental impact. HRAPs cost half as much as AS systems to construct, and use a third of the energy required by an AS system (Oswald 2003). They produce algal biosolids which are more attractive and valuable than the sludge typically produced by the AS method (Oswald 2003). Oswald gives a simple example to compare AS and HRAP systems:

> *"1.00 kg of BOD removed in an AS process requires 1.00 kWh of electricity for aeration, which produces 1.00 kg CO_2 from the power generation. By contrast, 1.00 kg of BOD removed by photosynthetic oxygenation requires no energy inputs and produces 1 kWh of electric power"* (Oswald 2003).

Most municipal water treatment plants use conventional AS systems to treat wastewater. This is the most expensive treatment method in terms of construction, operation costs and energy consumption. The initial anaerobic treatment of wastewater with a high organic load usually results in an effluent with high concentrations of various organic acids, phosphate, ammonia, nitrate and other low molecular weight substances (Ogbonna *et al.* 2000). High concentrations of organic acids can be inhibitory to many aerobic microorganisms, and hence the conventional AS method is often not efficient at treating undiluted high-strength wastewater (Ogbonna *et al.* 2000). There is also the need for disposal of sludge which requires large tracts of land, and is costly (Oswald 2003). Sludge is a potential nutrient resource that is wasted when it is disposed of. There has been considerable recent research on the potential of energy recovery from AS solids through AD (Appels *et al.* 2008) and pyrolysis gasification (Chun *et al.* 2011). HRAP sludge contains assimilated nutrient in algae, and can be directly applied as fertilizer or animal feed (Craggs *et al.* 2011). The algae in HRAPs recycle nutrients and produce a potentially valuable secondary product. Algae are a rich source of protein, lipids, and carbohydrates (Table 1.4.1.a, Becker 2007).

Table 1.4.1.a: The nutritional composition of several algal species (Becker 2007).

Alga	Protein (%)	Carbohydrates (%)	Lipids (%)
Anabaena cylindrica	43 - 56	25-30	4 - 7
Aphanizomenon flos-aquae	62	23	3
Chlamydomonas rheinhardii	48	17	21
Chlorella pyrenoidosa	57	27	2
Chlorella vulgaris	51 - 58	23 - 27	14 - 22
Dunaliella salina	57	32	6
Euglena gracilis	39 - 61	14 - 18	14 - 20
Porphyridium cruentum	28 - 39	40 - 57	9 - 14
Scenedesmus obliquus	50 - 46	10 - 17	12 - 14
Spirogyra sp.	6 - 20	33 - 64	11 - 21
Spirulina platensis	46 - 63	8 - 14	4 - 9
Synechococcus sp.	63	15	11

HRAPs and wetlands present wastewater producers with the opportunity to recycle effluent on-site. They are self-sustaining technologies that require low technical manpower, and do not require high capital and operating costs (Benemann *et al.* 2003). The practice of treating water with the conventional AS method in large regional plants actually makes it more difficult to re-use water afterward because of the difficulty in re-conveying it to the producer (Oswald 2003). What often happens is that the effluent is discharged into marine or freshwater systems which lead to a loss of valuable freshwater, and to pollution due to the residual nutrients in effluent (Oswald 2003). Wastewater treatment should become decentralised and move to smaller treatment plants that are

located at the point of production (i.e. a factory). This will make it easier to recycle water and nutrients, and will benefit the producer due to the reduced cost of treatment, additional water availability and savings on purchasing water (Oswald 2003). One example of such a system is in Ridgemark, California. A series of HRAPs were installed. The HRAP treated effluent was pumped into a ground-well and was used to irrigate golf-courses, parks and gardens. The ponds were visibly attractive, friendly to waterfowl and birds, and odourless. No hygienic problems were attributed to the use of the recycled water and it was situated in an upmarket urban environment (Oswald 2003).

Another important application of HRAPs is that of carbon sequestration and to reduce greenhouse gas emissions into the atmosphere (Oswald 2003, Craggs *et al.* 2011, Park *et al.* 2011). Steel-making plants and thermal power stations emit CO_2 concentrations that are about 500 times greater than the concentration found in the atmosphere (Yun *et al.* 1997). HRAPs can remove nitrogen from the wastewater and CO_2 from the effluent gas of a steel-making plant. This can decrease the amount of greenhouse gases that contribute to global warming (Yun *et al.*1997). The steel plant wastewater did not contain phosphorous, and so this element was added as a growth medium. This was a pilot project which investigated the removal of CO_2 and nutrients from wastewater, and to develop en economically feasible CO_2 fixation process with benchtop experiments. It concluded that, assuming that the total amount of NH_4-N discharged from the steel-making plant (818 kg/d) was completely used by algae, CO_2 could be fixed at a rate of 23 100 kg CO_2/day, and approximately 12 430 kg algal biomass could be produced per day. The algal biomass generated in a steel plant's effluent would most likely be unsuitable for human or animal consumption due to its large heavy metal concentration, but could potentially be used in the formulation of biotic heavy metal absorbents. There are considerable economic and technological obstacles that need to be overcome before this technology becomes economically feasible (Figure 1.4.1.a, Yun *et al.* 1997).

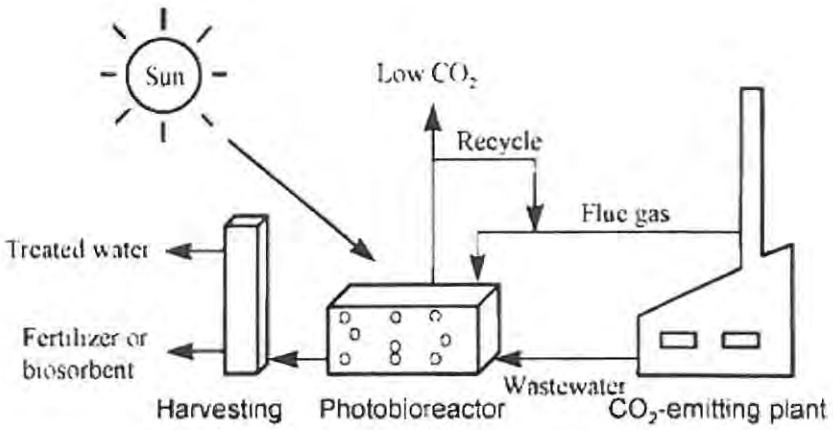

Figure 1.4.1.a: A conceptual system of carbon dioxide (CO_2) fixation using wastewater nutrients discharged from the site of a CO_2-emitting plant (Yun *et al.* 1997).

The environmental gains of using a HRAP system instead of an electromechanical wastewater treatment option have been clearly demonstrated (Shilton *et al.* 2008). HRAPs could save 35 million kWh over a 30-year design life for a town of 25,000 people in the English countryside. In the UK an average of 0.43 kg CO_2 is emitted per kWh of electricity produced. This amounts up to 500 tonnes CO_2 emitted per year, which would require 200 hectares of pine forest to soak it up. One tonne of algal biomass assimilates approximately 1.8 tonnes CO_2 (Shilton *et al.* 2008). Wastewater produced algae present an environmentally sound way of producing biofuels such as e.g. biogas, ethanol, biodiesel and crude bio-oil, as the land and capital that would have been required to set up an independent algae production unit is not required, as it is already incorporated in wastewater treatment facilities (Craggs *et al.* 2011). The additional procurement of water and fertilizers is also covered (Craggs *et al.* 2011). Once converted into biofuel it can offset CO_2 greenhouse gas emissions, depending on the type of fuel it replaces. HRAP wastewater treatment reduces the CO_2 emissions of the fossil fuel that would have been used in electromechanical treatment such as AS systems (Craggs *et al.* 2011, Park *et al.* 2011).

Algal biomass can be used as fuel to produce methane (CH_4) biogas through anaerobic digestion. Algae can be digested with a yield of 0.30 m^3 (0.20 kg) CH_4/kg algal biomass, with 50 – 60 % volatile solids removal (Oswald & Golueke 1960, Craggs *et al.* 2011). Co-digestion of algae with the breakdown of solids in primary wastewater treatment could double the CH_4 yield. Algae-derived biogas can produce electricity of 1 kWh electricity / kg algal volatile solids (Oswald & Golueke 1960, Craggs *et al.* 2011). CH_4 biogas can be used for heating or for electricity generation at 30 %

conversion efficiency (Oswald & Golueke 1960). CH_4 biogas can be cleaned or compressed for export or it can be used as transport fuel. One cubic metre of CH_4 biogas has the energy value equivalent to 1.00 L of petrol (Oswald & Golueke 1960, Craggs *et al.* 2011, Park *et al.* 2011). The brewery at the pilot site already produced CH_4 biogas in the anaerobic digester from the effluent it received, although it was not being used for anything in particular at the time of the study, and was therefore flared. CH_4 biogas could be applied as fuel to drive all the processes in an integrated HRAP treatment plant (Park *et al.* 2011).

Another important breakthrough connected to human health was the discovery that HRAPs were able to remove the ova of roundworms (*Helminth* sp.), whereas AD and AS methods were not (Oswald 2003). The presence of these ova in water that has not been properly disinfected has led to the death of many children across the globe (Oswald 2003). HRAPs have been applied in South Africa to successfully treat sewage water to remove *E. coli* bacteria and other nutrients such as nitrogen and phosphate from effluent (Gaigher *et al.* 1985). Algal growth causes a daily increase in the pH of a pond, which supports its use to remove pathogens such as *Helminth* ova and *E. coli* from wastewater (Oswald 2003). The removal of coliforms from sewage water is probably due to the high pH, light intensity and dissolved oxygen (DO) concentration that are typical of HRAPs. One dry weight unit of algal growth can produce one and a half as much DO. More research is needed to find out how to make use of the large amount of oxygen that is produced (Oswald 2003).

It is estimated that 30 % of the world's algae production is sold as animal feeds (Becker 2007). The efficiency of HRAP technology in the treatment of sago palm starch factory wastewater simultaneously produced *Spirulina* algae to use as animal feed in Malaysia (Phang *et al.* (2000). Results indicated that HRAPs were successful in treating wastewater from sago palm factory, with COD, ammonia and phosphate removal efficiencies of 94 %, 99 % and 93 % respectively (Phang *et al.* 2000). The biochemical composition of *Spirulina* biomass indicated that it could be used as a high-quality feedstock, especially for aquaculture, as well as a source of useful biochemicals. Thirty tonnes of *Spirulina* sp. was harvested from 50 000 m^2 in 1995 (Phang *et al.* 2000). Thirty percent of it was used for human consumption, and the rest was used as animal fodder (Phang *et al.* 2000). The study commented on the good use of *Spirulina* species for mass cultivation, its growth in alkaline conditions which eliminates other species, and its nutritional value that makes it a good option for animal feeds. Animal feedstock generally consists of about 12 – 15 % protein, and algae can contain up to 50 % protein, depending on the species (Dugan *et al.* 1972). The algal stock must therefore be diluted with low-cost carbohydrates and lipids (Dugan *et al.* 1972). Twenty per cent algal fortified mash has been used as a feedstock to rear baby chickens to full grown laying hens (Dugan *et al.* 1972). No disease transmission from humans to birds or from birds to birds was observed due to the

heat during the pasteurization process which was used (Dugan *et al.* 1972). Algae can be combined with barley in pelleting machines to produce a 12 % to 15 % protein feed for fish, chicken, cattle or swine (Green *et al.* 1996). There was no concern about disease transmission. Pelletizing occurs at high temperatures for several hours, which causes all pathogens to die off (Green *et al.* 1996).

To date, the major sales of micro-algal preparations can be found in the health food market (Becker 2007). The incorporation of micro-algal protein as a food or food substitute has not gained significant importance as yet due to the powdery form it comes in, its dark green colour, and its faintly fishy odour. There are socio-ethnological barriers towards eating unknown food sources (Becker 2007). The production costs for micro-algae are still too high to compete with conventional protein sources. By using the by-products of wastewater grown algae, it could potentially reduce the construction and operational costs of HRAP wastewater treatment systems..

To summarise, the potential benefits of the application of HRAPs in wastewater treatment include its low environmental impact, the recycling of nutrients that can be used as animal feeds or fertilizer, the production of algae that can be used as biofuels, the low cost of construction and operation, relatively low man power requirements, carbon sequestration and emissions offsets. HRAPs should not be seen just as "effluent treatment", but also as a means of recycling and beneficiation. This requires a change in the traditional management mind-set which just considers the brewing process and disposal of waste. HRAP technology is not "turn-key" technology, and a scientific research and development approach is required to optimise the various elements of the system.

The implementation of HRAPs is a developmental activity. Fortunately, SAB Ltd. was motivated by its sustainability policy and economic motivation to invest in the development of more sustainable production technologies and to reduce effluent disposal costs. It decided to support a pilot Research and Development (R&D) plant at iBhayi brewery to evaluate the efficacy of HRAPs in treating post-AD effluent with a view to recycle the water and nutrients, and so Project Eden came into existence.

1.5 Research approach and objectives

In the light of the situation described above, SAB Ltd., Rhodes University (Grahamstown, South Africa) and the South African Water Research Commission (known as the WRC) embarked on a research project named *Project Eden*, to develop wastewater treatment technology that would enable SABMiller to realize its sustainable development goals (Section 1.2.1). The study site was located on SAB Ltd. iBhayi Brewery grounds in Port Elizabeth, Nelson Mandela Metropolitan Municipality, South Africa. The pilot plant was an integrated effluent treatment system. This implies

that a number of processes were brought together in the optimal sequence to treat effluent (Green *et al.* 1996). Effluent from the brewery was treated in an AD (primary treatment), after which it was subsequently fed into a primary facultative pond (PFP, secondary treatment). The PFP decanted into two HRAP trains (tertiary treatment) and from the HRAPs it was pumped into a horizontal flow constructed wetland (quaternary treatment). Post-HRAP and post-wetland, treated effluent was used in aquaculture and hydroponic lettuce production (Chapter 3, Figures 1.5.a and 1.5.b). This thesis investigated the use of HRAPs in the treatment of brewery effluent.

A 10-month baseline "proof-of-concept" phase (May 2009 – February 2010) was implemented, followed by an 11-month "optimization phase" (March 2010 – January 2011) to test the application of high rate algal ponds and a constructed wetland in the treatment of brewery effluent.

The objectives of the baseline phase (Chapter 4) were to answer the following questions:

- What is the effect of season on HRAP performance?
- What is the effect of a relatively long, constant HRT on algal nutrient uptake efficiency?
- Would it be possible to treat wastewater to a quality that met the DWAF general limits for discharge into a natural water resource (Table 1, Appendix 1).

The objectives of the optimization phase (Chapter 5) of the study were to answer the following questions:

- What is the maximum volume of effluent that a HRAP can treat in autumn and summer?
- What are the possible biochemical pathways that could explain carbon, nitrogen and phosphate behaviour in HRAP treated wastewater?
- What role does HRT, light and temperature play in increasing algal productivity and subsequent nutrient uptake efficiency?

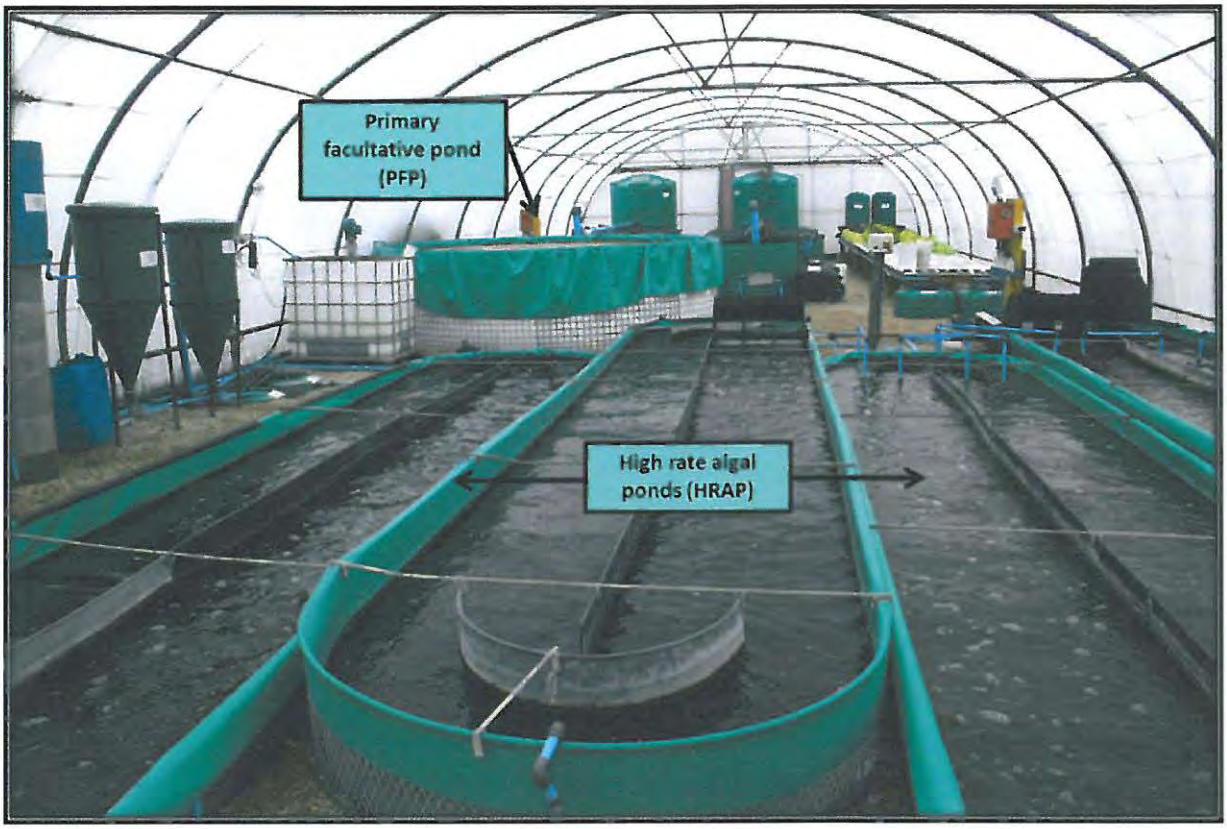

Figure 1.5.a: Project Eden: Anaerobically digested effluent was fed into a primary facultative pond (PFP) and high rate algal ponds (HRAP).

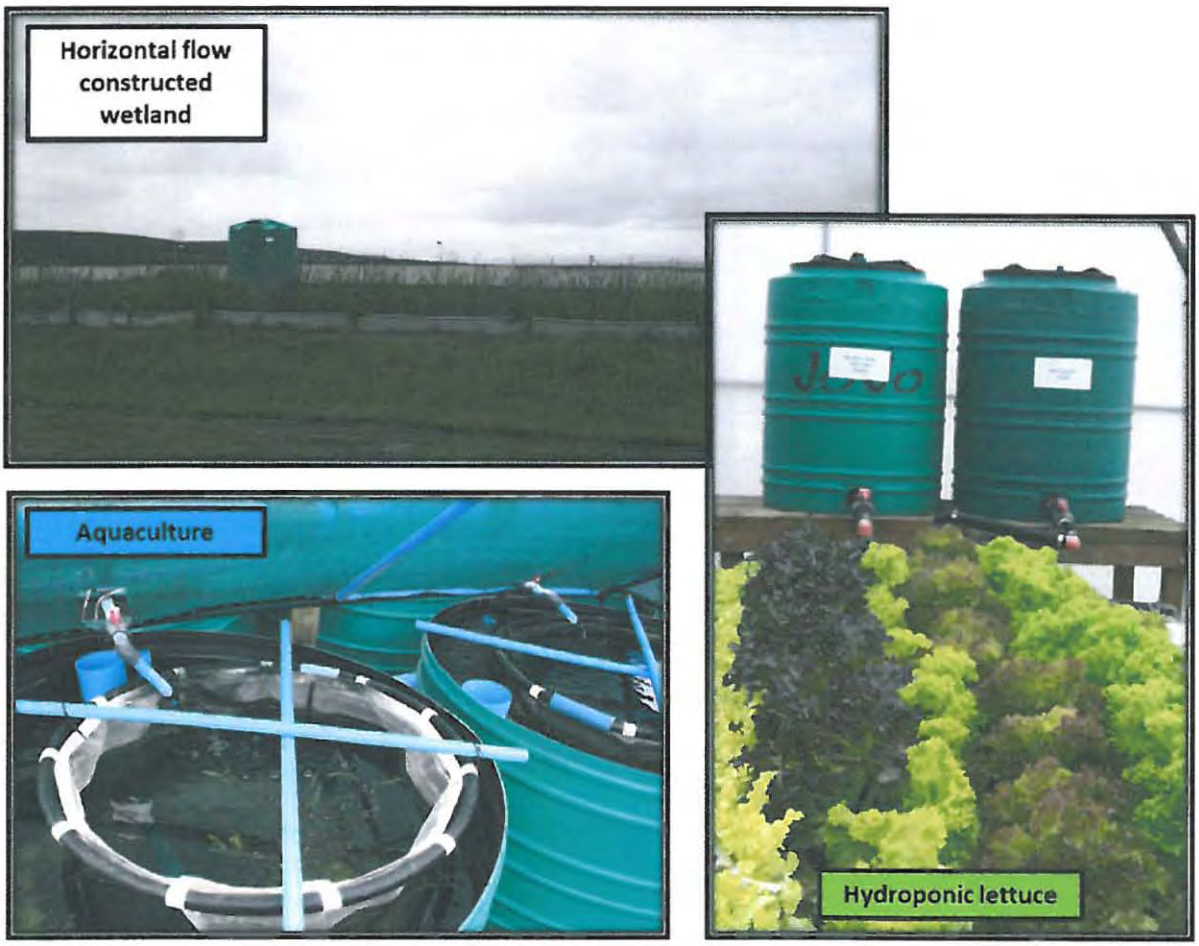

Figure 1.5.b: Project Eden: High rate algal pond and wetland treated effluent was used in aquaculture and hydroponic lettuce production.

Chapter two
An overview of integrated wastewater treatment systems

2.1 Introduction

Integrated treatment systems refer to a number of well-known and lesser known processes that are brought together in the optimal sequence for the optimal treatment of wastewater (Green *et al.* 1996). The end goal can be to produce water that is suitable for primary or for secondary use. Primary water use refers to drinking water, whilst secondary water use refers to water that is used in irrigation and cleaning processes. The design of the system and the quality standards of recycled effluent will depend on the application of treated effluent (Braeken *et al.* 2004, Simate *et al.* 2011).

Wastewater can be recycled and re-used with the combined benefit of the production of valuable secondary products with economic and social value. This is called the beneficiation of wastewater. Integrated systems work on the principle that the waste product of one process can be used as the fuel that sustains the next. Complex dissolved organics are broken down into simple dissolved organics that can subsequently be used by algae, fish, macrophytes or vegetables as the nutrient that supports their growth, and so the beneficiation of wastewater can be achieved (Gaigher *et al.* 1985).

Advanced integrated wastewater ponding systems that consist of a minimum of four ponds in series have been developed i.e. an advanced facultative pond in which anaerobic, aerobic digestion and sedimentation occurred in; high rate algal ponds; algal settling ponds and maturation ponds (Green *et al.* 1996). The proportions of different nitrogen species changed as it moved through the system. Eighty five per cent of fixed nitrogen was removed without special sedimentation (Figure 2.1.a).

An integrated system that consisted of a sedimentation tank, AD, HRAPs and an algal harvesting unit was used to treat effluent from a chicken factory (Dugan *et al.* 1972). The resultant algae were used as a feedstock for chickens in the form of dried algae that were fed back into the chickens' diet as a protein supplement (Dugan *et al.* 1972). The treated effluent was used to flush manure troughs (Figure 2.1.b, Dugan *et al.* 1972).

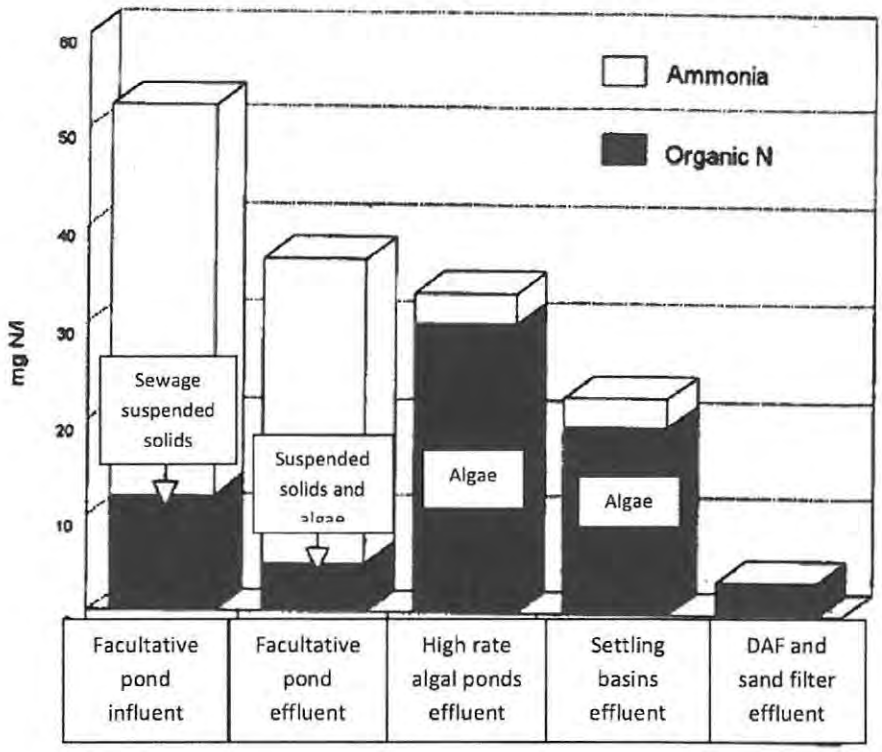

Figure 2.1.a: Nitrogen removal in different parts of an advanced integrated wastewater ponding system (Green *et al.* 1996).

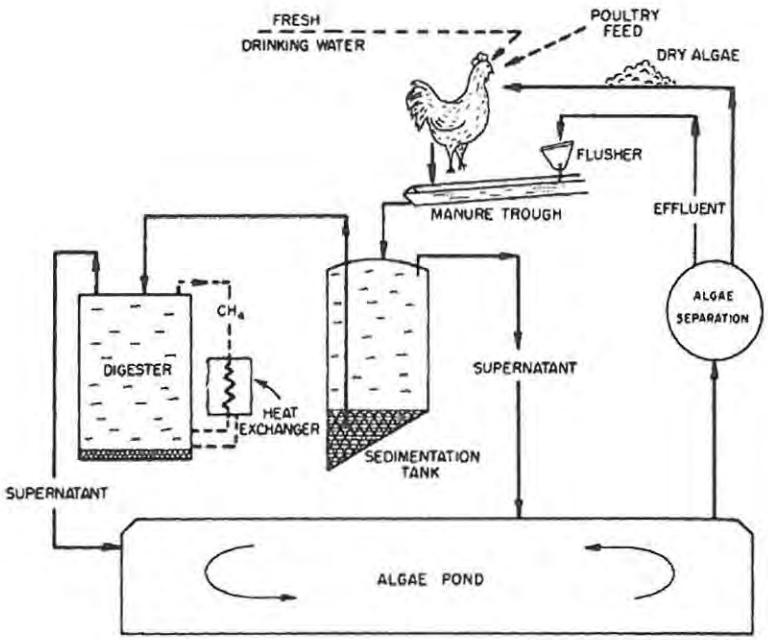

Figure 2.1.b: An example of an integrated system for the treatment of poultry waste (Dugan *et al.* 1972).

The integrated system at SAB Ltd. iBhayi brewery in Port Elizabeth consisted of a mechanical filter, an aerobic digester (AD), a primary facultative pond (PFP), two high rate algal ponding trains (HRAP), algal settling cones and a wetland.

It is useful to provide an overview some of the principles and applications of anaerobic digestion and HRAPs in this chapter, as these are the processes that facilitated the integrated treatment of brewery effluent at Project Eden. Anaerobic digestion was the first component in the integrated system at Project Eden. The general principles of an AD, the methane (CH_4) potential of wastes, the anaerobic digestion of microalgae and anaerobic denitrification are described in Section 2.2: Anaerobic digestion. A PFP was the second component in the system. Its main purpose was for the sedimentation of solids leaving the AD. Its use is discussed in Chapter 5, Section 5.2.3. Effluent from the PFP decanted into two HRAP trains, which formed the third component of the integrated wastewater treatment system at Project Eden. The factors that influenced the optimization of brewery effluent treatment in HRAPs and some methods for harvesting algae are described in Section 2.3.

2.2 Anaerobic digestion

Anaerobic digestion is a biological process that converts organic carbon into a gaseous mixture that is principally composed of CH_4, the most reduced carbon state, and carbon dioxide (CO_2), the most oxidized carbon state (Lyberatos & Skiadas 1999). The process happens through the concerted action of a highly integrated community of bacteria (Lyberatos & Skiadas 1999). The whole process happens in the absence of oxygen. Although the main products that form during anaerobic digestion are CO_2 and CH_4, other gases such as nitrogen, nitrogen oxides, hydrogen, ammonia, hydrogen sulphide and other volatile compounds, are also generated (Angelidaki & Sanders 2004).

Bacterial granules develop during anaerobic digestion (Lyberatos & Skiadas 1999). The precise mechanism of granule formation remains unknown as there are various factors that influence it under different conditions (Lyberatos & Skiadas 1999). The composition of granules and the factors that influence their formation on the other hand, are well understood (Lyberatos & Skiadas 1999). Granules contain bacteria in a 3-D arrangement of which the exact bacterial species depend on the wastewater composition. The formation of granules is affected by a number of process, physico-chemical and biological factors (Lyberatos & Skiadas 1999).

2.2.1 The general principles of anaerobic digestion

The biodegradation of organic compounds in the environment are affected by several physical, chemical and physiological factors (Angelidaki & Sanders 2004). Anaerobic digestion can become unstable due to a feed overload, a feed underload, the entrance of an inhibitor, or inadequate temperature control. Instability can be picked up by a drop in the CH_4 production rate, a drop in pH, or a rise in the volatile fatty acid concentration. Volatile fatty acid accumulation reduces the pH which causes a decrease in the free ammonia concentration and the inhibition of methanogenesis. When this happens, the usual course of action is to increase the HRT. When this fails the digester needs to be primed with sludge from a healthy digester (Lyberatos & Skiadas 1999).

Four important activities have been recognized within the anaerobic digestion process; each is affected by a different group of bacteria.

Hydrolysis is the break-up of insoluble organic matter into soluble intermediates such as amino acids, simple sugars and fatty acids. It is one of the most important chemical conversion processes that breaks down insoluble organic material into soluble organics. During the hydrolysis of macro-pollutants, polymers such as lipids, protein and carbohydrates are depolymerized into glycerol and long chain fatty acids, into amino acids, and into oligo- and monosaccharides, respectively (Angelidaki & Sanders 2004). The hydrolysis rate for the substrate depends on the surface area that is accessible to enzymes. The surface area of dissolved polymers is larger than particulate organics. The physical state and structure of the substrate and its accessibility to enzymes will therefore determine the rate of the process. The formation of a biofilm of organisms around particulate surfaces is necessary for the complete anaerobic digestion of a particle (Angelidaki & Sanders 2004).

Acidogenesis is the formation of volatile fatty acids or other organic acids such as propionate, butyrate and benzoate by acidogenic bacteria (Lyberatos & Skiadas 1999).

Acetogenesis is the formation of acetate and hydrogen gas (H_2) and/or CO_2 by acetogenic bacteria from acetogenic substrates such as propionate, butyrate and benzoate (Lyberatos & Skiadas 1999).

Methanogenesis is the formation of CH_4 and CO_2 from acetate by acetoclastic/methanogenic archaea bacteria. This process is preceded by a multi-step process in which subsequent groups use the products from the first groups of organisms in the chain as substrates. Methanogenesis is an exergonic reaction, and therefore releases energy. The amount of energy that is released depends on the H_2 partial pressure (Lyberatos & Skiadas 1999).

Other important conditions that can influence anaerobic digestion include redox conditions, temperature, enzyme activity, the inoculum, the medium, the pH and the presence of heavy metals.

Redox reactions happen during anaerobic digestion. During redox reactions the reductant transfers electrons to the oxidant. The oxidant removes electrons from another substance, whilst the reductant transfers electrons to another substance and becomes oxidised. Redox conditions, or oxidation-reduction processes, are created by the availability of electron acceptors such as nitrate, iron, sulphate or carbon dioxide, as oxygen is the best electron acceptor and not available under anaerobic conditions (Angelidaki & Sanders 2004). In a natural environment, redox conditions often occur in the bottom levels of a water body (Fogg 1991). The energy that is released through redox reactions is used to support the maintenance and growth of the microbial population (Angelidaki & Sanders 2004).

Temperature is the most important variable in controlling the rate of the microbial metabolism in anaerobic environments (Angelidaki & Sanders 2004). Anaerobic digestion occurs under three conditions of temperature: mesophilic (25 – 40 °C), thermophilic (45 – 60 °C) and psycrophylic (≤ 20 °C). If the temperature rises to above the optimum temperature, the result will be a sharp decrease of the bacterial growth rate (Angelidaki & Sanders 2004). The CH_4 potential of manure increased from 2.40 % at a temperature of 5 °C to 15.70 % at a temperature of 35 °C after 345 d of manure digestion (Kaparaju and Rintala 2003). Temperature has a significant impact on bacterial growth and the solubility of the substrate. It also influences the rate at which enzymes act on their substrate (Angelidaki & Sanders 2004).

The inoculum which is used must contain the full consortium of microorganisms that is necessary for the anaerobic digestion process. It is sometimes necessary to source an inoculum that is adapted to special conditions, such as in the case where high ammonia concentration prevails in the digester (Angelidaki & Sanders 2004).

The pH is another important factor to consider when maintaining an anaerobic digestion process. Anaerobic digestion happens in a pH range between 6.00 and 8.30. The optimum pH for most methanogens lies between 7.00 and 8.00, whilst acidogens prefer a lower pH (Angelidaki & Sanders 2004). The high protein content in algal biomass leads to high ammonia gas (NH_3) release, which will increase the pH due to higher alkalinity, and will cause the gas content to shift more to CH_4, as well as the inhibition of anaerobic microflora. If a substrate such as microalgae is therefore digested, codigestion with a nitrogen poor substrate might be the answer to prevent the inhibition of anaerobic bacteria (Golueke & Oswald 1959).

The presence of heavy metals can disrupt anaerobic digestion (Hayes & Theis 1978). This can lead to lowered gas production and CH_4 potential, with the subsequent accumulation of intermediate organic acid substrates through the inhibition of methanogenic bacteria (Hayes & Theis 1978).

2.2.2 The methane potential of wastes

Methane potential is an inherent property of substrate. Every type of waste has its own CH_4 potential. Several methods exist for determining the CH_4 potentials of waste, and are described in more detail by Angelidaki and Sanders (2004) and Sialve *et al.* (2009). The ability to realize the methane potential can be compromised under non-ideal conditions such as the presence of sulphate and nitrate reducers as they outcompete methanogens, the bioavailability of an organic substrate, problems during chemical oxygen demand (COD) determination, and the inhibition of the anaerobic digestion process (Angelidaki & Sanders 2004).

Oswald (2003) advocated the use of an AD as a primary wastewater treatment system with the formation of CH_4 from all the settleable solids. The process requires no mechanical aeration and therefore saves on costs and greenhouse emissions due to the energy that would have been required to aerate effluent.

The CH_4 potential of brewery effluent is significant as it contains a large amount of organic material that is converted into CH_4. Sierra Nevada Brewery generated approximately 25 – 40 % of its natural gas supplies with biogas from their anaerobic digester (AD) (Andrews *et al.* 2011). The anaerobic digester at SAB Ltd., iBhayi brewery, produced CH_4 daily which was flared into the atmosphere. It could potentially be used to heat algal ponds or to drive power generators for the generation of electricity.

2.2.3 The anaerobic digestion of microalgae

Algae can be used to feed into an AD to subsequently produce CH_4 biogas and to re-mineralise the nitrogen and phosphate in algae into the nutrients that are needed to maintain algal growth in HRAPs. As HRAPs produce algae, the anaerobic digestion of algae to produce CH_4 biogas is an option for beneficiation. Golueke and Oswald (1959) studied the biological conversion of light energy into the chemical energy of CH_4 biogas as an alternative energy source to fossil fuels and nuclear energy. The idea was to transform solar energy into the cellular energy of minute algal plants, which would in turn be converted into the chemical energy of CH_4 biogas through the anaerobic digestion of algae. They tested this idea by setting up a lab-scale experiment. Their results indicated a high rate of CH_4 biogas production when fed with a large amount of volatile matter after the culture first adapted to the high concentration of nutrient for some time. They also stipulated that unless a digester is operated at a temperature above the thermal death-point for algae, that some of the algae are likely to survive, which will result in the entire process to become less efficient. The algal

culture in their experiment died at temperatures above 40 ° C. The temperature in the AD should therefore be above 40 °C for the anaerobic digestion of algae (Golueke & Oswald 1959).

Sialve *et al.* (2009) described the anaerobic digestion of microalgae as a necessary step to make biodiesel sustainable. Microalgae (eukaryotic microalgae and prokaryotic cyanobacteria) can synthesize lipids that can be used in the production of biofuels (Sialve *et al.* 2009, Park *et al.* 2011). The proportions of proteins (6 – 52 %), lipids (7 – 23 %) and carbohydrates (5 -23%) in algae are strongly species dependent. Sialve *et al.* (2009) provided a table in which the gross composition of several microalgae species are shown, along with their theoretical CH_4 biogas potential and NH_3 release during the anaerobic digestion of the total algal biomass.

Table 2.2.3.a: The gross composition of several microalgae and calculated theoretical methane (CH_4) potential and ammonia ($N\text{-}NH_3$) release (Sialve *et al.* 2009).

Species	Proteins (%)	Lipids (%)	Carbohydrates (%)	CH_4 (L CH_4/g VS)	$N\text{-}NH_3$ (mg/g VS)
Euglena gracilis	39-61	14-20	14-18	0.53-0.80	54.30-84.90
Chlamydomonas reinhardtii	48	21	17	0.69	44.70
Chlorella pyrenoidosa	57	2	26	0.80	53.10
Chlorella vulgaris	51-58	14-22	12-17	0.63-0.79	47.50-54.00
Dunaliella salina	57	6	32	0.68	53.10
Spirulina maxima	60-71	6-7	13-16	0.63-0.74	55.90-66.10
Spirulina platensis	46-63	4-9	8-14	0.47-0.69	42.80-58.70
Scenedesmus obliquus	50-56	12-14	10-17	0.59-0.69	46.60-42.20

The mineral composition of microalgae meets the requirements for anaerobic microorganisms. Apart from carbon, nitrogen and phosphorus, microalgae also contain oligo-nutrients such as iron, cobalt and zinc, and are known to stimulate methanogenesis. The accumulation of metals may inhibit anaerobic digestion and the process therefore needs to be monitored closely to prevent its inhibition (Sialve *et al.* 2009).

By using the average chemical composition of microalgae of $CO_{0.48}H_{1.83}N_{0.11}P_{0.01}$, the nitrogen and phosphorous requirements that is required to cultivate algae per unit area and per year can be estimated. Based on this formula, algae require large amounts of nitrogen and phosphorus to grow (Sialve *et al.* 2009). A process that recycles the nitrogen and phosphorus that is contained in algal waste is needed to reduce the use of fertilizers in algal production. This process is the anaerobic digestion of microalgae. The mineralised algal waste will contain phosphorus and nitrogen, which can subsequently be used as substrate for microalgae to grow in (Sialve et al. 2009).The algae that were produced in the HRAPs at Project Eden could be fed back into an AD in a similar way to recycle the nutrients in HRAP algae, as well as to produce more CH_4 biogas.

2.2.4 Anaerobic denitrification

Conventional anaerobic ponds can emit almost as much nitrogen gas (N_2) as CH_4 and H_2 gas. N_2 gas can develop in the bottom of the sludge through heterotrophic nitrification facilitated by bacteria of the genus *Arthrobacter*. The bacteria can permit undetectable amounts of O_2 to facilitate nitrification (Verstraete & Alexander 1973). Heterotrophic microorganisms are capable of oxidising ammonium if grown on a medium with reduced nitrogen forms. Amino acids and ammonium sulphate can serve as sources for the formation of hydroxylamine, nitrite (NO_2-N) and nitrate (NO_3-N) (Verstraete & Alexander 1973). Both inorganic and organic substrates can be metabolized during the process of heterotrophic nitrification (Verstraete & Alexander 1973).

NO_2-N and NO_3-N can be converted into N_2 gas through denitrifcation. Algal biomass can be used as a carbon source in anaerobic denitrification processes (Green *et al.* 1996). Neba and Rose (2004) designed an algal tertiary treatment (ATT) denitrification unit to compliment the nitrogen removal capacity in HRAPs when the NO_3-N concentration post-HRAP exceeded 90 mg/L (Figure 2.2.4.a). It consisted of a 1000 L anaerobic submerged trickle filter with small rocks in it and was fed with algal biomass. The ATT facilitated the heterotrophic bacterial denitrification of NO_2-N and NO_3-N containing effluent. NO_3-N and NO_2-N removal efficiencies of 80 % and 95 % respectively were recorded from initial concentrations of between 60 and 90 mg/L NO_3-N, and between 1 and 3 mg/L NO_2-N (Neba & Rose 2004). The system reached a stable phase at Day 60, and NO_2-N and NO_3-N removal efficiency improved with time. The main disadvantage of the ATT system was that NH_4-N and PO_4-P concentrations increased, and that it subsequently needed to be removed downstream. NH_4-N increased from 67.1 % to 92.9 % during the steady state conditions (Day 60), and PO_4-P increased with 76 %. An increase in NH_4-N concentrations could be due to a drop in pH below 8.0 that inhibited NH_4-N volatilization, as well as the bacterial decomposition of algal matter that released NH_4-N. An increase in PO_4-P concentration might be due to a low pH that causes the dissociation of PO_4-P precipitates, as PO_4-P generally precipitates at a pH above 9.0 (Dekker 2002, Knud-Hansen 1998).

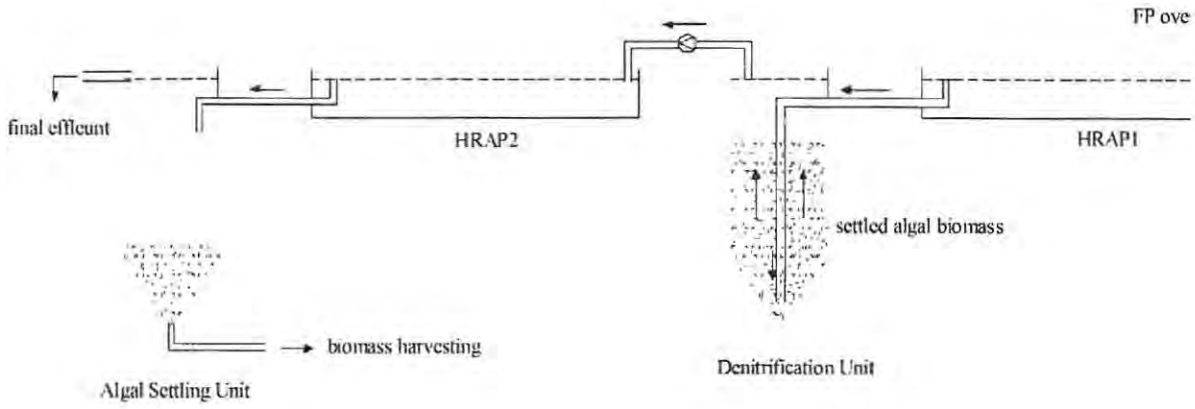

Figure 2.2.4.a: An example of a high rate algal ponding (HRAP) system that was operated to achieve denitrification after HRAP 1, followed by ammonia volatilization and phosphate precipitation in HRAP 2 (Dekker 2002).

2.3 High rate algal ponds

2.3.1 Algal growth: What makes them tick?

Algae and heterotrophic bacteria exist symbiotically in HRAPs. Algae fix carbon into biomass through photosynthesis and subsequently provide the oxygen (O_2) that is needed for the aerobic breakdown of organic compounds by bacteria (Johnson 2010). Heterotrophic bacteria utilise O_2 produced by algae to oxidise complex organic compounds into simple organic compounds with exo-enzymes (Craggs *et al.* 2011). The optimization of algal productivity as a management goal in HRAP effluent treatment was important from a nutrient removal efficiency and space requirement perspective (Knud-Hansen 1998, Johnson 2010). Algal productivity can be affected by a number of variables, which included abiotic, biotic and operational factors (Table 5.2.1, Johnson 2010). HRT can also have a significant impact on algal productivity (Azov and Shelef 1982).

Table 5.2.1.a: Factors that influence algal productivity (Johnson 2010).

Abiotic	Biotic	Operational
Temperature	Algae competition	Turbulence
Light	Grazers	Depth (influences lights intensity)
Nutrient concentration	Pathogents	Hydraulic retention time (HRT)
CO_2 and pH		Dilution rate

In natural marine and freshwater environments, algae exist in a world where there are intimate relationships between them and the surrounding bacteria, viruses and zooplankton. Their physiology therefore cannot be described in isolation (Fogg 1991). Algae are photosynthetic organisms that

convert light energy into cellular energy (Oswald & Golueke 1960). Water movement, temperature, light and nutrients are the most important factors that determine algal productivity.

As light penetrates into the water surface it decreases exponentially so that photosynthesis cannot happen below 100 metres (Fogg 1991). On the other hand, nutrients that are available in particulate form are likely to sink to the bottom of the water column through sedimentation. Phytoplankton therefore exists precariously between a shortage of light below on the one hand, and a shortage of nutrients above on the other (Fogg 1991). Water movement controls both of these factors and is therefore a crucial factor in their lives. In natural environments this can happen through wave action, eddies and turbulence, whilst mixing induces water movement in artificial HRAPs (Fogg 1991).

Mixing is required to keep the algal cells from settling to the bottom of the pond, which would inhibit nutrient uptake and reduce the system's efficiency. Mixing was introduced through the use of paddle-wheels (Chapter 3, Section 3.1). In natural conditions, algae larger than 10μm in diameter are able to assimilate nutrients through their relative motion to the water which is brought about by rising, swimming, or sinking (Fogg 1991). This steepens the concentration gradient and enhances nutrient uptake (Fogg 1991). Artificial water movement in HRAPs through mixing promotes nutrient uptake through a similar mechanism. During the day, mixing moves cells in and out of the light zone and improves the mass transfer between the cells and the medium, supplies CO_2 to the culture, degases photosynthetically accumulated O_2, and prevents sedimentation (Ogbonna & Tanaka 1996). Specific algal productivity is the greatest in stirred cultures (Martinez *et al.* 2000). Stirring reduces the time that is needed to reach the highest levels of nutrient removal under similar temperature and light conditions (Martinez *et al.* 2000). The continuous flow and gentle mixing of a culture allows algae and algal flocs to remain in suspension near the surface and within the depth of light penetration. Larger bacterial flocs uses photosynthetically produced dissolved oxygen (DO) to oxidize the influent BOD, and generally moves more slowly along the bottom of a HRAP (Green *et al.* 1995).

Energy is required for simulated HRAP mixing and recirculation pumping in an advanced integrated wastewater ponding system (Green *et al.* 1995). Electrical energy is required to drive paddle wheels (Green *et al.* 1995). The optimal velocity is approximately 15 cm/s (Oswald 1988). Electricity generated at 30 % CH_4 efficiency from recovered CH_4 would satisfy all of the energy requirements for HRAP paddle wheel mixing, recirculation pumping, and supplemental surface aeration (Green *et al.* 1995).

Different algal species have different specific optimum temperatures at which productivity is maximal (Raven & Geider 1988, Fogg 1991). When light is the limiting factor, the phenotypic effect of suboptimal temperatures on growth is less distinct than when growth is light saturated (Raven & Geider 1988). This applied to resource limited growth. When a chemical nutrient is limiting, the temperature effect on growth will become less (Raven & Geider 1988). When light is limiting, the growth rate will be low due to temperature insensitive reactions that are affected such as light absorption, excitation energy transfer and primary photochemistry (Raven & Geider 1988). Light limited growth can be sensitive to temperature due to a decrease in absorptance as a result of lower pigment content per cell at low growth temperatures. A decrease in temperature has one common response: the ratio of catalyst dealing with steps of photosynthesis from light absorption through primary photochemistry, i.e. those which are temperature insensitive, to those that are temperature sensitive reactions, is decreased (Raven & Geider 1988).

The HRT is the primary variable used to manage HRAP systems. Temperature determines the optimal HRT that can be used, whilst light intensity determines the maximum algal productivity (Azov & Shelef 1982). HRT plays an important role in the amount of light cells receive. Algal productivity declines when there is a shortage of light. When the amount of light available to algae was increased by reducing the HRT and diluting the culture, a 33 % increase in productivity was witnessed (Phang *et al.* 2000). A higher HRT is achieved by supplying ponds with a smaller volume effluent, and vice versa.

The first photosynthesis light saturation model was developed by Shelef *et al.* in 1968. This model assumed a linear relationship between photosynthesis and light intensity until a maximum rate was attained where after the rate remained constant (Azov & Shelef 1982, Figure 2.3.1.a). The Photosynthetic rate/Irradiance (PI) curve represents a typical growth response to substrate availability. At first, the initial rate of response to increasing substrate availability is the highest. Soon the rate decreases, signifying the initiation of a saturation process, in which an increasingly higher flux is needed to affect a given response. Finally, there is no more net response, and the photosynthetic machinery of all cells become fully light saturated. If the light flux increases much above the saturation, photoinhibition can become evident. This can lead to culture deterioration (Richmond 2000). Photosynthetic efficiency is the fraction of light energy that is converted into chemical energy. It is highest at very low light intensities and decreases as soon as the light flux saturates the photosynthetic apparatus (Richmond 2000). At strong light intensities (e.g. sunlight at midday) the efficiency can approach 20 % of its peak obtained under low light intensities, according to the PI curve (Richmond 2000).

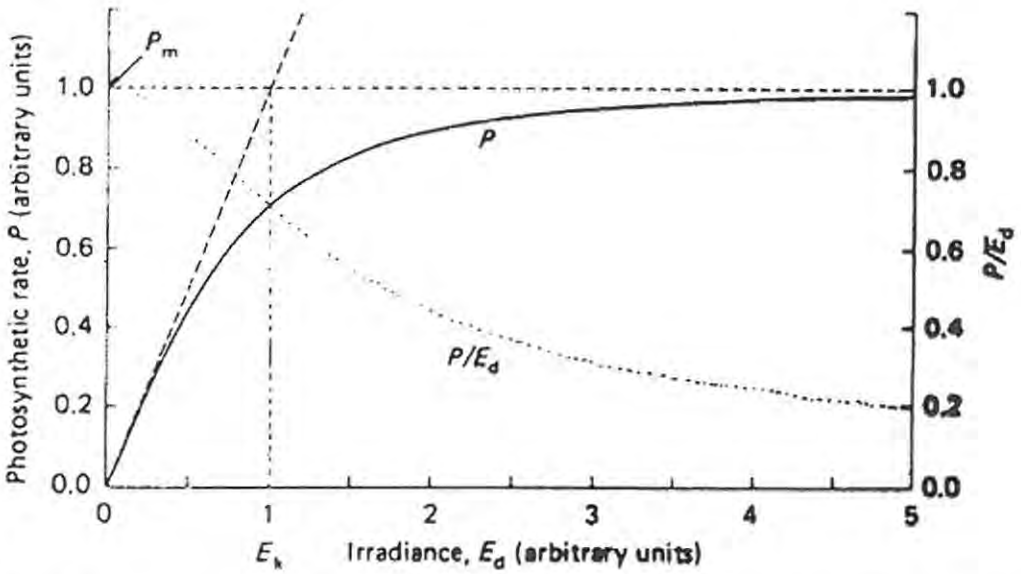

Figure 2.3.1.a: Idealized curve of specific photosynthetic rate (P) as a function of irradiance (E_d). The maximum photosynthesis rate, P_m, and the saturation onset parameter, E_k, are illustrated. The variation of P/Ed is a measure of the efficiency of utilization of incident light (Richmond 2000).

The practicality of these 'light curve concepts' to the production physiology of mass cultures outdoors is as a rule of little use (Richmond 2000).The limitation of the light curve model is that it is limited to the response to light of only optically thin cultures in which there is no mutual shading effect, and in which all cells receive light continuously (Richmond 2000). This situation is non-existent in mass cultures, where the major objective is maximal productivity of biomass at the available irradiance (Richmond 2000). The effective use of strong light requires relatively high cell densities in which mutual shading is most pronounced (Richmond 2000). Cells in mass cultures receive light intermittently, which is the most practical mode of diluting strong light. (Richmond 2000).

Briefly summarised, intermittent light exposes culture cells to cyclic periods of light in the photic volume, and of darkness in the dark volume (Richmond 2000). These cycles may take milliseconds or a few seconds to complete, and are reminiscent of a "flashing light effect" (Richmond 2000). Light intermittence is therefore associated with two basic parameters i.e. 1) the ratio between the light and the dark period in a cycle; and 2) the frequency of the light-dark (L-D) cycle. In most open HRAPs, the photic volume occupies 15 % of the reactor volume (Richmond 2000). Thus, at any instant, approximately 85 % of the cells are in the dark (Richmond 2000). Intermittent light results in diluted light for the average cell, and is without exception mandatory for the effective utilisation of strong light. The shading effect of many cells in suspension cause a low level of light to be available

per cell (Ogbonna and Tanaka 1996). These factors, together with the light source, create the cells' "light regime" or "light climate", which relates to the state of light available to a single cell. The light regime is the most important single factor that controls the productivity of mass cultures, and it cannot be defined quantitatively in terms of a single parameter (Richmond 2000).

The biomass concentration and carbohydrate content in *Chlorella* cells reportedly decreases at night, whereas the protein content increases (Ogbonna & Tanaka 1996). In the absence of light intracellular carbohydrates are metabolized as an energy source for cell maintenance and protein synthesis (Ogbonna & Tanaka 1996). Cells did not grow during the night, but respired to maintain themselves in the absence of light energy or another metabolizable organic carbon sources (Ogbonna & Tanaka 1996). Up to 35 % of the biomass produced during the day may be lost through respiration at night (Ogbonna & Tanaka 1996). Light intensity and temperature affected night biomass loss due to their influence on the cells' biochemical composition. Loss could be minimised by decreasing the nocturnal temperature (if possible), and by avoiding mixing at night in order to reduce the cells' metabolic rate (Ogbonna & Tanaka 1996).

2.3.2 Methods for harvesting algae

Harvesting algae is a challenging part of maintaining HRAPs and in producing an effluent that meets discharge standards, as the presence of algae can influence water quality parameters such as pH and COD (Park *et al.* 2011). The aim of harvesting is to concentrate the algae biomass 50 to 200-fold, in order to obtain a slurry of between 5 – 15 % dry solids (Grima et al, 2003). Various methods have been developed to harvest algae, although it still remains quite expensive (Craggs *et al.* 2011). The type of method that is used to harvest algae depends on what it will be used for after harvesting.

Bioflocculation refers to algal settling through gravitation, and this method was used to harvest algae in this study. It is the most cost effective technology that can produce an effluent that meets effluent discharge standards, as well as to harvest algae for biofuel production (Park *et al.* 2011). Sedimentation without chemical addition is the option most widely applied in full-scale facilities (Garcia *et al.* 2000). The algae from an advanced integrated wastewater ponding system accumulated in a settling pond with a HRT of 1 to 2 d (Green *et al.* 1996). Between 50 – 80 % algae could be removed in this way (Green *et al.* 1996). Settled algae have a very low respiration rate, do not release much nutrients, and can remain there for many years, however, in most cases it would be better to recover the algae to get the highest nutrient and protein value from its biomass (Green *et al.* 1996). For low-value products, gravity sedimentation preceded by flocculation might be the method of choice (Figure 2.3.2.a).

Figure 2.3.2.a: High rate algal ponds used in piggery effluent treatment (Singapore, Malaysia). Sedimentation tanks are in the foreground (Phang 1991).

Flocculation can be used to aggregate algal cells to increase the effective particle size and improve sedimentation, centrifugal recovery and filtration. Algal cells are negatively charged, and this can prevent aggregation (Grima *et al.* 2003). The fact that actively growing algal surfaces are negatively charged can also facilitate its harvesting by using chemical or electrolytic flocculation (Oswald 2003). Van Vuuren & Van Duuren (1965) investigated the removal of algae from wastewater effluent and tested various separation techniques. The major outcomes of their study were that 1) chemical coagulation of algae laden sewage could yield an acceptably clear and colourless water; 2) polyelectrolytes were found to be indifferent flocculants; 3) alum and excess lime both yielded good results when used as flocculants; and 4) the addition of activated silica when alum was used resulted in a stable algae blanket at the water surface (Figure 2.3.2.b).

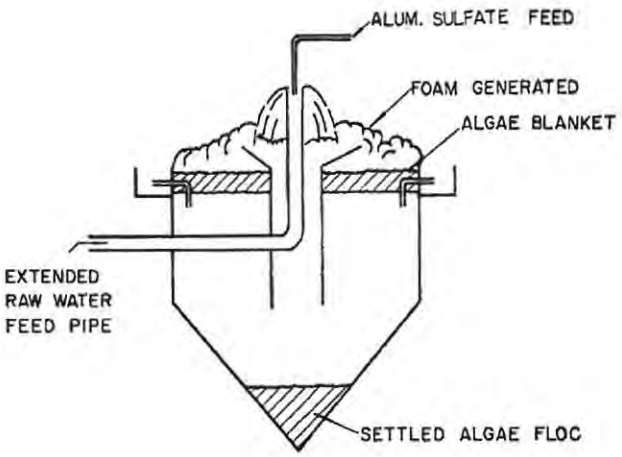

Figure 2.3.2.b: Design of a sedimentation tank with chemical flocculation (Van Vuuren & Van Duuren 1965).

Flocculation can be achieved simply by changing the pH. Effective flocculation can occur between pH of 11.80 and 12.00 (Grima *et al.* 2003). Extreme pH can also cause sedimented cells to lyse and to subsequently release all their valuable intracellular products into the sludge (Grima *et al.* 2003). The addition of multivalent cations and cationic polymers can neutralize or reduce the cell surface (Grima *et al.* 2003). Multivalent metal salts are effective flocculants (Grima *et al.* 2003). The most commonly used chemical flocculants include ferric oxide ($FeCl_3$), aluminum sulphate ($Al_2(SO_4)_3$, alum) and ferric sulphate ($Fe_2(SO_4)_3$) (Grima *et al.* 2003). Polyferric sulfate is a better flocculant than traditional nonpolymerized metal salt flocculants as it operates over a wide pH range (Grima *et al.* 2003). Alum has been widely applied to flocculate algae produced in wastewater (Van Vuuren & Van Duuren 1965). Dissolved air flotation with an aluminium sulphate flocculant was used to obtain a slurry that contained 2 - 3 % dry solids, and with a polyelectrolyte a slurry that contained 8 % solids. If this method was used with photosynthetically produced oxygen to effect spontaneous flotation the cost of separation, dewatering and drying could be reduced significantly (Viviers & Briers 1982).

Electrolytic flocculation is another effective method (Poelman *et al.* 1997) and not require chemical flocculants. It needs little electricity to flocculate micro-algae from suspension and subsequently float algae to the surface (Poelman *et al.* 1997). The principle is based on the movement of electrically charged particles in an electric field (Poelman *et al.* 1997). Algae have a negative surface charge that causes them to be attracted to the anode during the electrolyses of the algal suspension (Poelman *et al.* 1997). Once they reach the positive anode they lose their charge, which enables aggregation (Poelman *et al.* 1997). During the electrolysis of water, H_2 and O_2 are produced at the electrodes. These rise as bubbles to the surface, taking with them algal aggregates. Algal flocs can subsequently be skimmed off easily (Poelman *et al.* 1997). The method can be used to separate several different taxonomic groups of algae. As there are no chemical flocculants involved, harvested

algae are suitable for animal consumption or fertilizer. The method resulted in 90 % separation of algae (Poelman *et al.* 1997).

Dissolved air flotation is by far the most economical and efficient harvesting method, and if this process is followed by filtration and UV disinfection, the effluent quality will be suitable for unrestricted use (Grima *et al.* 2003). Filtration is another effective harvesting method (Grima *et al.* 2003). An example is a filter press, which operates under pressure/vacuum, and is a satisfactory method for recovering relatively large microalgae such as *Coelastrum*, *Proboscideum* and *Spirulina platensis*, but less effective in recovering smaller organisms that approach bacterial dimensions.Separation technologies can be categorized according to their fundamental principles (mechanical, physical, thermal or chemical) and have different process costs (Figure 2.4.7.c, Keller *et al.* 2001).

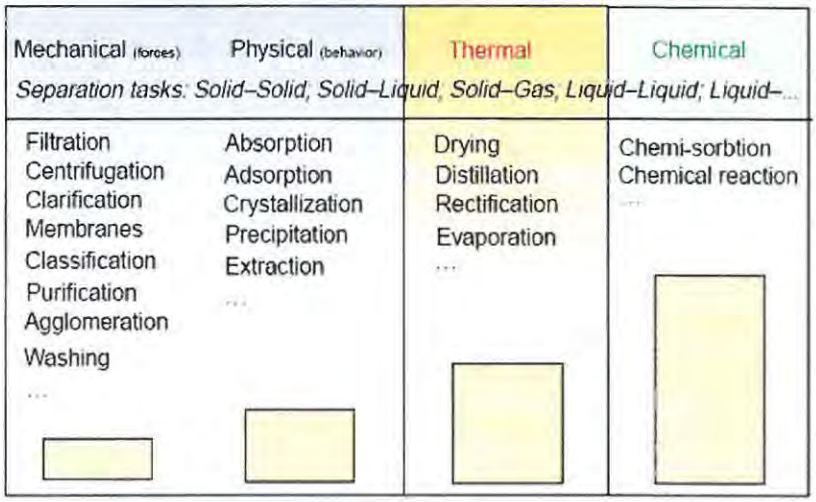

Figure 2.3.2.c: Separation technologies categorised according to fundamental principles (Keller *et al.* 2001).

2.3.3 The use of algae in brewery effluent treatment

The use of integrated systems that consist of bacteria, algae, fish and wetlands for the treatment of brewery effluent was tested in South Africa in as early as 1981 (Gaigher *et al.* 1985). This is the only known study to have been conducted on pilot integrated AD/algal systems in the treatment of brewery effluent, specifically. This study was carried out at the Hamilton Beer Brewery in the industrial area of Bloemfontein (February to December 1981). The project evaluated the stability of the treatment process and highlighted certain practical problems, but did not investigate the chemical processes in the system in any depth. The wastewater originated from the washings of fermenters, packing trucks and packing sheds. Only wastewater free from sodium hydroxide (NaOH)

was used in the system, which was only used from time to time for cleaning purposes. Effluent from the Hamilton brewery was deficient in nitrogen, and most of the nutrient uptake took place in the photosynthetic bacterial ponds, whilst the algal ponds contributed very little, probably due to an insufficient nitrogen concentration in the effluent (Gaigher *et al.* 1985).

The experimental unit was based inside a plastic tunnel, with a primary facultative pond (called a storage reservoir in this study), and three ponds with paddle wheels in which photosynthetic bacteria and algae were cultured separately (Gaigher *et al.* 1985). The second bacterial pond overflowed into a HRAP which was naturally dominated by *Closterium* algae. An increase in pH from 6.50 (wastewater pH) to 8.00 in HRAPs indicated that the system was operating successfully (Gaigher *et al.* 1985).

The overflow from the HRAP was fed into two treated fish tanks that were stocked with Tilapia (*Oreochromis mossambicus*) which were cleaned at two to three week intervals. During the first six months of the study the pH, DO and total dissolved solids concentrations were determined daily (Gaigher *et al.* 1985). From August 1981, the treated effluent was analysed once a week for electrical conductivity, alkalinity, NH_4-N, inorganic NO_2-N and NO_3-N, inorganic phosphate (PO_4-P) and unfiltered COD (Gaigher *et al.* 1985).

The success of the system was dependent on the efficient growth of photosynthetic bacteria (mainly *Rhodopseudomonas* sp.) and algae at a relatively short HRT in order to treat the largest possible volume effluent (Gaigher *et al.* 1985). Both cultures ceased growing from time to time, in which case it became necessary to increase the HRT to 25 d to allow the culture to recover (Gaigher *et al.* 1985). Water quality parameters were compared from the point at which the raw effluent entered the system, through the two bacterial ponds, the algal pond, the fish pond, and finally into the macrophyte pond (wetland). NH_4-N increased from 0.03 mg/L in the raw effluent to 0.1 mg/L in the macrophyte pond. NO_2-N and NO_3-N remained below 0.001, phosphate decreased from 20 mg/l in the raw effluent to 5 mg/l in the macrophyte pond, and the COD decreased sharply from 4000 mg/l to 1000 mg/l in the first bacteria pond, after which it gradually decreased to 100 mg/l in the macrophyte pond. The efficacy of bacteria and algae to reduce the COD was clearly demonstrated (Gaigher *et al.* 1985). The key process in the system was the conversion of acids in the effluent by photosynthetic bacteria to create conditions suitable for the growth of other organisms like algae (Gaigher *et al.* 1985).

Other studies that have been conducted on the use of algae in the treatment of brewery effluent using lab experiments include Filomena *et al.* (2010) and Mata *et al.* (2012). Filomena et al. (2010) investigated the use of microalgae for brewery effluent treatment and possible applications for produced biomass. Results indicated a 27 % reduction in BOD from an initial concentration of 2172 mg/L O_2/L, and a 15 % reduction in COD from an initial concentration of 1340 mg O_2/L in raw effluent. The rate of nitrogen removal was much higher than the rate of phosphate removal. Mata *et al.* (2012) performed a parametric study of brewery effluent treatment using microalgae *Scenedesmus obliquus*. This work studied the influence of light intensity (4500 vs 12000 Lux), light/dark photoperiod (12/12 vs 24/0 h) and culture aeration with an average rate of 4 mL/s and without aeration. Synthetic effluent was prepared and used as the medium to grow *S. obliquus* in under light/dark photoperiods and room temperature (30 ± 3 °C). Conclusions were that the best operating conditions were aerated cultures that were exposed to a 12 h period of daylight at the higher light intensity. The final COD concentrations were 1692 mg/L. This means that for microalgae to be used as a treatment method, it can be combined with other treatment technologies and used as a secondary or tertiary method. Other findings were that the presence of other microorganisms apart from microalgae inhibited their growth, but did not significantly change treatment effectiveness in terms of COD and phosphate removal. In stressful conditions, algae used available nutrients to produce lipids and carbohydrates rather than proteins. The study concluded that the efficiency of wastewater treatment by microalgae depended on their productivity, and hence algal productivity should be optimized for most efficient nutrient removal.

2.4 Conclusion

This review sketched the basic principles and applications of integrated wastewater treatment systems with a special focus on anaerobic digestion, HRAP systems and algal harvesting techniques to provide a theoretical background to the thesis research objectives. Integrated systems present an environmentally sustainable approach to treating wastewater, with many positive attributes such as reduced greenhouse gas production, the recovery of useful products from algae such as protein supplements for use in the animal feed industry, the purification and recycling of wastewater, the disinfection of water, the production of CH_4 biogas and the recycling of wastes, amongst others. It can be a more energy efficient way to treat wastewater, and hence more cost-efficient. The use of integrated systems in the treatment of wastewater and constructed wetlands is strongly supported in this review, and there is potential for the development of mainstream treatment methods for wastewater, using these technologies, on a large scale in South Africa.

Chapter three
Materials and Methods

3.1 System design

Effluent from the brewery was treated by first running it through a drum filter (Autrex Industrial Screening, Serial no. A 140/02, Approximate size U-5/3.04, Model no. R 015) to remove solid waste such as paper, bottle caps or spent hops (Figure 3.1.a). After the grit screen, effluent was treated in the iBhayi brewery anaerobic digester (AD) (Figure 3.1.b). A 25 mm polyvinylchloride (PVC) pipe was connected to the iBhayi brewery AD, which directed approximately 1 m^3/d effluent into the pilot plant AD, a closed 5000 L PVC tank (Figure 3.1.c and Figure 3.1.d).

Effluent was pumped from the pilot plant AD into a primary facultative pond (PFP) using a submersible pump (LifeTech AP5800 Water Pump, AC220-240V 50 Hz 360 W, Hmax: 5.6 m, Qmax: 12000 L/h) (Figure 3.1.e). The hydraulic retention time (HRT) was controlled at the inlet valve into the PFP (Figure 3.1.f). The PFP was 1.08 m deep and its volume was 16.73 m^3. Post-PFP, effluent overflowed into a splitter box (Figure 3.1.g) that divided the incoming effluent into two streams. Each stream flowed by gravity into a series of high rate algal ponding (HRAP) trains, train-A and train-B. Each train consisted of two ponds in series (Figure 3.1.h).

The HRAPs were made from green plastic PVC pond liner and were supported by a galvanised metal frame. Each HRAP train consisted of a deep pond (HRAP A1 and B1) that decanted into a shallower pond (HRAP A2 and B2) (Table 3.1.a, Figure 3.1.i). A stainless steel paddle wheel driven by 0.45 kW electrical motors kept the algae in suspension in each HRAP. Paddle wheels moved HRAP effluent at an approximate velocity of 4.15 m/s (HRAP A1 and B1), and 6.10 m/s (HRAP A2 and B2) (Figures 3.1.j).

Table 3.1.a: The surface area, volume and depth of high rate algal ponds (HRAP) A1, A2, B1 and B2.

HRAP	Surface area (m^2)	Volume (m^3)	Depth (cm)
A1	14.84	3.64	24.50
A2	14.89	1.82	12.25
B1	14.77	3.73	25.25
B2	15.17	1.67	11.00

A heating system was installed in HRAP train-A a month after the start of the optimization trials to test the effect of heating on algal nutrient uptake efficiency (April 2010 to January 2011). A heat pump was installed on the outside of the tunnel. It pumped heated water into an insulated 500 L tank that served as a geyser to store the heated water. Heated water was transferred into the HRAP through an installed 25 mm PVC pipe network that served as a heating element. The heating temperature on the pump was set at 52 °C (the maximum temperature that the heat pump could reach) to heat up the HRAP effluent to the highest temperature possible. This resulted in a circulation temperature in the heating geyser of 36 - 37 °C (Figure 3.1.k).

Effluent from HRAP A2 and B2 decanted into a 500 L submersed collection sump (Figure 3.1.l). From there, effluent was pumped into two elevated algal settling cones with a submersible pump (LifeTech AP5800 Water Pump, AC220-240V 50 Hz 360 W, Hmax: 5.6 m, Qmax: 12000 L/h) (Figure 3.1.m). The algal settling cones facilitated the settling of algae suspended in the HRAP treated effluent stream. The valves at the bottom of each algal settling cone were opened weekly to discharge the algal slurry into a 500 L recessed slurry collection tank (Figure 3.1.l). The clarified effluent stream drained into a 1000 L collection tank (Figure 3.1.n).

Post-HRAP treated effluent was pumped into a horizontal flow constructed wetland (Figure 3.1.o) with a submersible pump (same model as mentioned before) for further polishing of the effluent. Effluent treated in the wetland was used in aquaculture tanks for the rearing of fish, i.e. swordtail (*Xiphopohorus helleri)* and Mozambique tilapia (*Oreochromus mossambicus*) (Figure 3.1.q), as well as in a hydroponic system for the production of lettuce (*Lactuca* sp.) (Figure 3.1.p). These species were also used as bioindicators to test the treated effluent's quality.

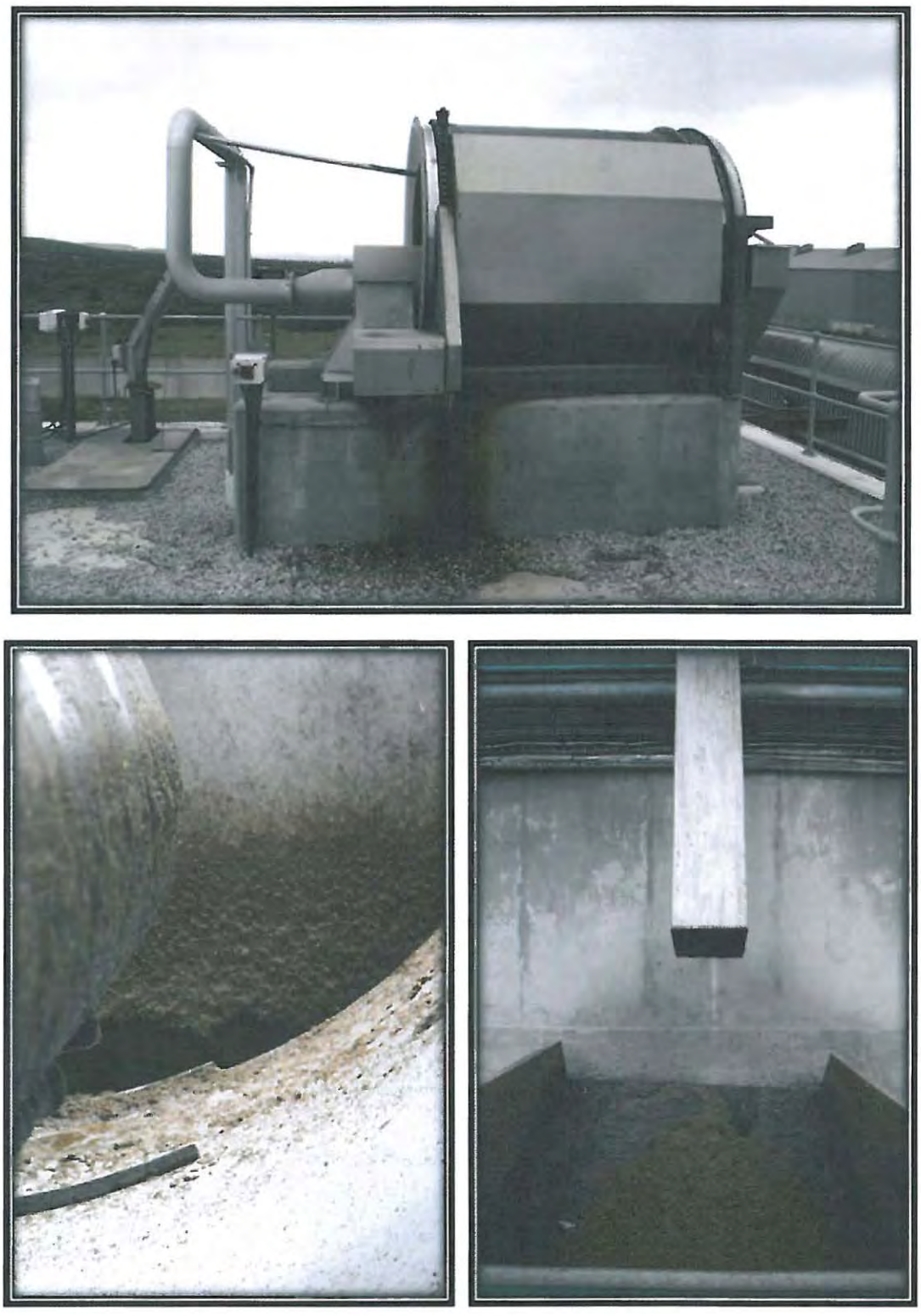

Figure 3.1.a: A drum filter screen (top) inside a drum filter (bottom left). Solids moved through the filter where it was collected at an outlet point (bottom right).

Figure 3.1.b: A view on top of the iBhayi brewery anaerobic digester.

Figure 3.1.c: The connection point that diverted approximately 1 m^3/d anaerobically digested effluent from the iBhayi brewery anaerobic digester into the pilot plant anaerobic digester.

Figure 3.1.d: The pilot plant anaerobic digester.

Figure 3.1.e: The primary facultative pond.

Figure 3.1.f: The inlet valve used to control the hydraulic retention time in the primary facultative pond and high rate algal ponds.

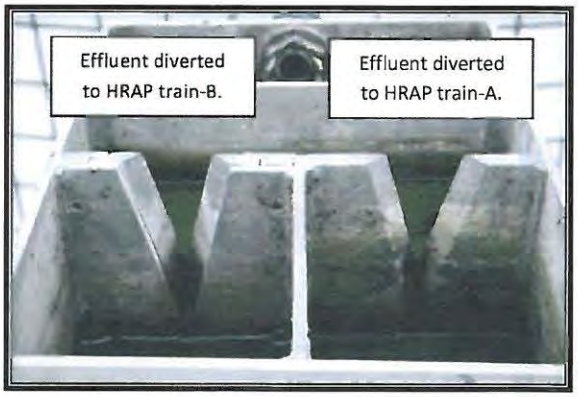

Figure 3.1.g: The splitter box diverted post-primary facultative pond effluent into high rate algal ponds (HRAP) trains-A and –B.

Figure 3.1.h: Effluent was diverted from the primary facultative pond into high rate algal ponds train-A and train-B.

Figure 3.1.i: High rate algal ponds (HRAP) A1, A2, B1 and B2.

Figure 3.1.j: An example of one of the paddle wheels used to keep algae in suspension.

Figure 3.1.k: The heating system that was installed in high rate algal pond train-A. The insulated drum geyser that stored heated water from the heat pump (insert).

Figure 3.1.l: The submersed collection tank for high rate algal pond A2 and B2 effluent (right). The recessed slurry collection tank into which algae from harvesting tanks were drained weekly (left).

Figure 3.1.m: The algal settling cones used to harvest algae from the high rate algal ponds.

Figure 3.1.n: The 1000 L collection tank used to collect clarified high rate algal pond treated effluent.

Figure 3.1.o: The horizontal flow constructed wetland that polished high rate algal pond treated effluent.

Figure 3.1.p: Hydroponic lettuce production with high rate algal pond and wetland treated effluent.

Figure 3.1.q: The aquaculture system in which high rate algal pond treated effluent was used in the rearing of Sword tail (*Xiphopohorus helleri)* and Mozambique tilapia (*Oreochromus mossambicus*) fish. Watercress can be seen floating on top.

3.2 Growing the algal culture

The algal culture which was used to grow a new culture at the study site (iBhayi brewery) was obtained from the Institute of Environmental Biotechnology at Rhodes University (EBRU), Grahamstown. One hundred litres (100 L) of the algal culture from the ponds at EBRU was mixed with five hundred litres (500 L) municipal water with double strength artificial growth medium added (Table 3.2.a). The algal culture was grown in a 1000 L container on-site. Algal cells were kept in suspension by mixing, and cultivated under ambient conditions for ten days. After ten days the culture was inoculated into the HRAPs and fed with post-PFP effluent (150 L/d) for two weeks until the HRAPs were full. When this stage was reached it was possible to start the experiment and adjust HRTs (Figure 3.2.a).

Table 3.2.a: The recipe used to make up the artificial growth medium in which the algal culture was grown before it was inoculated into high rate algal ponds (HRAP).

Nutrients	mg/L	g/L	g/m^3	Double strength g/m^3
$NaNO_3$	75.0	0.8	750.0	1500.0
$NaCl_2$	2.5	0.0	25.0	50.0
$CaCl_2.2H_2O$	2.5	0.0	25.0	50.0
$MgSO_4.7H_2O$	7.5	0.1	75.0	150.0
K_2HPO_4	7.5	0.1	75.0	150.0
KH_2PO_4	7.5	0.1	75.0	150.0

Figure 3.2.a: The algal culture with added post-anaerobically digested effluent before inoculation into the high rate algal ponds.

The procedure of inoculating the ponds with a new algal culture was performed twice: The first time in March 2009 after the construction of the pilot plant, and the second time on the 27th of October 2010. The reason for re-inoculation in October 2010 was because the algal culture had become senescent and was subjected to heavy grazing. Microscopic examination revealed that there was considerable algal debris in the culture, and little evidence of *Chlorella*, which is generally an indicator of a healthy algal culture in wastewater treatment.

The algal cultures in HRAP train-A and train-B were mixed together on two occasions to ensure that the algal culture remained homogenous. The first time this was done was on the 6th of July 2010, and the second time was on the 26th of August 2010. As the ponds were subjected to increased volumes of effluent and heating (HRAP train-A), algal productivity, mortality and species composition between the ponds could differ in their ability to take up nutrient (pond effects).

3.3 Data collection for water quality parameters

Data was collected for temperature (Section 3.3.1), pH (Section 3.3.2), electrical conductivity – EC (Section 3.3.3), dissolved oxygen - DO (Section 3.3.4), chemical oxygen demand - COD (Section 3.3.5), ammonia – NH_4-N (Section 3.3.6), phosphate – PO_4-P (Section 3.3.7), nitrate – NO_3-N (Section 3.3.8), nitrite – NO_2-N (Section 3.3.9), and chloride – Cl^- (Section 3.3.10) concentrations.

Post-AD samples were collected from the outlet pipe that transported effluent from the pilot plant AD into the PFP (Figure 3.1.f). Post-PFP samples were collected from the splitter box (Figure 3.1.g). HRAP A1 and B1 samples were collected from the overflow pipes that led treated effluent from HRAP A1 and B1, into HRAP A2 and B2, respectively. Samples for HRAP A2 and B2 were collected from the outlet points that led effluent from the overflow pipes in HRAP A2 and B2 into the submersed collection sump (Figure 3.1.l, right). Post-HRAP samples were collected in the 1000 L collection tank that received the clarified effluent from all four HRAPs (Figure 3.1.n). Temperature, EC, pH and DO readings were taken at 09h00 in post-AD, PFP and HRAP treated effluent.

Quality control and assurance of the results were effected after the author followed training in the Talbot and Talbot water quality lab at iBhayi brewery.

Two models of spectrophotometers were used to determine the concentration of the parameter being measured. A Lovibond System PC Multidirect spectrophotometer was used from 1 May 2009 until the 19th of July 2010, and a Merck Spectroquant Pharo 100 spectrophotometer (product number 100706, Darmstadt, Germany) was used from the 22nd of July 2010 until the 16th of January 2011.

3.3.1 Temperature

Temperature readings were taken with a Hanna temperature probe in degrees Celsius (°C) (Hanna, HI 98129, United Kingdom) (Table 3.3.1.a).

Table 3.3.1.a: The sampling procedure used to collect temperature data in post-pilot plant anaerobic digester (AD), primary facultative pond (PFP), high rate algal pond (HRAP) A1, A2, B1, B2 and mixed post-HRAP effluent from 1 May 2009 until 16 January 2011.

Start date	End date	Sampling frequency	System components sampled	Phase
01-May-09	12-May-09	No samples		Baseline, Ch. 4
13-May-09	24-May-09	Morning samples three times/week	Post-AD, PFP, HRAP A1, A2, B1, B2, Post-HRAP	Baseline, Ch. 4
25-May-09	22-Jul-09	Morning samples five times/week	Post-AD, PFP, HRAP A1, A2, B1, B2, Post-HRAP	Baseline, Ch. 4
23-Jul-09	31-Aug-09	No samples		Baseline, Ch. 4
01-Sep-09	23-Oct-09	Morning samples five times/week	PFP, HRAP A1, A2, B1, B2, Post-HRAP	Baseline, Ch. 4
26-Oct-09	28-Feb-10	Morning samples five times/week	Post-AD, PFP, HRAP A1, A2, B1, B2, Post-HRAP	Baseline, Ch. 4
01-Mar-10	16-Jan-11	Morning and afternoon samples five time/week	Post-AD, PFP, HRAP A1, A2, B1, B2, Post-HRAP	Optimization, Ch. 5

3.3.2 The pH

The pH of treated effluent was measured with a Hanna pH probe (Hanna Model HI 98129, United Kingdom) (Table 3.3.2.a).

Table 3.3.2.a: The sampling procedure used to collect pH data in post-pilot plant anaerobic digester (AD), primary facultative pond (PFP), high rate algal pond (HRAP) A1, A2, B1, B2 and mixed post-HRAP effluent from 1 May 2009 until 16 January. 2011.

Start date	End date	Sampling Frequency	System components sampled
01-May-09	12-May-09	No samples	
03-May-09	22-Jul-09	Morning samples five times/week	Post-AD, PFP, HRAP A1, A2, B1, B2, Post-HRAP
23-Jul-09	31-Aug	No samples	
01-Sep-09	29-Feb-10	Morning samples five times/week	Post-AD, PFP, HRAP A1, A2, B1, B2, Post-HRAP
01-Mar-10	16-Jan-11	Morning and afternoon samples five times/week	Post-AD, PFP, HRAP A1, A2, B1, B2, Post-HRAP

3.3.3 Electrical conductivity

The EC of treated effluent in different components of the system was measured with a Hanna EC probe in µS/cm (Hanna, HI 98130, United Kingdom) (Table 3.4.3.a).

Table 3.3.3.a: The sampling procedure used to collect electrical conductivity data in post-pilot plant anaerobic digester (AD), primary facultative pond (PFP), high rate algal pond (HRAP) A1, A2, B1, B2 and mixed post-HRAP effluent from 1 May 2009 until 16 January 2011.

Start date	End date	Sampling frequency	System components sampled
01-May-09	12-May-09	No samples	
13-May-09	23-May-09	Morning samples three times/week	Post-AD, PFP, HRAP A1, A2, B1, B2, Post-HRAP
25-May-09	22-Jul-09	Morning samples four to five times/week	Post-AD, PFP, HRAP A1, A2, B1, B2, Post-HRAP
23-Jul-09	30-Aug-09	Morning samples two times/week	Post-AD, PFP, HRAP A1, A2, B1, B2, Post-HRAP
01-Sep-09	26-Feb-10	Morning samples five times/week	Post-AD, PFP, HRAP A1, A2, B1, B2, Post-HRAP
01-Mar-10	16-Jan-11	Morning and afternoon samples five times/week	Post-AD, PFP, HRAP A1, A2, B1, B2, Post-HRAP

3.3.4 Dissolved oxygen

The DO concentration was measured with an electronic dissolved oxygen metre (Oxyguard Handy Polaris 1 portable DO meter, Los Angeles, United States of America). DO was not measured during the baseline phase. The percentage saturation and DO concentration (mg/L) were measured in post-pilot plant AD, PFP and HRAP A1, A2, B1 and B2 effluent at 09h00 in the morning 5.00 times a week from the 19th of April 2010 until the 16th of January 2011.

3.3.5 Chemical oxygen demand

3.3.5.1 Lovibond PC Multidirect Spectrophotometer (1 May 2009 – 19 July 2010)

The COD medium range tube kit was used (VARIO reagent, Product number 420722, range: 0 – 1500 mg/L O_2, Dortmund, Germany). The method used was the Dichromate/H_2SO_4 method (Lovibond Spectrophotometer System Multidirect Instruction Manual, www.tintometer.de, 12 September 2011).

3.3.5.1.a Sample preparation

All samples were filtered through 8 µm filter paper (Whatman 40 Ashless Circles, 125 mm diameter, Cat no. 1440 125). A blank was prepared by adding 2.00mL deionised water into a vial. A sample was prepared by adding 2.00 mL of the filtered sample to another vial. Vials were capped and gently inverted several times to mix the contents. The vials were heated for 120 min in a preheated digester reactor at a temperature of 150 ^{o}C. After 120 min the vials were removed from the heating block. The contents were mixed carefully by inverting each of the tubes several times while they were still hot and left to cool down to 60 ^{o}C or less for at least 30 min (Lovibond Spectrophotometer System Multidirect Instruction Manual. www.tintometer.de, 12 September 2011).

3.3.5.1.b Sample analysis

COD analysis was performed by colorimetric determination at 610 nm using a Lovibond PC Multidirect spectrophotometer programmed for COD analysis (programme no. 131) (Lovibond spectrophotometer System Multidirect instruction manual. www.tintometer.de, 12 September 2011).

3.3.5.2 Merck Pharo 100 Spectroquant spectrophotometer (22 July 2010 – 16 January 2011)

The potassium dichromate solution method was used. The water sample is oxidised with a hot sulphuric solution of potassium dichromate with silver sulphate as the catalyst. Chloride is masked with mercury sulphate. The concentration of unconsumed $Cr_2O_7^{2-}$ ions was determined spectrophotometrically. The method was analogous to EPA 410.4, US Standard Methods 5220D, and ISO 15705 (product number 109119, range: 0 - 1500 mg/L O_2, Merck Chemicals, Darmstadt, Germany, http://www.merck-chemicals.com/potassium-dichromate-solution, 12 September 2011).

3.3.5.2.a Sample preparation

The sample preparation was the same as for COD sample preparation in Section 3.3.5.1.a.

3.3.5.2.b Sample analysis

Analysis of COD medium range was performed by colorimetric determination using the Merck Pharo 100 Spectroquant spectrophotometer (product number 100706, Darmstadt, Germany).

Table 3.3.5.a: The sampling procedure used to collect chemical oxygen demand data in post-pilot plant anaerobic digester (AD), primary facultative pond (PFP), high rate algal pond (HRAP) A1, A2, B1, B2 and mixed post-HRAP effluent from 1 May 2009 until 16 January 2011.

Start date	End date	Sampling frequency	System components analysed	Phase
01-May-09	01-Mar-10	One or two times/week	Post-AD, Post-PFP, Post-HRAP	Baseline, Ch. 4
01-Mar-10	28-Mar-10	One or two times/week	Post-AD, Post-PFP, Post-HRAP	
29-Mar-10	30-Apr-10	Five times/week	Post-AD, Post-PFP, Post-A2, Post-B2, Post-HRAP	Optimization, Ch. 5
01-May-10	13-Jul-10	Two times/week	Post-AD, Post-PFP, Post-A2, Post-B2	Optimization, Ch. 5
21-Jul-10	25-Jul-10	Three times/week	Post-AD, Post-PFP, Post-A1, Post-A2, Post-B1, Post-B2	Optimization, Ch. 5
26-Jul-10	01-Oct-10	Three times/week	Post-AD, Post-PFP, Post-A1, Post-A2, Post-B1, Post-B2	Optimization, Ch. 5
10-Nov-10	16-Jan-10	Three times/week	Post-AD, Post-PFP, Post-A1, Post-A2, Post-B1, Post-B2	Optimization, Ch. 5

3.3.6 Ammonia

Ammonia nitrogen (NH_4-N) high range (0 - 50 mg/L) tests were used to measure NH_4-N concentrations in post-pilot plant AD and post-PFP samples, as these samples always contained more than 4.00 mg/l NH_4-N. NH_4-N low range (0 - 4 mg/L) tests were used for HRAP samples, as NH_4-N was mostly lowered to below 4.00 mg/L post-HRAP. When over-range readings were observed, high range tests were subsequently used to determine the NH_4-N concentration (Table 3.3.6.a).

3.3.6.1 Ammonia high range analysis

3.3.6.1.a Lovibond PC Multidirect Spectrophotometer (1 May 2009 – 19 July 2010)

The NH_4-N high range (HR) tube test kits were used for a range of 0 – 50 mg/L NH_4-N (VARIO reagent, Product number 535650, Dortmund, Germany). The salicylate method was used (Lovibond Spectrophotometer System Multidirect Instruction Manual, www.tintometer.de, 12 September 2011).

All samples were filtered through 8 µm filter paper (Whatman 40 Ashless Circles, 125 mm diameter, Cat no. 1440 125). A blank was prepared by adding 0.1 mL deionised water into a premixed tube test. A sample was prepared by adding 0.1 mL of the filtered sample into another premixed tube test. The contents of one pack of Vario Ammonia Salicylate Powder Pack (Product number 535650,

Dortmund, Germany) and one pack of Vario Ammonia Cyanurate F5 Powder Pack (Product number 535650, Dortmund, Germany) were added straight from the foil into each vial. Vials were left to stand for the reaction period of 20 min. Sample analyses were carried out in the same way as described in Section 3.3.5.1.b (programme no. 66, wavelength 660 nm) (Table 3.3.6.a).

3.3.6.1.b Merck Pharo 100 Spectroquant spectrophotometer (22 July 2010 – 16 January 2011)

Ammonia nitrogen (NH_4 – N) occurs partly in the form of ammonium ions and partly as ammonia. A pH-dependent equilibrium exists between the two forms. In strongly alkaline solution ammonium nitrogen is present almost entirely as ammonia, which reacts with hypochlorite ions to form monochloramine. This in turn reacts with a substituted phenol to form a blue indophenol derivative that can be measured spectrophotometrically. The method was analogous to EPA 350.1, US Standard Methods 4500 – NH_3 D, and ISO 7150/1 (NH_4-N HR, Product number 1.14559.001, range: 4.0 – 80 mg/L NH_4 – N, Merck Chemicals, Darmstadt, Germany, http://www.merck-chemicals.com/ammonium-cell-test, 12 September 2011).

All samples were filtered through 8 µm filter paper (Whatman 40 Ashless Circles, 125 mm diameter, Cat no. 1440 125) and a 0.1 mL aliquot from each sample was added to a tube test. The reagent was added to each tube test and the tubes were closed and shaken vigorously until all the granules were dissolved. The tubes were left to stand 15 min. Sample analyses were carried out in the same way as described in Section 3.3.5.2.b (Table 3.3.6.a).

3.3.6.2. Ammonia low range analysis

3.3.6.2.a Lovibond PC Multidirect Photometer (1 May 2009 – 19 July 2010)

The NH_4-N low range (LR) tube test was used for a range of 0 – 2.5 mg/L N. (VARIO reagent, Product number 512581, Dortmund, Germany). The salicylate method was used (Lovibond Spectrophotometer System Multidirect Instruction Manual, www.tintometer.de). Sample preparation and analyses were the same as described in Section 3.3.6.1.a (programme no. 65, wavelength 660 nm).

3.3.6.2.b. Merck Pharo 100 Spectroquant spectrophotometer (22 July 2010 – 16 January 2011)

The method is identical to the high range method, except that it is more sensitive. The method was analogous to EPA 350.1, US Standard Methods 4500 – NH_3 D, and ISO 7150/1 (NH_4-N LR Product

number 1.14752.001, range: 0.010 – 3 mg/l NH_4 – N, Merck Chemicals, Darmstadt, Germany, http://www.merck-chemicals.com/ammonium-test, 12 September 2011).

All samples were filtered through 8 µm (Whatman 40 Ashless Circles, 125 mm diameter, Cat no. 1440 125) filter paper, and a 5 mL aliquot from each sample was added to a clean 10 mL glass sampling vial with a pipette. 0.6 mL of Reagent 1 was added into the sample bottle, as well as one level blue micro spoon of Reagent 2. The cap was closed and the vial shaken vigorously to dissolve granules. The sample was left to stand for 5 min. Four drops of Reagent 3 were added and the sample left to react for another 5 min. Sample analyses were carried out in the same way as described in Section 3.3.5.2.b (Table 3.3.6.a).

Table 3.3.6.a: The sampling procedure that was used to collect ammonia concentration data in post-pilot plant anaerobic digester (AD), post-primary facultative pond (PFP), post-high rate algal pond (HRAP) A1, A2, B1, B2 and mixed post-HRAP effluent from 1 May 2009 until 16 January 2011.

Start date	End date	Frequency	System components analysed	Phase
01-May-09	01-Mar-10	Two - three times/week	Post-AD, Post-PFP, Post-HRAP	Baseline, Ch. 4
04-Mar-10	30-Apr-10	Five times/week	Post-AD, Post-PFP, Post-A2, Post-B2, Post-HRAP	Optimization, Ch. 5
04-May-10	22-Jun-10	Three times/week	Post-AD, Post-PFP, Post-A1, Post-A2, Post-B1, Post-B2, Post-HRAP	Optimization, Ch. 5
22-Jun-10	20-Jul-10	No readings		Optimization, Ch. 5
21-Jul-10	01-Oct-10	Three times/week	Post-AD, Post-PFP, Post-A1, Post-A2, Post-B1, Post-B2	Optimization, Ch. 5
10-Nov-10	16-Jan-10	Three times/week	Post-AD, Post-PFP, Post-A1, Post-A2, Post-B1, Post-B2	Optimization, Ch. 5

3.3.7 Nitrate

3.3.7.a Lovibond PC Multidirect Photometer (1 May 2009 – 19 July 2010)

The nitrate (NO_3-N) tube test was used for a range of 1 – 30 mg/L NO_3-N. (VARIO reagent, Product number 535580, Dortmund, Germany). The chromotropic acid method was used (Lovibond Spectrophotometer System Multidirect Instruction Manual, www.tintometer.de, 12 September 2011).

All samples were filtered through 8 µm filter paper (Whatman 40 Ashless Circles, 125 mm diameter, Cat no. 1440 125). One reaction vial was filled with deionised water and served as the blank. A filtered sample of 1 mL was added to another reaction vial. The content of one Vario Nitrate Chromatographic Powder Pack (VARIO reagent, Product number 535580, Dortmund, Germany) was added straight from the foil into each vial. The vials were closed and gently inverted several times to mix the contents. It was left standing for the reaction time of 5 min. Sample analyses were the same as described in Section 3.3.5.1.b (programme no. 265, wavelength 430 nm).

3.3.7.b Merck Pharo 100 Spectroquant spectrophotometer (22 July 2010 – 16 January 2011)

All samples were filtered through 8 µm (Whatman 40 Ashless Circles, 125 mm diameter, Cat no. 1440 125) filter paper. 4 mL of Reagent 1 was added into a clean 10 mL dry glass sampling vial with a pipette. A 0.5 mL aliquot of filtered sample was added with a pipette after which 0.5 mL of Reagent 2 was added. Vials were capped and shaken to mix their contents. The samples were left to stand for the reaction time of 10 min. Sample analyses were the same way as described in Section 3.3.5.2.b (Table 3.3.7.a).

Table 3.3.7.a: The sampling procedure used to collect nitrate concentration data in post-pilot plant anaerobic digester (AD), post-primary facultative pond (PFP), post-high rate algal pond (HRAP) A1, A2, B1, B2 and mixed post-HRAP effluent from 1 May 2009 until 16 January 2011.

Start date	End date	Sampling frequency	System components analysed	Phase
01-May-09	03-Aug-09	Two - three times/week	Post-AD, Post-PFP, Post-HRAP	Baseline, Ch. 4
03-Aug-09	25-Aug-09	No tests		Baseline, Ch. 4
25-Aug-09	22-Oct-09	Two times a week	Post-AD, Post-PFP, Post-HRAP	Baseline, Ch. 4
23-Oct-09	15-Nov-09	No tests		Baseline, Ch. 4
16-Nov-09	01-Mar-09	Two times/week	Post-AD, Post-PFP, Post-HRAP	Baseline, Ch. 4
08-Mar-09	16-Apr-09	Five times/week	Post-AD, Post-PFP, Post-A1, Post-A2, Post-B1, Post-B2	Optimization, Ch. 5
17-Apr-09	10-May-09	No tests		Optimization, Ch. 5
11-May-09	22-Jun-09	Two times/week	Post-AD, Post-PFP, Post-A1, Post-A2, Post-B1, Post-B2, Post-HRAP	Optimization, Ch. 5
23-Jun-10	08-Jul-10	No tests		Optimization, Ch. 5
09-Jul-10	26-Jul-10	Three times/week	Post-AD, Post-PFP, Post-A1, Post-A2, Post-B1, Post-B2	Optimization, Ch. 5
28-Jul-10	01-Oct-10	Three times/week	Post-AD, Post-PFP, Post-A1, Post-A2, Post-B1, Post-B2	Optimization, Ch. 5
10-Nov-10	16-Jan-10	Three times/week	Post-AD, Post-PFP, Post-A1, Post-A2, Post-B1, Post-B2	Optimization, Ch. 5

3.3.8 Nitrite

3.3.8.a Lovibond PC Multidirect Photometer (1 May 2009 – 19 July 2010)

The nitrite (NO_2-N) tablet test was used for a range of 0.01 – 0.5 mg/L NO_2-N (VARIO reagent, Product number 535680, Dortmund, Germany). The N-(10Naphtyl)-ethylendiamine method was used (Lovibond Spectrophotometer System Multidirect Instruction Manual, www.tintometer.de, 12 September 2011).

All samples were filtered through 8 µm filter paper (Whatman 40 Ashless Circles, 125 mm diameter, Cat no. 1440 125). A clean 10 mL glass vial was filled with 10 mL deionised water and capped. This served as the blank. Another vial was filled with 10 mL sample. One NO_2-N low range tablet (VARIO reagent, Product number 535680, Dortmund, Germany) was added straight from the foil onto a clean plastic plate and was crushed with the round edge of a metal spoon. The crushed tablet was

added to the vial's contents and the vial closed tightly. The vials were swirled around several times until the tablets were dissolved and left to stand for the reaction time of 10 min. Sample analyses were the same as described in Section 3.3.5.1 (programme no. 270, wavelength 560 nm).

3.3.8.b Merck Pharo 100 Spectroquant spectrophotometer (22 July 2010 – 16 January 2011)

In acidic solution nitrite ions react with sulfanilic acid to form a diazonium salt, which in turn reacts with N-(1-naphtyl) ethylenediamine dihydrochloride to form a red-violet azo dye. The dye can be measured spectrophotometrically. The method was analogous to EPA 354.1, US Standard methods 4500-NO_2^- B and EN 26777 (NO_2-N product number 1.14776.001, range: 0.002 – 1.00 mg/L NO_2 – N, http://www.merck-chemicals.com/nitrite-test, 12 September 2011).

All samples were filtered through 8 µm filter paper (Whatman 40 Ashless Circles, 125 mm diameter, Cat no. 1440 125). A 5 mL aliquot of sample was added into a clean 10 mL glass sampling vial with a pipette. One level micro spoon Reagent 1 was added into the vial. The vials were capped and shaken vigorously to allow the reagent to dissolve. The samples were left to stand for the reaction time of 10 min. Sample analyses were the same as described in Section 3.3.5.2.b (Table 3.3.8.a).

Table 3.3.8.a: The sampling procedure used to collect nitrite concentration data in post-pilot plant anaerobic digester (AD), post-primary facultative pond (PFP), post-high rate algal pond (HRAP) A1, A2, B1, B2 and mixed post-HRAP effluent from 1 May 2009 until 16 January 2011.

Start date	End date	Sampling frequency	System components analysed	Phase
01-May-09	03-Aug-09	Two - three times/week	Post-AD, Post-PFP, Post-HRAP	Baseline, Ch. 4
03-Aug-09	25-Aug-10	No tests		Baseline, Ch. 4
25-Aug-09	22-Oct-09	Two times a week	Post-AD, Post-PFP, Post-HRAP	Baseline, Ch. 4
23-Oct-09	15-Nov-09	No tests		Baseline, Ch. 4
16-Nov-09	01-Mar-10	Two times/week	Post-AD, Post-PFP, Post-HRAP	Baseline, Ch. 4
08-Mar-10	16-Apr-10	Five times/week	Post-AD, Post-PFP, Post-A1, Post-A2, Post-B1, Post-B2	Optimization, Ch. 5
17-Apr-10	10-May-10	No tests		Optimization, Ch. 5
11-May-10	22-Jun-10	Two times/week	Post-AD, Post-PFP, Post-A1, Post-A2, Post-B1, Post-B2, Post-HRAP	Optimization, Ch. 5
23-Jun-10	08-Jul-10	No tests		Optimization, Ch. 5
09-Jul-10	26-Jul-10	Three times/week	Post-AD, Post-PFP, Post-A1, Post-A2, Post-B1, Post-B2	Optimization, Ch. 5
28-Jul-10	16-Jan-10	Three times/week	Post-AD, Post-PFP, Post-A1, Post-A2, Post-B1, Post-B2	Optimization, Ch. 5

3.3.9 Phosphate

3.3.9.a Lovibond PC Multidirect Photometer (1 May 2009 – 19 July 2010)

In sulphuric solution orthophosphate ions react with ammonium vanadate and ammonium heptamolybdate to form orange-yellow molybdovanadophosphoric acid that can be determined spectrophotometrically ("VM" method). The method was analogous to US Standard methods 4500-PC (PO_4-P product number 1.14842.001, range: 0.5 – 30 mg/L PO_4 – P, Darmstadt, Germany, http://www.merck-chemicals.com/phosphate-test, 12 September 2011).

All samples were filtered through 8 µm (Whatman 40 Ashless Circles, 125 mm diameter, Cat no. 1440 125) filter paper, and a 5 mL aliquot from each sample was added to a 10 mL clean glass sampling vial with a pipette. 1.2 mL of Reagent 1 was added into the sample bottle, after which the sample was ready for analysis. Sample analyses were the same way as described in Section 3.3.5.2.b (Table 3.3.9.a).

3.3.7.b Merck Pharo 100 Spectroquant spectrophotometer (22 July 2010 – 16 January 2011)

In sulphuric solution orthophosphate ions react with ammonium vanadate and ammonium heptamolybdate to form orange-yellow molybdovanadophosphoric acid that can be determined spectrophotometrically ("VM" method). The method was analogous to US Standard methods 4500-PC (PO_4-P product number 1.14842.001, range: 0.5 – 30 mg/L PO_4 – P, Darmstadt, Germany, http://www.merck-chemicals.com/phosphate-test, 12 September 2011).

All samples were filtered through 8 µm (Whatmans GF/A) filter paper, and a 5 mL aliquot from each sample was added to a 10 mL clean glass sampling vial with a pipette. 1.2 mL of Reagent 1 was added into the sample bottle after which the sample was ready for analysis. Sample analyses were the same as described in Section 3.3.5.2.b (Table 3.3.9.a).

Table 3.3.7.a: The sampling procedure used to collect phosphate concentration data in post-pilot plant anaerobic digester (AD), post-primary facultative pond (PFP), post-high rate algal pond (HRAP) A1, A2, B1, B2 and mixed post-HRAP effluent from 1 May 2009 until 16 January 2011.

Start date	End date	Sampling frequency	System components analysed
01-May-09	03-Aug-09	Two - three times/week	Post-AD, Post-PFP, Post-HRAP
03-Aug-09	25-Aug-09	No tests	
25-Aug-09	Oct-09	Two times/week	Post-AD, Post-PFP, Post-HRAP
23-Oct-09	15-Nov-09	No tests	
16-Nov-09	01-Mar-10	Two times/week	Post-AD, Post-PFP, Post-HRAP
08-Apr-10	16-Apr-10	Five times/week	Post-AD, Post-PFP, Post-A1, Post-A2, Post-B1, Post-B2
17-Apr-10	10-May-10	No tests	
11-May-10	22-Jun-10	Two times/week	Post-AD, Post-PFP, Post-A1, Post-A2, Post-B1, Post-B2, Post-HRAP
23-Jun-10	08-Jul-10	No tests	
09-Jul-10	26-Jul-10	Three times/week	Post-AD, Post-PFP, Post-A1, Post-A2, Post-B1, Post-B2
28-Jul-10	01-Oct-10	Three times/week	Post-AD, Post-PFP, Post-A1, Post-A2, Post-B1, Post-B2
10-Nov-10	16-Jan-11	Three times/week	Post-AD, Post-PFP, Post-A1, Post-A2, Post-B1, Post-B2

3.3.10 Chloride

3.3.10.1 Merck Pharo 100 Spectroquant spectrophotometer

Chloride (Cl^-) ions react with mercury(II)thiocyanate to form slightly dissociated mercury(II)chloride. The thiocyanate released in the process in turn reacts with iron(III) ions to form red iron(III)thiocyanate that can be measured spectrophotometrically. The method was analogous to EPA 325.1 and US Standard Methods 4500-CL^- E. (Cl^- product number 1.14897.001, range: 2.5 – 250 mg/L Cl^-, http://www.merck-chemicals.com/chloride-test, 12 September 2011).

All samples were filtered through 8 µm filter paper (Whatman 40 Ashless Circles, 125 mm diameter, Cat no. 1440 125). Chloride samples were diluted 1:2 as the chloride concentration exceeded the maximum range of 250 mg/l. A diluted sample of 2.00 mL of diluted sample was added to a vial. A volume (2.50 mL) of Reagent 1 was then added to the vial followed by 0.50 mL of Reagent 2. The vials were capped and the mixtures left to stand for the reaction time of one min. Sample analyses were the same as described in Section 3.3.5.2.b (Table 3.3.10.a).

Table 3.3.10.a: The sampling procedure used to collect chloride concentration data in post-pilot plant anaerobic digester (AD), post-primary facultative pond (PFP), post-high rate algal pond (HRAP) A1, A2, B1, B2 and mixed post-HRAP effluent from 1 May 2009 until 16 January 2011.

Start date	End date	Sampling frequency	System components analysed	Phase
02-May-09	02-Aug-09	Two - three times/week	Post-AD, Post-PFP, Post-HRAP	Baseline, Ch. 4
03-Aug-09	24-Aug-09	No tests		Baseline, Ch. 4
25-Aug-09	22-Oct-09	Two times a week	Post-AD, Post-PFP, Post-HRAP	Baseline, Ch. 4
16-Nov-09	01-Mar-09	Two times/week	Post-AD, Post-PFP, Post-HRAP	Optimization, Ch. 5
08-Mar-10	16-Apr-10	Five times/week	Post-AD, Post-PFP, Post-A1, Post-A2, Post-B1, Post-B2	Optimization, Ch. 5
17-Apr-10	10-May-10	No tests		Optimization, Ch. 5
11-May-10	22-Jun-10	Two times/week	Post-AD, Post-PFP, Post-A1, Post-A2, Post-B1, Post-B2,	Optimization, Ch. 5
23-Jun-10	08-Jul-10	No tests		Optimization, Ch. 5
09-Jul-10	26-Jul-10	Three times/week	Post-AD, Post-PFP, Post-A1, Post-A2, Post-B1, Post-B2	Optimization, Ch. 5
28-Jul-10	01-Oct-10	Three times/week	Post-AD, Post-PFP, Post-A1, Post-A2, Post-B1, Post-B2	Optimization, Ch. 5
10-Nov-10	16-Jan-10	Three times/week	Post-AD, Post-PFP, Post-A1, Post-A2, Post-B1, Post-B2	Optimization, Ch. 5

3.4 Experimental design for the baseline phase

The baseline phase was a "proof of concept phase" to monitor the seasonal performance of the HRAPs. A conservative HRT was used to test whether HRAPs could treat brewery effluent and to evaluate whether the water quality of HRAP treated brewery effluent would meet the Department of Water Affairs and Forestry's (DWAF) general limits for discharge into a natural water resource of 1998 (Table 1, Appendix 1). Temperature, pH, EC, COD, NH_4-N, PO_4-P, NO_3-N, NO_2-N and Cl^-nutrient data were collected as described in Section 3.3. Nutrient concentration samples were collected and compared between post-pilot plant AD effluent, post-PFP and post-HRAP A1, A2, B1 and B2 mixed treated effluent.

The HRT was checked daily and was measured in days (d). The HRT refers to length of time that effluent remained in a pond. The HRT was determined by measuring the time it took to fill a one litre container at the inflow point into the PFP. A stopwatch was used to measure the number of seconds it took to fill up the one litre container. This resulted in a seconds (s)/litre (L) figure. This figure was converted to a HRT figure in days (d) by using the formula:

HRT = Volume of pond (m^3)/flow rate influent (m^3/day) (1)

The HRT was calculated from the volume that was treated per day. A timer switch located at the inflow point into the PFP determined the number of hours that the system was operated in continuous flow. The system operated continuously for eight hours during the baseline phase.

An example of how the HRT was calculated follows:

Flow rate (s/L) = 24 s/L into a HRAP. There were 36 000 seconds in a 10-hour day during autumn in April 2010, for example. It follows that that the flow rate in L/d was 1500 L/d. This figure was converted into a m^3/d figure to calculate the HRT in days (d). There are 1000 L in 1 m^3, so the flow rate in m^3/d was 1.5 m^3/d. The HRT was calculated by using formula (1) provided above. The volume of HRAP train-A was 5.46 m^3. The HRT in HRAP train-A was therefore 5.46 m^3/1.5 m^3/d = 3.64 d.

The HRTs that were used fluctuated between 17.29 d and 18.29 d in the PFP and between 11.16 d and 12.00 d in the HRAPs. The HRAP system received effluent for eight hours per day during the whole course of the baseline phase, after which the amount of hours were adjusted based on the amount of daylight hours the system was running for during the optimization phase.

3.5 Experimental design for the optimization phase

The objective of the optimization phase of the experiment was to manipulate the HRT to achieve the maximum treatment volume that met the DWAF general limits for discharge into a natural water resource of 1998 (Table 1, Appendix 1) by using nitrogen (NH_4-N, NO_3-N) removal efficiency as an indicator of nutrient removal success.

The optimal HRT was required to facilitate the planning of a commercial pilot plant, as it could provide an estimate of the total volume that could be treated per day and the subsequent area that would be required to treat a certain percentage of the brewery's effluent. The HRT in both HRAP train-A and train-B were adjusted during the course of the optimization phase, to determine alternately, the optimal HRT for NH_4-N and NO_3-N removal (Table 3.5.a).

Table 3.5.a: The hydraulic retention time (HRT) and daily running time (hours per day – h/d) in the primary facultative pond (PFP) and the two high rate algal ponds (HRAP) trains during the optimization phase.

Begin date	End date	H/d	HRT (d) PFP	HRT (d) HRAP train-A	HRT (d) HRAP train-B	Total HRT (d) (PFP + HRAP)
01-May-09	01-Mar-10	8.00	18.58	12.13	12.00	30.58
02-Mar-10	15-Mar-10	8.00	14.26	6.98	13.23	21.24
16-Mar-10	05-Apr-10	12.00	8.86	3.83	9.32	12.69
06-Apr-10	12-Apr-10	12.00	6.12	3.61	4.15	9.73
13-Apr-10	15-Apr-10	12.00	4.41	3.53	2.63	7.94
20-Apr-10	17-May-10	10.00	7.25	4.96	8.00	12.21
18-May-10	04-Jun-10	8.00	16.01	10.62	10.55	26.63
05-Jun-10	20-Jul-11	8.00	23.57	15.89	14.85	39.46
21-Jul-10	10-Aug-10	8.00	21.44	13.83	13.55	35.27
11-Aug-10	25-Aug-10	8.00	28.75	18.49	18.30	47.24
26-Aug-10	06-Sep-10	10.00	22.37	14.58	14.42	36.95
07-Sep-10	17-Sep-10	10.00	17.69	11.54	11.42	29.23
20-Sep-10	01-Oct-10	10.00	17.11	11.17	11.04	28.28
26-Nov-10	10-Dec-10	12.00	8.37	5.40	5.40	10.80
12-Dec-10	28-Dec-10	12.00	5.58	3.60	3.60	7.20
29-Dec-10	02-Jan-11	12.00	4.69	3.02	3.02	7.71
03-Jan-11	05-Jan-11	12.00	3.80	2.45	2.45	6.25
06-Jan-11	09-Jan-11	12.00	3.35	2.16	2.16	4.32
10-Jan-11	16-Jan-11	12.00	3.80	2.45	2.45	4.90

Initially, the HRT of HRAP trains-A and -B were adjusted independently to arrive at the optimal HRT for NH_4-N removal (2nd of March to the 5th of April 2010). A heating system was installed in HRAP train-A on the 6th of April 2010 (Section 3.1, Figure 3.1.k). The effect of heating in HRAP train-A was tested for the remainder of the study period. The HRT in both HRAP trains were set at similar rates from this point onwards. The HRT was adjusted to determine the optimal HRT for NH_4-N removal in

autumn (2nd of March to the 17th of May 2010) and summer (26th of November 2010 to the 16th of January 2011). Suboptimal system functioning was indicated by an NH_4-N concentration that exceeded the DWAF discharge limit, after which the HRT was adjusted to allow ammonia concentrations to decrease to within the allowed limit of 6.00 mg/L (Table1, Appendix 1). The HRT was adjusted to determine the optimal HRT for NO_3-N removal in winter and spring (18th of May to the 1st of October 2010). This determined whether a longer HRT could facilitate improved NO_3-N removal from the HRAPs.

3.6 Algal biomass concentration

Algal biomass concentration data was collected during the optimization phase (2 March 2010 – 16 January 2011), and not during the baseline phase. Four 250 mL samples were collected from the outlets of HRAPs A1, A2, B1 and B2 using a 250 mL measuring cylinder. Each algal culture sample was filtered through 8 µm filter paper (Whatman 40 Ashless Circles, 125 mm diameter, Cat no. 1440 125) with a water-driven vacuum flask that was connected to a tap. After filtration the algal biomass remained on the filter paper. Filter papers and algal biomass were placed on four petri-dishes inside an oven at 80 °C for 24 hours. Once dry, each filter paper with dried algal biomass was weighed on a 4-digit analytical balance, and the weights were recorded. The frequency at which these measurements were taken varied from two to 5.00 times weekly, except for October 2010, during which no productivity measurements were taken.

3.7 Algal productivity

Algal productivity was calculated during the optimization phase (2 March 2010 – 16 January 2011), and not during the baseline phase. An Excel spread sheet with a formula that calculated algal productivity was used to calculate algal productivity (Figure 3.7.a).

The weights of the filter paper and algae were inserted into an Excel spread sheet, and subtracted from the average weight of the filter paper. The average weight of the filter paper was determined by weighing 5.00 oven-dried filter papers on 5.00 different days and by taking the average. The average weight of the filter paper was 1.1 g. The formula used to calculate algal productivity in, for example, HRAP A1, follows:

Weight of algal biomass

Today's weight of sample A1 and filter paper = A (g)

Today's weight of filter paper = B (g)

Today's sample weight A1 = A – B = C (g)

Yesterday's weight of sample A1 and filter paper = D (g)

Yesterday's weight of filter paper = E (g)

Yesterday's sample weight A1 = D – E = F (g)

Dry weight leaving Pond A1 = L (kg)

Input Data:

Volume filtered: 0.25 L

Hourly flow rate: L/hr (varied)

Number of hours of flow per day: (varied between 8-12 h)

Pond volume: m^3 (A1 = 3.87 m^3, A2 = 1.72 m^3)

Calculated Data:

Today's sample weight = C (g)

Today's culture density = Sample weight (C)/Volume filtered (0.25 L) = G g/L (kg/m^3)

Today's pond inventory = Today's culture density (G)/Pond volume (Different for A1 and A2) = H kg

Yesterday's culture density = F/Volume filtered (0.25 L) = I g/L (kg/m^3)

Yesterday's pond inventory = Yesterday's culture density (I)/Pond volume (Different for A1 and A2) = J kg

Daily flow rate in last 24 hours = (L/h) / 100 x number of hours flow/day = K (m^3/d)

Dry weight leaving ponds/day = Average Today and Yesterday's pond inventory [(H+J)/2] x daily flow rate (K) = L kg

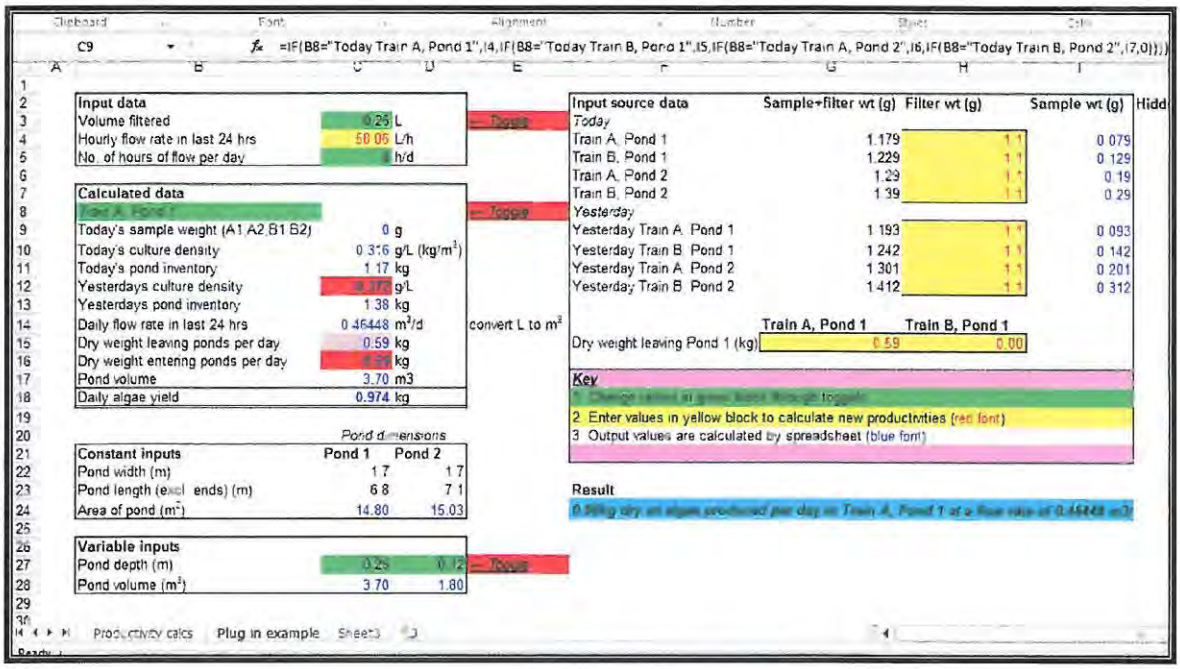

Figure 3.7.a: The Excel spread sheet that was designed by Mr. Richard Laubscher (Institute of Environmental Biotechnology, Rhodes University, Grahamstown) to calculate algal productivity.

3.8 Statistical analyses

All analyses of variance (ANOVA), scatterplots and multiple regression analyses were carried out using Statistica Version 10 and Microsoft Excel. A Pearson r correlation coefficient (r) of -1.00 represented a perfect negative correlation, while a value of +1.00 represented a perfect positive correlation. A value of 0.00 represented a lack of correlation. R^2 was the coefficient that represented the proportion of common variation in the two variables (i.e. the "strength" or "magnitude" of the relationship). An R^2 – value of 0.4 meant that the variability of the y-values explained 40 % of the original variability. The R^2 – value was an indicator of how well the model fitted the data. The p-value represented a decreasing index of reliability to determine the statistical significance of relations between variables. The confidence interval was set at 0.05 for all analyses. The regression line expressed the best prediction of the dependent variable (Y), given the independent variables (X). However, nature is rarely (if ever) perfectly predictable, and usually there was substantial variation of the observed points around the fitted regression line. Regression confidence intervals (the dotted line) were set at a 95% (Chapter 4, Chapter 5, Statistica Version 10 User Manual).

3.9 The effect of evaporation

The percentage increase in EC and normalised COD were calculated for the HRAPs to address the effect that evaporation had on these parameters. The effect of evaporation on the EC and COD (mg/L) was calculated using the formula:

$C1/V1 = C2/V2$ (2)

where:

C1 = concentration dissolved salts (EC) at the inflow point (post-PFP)

V1 = volume of sample (one litre)

C2 = concentration dissolved salts (EC) at the final outlets (HRAP A2 and B2)

V2 = original volume of sample (unknown)

An example follows:

The EC post-PFP is 3267.70 µS/cm in a one litre sample. The EC post-HRAP A2 is 3361.30 µS/cm in a one litre sample. If no evaporation occurred, the EC at the inlet should be the same as at the outlet. Next, the volume that is proportional to the increase in EC at the outlet is calculated using formula 2:

$V2 = (V1/C1) \times C2 = 0.97$

If effluent with an EC of 3362.30 µS/cm is left in HRAPs, then the amount of water lost in one litre through evaporation can be calculated as follows: 1 – 0.97 (V1) = 0.03 L. The normalised volume is the original sample volume with the amount of water lost through evaporation added (1.03 L). The percentage increase in EC can be calculated as follows: 100 – (3267.70/3361.30 x 100) = 2.78 %. The loss of water through evaporation will also have an effect on the interpretation of COD measurements, and this must be accounted for. As an example to illustrate this process, the COD in a one litre sample was given as 180.65 mg/L. The COD that was measured in a one litre sample has to be divided by the sum of the sample volume and the volume of water lost through evaporation (1 L + 0.03 L = 1.03 L; 180.65/1.03 = 175.38 mg/L). The same principle obviously applies to other nutrient concentrations as well, but nutrient concentration measured at the outlet points of HRAPs was so low, that the effects of evaporation would add very little meaning to the final results.

Chapter four
The baseline phase

4.1 Results

The objective of the baseline "proof of concept" phase of the experiment (May 2009 – March 2010) was to monitor the seasonal performance of the HRAPs at a HRT that fluctuated between 17.29 d and 18.29 d in the PFP and between 11.16 d and 12.00 d in the HRAPs, and to evaluate whether the water quality of the treated brewery would meet the Department of Water Affairs and Forestry's (DWAF) general limits for discharge into a natural water resource of 1998 (Table 1, Appendix 1) (Figure 4.1.a).

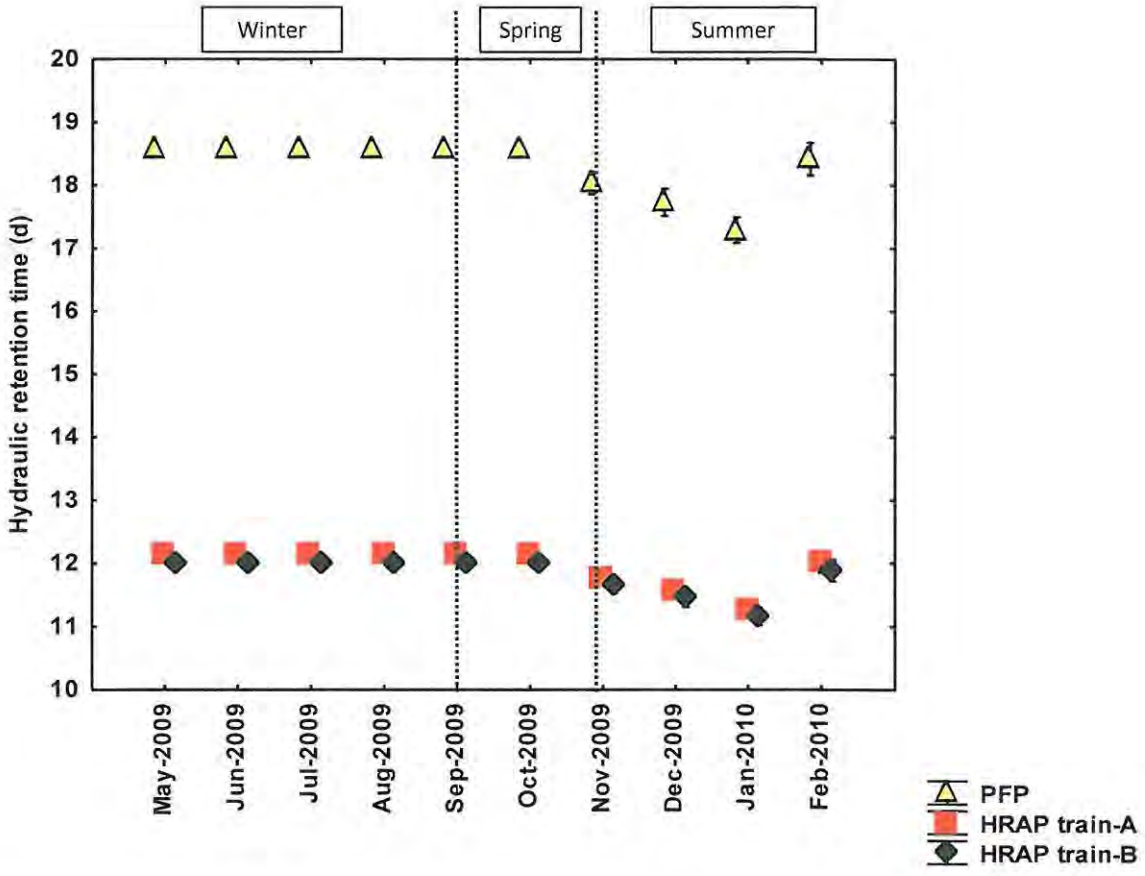

Figure 4.1.a: The mean (± standard error) hydraulic retention time (days) in the primary facultative pond (PFP), high rate algal pond (HRAP) train-A and HRAP train-B from May 2009 until the end of February 2010.

4.1.1 A summary of the results

The effluent temperature decreased as it moved through the pilot plant anaerobic digester (AD) into the PFP and the HRAPs (Section 4.1.2). The pH in the effluent increased as it moved through the pilot plant anaerobic digester (AD), the primary facultative pond (PFP) and finally through the high rate algal ponds (HRAP) (Section 4.1.3). The average chemical oxygen demand (COD) increased as effluent moved through the pilot plant AD, PFP and HRAPs (Section 4.1.4). The average ammonia (NH_4-N) concentration decreased from 46.59 ± 2.47 mg/L in post-pilot plant AD effluent, to 1.08 ± 0.12 mg/L NH_4-N in post-HRAP effluent (Section 4.1.5). The nitrite (NO_2-N) concentration in post-pilot plant AD, post-PFP and post-HRAP effluent remained below 1.00 mg/L NO_2-N. The mean phosphate (PO_4-P) concentration in post-pilot plant AD effluent decreased from 29.81 ± 1.39 mg/L, to 17.30 ± 1.16 mg/L post-HRAP (Section 4.1.6). The electrical conductivity (EC) increased as effluent moved through the AD, PFP and HRAPs (Section 4.1.7, Table 4.1.1.a).

Table 4.1.1.a: The performance characteristics of post-pilot plant anaerobic digester (AD) effluent, primary facultative pond (PFP) effluent and post-high rate algal pond (HRAP) treated effluent during the baseline phase of the experiment (1 May 2009 – 1 March 2010). The Department of Water Affairs and Forestry's (DWAF) general limits for discharge into a natural water resource of 1998 (Table 1, Appendix 1, DWAF limit-right hand column of this table) were used as a benchmark of nutrient removal success. Data are presented here as mean ± standard error, and N-values (number of samples).

Parameter	AD effluent			PFP effluent			HRAP effluent			DWAF limit
	Mean	Std. Err.	N	Mean	Std. Err.	N	Mean	Std. Err.	N	
Temperature (°C)	24.33	0.32	109	20.67	0.26	160	22.31	0.27	85	
pH	7.57	0.03	149	8.24	0.03	163	9.82	0.04	147	5.5 - 9.5
Chemical oxygen demand (mg/L)	130.12	6.94	42	163.46	8.66	54	171.21	7.99	54	75.00
Ammonia (mg/L)	46.59	2.47	66	29.72	1.15	73	1.08	0.12	72	6.00
Nitrite (mg/L)	0.01	0.00	19	0.03	0.01	9	0.01	0.00	24	15.00
Phosphate (mg/L)	29.81	1.39	61	24.87	1.07	71	17.30	1.16	69	10.00
Chloride (mg/L)	294.43	30.91	14	309.36	13.47	25	417.65	10.89	20	0.25
Electrical conductivity (µS/cm)	2924.61	30.94	152	2767.42	26.17	162	3488.02	42.65	147	700.00

4.1.2 Temperature

Effluent left the iBhayi brewery AD at 35 °C, and cooled down as it moved through the pilot plant AD, PFP and algal ponds. The mean ambient water temperature post-pilot plant AD was 24.33 ± 0.32 °C, which decreased to 20.67 ± 0.26 °C post-PFP, and to 18.79 ± 0.27 °C in HRAP train-A and 18.56 ± 0.26 °C in HRAP train-B (Table 4.1.2.a, Figure 4.1.2.a).

The ambient water temperature post-pilot plant AD, PFP and HRAPs fluctuated seasonally, with cooler temperatures observed in winter and warmer temperatures in summer. The temperature in the HRAPs varied between 14.25 °C and 17.40 °C in winter (May – August 2009), between 15.87 °C and 18.79 °C in spring (September – October 2009), and between 19.36 °C and 22.72 °C in summer (November 2009 – February 2010) (Figure 4.1.2.a).

Table 4.1.2.a: The temperature (°C) in post-pilot plant anaerobically digested (AD) effluent, primary facultative pond (PFP) effluent, high rate algal ponds (HRAP) train-A and train-B effluent and post-HRAP A1, A2, B1 & B2 mixed effluent from 1 May 2009 until 1 March 2010. Data are presented here as mean (± standard error), minimum, maximum and N-values.

System	Mean	Std.Err.	Min.	Max	N
Post-AD	24.33	0.32	15.70	32.10	109
PFP	20.67	0.26	13.00	28.50	160
HRAP train-A	18.79	0.27	10.95	27.05	162
HRAP train-B	18.56	0.26	10.75	27.05	162
Post-HRAP	22.31	0.27	18.10	29.70	85

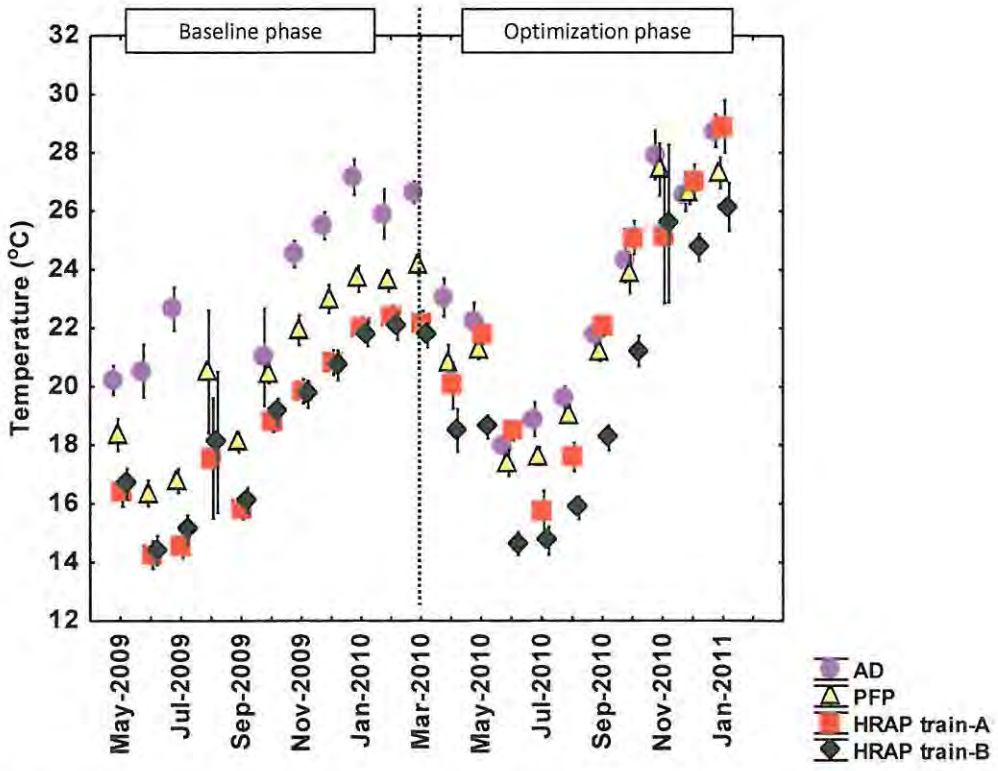

Figure 4.1.2.a: The mean (± standard error) monthly temperature (°C) in post-pilot plant anaerobically digested (AD) brewery effluent, primary facultative pond effluent (PFP), high rate algal pond (HRAP) train-A and HRAP train-B effluent from May 2009 until January 2011.

4.1.3 The pH

The pH of the system increased as brewery effluent moved through the pilot plant AD (7.57 ± 0.03) into the PFP (8.24 ± 0.03) and into the HRAPs (10.05 ± 0.03). The pH in the HRAPs decreased in winter (May – August 2009) and increased in summer. The pH decreased from 10.50 to 9.50 (May – August 2009, HRAP A2 and B2). It increased from 9.50 to 10.50 (August - December 2009, HRAP A2 and B2). The pH fluctuated between 10.00 and 10.50 (December 2009 - 1 March 2010). The pH was lower in the first ponds in series (HRAP A1 and B1) than in the second (HRAP A2 and B2) (Table 4.1.3.a, Figure 4.1.3.a).

Table 4.1.3.a: The pH in post-pilot plant anaerobically digested (AD) treated brewery effluent, in primary facultative pond (PFP) effluent, in high rate algal ponds (HRAP) A1, A2, B1 and B2 effluent, and in mixed HRAP effluent from 1 May 2009 until 1 March 2010. Data are presented here as mean (± standard error), minimum, maximum and N-values.

System	Mean	Std. Err.	Min.	Max.	N
AD	7.57	0.03	6.82	10.02	149
PFP	8.24	0.03	7.40	9.89	163
HRAP A1	9.38	0.03	8.05	10.20	160
HRAP A2	10.06	0.03	9.33	10.96	160
HRAP B1	9.43	0.02	8.68	10.02	160
HRAP B2	10.04	0.03	9.02	10.93	160
Post-HRAP	9.82	0.04	6.64	10.50	147

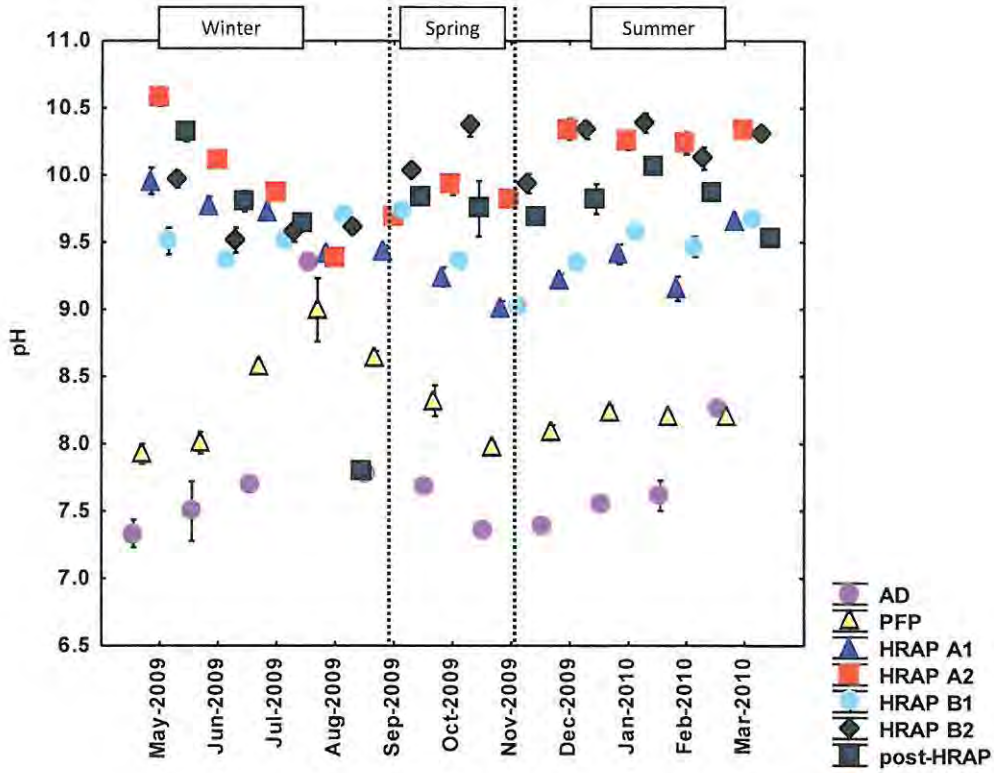

Figure 4.1.3.a: The mean (± standard error) monthly pH in post-pilot plant anaerobically digested (AD) effluent, primary facultative pond (PFP) effluent, high rate algal ponds (HRAP) A1, A2, B1, B2 effluent and mixed HRAP effluent from 1 May 2009 until 1 March 2010.

4.1.4 Chemical oxygen demand

The HRAPs were only effective at lowering the COD in July 2009. A decrease from 210.00 mg/L (post-pilot plant AD) to 114.45 mg/L (post-HRAP) was observed in July 2009 (45.50 % COD removal efficiency). For the rest of the baseline phase, the COD in post-HRAP effluent was higher than in post-pilot plant AD effluent. The COD in post-HRAP effluent was higher than in post-pilot plant AD effluent from August 2009 until January 2010. The COD in post-pilot plant AD treated effluent was 109.5 ± 9.43 mg/L, compared to 250.00 ± 26.73 mg/L in post-HRAP treated effluent (October 2009). COD post-HRAP decreased from October 2009 until March 2010 (Table 4.1.4.a, Figure 4.1.4.a).

Table 4.1.4.a: The chemical oxygen demand (COD) removal efficiency (%) in high rate algal ponds (HRAP) compared to post-pilot plant anaerobic digester (AD) effluent. Negative values in the right hand column indicate times when the COD in post-HRAP treated brewery effluent was higher than in post-pilot plant AD treated brewery effluent.

Month	Mean COD post-AD (mg/L)	Std. Err	N	Mean COD post-HRAP (mg/L)	Std. err.	N	% COD reduction post-HRAP compared to post-AD
Jun-09	100.33	19.01	3	97.50	4.37	5	2.82
Jul-09	210.00		1	114.45	11.66	4	45.50
Aug-09	176.67	30.99	3	199.60	32.33	5	-12.98
Sep-09	125.75	26.06	4	163.33	24.66	3	-29.89
Oct-09	109.50	9.43	6	250.00	26.73	5	-128.31
Nov-09	123.33	6.69	3	219.00	9.51	6	-77.57
Dec-09	156.38	7.50	8	208.80	21.46	5	-33.53
Jan-10	93.75	16.50	8	155.89	13.89	9	-66.28
Feb-10	143.00	20.04	5	136.17	13.07	6	4.78
Mar-10	178.00		1	164.00	13.01	6	7.87

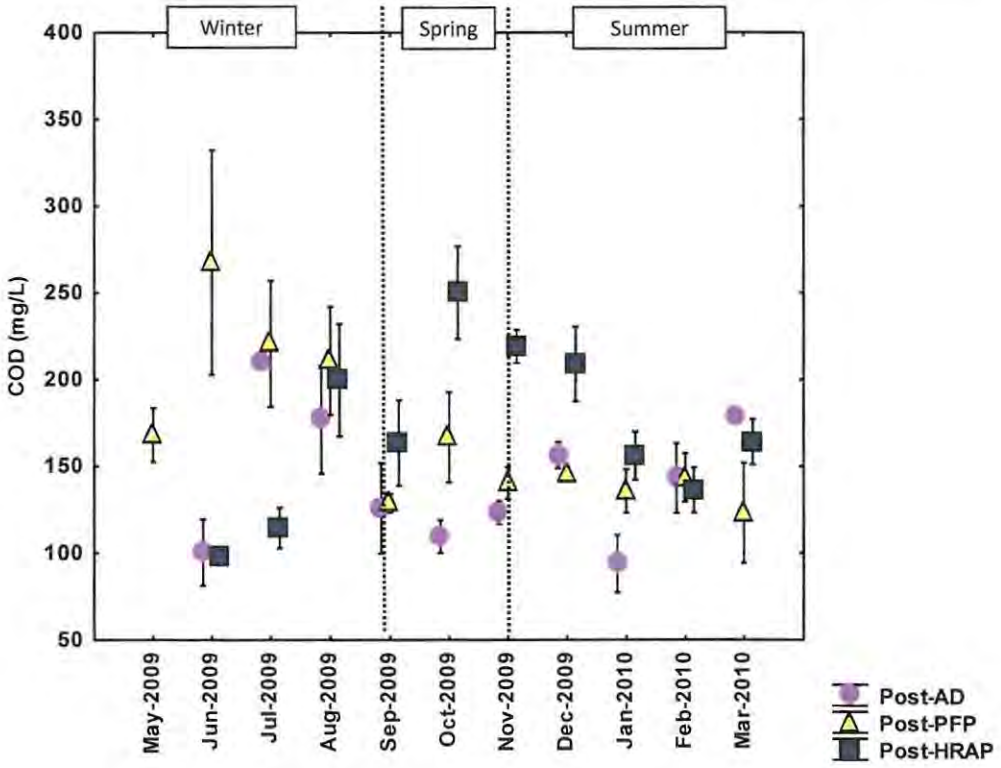

Figure 4.1.4.a: The mean (± standard error) monthly chemical oxygen demand (COD in post-pilot plant anaerobically digested (AD) brewery effluent, post-primary facultative pond (PFP) effluent and mixed post-high rate algal pond (HRAP) A1, A2, B1 & B2 effluent from 1 May 2009 until 30 March 2010.

4.1.5 Ammonia

The HRAP system was highly efficient at removing ammonia (NH_4-N) from post-pilot plant effluent. The mean NH_4-N concentration decreased from 44.04 ± 1.31 mg/L NH_4-N post-pilot plant AD, to below 2.00 mg/L NH_4-N at the outlets of the HRAPs (May 2009 - 1 March 2011). A mean percentage

of 36 % NH_4-N was removed in the PFP, whilst 97.68 % of the incoming NH_4-N concentration from the AD was removed in the HRAPs (Table 4.1.5.b, Figure 4.1.5.a).

Table 4.1.5.a: The ammonia concentration (mg/L) in post-pilot plant anaerobically digested (AD) brewery effluent, post-primary facultative pond (PFP) treated effluent and in mixed post-high rate algal pond (HRAP) A1, A2, B1 and B2 treated effluent from 1 May 2009 until 30 March 2010. Data are presented as mean ± standard error, minimum, maximum and N-values.

System	Mean (mg/L)	Std. err.	Min.	Max.	N
Post-AD	46.59	2.47	8.40	103.00	66
Post-PFP	29.72	1.15	10.90	76.00	73
Post-HRAP	1.08	0.12	0.00	5.00	72

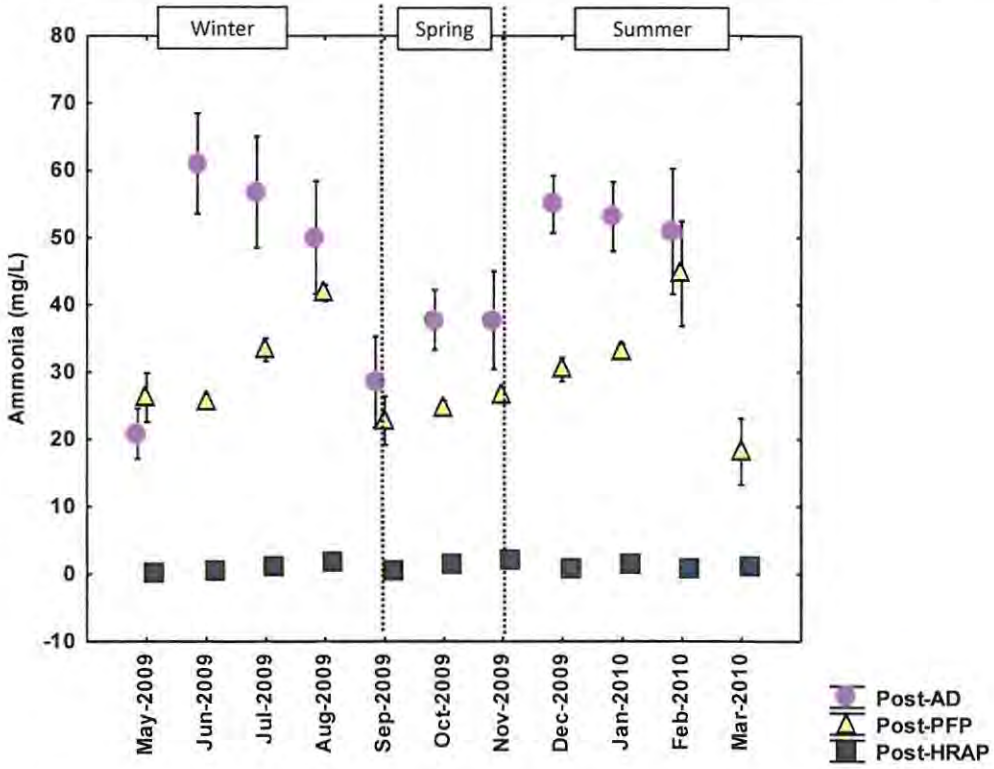

Figure 4.1.5.a: The mean (± standard error) monthly ammonia concentrations in post-pilot plant anaerobically digested (AD) brewery effluent, in post-primary facultative pond (PFP) treated effluent, and in mixed high rate algal pond (HRAP) A1, A2, B1 and B2 treated effluent from 1 May 2009 until 30 March 2010.

4.1.6 Phosphate

The HRAP system was inconsistent in its ability to remove phosphate (PO_4-P) from AD-treated effluent. The HRAPs removed PO_4-P from pilot plant AD-treated effluent from May to August 2009 (winter), although the PO_4-P removal efficiency in HRAPs steadily declined. PO_4-P removal efficiency decreased from 94.57 % (May 2009) to -22.97 % (September 2009). The PO_4-P concentrations post-pilot plant AD and post-HRAP were in a similar range from September until November 2009

(summer). PO_4-P removal efficiency increased from 6.23 % in November 2009, to 46.98 % in February 2010. The PO_4-P concentration post-HRAP was 19.32 ± 7.02 mg/L in March 2010 (Table 4.1.6.a, Figure 4.1.6.a).

Table 4.1.6.a: The phosphate (PO_4-P) removal efficiency (%) in high rate algal ponds (HRAP) compared to post-pilot plant anaerobic digester (AD) effluent. Negative values in the right hand column indicate times when the PO_4-P concentration in post-HRAP treated brewery effluent was higher than in post-pilot plant AD treated brewery effluent.

Month	Mean post-AD (mg/L)	Std. err	N	Mean post-HRAP (mg/L)	Std. err.	N	% Phosphate reduction post-HRAP
May-09	30.00		2	1.63		1	94.57
Jun-09	33.82	4.56	9	4.15	1.14	8	87.74
Jul-09	38.84	4.96	5	13.36	1.54	9	65.61
Aug-09	23.16	5.10	5	17.66	0.37	5	23.75
Sep-09	19.73	3.46	6	24.27	1.39	3	-22.97
Oct-09	26.60	2.86	9	21.39	3.81	10	19.59
Nov-09	29.97	2.29	3	28.10	1.21	6	6.23
Dec-09	35.94	1.08	8	24.04	1.14	5	33.11
Jan-10	27.65	1.44	8	15.84	0.65	9	42.70
Feb-10	31.28	7.33	6	16.59	1.51	7	46.98
Mar-10			0	19.32	7.02	6	

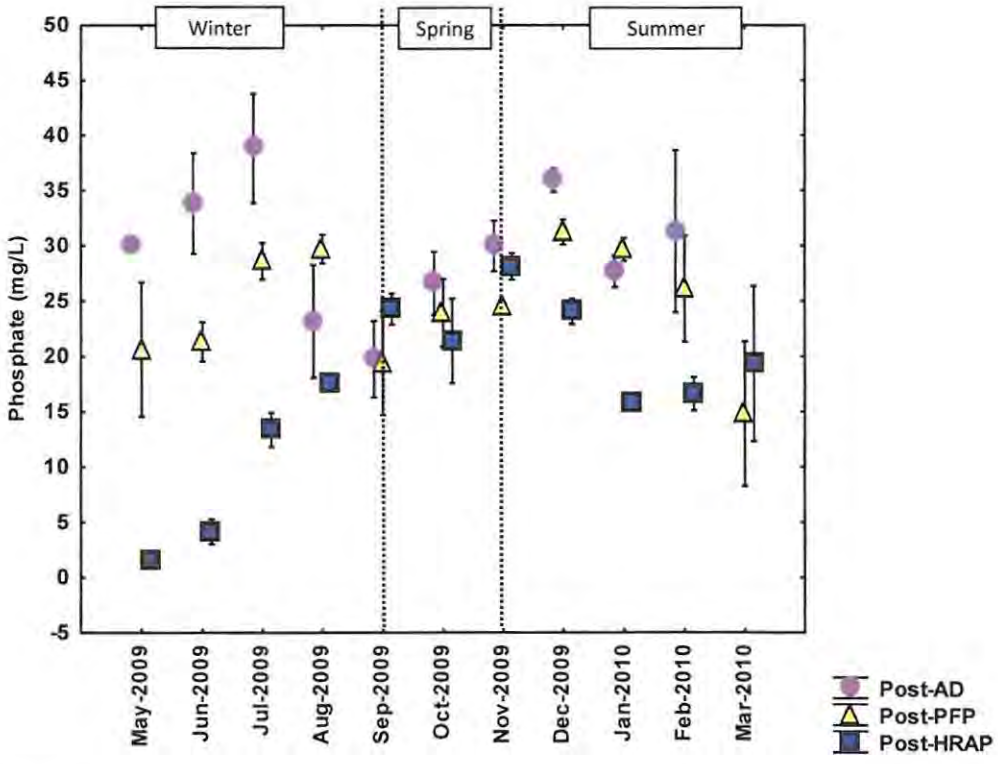

Figure 4.1.6.a: The mean (± standard error) monthly phosphate concentration in post-pilot plant anaerobically digested (AD) brewery effluent, in post-primary facultative pond (PFP) treated effluent, and in mixed post-high rate algal pond (HRAP) A1, A2, B1 & B2 treated brewery effluent from 1 May 2009 until 30 March 2010.

4.1.7 Electrical conductivity

Electrical conductivity (EC) followed a seasonal trend in all the system components. The EC increased from 2000 µS/cm, to 3200 µS/cm in PFP effluent (May 2009 - March 2010). The EC increased from 2200 µS/cm to 3800 µS/cm in HRAPs (May 2009 - November 2009), after which it declined to 3400 µS/cm (February 2010). The EC was higher in HRAP effluent compared to post-pilot plant AD effluent from September 2009 until March 2010 (Figure 4.1.7.a).

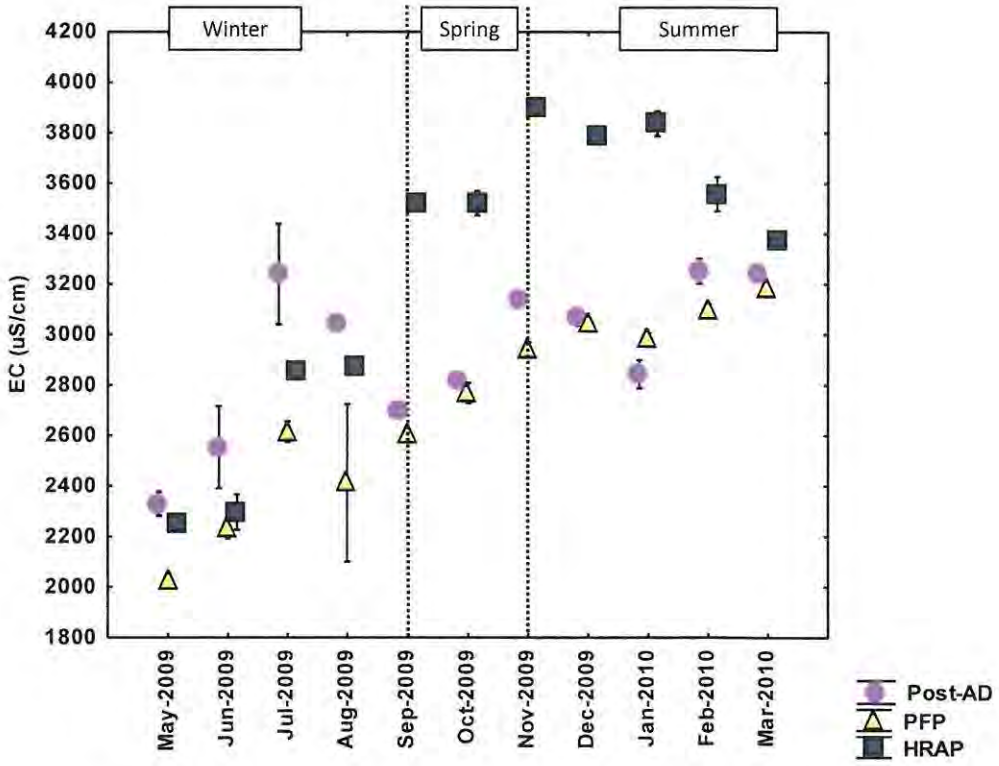

Figure 4.1.7.a: The mean (± standard error) monthly electrical conductivity (EC) in post-pilot plant anaerobically digested (AD) brewery effluent, primary facultative pond (PFP) treated effluent, and in mixed high rate algal pond (HRAP) A1, A2, B1 and B2 treated effluent from 1 May 2009 until 30 March 2010.

5.1.8 The effect of evaporation

The percentage increase in the salt concentration between post-pilot plant AD effluent and HRAP effluent ranged from 2.91 % (June 2009, winter) to 26.05 % (September 2009, spring). The medium was approximately 20 % more concentrated during the baseline phase than during the optimization phase. The effect of evaporation on the COD was more during the baseline phase than during the optimization phase. The seasonal effect of evaporation at a longer HRT was clearly visible during the baseline phase. The increase in EC was less in winter, increased in summer, and decreased in autumn. The maximum percentage increase in dissolved salt concentration was 24.55 % in HRAP effluent (November 2009, summer) when a HRT of 12 d was being used in the HRAPs (Table 4.1.8.a).

Table 4.1.8.a: The effect of evaporation on the electrical conductivity (EC) and chemical oxygen demand (COD) in high rate algal pond (HRAP) treated effluent (May 2009 – February 2010). The evaporation formula was used to calculate the values in Columns 4 - 9 (Chapter 3, Materials and Methods).

Month	EC PFP	EC HRAP	Volume V2	Volume lost per one L	Normalised volume (1 L + volume lost through evaporation)	% Increase in EC	COD post-HRAP (mg/L)	Normalised COD post-HRAP
May-09	2023.25	2248.33	0.90	0.10	1.10	10.01		
Jun-09	2229.44	2296.33	0.97	0.03	1.03	2.91	97.50	94.74
Jul-09	2615.31	2851.78	0.92	0.08	1.08	8.29	114.45	105.69
Aug-09	2412.50	2868.00	0.84	0.16	1.16	15.88	199.60	172.24
Sep-09	2605.14	3522.70	0.74	0.26	1.26	26.05	163.33	129.58
Oct-09	2768.58	3520.79	0.79	0.21	1.21	21.36	250.00	205.99
Nov-09	2944.19	3902.29	0.75	0.25	1.25	24.55	219.00	175.83
Dec-09	3042.71	3786.24	0.80	0.20	1.20	19.64	208.80	174.53
Jan-10	2979.20	3836.60	0.78	0.22	1.22	22.35	155.89	127.41
Feb-10	3092.15	3556.65	0.87	0.13	1.13	13.06	136.17	120.44
Mar-10	3178.00	3375.00	0.94	0.06	1.06	5.84	164.00	154.96

4.2 Discussion

The objective of the baseline "proof of concept" phase of the experiment (May 2009 – March 2010) was to monitor the seasonal performance of the HRAPs at a HRT that fluctuated between 17.29 d and 18.29 d in the PFP and between 11.16 d and 12.00 d in the HRAPs, and to evaluate whether the water quality of the treated brewery would meet the DWAF general limits for discharge into a natural water resource (Table 1, Appendix 1).

The baseline study was initiated in the winter of 2009 and was completed at the end of summer in 2010. The HRT that was used during the baseline phase was 30.58 d in the PFP and HRAPs. Gaigher *et al.* (1985) used a total HRT of 25 d whilst testing the treatment of brewery effluent in an integrated wastewater treatment plant in Bloemfontein that consisted of bacteria, algae, fish and a wetland. The HRT was comparatively long compared to Moutin *et al.* (1992) who used a HRT of 4 to 8 d to promote optimal algal growth in HRAPs, and Wells and Rose (2008) who used a HRT of 3 to 6 d in HRAPs. Garcia *et al.* (2000) recommended a HRT of 4 d in spring and summer and 10 d in autumn and winter. The reason for the relatively long HRT that was used was that the baseline phase was an exploratory phase of the study to test whether the HRAP system could treat brewery effluent, and so a conservative HRT was used. In retrospect a shorter HRT could have been used.

The COD post-HRAP was not maintained within the DWAF general limits for discharge into a natural water resource of 75 mg/L (Table 1, Appendix 1), as the mean COD post-HRAP was 171.21 ± 7.99 mg/L, compared to a mean of 130.12 ± 6.94 mg/L in post-pilot plant AD effluent. Although the algal biomass concentration and productivity during the baseline phase was not measured, one reason for the high COD was ascribed to the dense algal culture at the high HRT that was used. The increased COD post-HRAP compared to post-AD could have been due to the presence of small algal cells that were not removed when HRAP samples were filtered. Higher COD were found in unfiltered HRAP samples compared to filtered HRAP samples (Wells 2005). HRAP COD readings from algal settling pond samples were lower when the algal cells settled to the bottom and were therefore not included in samples. This illustrates the contribution of algae to COD readings. If the algal culture was thick during the baseline phase with the long HRT that was used, the contribution of algal cells to the COD measured might have been significant. Other factors that might have contributed to high COD post-HRAP included:

- the mineralization of organic carbon and its appearance as intermediary products similar to the way this occurs in anaerobic digestion (Angelidaki & Sanders 2004);
- natural algal release into the medium from cells that died or ruptured (Martinez *et al.* 2000); and

- evaporation that made the medium more concentrated (Borowitzka, 1999).

NH_4-N consistently decreased from 44.04 ± 1.31 mg/L NH_4-N in post-AD effluent, to below 2.00 mg/L NH_4-N in post-HRAP effluent. The HRAPs were successful at producing an effluent that met the DWAF general limits for discharge into a natural water resource for NH_4-N of 6.00 mg/L (Table 1, Appendix 1) during the baseline phase. NH_4-N could have been removed by two mechanisms: NH_4-N volatilization at high pH, and through the assimilation of NH_4-N into algal biomass (Knud-Hansen 1998, Dekker 2002). As neither NH_4- N volatilization nor algal uptake was specifically measured, both mechanisms need to be taken into account as possible means of NH_4- N removal in HRAPs.

Two points can be made here regarding NH_4-N volatilization: (1) NH_4-N volatilization could have been significant in NH_4-N removal from the effluent in HRAPs as the pH was high enough to facilitate NH_4-N volatilization; and (2) more NH_4-N volatilization would have occurred in the second ponds of each train as the pH was higher in HRAP A2 and B2. The pH decreased from 10.50 in May 2009, to 9.50 in August 2010 (HRAP A1, A2, B1 & B2). From September 2009 until March 2010 the pH fluctuated between 9.50 and 10.50 (HRAP A2 and B2), and between 9.00 and 9.50 (HRAP A1 and B1).The decant of algal cells from the first into the second ponds contributed to the accumulation of algal biomass, resulting in higher net photosynthesis in HRAP A2 and B2. At a pH of 7.00, nearly all NH_4-N will be present in its ionised form (NH_4-N), whereas at a pH of 11.50, the majority of NH_4-N will be present in its unionised gaseous form, NH_3 (Idelovitch *et al.* 1981). The amount of NH_4-N that left the HRAP system through NH_4-N volatilization could have decreased as the pH decreased in winter, even though most of the incoming NH_4-N from the pilot plant AD still decreased to below 2.00 mg/L in the HRAPs. The removal of NH_4- N through assimilation into algal biomass would have required a short HRT, as shorter HRT stimulates increased algal productivity (Park *et al.* 2011). Azov and Shelef (1982) described the influence of HRT on algal productivity. Algal production increases by increasing the dilution rate until maximum productivity is attained in the algal culture. At the point of wash-out the dilution rate exceeds maximal algal productivity. A wash-out occurs when more algae are being washed out of the pond than are being produced in-pond (Azov & Shelef 1982). As the HRT during the baseline phase was relatively high, algal productivity was probably below optimum, as the algal biomass concentration was dense, thus promoting self-shading. NH_4-N volatilization and algal assimilation are acknowledged here as two possible means of nitrogen removal. As algal productivity was not measured during the baseline phase, the exact proportions could not be determined.

The HRAPs' PO_4-P removal efficiency was variable with the long HRTs that were used. The mean PO_4-P concentration post-HRAP was 17.29 mg/L, and did therefore not meet the DWAF general limits for

discharge into a natural water resource for PO_4-P of 10 mg/L (Table 1, Appendix 1). As the pH was high enough, the main mechanism for PO_4-P removal might have been through the precipitation of PO_4-P if calcium, magnesium or iron was present in effluent (Dekker 2002). The fact that the PO_4-P removal efficiency decreased in winter and increased in summer might have suggested lower removal efficiency in winter due to lower algal productivity at cooler temperatures (Raven & Geider 1988). However, as the HRT was relatively lengthy, algal productivity was probably sub-optimal. As a result, PO_4-P removal through precipitation or adsorption might have been more prominent than anticipated (Knud-Hansen 1998). Luxury or surplus uptake of PO_4-P by algae is also a recognised mechanism (Powell et al 2008). The mechanisms of PO_4-P removal was not measured in this study, however, the effect of a PO_4-P replete algal population on low PO_4-P removal, is acknowledged. If the algal population in the HRAPs were light-limited rather than temperature regulated, a shorter HRT may have stimulated higher algal productivity and subsequently increased PO_4-Puptake (Craggs *et al.* 2011).

Dissolved salts were not removed in the HRAPs. The EC in the HRAPs increased from 2200 µS/cm (May 2009) to 3800 µS/cm in winter, and exceeded that in the AD and PFP from September 2009 until January 2010. The EC in the HRAPs became increasingly concentrated through evaporation at long HRT (characterised by a slow dilution rate) and high temperatures in summer (September 2009 - January 2010). The EC decreased when temperatures cooled down and less evaporation occurred (February – March 2010). Salinity removal was reported to be ineffective in HRAPs treating distillery effluent, and apart from salinity, a quality effluent could be produced that met the DWAF general limits for discharge into a natural water resource (Dekker 2002). Results from the baseline phase confirmed ineffective dissolved salt removal in HRAPs at long HRT.

In conclusion, nutrient removal efficiencies in the HRAPs met the DWAF general limits for discharge into a natural water resource (Table 1, Appendix 1) for NH_4-N, but failed to meet them for pH, COD, PO_4-P, and dissolved salts concentration. The COD in post-HRAP effluent was higher compared to post-AD effluent, perhaps due to the presence of algal cells and dissolved organic carbon in the medium, or through evaporation losses that concentrated the effluent. While the "proof-of-concept" baseline phase demonstrated that the system was efficient at removing NH_4-N, the seasonal effects of temperature and daylength on nutrient uptake efficiency and the effect of length of HRT on nutrient uptake efficiency were evident. The subsequent "optimization" phase was designed to test nutrient removal efficiencies at shorter HRTs at different times of the year.

Chapter five
The optimization phase

5.1 Results

The performance of the PFP/HRAP system was optimized for nitrogen removal over a 44-week period (1 March 2010 – 16 January 2011) under different hydraulic retention times (HRT). An NH_4-N concentration in excess of 6.00 mg/L was used as a proxy of treatment success that met the Department of Water Affairs and Forestry's (DWAF) general limits for discharge into a natural water resource of 1998 (Table 1, Appendix 1). The objective of the optimization phase of the experiment (2 March 2010 – 16 January 2011) was to manipulate the HRT to achieve the maximum treatment rate that met these standards by using nitrogen removal efficiency as an indicator of nutrient removal success.

The system HRT was varied to determine the optimal HRT for different times of the year (the maximum treatment volume per smallest unit area). The total nitrogen concentration was used as an indicator of nutrient removal efficiency, and the HRT was adjusted to maintain ammonia (NH_4-N) and nitrate (NO_3-N) concentrations to below the DWAF general limits for discharge into a natural water resource (Table 1, Appendix 1). These limits were 6.00 mg/L for NH_4-N, and 15.00 mg/L for NO_3-N (Table 1, Appendix 1). Nitrite (NO_2-N) and phosphate (PO_4-N) played no role in determining the HRT. The HRT was gradually shortened until an an NH_4-N concentration that exceeded 6.00 mg/L NH_4-N post-HRAP in autumn (March – April 2010) and summer (November 2010 – January 2011) was observed. The HRT was subsequently increased until the NH_4-N concentration returned to below 6.00 mg/L post-HRAP A2 and B2. The optimal HRT presented in this thesis is a slightly longer HRT than the HRT at which the NH_4-N concentration increased to above 6.00 mg/L(Figure 5.1.a, Table 5.1.a, Table 5.1.b).

This point in autumn occurred at a HRT of 3.53 d (HRAP train-A) and 2.63 d (HRAP train-B) (13 – 20 April 2010). A HRT of 3.61 d (HRAP train-A) and 4.15 d (HRAP train-B) was used in the week before NH_4-N concentrations that exceeded the DWAF limit of 6.00 mg/L were observed. The NH_4-N concentration remained below the acceptable limits during that week (6 – 12 April 2010). The HRTs that were implemented after NH_4-N concentration exceeded the DWAF limit, and at which it returned to acceptable limits, was 4.96 d (HRAP train-A) and 4.91 d (HRAP train-B) (20 – 27 April 2010). The optimal HRT in autumn therefore ranged between 3.61 d and 4.96 d (the minimum and

maximum HRT at which the NH_4-N concentration remained below 6.00 mg/L in autumn). The average of these two values was 4.30 d, which provides a useful benchmark for determining the optimal HRT in autumn (Figure 5.1.a, Table 5.1.a, Table 5.1.b).

The HRT at which NH_4-N concentration increased to above 6.00 mg/L in summer was 2.16 d (HRAP train-A and train-B). A HRT of 3.02 d was used in HRAP train-A and train-B in the week prior to the increase in NH_4-N concentration(29 December 2010 – 5 January 2011). The HRT that was implemented after the increase in NH_4-N concentration was observed which allowed it to return to below 6.00 mg/L, was 2.45 d in HRAP train-A and train-B. The optimal HRT for summer could therefore lie between 2.45 d and 3.02 d in summer. The average of these values was 2.74 d. This value could be a useful benchmark for determining the optimal HRT in summer (Figure 5.1.a, Table 5.1.a, Table 5.1.b).

The nitrate (NO_3-N) concentration was used as an additional indicator to adjust the HRT from June 2010 until October 2010. Concentrations that exceeded the DWAF general limits for discharge into a natural water resource of 15 mg/L for NO_3-N (Table 1, Appendix 1) were observed in the effluent emerging from the HRAP system, and hence contributed to the ability to effectively reduce the total nitrogen concentrations. The HRTs in HRAPs were increased to between 10 and 18 d in winter and spring to test whether it would lower NO_3-N concentrations in the emerging effluent (Figure 5.1.a, Table 5.1.a, Table 5.1.b).

The HRT was negatively correlated with temperature; shorter HRTs could be implemented in warmer ambient temperatures and vice versa (Figure 5.1.b). HRT was observed to influence algal productivity; longer HRTs were associated with reduced algal productivity (Section 5.1.4). As HRT influenced algal productivity, it subsequently influenced nutrient uptake efficiency. Results in this chapter are presented in the context of fluctuating HRTs and their effects on algal productivities and nutrient removal in treated brewery effluent.

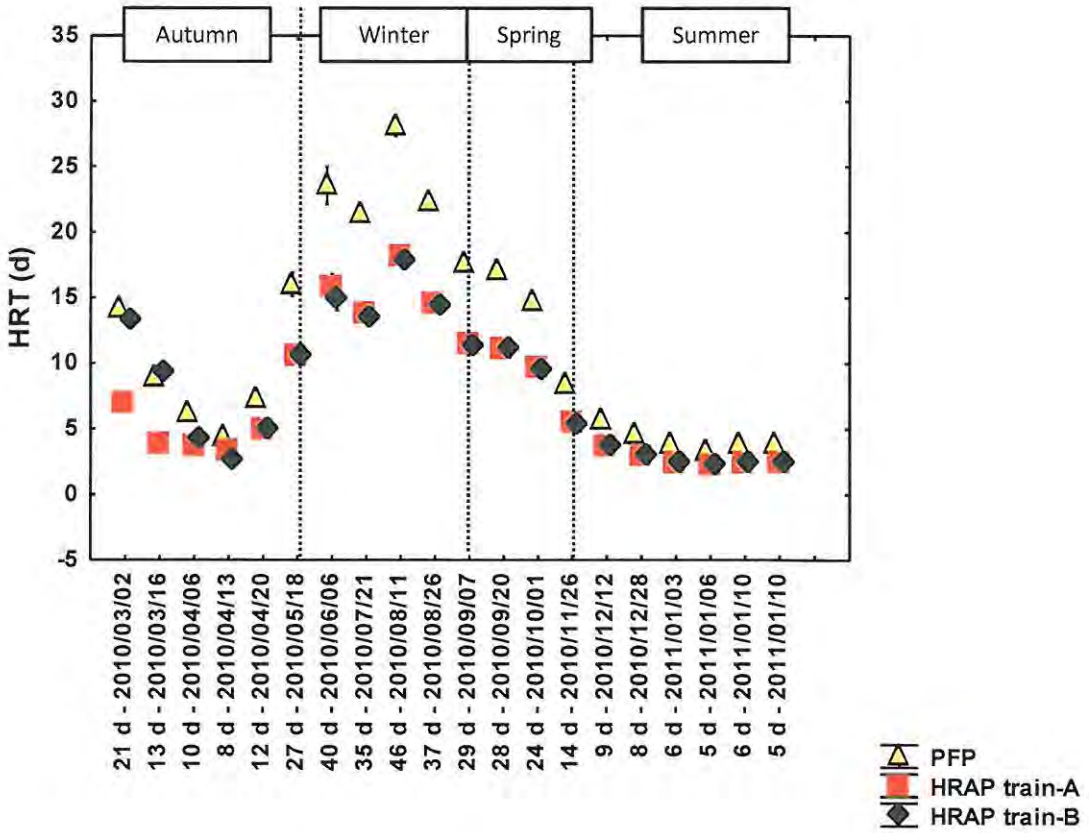

Figure 5.1.a: The hydraulic retention time (HRT) (mean ± standard error) in the primary facultative pond (PFP), and high rate algal pond (HRAP) train-A and train-B from 2 March 2010 until 16 January 2011. The length of the combined hydraulic retention time (HRT) in the PFP and HRAPs appears below the dates on the x-axis in days (d). Each date indicates the start of a new HRT.

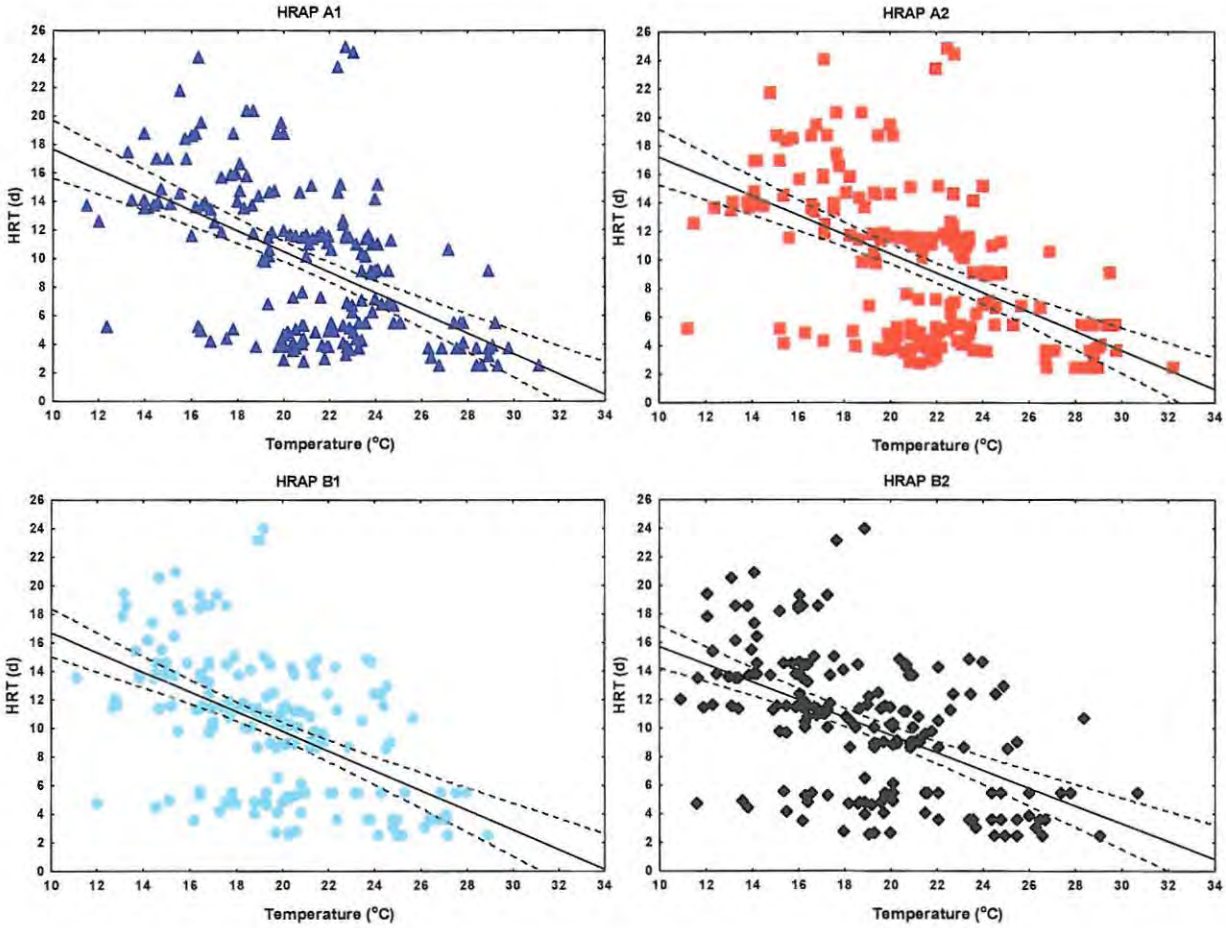

Figure 5.1.b: A multiple linear regression analysis of the relationship between the hydraulic retention time (HRT) and temperature in high rate algal pond (HRAP) A1, A2, B1 and B2. HRAP A1: r = -0.53, R^2 = 0.28, p < 0.0001 and F = 67.97. HRAP A2: r = -0.52, R^2 = 0.27, p < 0.0001 and F = 66.27. HRAP B1: r = -0.52, R^2 = 0.27, p < 0.0001 and F = 66.78. HRAP B2: r = -0.51, R^2 = 0.26, p < 0.0001 and F = 63.96.

Table 5.1.a: The response in ammonia (NH_4-N) and nitrate (NO_3-N) concentrations in high rate algal pond (HRAP) train-A and train-B to adjustments in the hydraulic retention time (HRT) which was measured in days (d). The number of hours that ponds were fed per day (h/d) fluctuated seasonally (2 March - 18 May 2010).

Date	NH_4-N concentration		NO_3-N concentration		Hydraulic retention time (d)			h/d	HRT action
	HRAP A2	HRAP B2	HRAP A2	HRAP B2	PFP	HRAP train-A	HRAP train-B		
01/03/2010	0.00	0.00	0.00	0.00					
02/03/2010					14.26	6.98	13.23	8.00	**Shortened** HRT in PFP. HRAP train-A received double the effluent volume effluent of HRAP train-B.
15/03/2010	0.00	0.18							
16/03/2010					8.86	3.83	9.32	12.00	**Shortened** HRT in HRAP train-A based on NH_4-N concentration < 6 mg/L, HRAP train-A received double the effluent volume of HRAP train-B.
05/04/2010	0.04	0.02	8.40	3.10					
06/04/2010					6.12	3.61	4.15	12.00	**Shortened** HRT based on NH_4-N concentration < 6 mg/L. HRAP train-A and B identical HRT.
12/04/2010	0.23	0.23	7.70	11.80					
13/04/2010					4.41	3.53	2.63	12.00	**Shortened** HRT based on NH_4-N concentration < 6 mg/L. **Wash-out.**
15/04/2010	9.30	12.90	15.50	5.70					
20/04/2010					7.25	4.96	4.91	10.00	**Increased** HRT based on NH_4-N concentration > 6 mg/L and shorter daylength.
17/05/2010	5.20	6.80	19.90	19.70					
18/05/2010					16.01	10.62	10.55	8.00	**Increased** HRT based on NH_4-N concentration > 6 mg/L and shorter daylength.
04/06/2010	0.50	0.50	24.60	26.50					
05/06/2010					23.57	15.89	14.85	8.00	**Increased** HRT based on NO_3-N concentration > 15 mg/L.
15/06/2010	0.00	0.00	23.70	18.70					
21/07/2010					21.44	13.83	13.55	8.00	**Shortened** HRT based on NO_3-N concentration > 15 mg/L.
10/08/2010	0.09	0.21	8.90	17.50					
11/08/2010					28.75	18.49	18.30	8.00	**Increased** HRT based on NO_3-N concentration > 15 mg/L.
25/08/2010	0.00	0.00	14.00	8.00					

Table 5.1.b: The response in ammonia (NH_4-N) and nitrate (NO_3-N) concentrations in high rate algal pond (HRAP) train-A and train-B to adjustments in the hydraulic retention time (HRT) which was measured in days (d). The amount of hours that ponds were fed with per day (h/d) fluctuated seasonally (7 September 2010 – 16 January 2010).

Date	NH_4-N concentration (mg/L)		NO_3-N concentration (mg/L)		HRT (d)			h/d	HRT action
	HRAP A2	HRAP B2	HRAP A2	HRAP B2	PFP	HRAP train-A	HRAP train-B		
					17.69	11.54	11.42	10.00	**Shortened** HRT based on NO_3-N concentration.
07/09/2010									
17/09/2010	0.09	0.11	14.70	13.80					
					17.11	11.17	11.04	10.00	**Shortened** HRT based on NO_3-N concentration
20/09/2010									
01/10/2010	0.20	0.32	16.60	22.80					
					8.37	5.40	5.40	12.00	**Re-inoculated** and started with **half the HRT** of that in October.
26/11/2010									
10/12/2010	0.08	0.08	5.20	6.30					
					5.58	3.60	3.60	12.00	**Shortened** HRT based on NH_4-N concentration < 6 mg/L.
12/12/2010									
	0.39	0.21	14.80	9.60					
					4.69	3.02	3.02	12.00	**Shortened** HRT based on NH_4-N concentration.
29/12/2010									
03/12/2010	0.08	0.26	18.50	11.20					
					3.80	2.45	2.45	12.00	**Shortened** HRT based on NO_3-N > 15 mg/L.
03/01/2011									
06/01/2011	0.09	0.25	22.00	15.40					
					3.35	2.16	2.16	12.00	**Shortened** HRT based on NH_4-N concentration < 6 mg/L. **Wash-out.**
06/01/2010									
07/01/2010	10.10	0.65	10.80	11.20					
					3.80	2.45	2.45	12.00	**Increased** HRT based on NH_4-N concentration > 6 mg/L.
10/01/2011									
16/01/2011	0.34	1.12	7.80	14.40					

5.1.1 A summary of the results

As the effluent moved through the pilot plant AD, the PFP and HRAPs (Section 5.1.2), the temperature decreased and pH and dissolved oxygen (DO) increased (Sections 5.1.6 and 51.7). The chemical oxygen demand (COD) of the HRAPs effluent was lower than that in post-pilot plant AD effluent (Section 5.1.8). The HRAPs produced an effluent in which the NH_4-N concentration was below 2.00 mg/L post-HRAP, except when the HRT was shortened to a point when the system lost its ability to successfully lower NH_4-N concentrations (Section 5.1.9). The NO_2-N concentration in post-HRAP effluent remained below 10 mg/L for the entire duration of the optimization phase (Section 5.1.10). The NO_3-N concentration increased from less than 5.005.00 mg/L in post pilot plant AD effluent, to between 10 and 25 mg/L in post-HRAP treated effluent (Section 5.1.11). The PO_4-P concentration was lowered from 35 mg/L in post-pilot plant AD effluent to less than 15 mg/L in post-HRAP treated effluent, when PO_4-P could be detected. PO_4-P was undetectable for the first four months of the optimization phase (Section 5.1.12) (Table 5.1.1.a).

5.1.2 Temperature

Effluent temperatures fluctuated seasonally and cooled as it moved through the integrated system (Figure 5.1.2.a). Raw brewery effluent temperature was averagely 35 °C. This temperature decreased to 21.10 ± 0.30 °C in HRAP train-A (heated) and to 19.18 ± 0.29 °C in HRAP train-B (ambient) (Table 5.1.2.a, Figure 5.1.2.a on page 107).

The heating system in HRAP train-A was successful at raising the ambient water temperature by an average of 2.14 ± 1.38 °C compared to HRAP train-B (April 2010 to January 2011). It was least effective at raising the temperature during July and August 2010, the coldest times of the year. The maximum temperature elevation that was achieved was 3.66 °C in October 2010 (spring). The heating system was not operational during November 2010 when the ponds were being cleaned. It resumed operations from December 2010 (Figure 5.1.2.b, Figure 5.1.2.c on page 108). The temperature elevation in HRAP train-A did not appear to have a significant effect on algal nutrient uptake efficiency, as similar nutrient uptake efficiencies were recorded in heated and unheated algal ponds.

Table 5.1.2.a: Seasonal temperature ranges in the pilot plant anaerobic digester (AD), primary facultative pond (PFP) and high rate algal pond (HRAP) train-B under ambient conditions. Data are presented as minimum and maximum temperatures for the different seasons.

Season	Temperature (°C) min/max		
	AD	PFP	HRAP train-B
Autumn 2009	20	18	16
Winter 2009	20 -23	16 - 20	14 - 18
Spring 2009	21	18 - 22	16 - 20
Summer 2010	24 - 27	22 - 24	20 - 22
Autumn 2010	22 - 23	21 - 24	19 - 22
Winter 2010	18 - 22	19 - 23	14 - 18
Spring 2010	20 - 22	21 - 27	18 - 26
Summer 2011	24 - 29	26 - 28	25 - 26

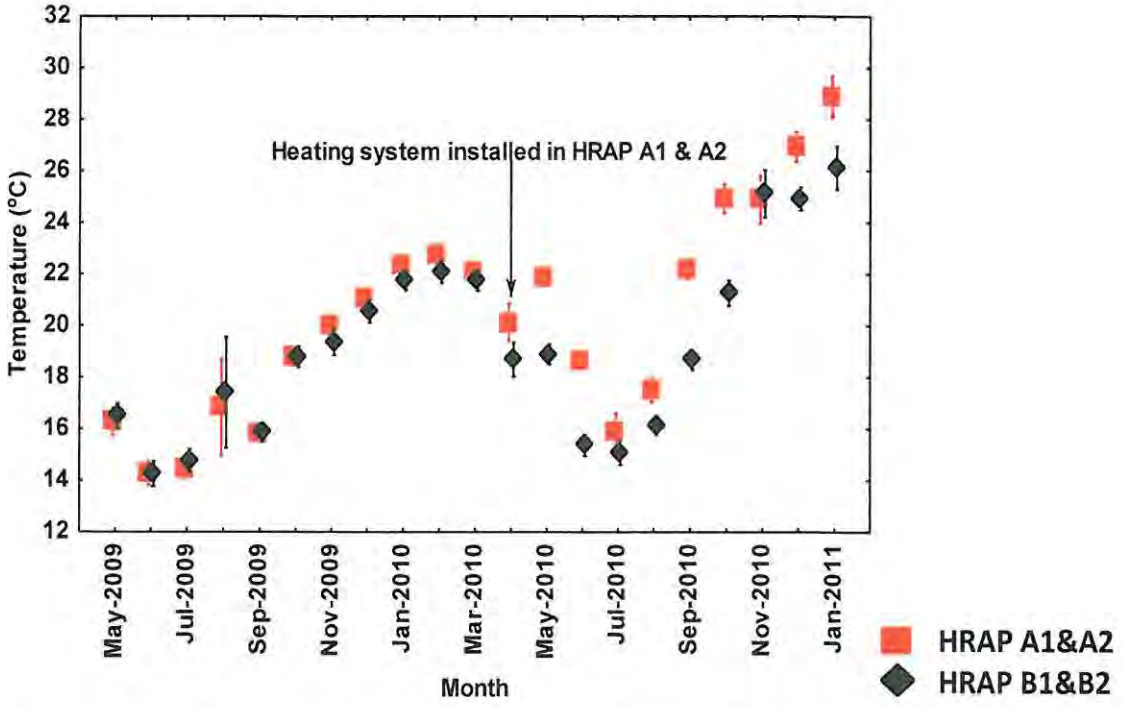

Figure 5.1.2.b: The mean (± standard error) monthly temperature in high rate algal ponds (HRAP) A1 & A2 and HRAP B1 & B2 from May 2009 until January 2011 to illustrate the effect of heating.

Table 5.1.1.a: A performance characteristics summary of the pilot plant anaerobic digester (AD) effluent, primary facultative pond (PFP) effluent, and high rate algal pond (HRAP) A2 and B2 effluent during the optimization phase of the experiment (2 March 2010 – 16 January 2011). The Department of Water Affairs and Forestry's (DWAF) general limits for discharge into a natural water resource of 1998 (Table 1, Appendix 1, DWAF limit-right hand column of this table) were used as a benchmark to evaluate the HRAPs' nutrient removal success. Data are presented as mean ± standard error, and N-values (number of samples).

Parameter	AD effluent			PFP effluent			HRAP A2 effluent			HRAP B2 effluent			DWAF limit
	Mean	Std. Err.	N	Mean	Std. Err.	N	Mean	Std. Err.	N	Mean	Std. Err.	N	
Temperature (°C)	22.69	0.29	193	21.71	0.27	191	21.07	0.31	185	18.78	0.30	185	
pH	7.82	0.03	188	8.25	0.02	189	9.68	0.04	182	9.84	0.04	182	5.5 - 9.5
Dissolved oxygen (mg/L)	2.30	0.16	152	1.53	0.15	150	10.26	0.19	145	11.88	0.82	144	
COD (mg/L)	153.21	4.79	89	135.74	4.11	89	95.00	3.75	82	100.82	5.93	82	75.00
Ammonia (mg/L)	42.53	1.38	100	39.49	0.88	106	1.77	0.79	104	1.70	0.83	104	6.00
Nitrite (mg/L)	0.07	0.01	85	0.08	0.01	86	1.72	0.39	77	2.18	0.43	77	15.00
Nitrate (mg/L)	1.97	0.19	71	1.82	0.17	72	13.82	0.77	70	12.37	0.76	69	15.00
Phosphate (mg/L)	12.49	1.44	86	9.50	1.12	93	5.23	0.68	90	5.53	0.86	89	10.00
Chloride (mg/L)	482.25	14.30	86	486.62	12.78	86	546.53	15.77	80	542.97	15.38	79	0.25 (free chlorine)
Electrical conductivity (µS/cm)	2761.85	26.32	189	2761.50	19.87	187	2867.51	24.53	181	2869.92	35.64	181	700.00
Algal productivity (kg/d)							1.19	0.12	161	1.23	0.11	161	

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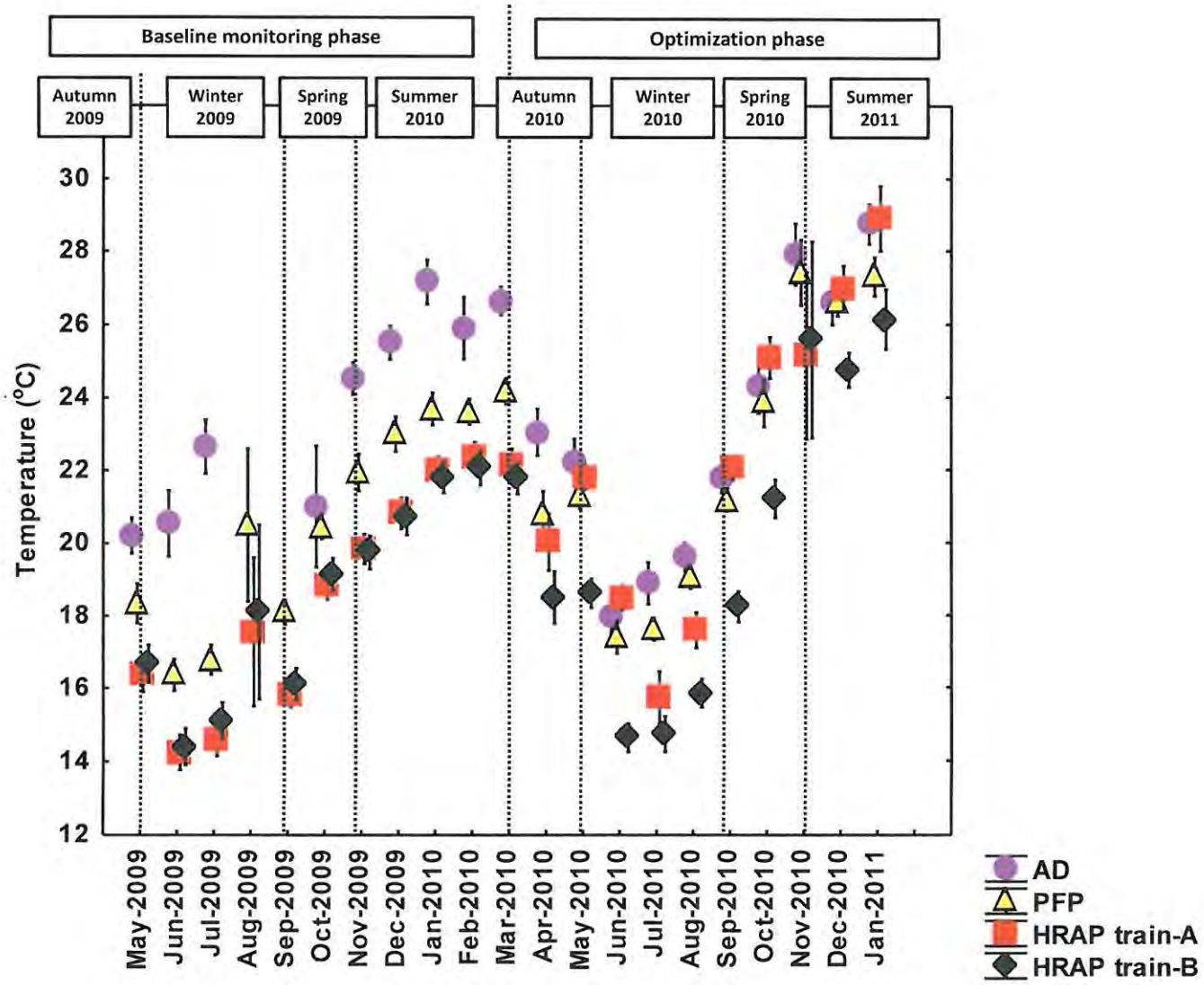

Figure 5.1.2.a: The mean (± standard error) seasonal temperature fluctuations in the pilot plant anaerobic digester (AD) treated brewery effluent, primary facultative pond (PFP) treated effluent, and high rate algal pond (HRAP) train-A (heated) and train-B (ambient) treated effluent from May 2009 until January 2011.

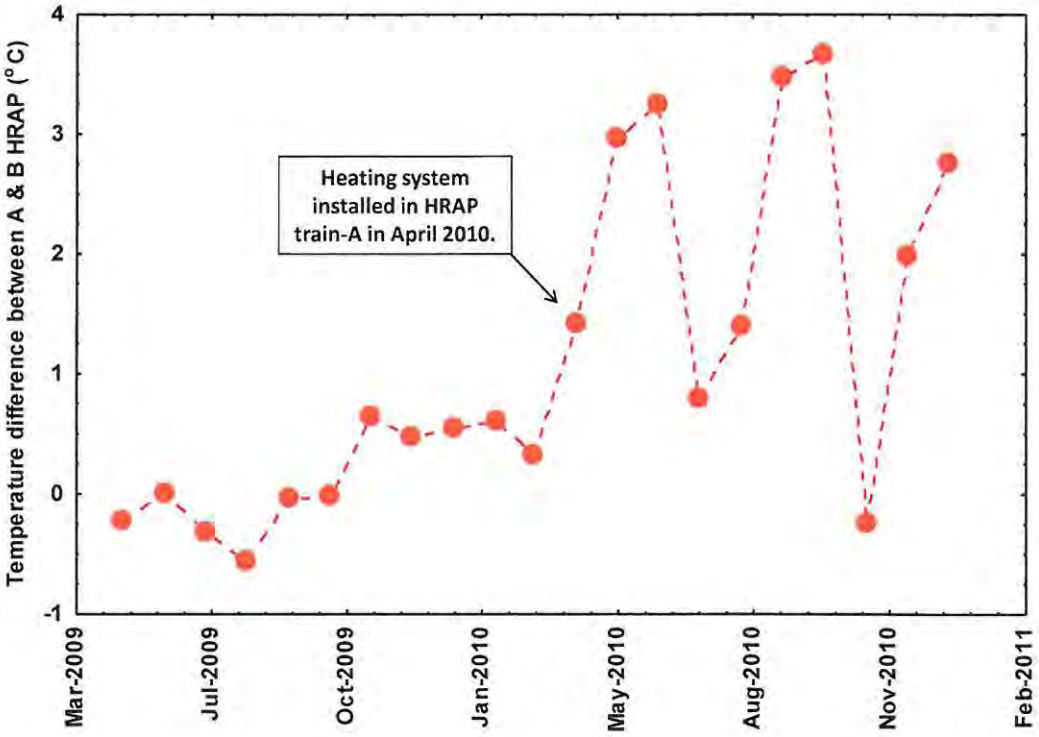

Figure 5.1.2.c: The difference in average monthly temperature (°C) between high rate algal pond (HRAP) train-A (heated) and HRAP train-B (ambient) from May 2009 to January 2011.

5.1.3 The algal biomass concentration

The mean algal biomass concentration in the HRAPs ranged between 0.0 g/L and 1.0 g/L throughout the optimization phase. The algal biomass concentration decreased from 0.5 to 0.3 g/L post-HRAP A2, and from 0.9 g/L to 0.3 g/L post-HRAP B2 in autumn, when the HRT was shortened (March – April 2010). The algal biomass concentration increased from 0.2 g/L (mid-May 2010) to 0.6 g/L (end-August 2010) in both HRAP trains in winter when the HRT was lengthened. The algal biomass concentration remained constant in both HRAP trains at approximately 0.6 g/L during spring (September – October 2010). A fresh algal culture was inoculated into the HRAPs on the 16th of November 2010. The algal biomass concentration of 1.6 g/L post-HRAP A2 and B2 in November 2010 was the highest recorded in the experiment. It decreased when the HRT was shortened and the dilution rate was increased. The algal biomass concentration was generally higher in HRAP A2 and B2 during winter and spring when longer HRTs were utilised (Figure1.3.a).

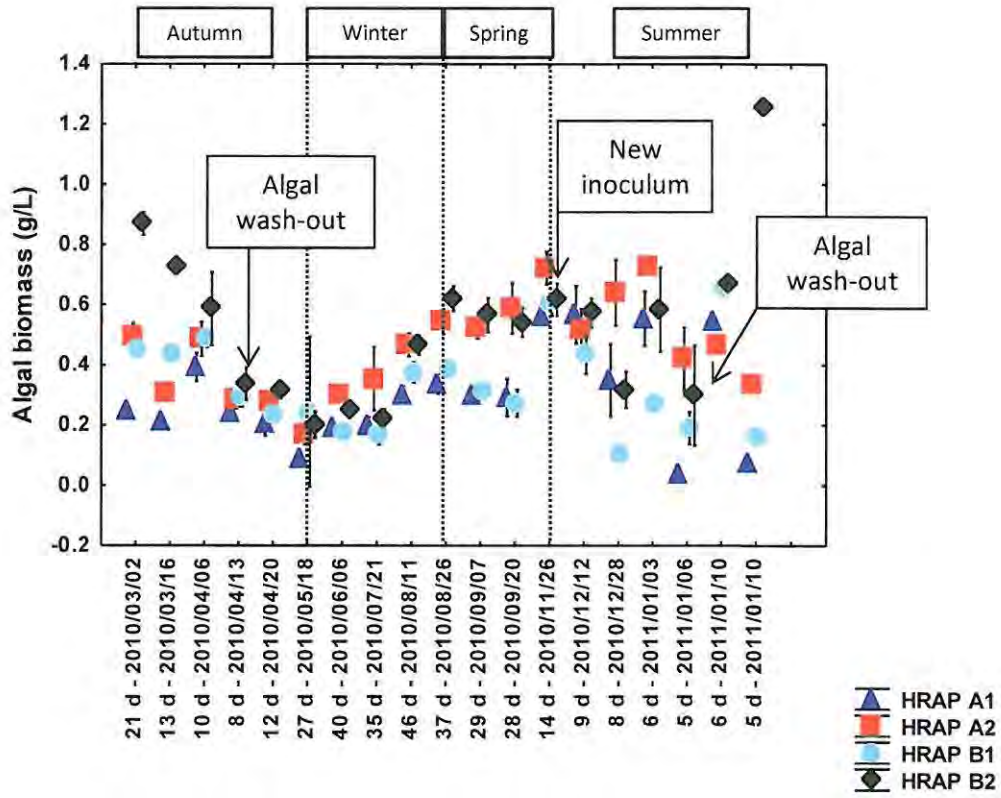

Figure 5.1.3.a: The mean (± standard error) algal biomass concentration (g/L) in post-high rate algal pond (HRAP) A1, A2, B1 & B2 effluent from 2 March 2010 until 16 January 2011. The length of the combined hydraulic retention time (HRT) in the primary facultative pond (PFP) and HRAPs appears below the dates on the x-axis in days (d). Each date indicates the start of a new HRT.

5.1.4 Algal productivity

Algal productivity was lower during winter and spring when a longer HRT was used and the algal culture was denser. Algal productivity increased in autumn (April 2010) and summer (November 2010 – January 2011) when shorter HRTs were used. Exceptionally high productivity was observed in summer (November 2010 – January 2011), after a newly grown algal inoculum had been inoculated into the HRAPs (Figure 5.1.4.a).

Algal productivity was positively correlated with warmer water temperatures (Figure 5.1.4.b) and negatively correlated with shorter HRTs (Figure 5.1.4.c) in all four algal ponds.

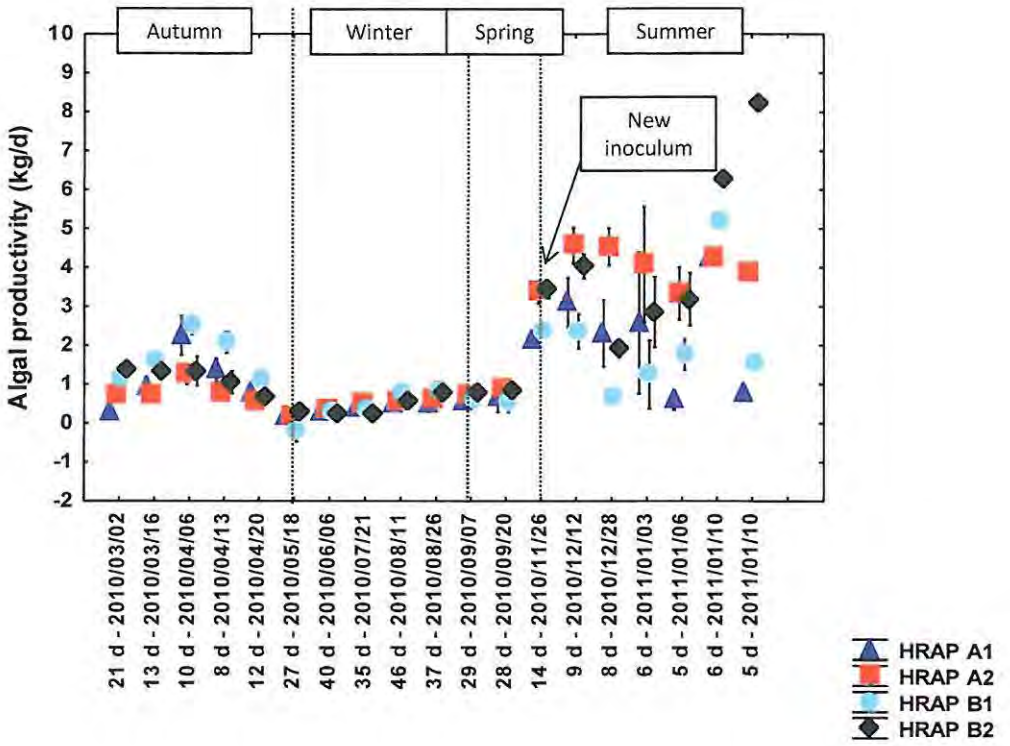

Figure 5.1.4.a: The mean (± standard error) algal productivity (kg/d) in high rate algal pond (HRAP) A1, A2, B1 & B2 from 2 March 2010 until 16 January 2011. The length of the combined hydraulic retention time (HRT) in the primary facultative pond (PFP) and HRAPs appears below the dates on the x-axis in days (d). Each date indicates the start of a new HRT.

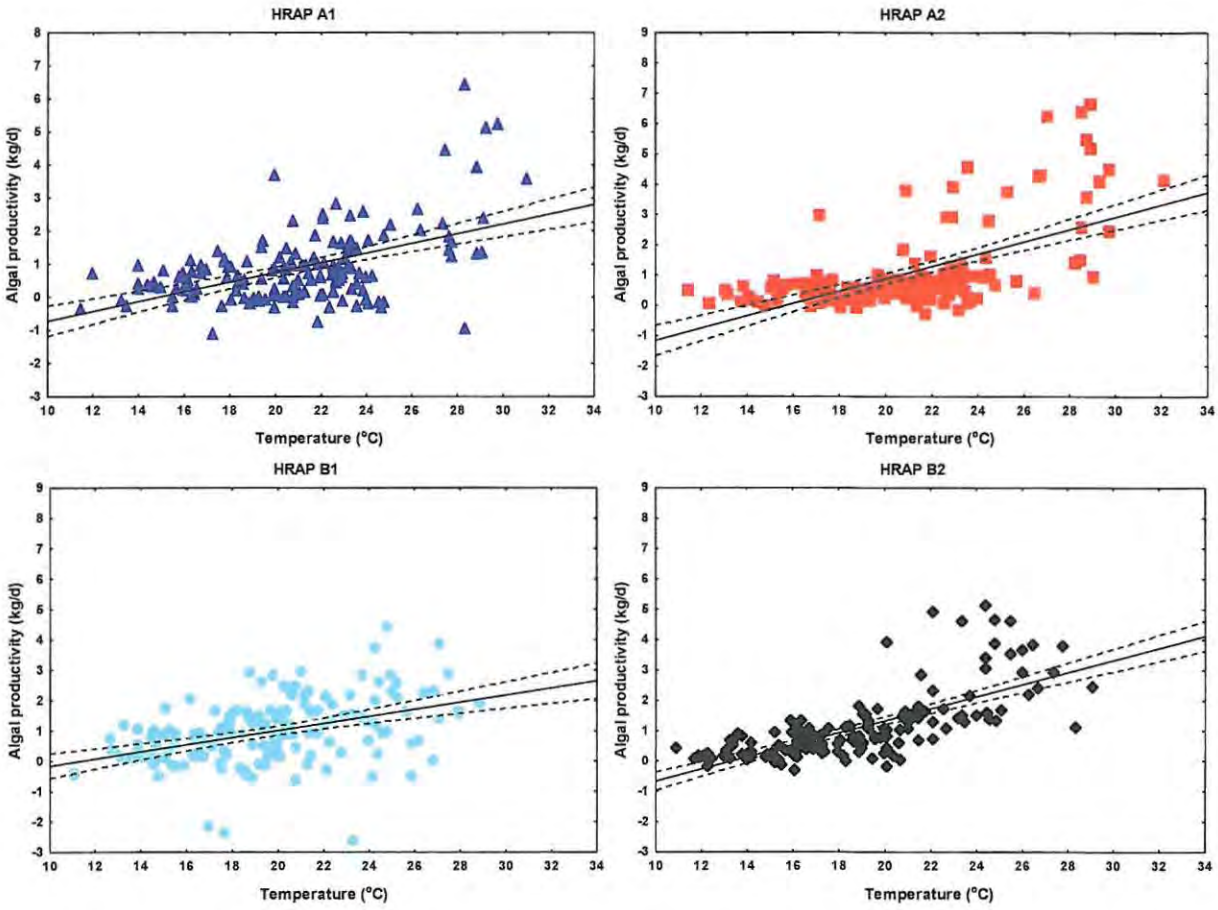

Figure 5.1.4.b: A multiple linear regression analysis of the relationship between algal productivity and water temperature in high rate algal pond (HRAP) A1, A2, B1 and B2. HRAP A1: $r = 0.51$, $R^2 = 0.21$, $p < 0.0001$, $F = 40.15$. HRAP A2: $r = 0.61$, $R^2 = 0.37$, $p < 0.0001$ and $F = 88.06$. HRAP B1: $r = 0.42$, $R^2 = 0.18$, $p < 0.0001$ and $F = 34.26$. HRAP B2: $r = 0.72$, $R^2 = 0.51$, $p < 0.0001$ and $F = 156.94$.

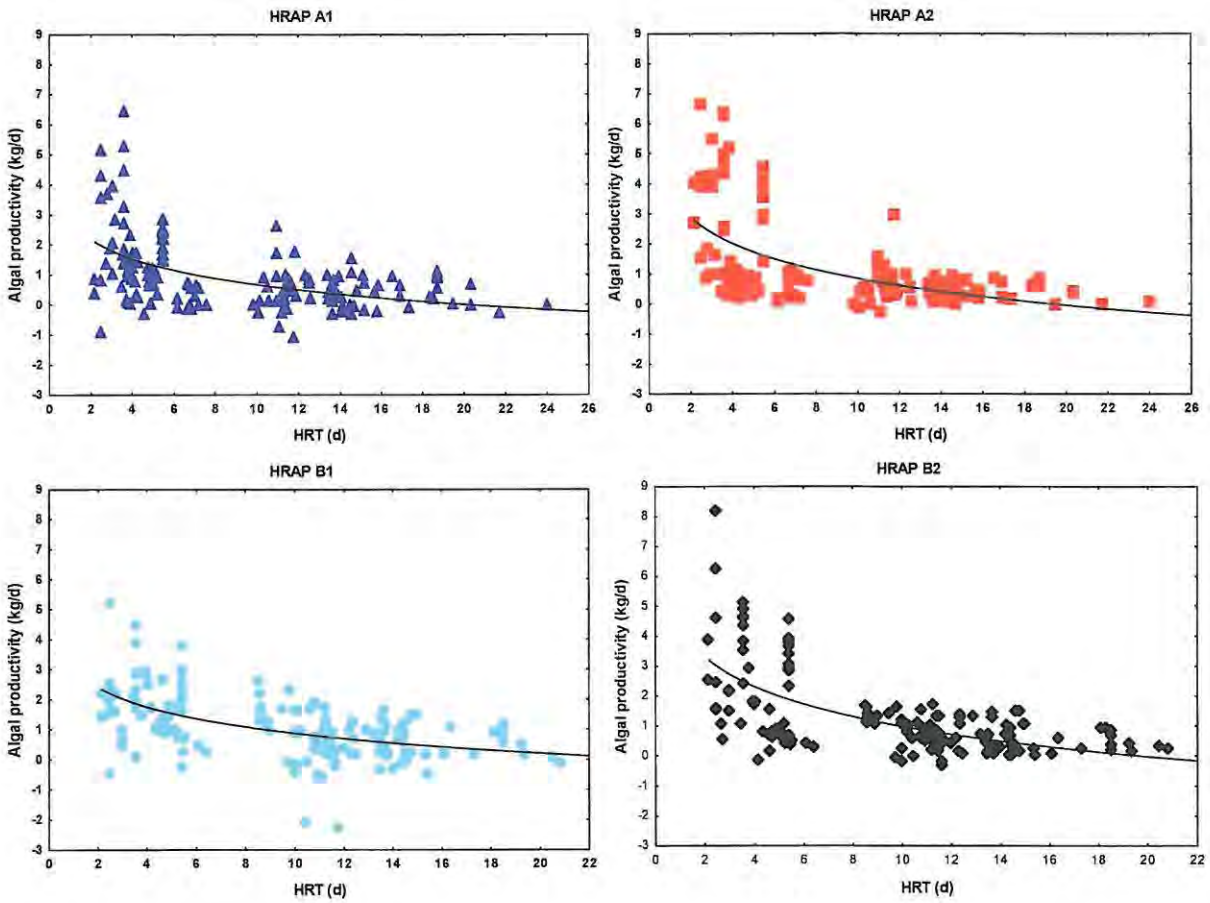

Figure 5.1.4.c: A multiple linear regression analysis of the relationship between algal productivity and hydraulic retention time (HRT) in high rate algal pond (HRAP) A1, A2, B1 and B2. HRAP A1: $r = -0.46$, $R^2 = 0.27$, $p < 0.0001$, $F = 44.34$. HRAP A2: $r = -0.49$, $R^2 = 0.30$, $p < 0.0001$ and $F = 50.59$. HRAP B1: $r = -0.52$, $R^2 = 0.26$, $p < 0.0001$ and $F = 59.38$. HRAP B2: $r = 0.58$, $R^2 = 0.37$, $p < 0.0001$ and $F = 79.36$.

5.1.5 Algal culture composition

Algae were sourced from technical scale HRAPs at the Institute of Environmental Biotechnology at Rhodes University (EBRU), which are situated at the Municipal disposal works in Grahamstown. These were used to inoculate the HRAPs of Project Eden. The typical algal species in these HRAPs were photographed by staff of EBRU in 2008. Similar algal species were present in the inoculum, and typically consisted of *Chlorella (Chlorellaceae)*, *Scenedesmus* (*Coelastraceae*), *Selanastrum* (*Selenastraceae*), *Haematococcus* (*Sphaerellaceae*), *Euglena (Euglenineae)*, *Arthrospira* and *Oscillatoria* (*Oscillatoriaceae*), *Kirchneriella*, many species of *Pediastrum* (*Hydrodictyaceae*), *Micractinium* (*Chlorellaceae*) species, other blue-green algae, and diatoms (Johnson HE 2010, Richard Laubscher, *pers. comm.*,senior researcher at EBRI July 2011, Figure 5.1.5.a).

After more than a year of operation, a build-up of detritus, aggregates of algae, bacteria, fungi and grazers was observed in September 2010. The presence of these aggregates and zooplanktonic grazers (protozoa and *Ostracods*) supported the decision to revitalise the algae community in the

HRAPs by re-inoculating them with an actively growing algal community sourced from EBRU, on the 16th of November 2010. No photomicrographs were taken after re-inoculation so it was not possible to determine the species composition or whether algal debris was still present after re-inoculation. However, the ponds were inoculated from the ponds at EBRU, where the dominant algal species are represented by species of *Pediastrum*, *Scenedesmus*, *Micractinium* and *Chlorella* (Johnson 2010, Richard Laubscher, *pers. comm.*,senior researcher at EBRI July 2011) (Figure 5.1.5.a).

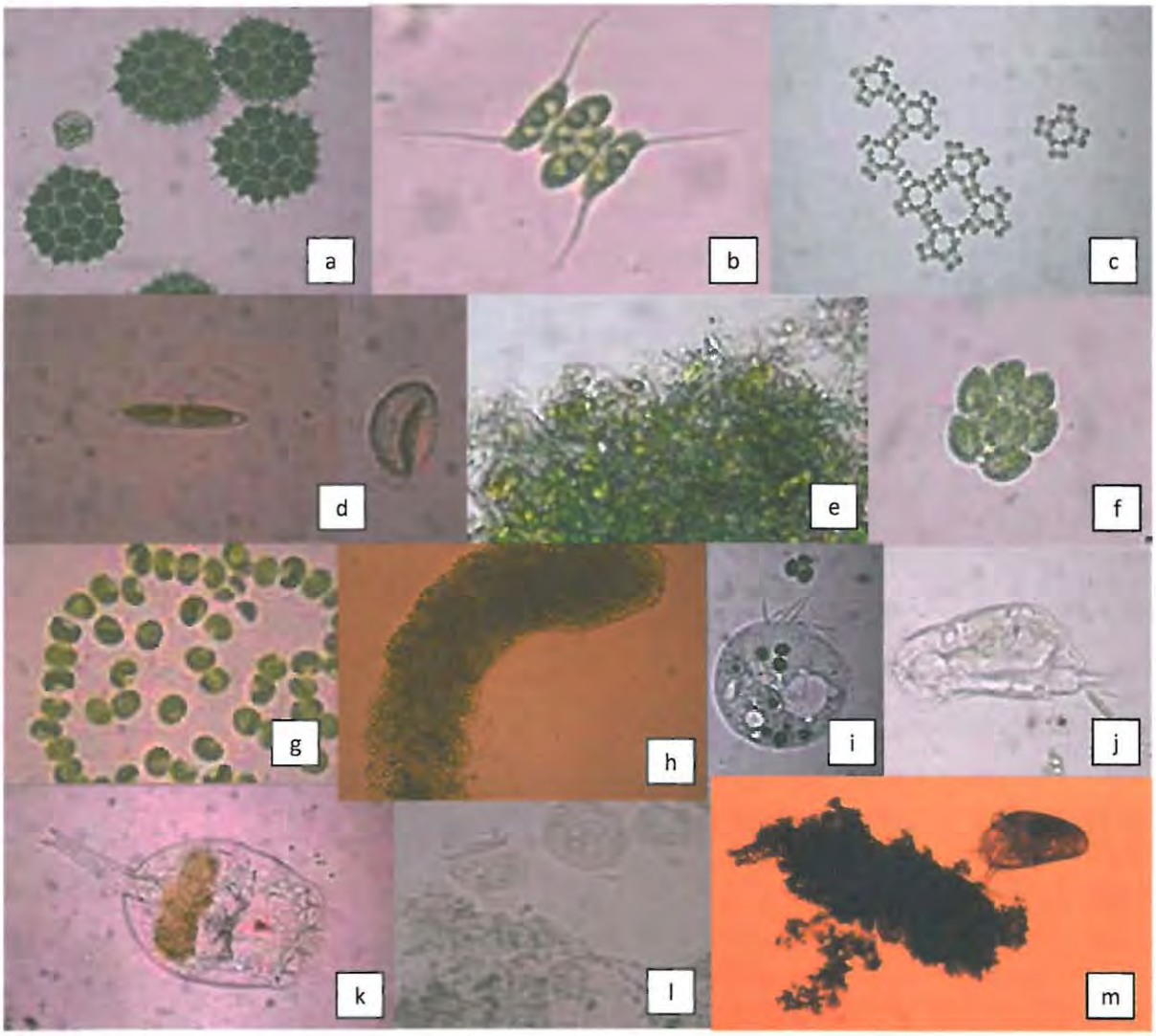

Figure 5.1.5.a: Microflora and fauna that were observed in HRAP samples from the Institute of Environmental Biotechnology in Grahamstown and HRAPs at Project Eden. Microalgae: a. *Pediastrum* in HRAPs at EBRU in 2008 (40 x magnification), b. *Scenedesmus* in HRAPs at EBRU in 2008 (40 x magnification), c. *Micractinium* in HRAPs at EBRU in 2008 (40 x magnification), d. Diatoms in HRAPs at EBRU in 2008 (40 x magnification), e. Blue-greens in HRAP at EBRU in 2008 (40 x magnification), f. *Pyrobotrys* in the HRAPs at EBRU in 2008 (40 x magnification), g. *Dictyosphaerium* in the HRAPs at EBRU in 2008, h. Algal/bacterial aggregate in HRAPs at Project Eden on the 16th of September 2010 (4 x magnification). Zooplankton: i. *Cyclidium* in HRAPs at EBRU in 2008 (40 x magnification), j. *Lecane* in HRAPs at EBRU in 2008 (40 x magnification), k. *Brachionus* in HRAPs at EBRU in 2008 (40 x magnification), l. Conochilus in HRAPs at EBRU in 2008 (40 x magnification), m. *Ostracod* feeding on detritus in HRAPs at Project Eden on the 16th of September 2010, (4 x magnification) (Johnson HE 2010).

5.1.6 The pH

The pH of the system increased as the effluent flowed through the pilot plant AD (7.82 ± 0.03), the PFP (8.25 ± 0.02) and the HRAPs (9.15 ± 0.03). The pH in HRAP A1 and B1 was lower than in HRAP A2 & B2. The pH decreased from 10.00 to 9.00 (HRAP A2 & B2) and from 9.50 to 8.00 (HRAP A1 & B1) when the HRT was shortened to a minimum of 4.3 d in autumn (March – April 2010). The pH increased from 9.00 to 10.00 in both HRAP trains during winter (May 2010 - August 2010), and decreased again from 10.20 to 9.40 (HRAP train-A), and from 10.40 to 9.90 (HRAP train-B) in spring (September – October 2010). The pH decreased from 11.00 to 10.00 in summer (November 2010) when HRAPS were re-inoculated with a new culture, and these pH levels persisted until the end of the optimization phase (mid-January 2011).

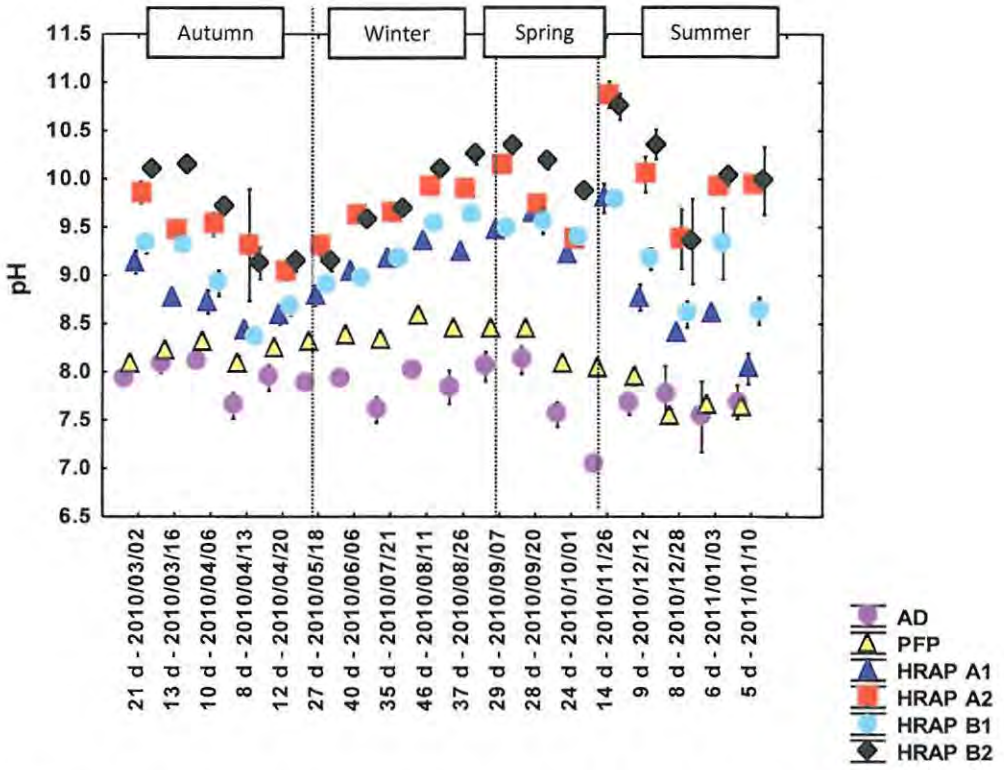

Figure 5.1.6.a: The mean (± standard error) monthly pH in pilot plant anaerobically digested (AD) brewery effluent, in primary facultative pond (PFP) treated effluent and in high rate algal pond (HRAP) A1, A2, B1 and B2 treated effluent from 2 March 2010 until 16 January 2011. The length of the combined hydraulic retention time (HRT) in the PFP and HRAPs appears below the dates on the x-axis in days (d). Each date indicates the start of a new HRT.

Table 5.1.6.a: The pH in post-pilot plant anaerobically digested (AD) effluent, primary facultative pond (PFP) effluent and high rate algal pond (HRAP) A1, A2, B1 & B2 treated effluent from 2 March 2010 until 16 January 2011. Data are presented as mean (± standard error), minimum, maximum and N-values.

System	Mean	Std.Err.	Min.	Max	N
Post-AD	7.82	0.03	6.73	9.1	188
PFP	8.25	0.02	7.46	9.57	189
HRAP A1	9.07	0.03	7.87	10.67	191
HRAP A2	9.68	0.04	8.17	11.6	182
HRAP B1	9.22	0.03	8.03	10.97	191
HRAP B2	9.84	0.04	8.56	11.38	182

5.1.7 Dissolved oxygen

The dissolved oxygen (DO) concentration in the post-pilot plant AD effluent was low (2.3 ± 0.16 mg/l O_2), and increased due to the high photosynthetic activity of algae in the HRAP system to more than 12 mg/L (HRAP A2 & B2). DO concentration decreased from 12 mg/L to 10 mg/L O_2 when increasingly shorter HRTs were being employed in autumn (HRAP A2 and B2). DO concentration increased from eight mg/L to 12 mg/L O_2 in winter (mid-May 2010 - end-August 2010). DO concentration remained at 12 mg/L (HRAP train-B), and decreased from 10.2 mg/L to 9.5 mg/L (HRAP train-A) in spring (September 2010 - October 2010). DO concentrations began high in summer at 16 mg/L (HRAP A2 and B2) when the new culture was inoculated into HRAPs. It decreased to concentrations between 7.00 and 12.00 mg/L by January 2011 (HRAP A2 and B2).

Table 5.1.7.a: The dissolved oxygen concentration (mg/L) in post-pilot plant anaerobically digested (AD) brewery effluent, in primary facultative pond (PFP) treated effluent and in high rate algal pond (HRAP) A1, A2, B1 and B2 treated effluent from 2 March 2010 until 16 January 2011. Data are presented here as mean (± standard error), minimum, maximum and N-values.

System	Mean	Std.Err.	Min.	Max	N
Post-AD	2.30	0.16	0.20	8.40	152
PFP	1.53	0.15	0.10	13.30	150
HRAP A1	8.52	0.26	0.10	21.40	153
HRAP A2	10.26	0.19	0.70	18.40	145
HRAP B1	9.20	0.26	0.60	24.00	153
HRAP B2	11.88	0.82	3.10	125.00	144

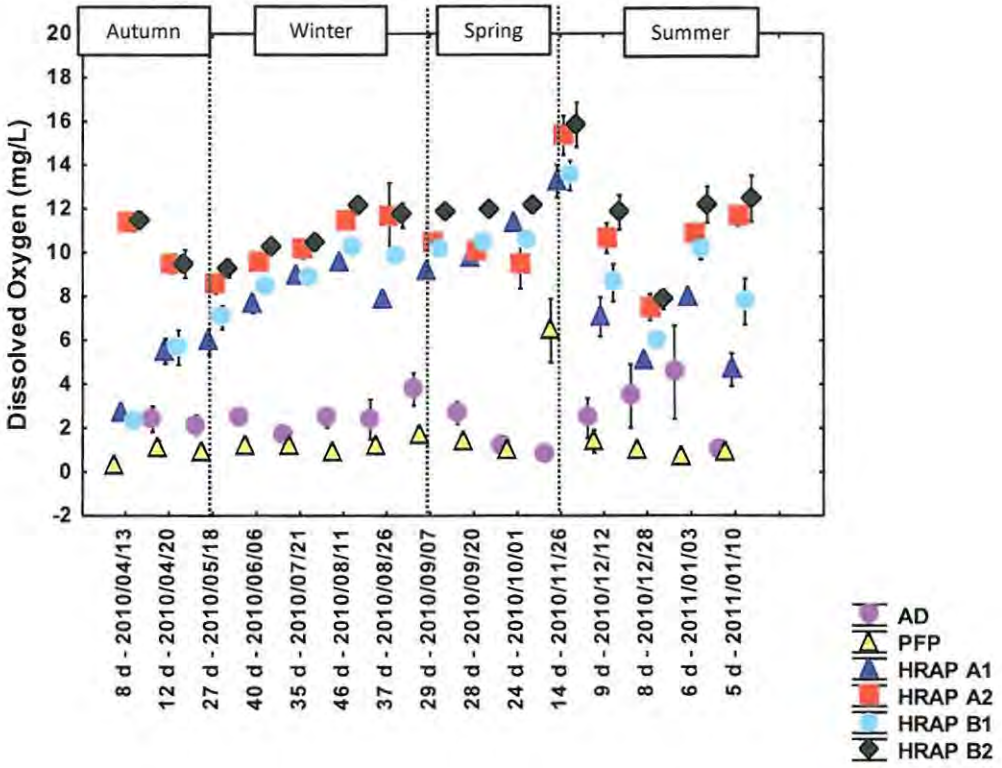

Figure 5.1.7.a: The mean (± standard error) monthly dissolved oxygen concentration (mg/L) in post-pilot plant anaerobically digested (AD) brewery effluent, in primary facultative pond (PFP) effluent and in high rate algal pond (HRAP) A1, A2, B1 and B2 treated effluent from 2 March 2010 until 16 January 2011. The length of the combined hydraulic retention time (HRT) in the PFP and HRAPs appears below the dates on the x-axis in days (d). Each date indicates the start of a new HRT.

5.1.8 Chemical oxygen demand

A consistent reduction in the chemical oxygen demand (COD) was observed in effluent leaving the pilot plant AD, to effluent leaving HRAP A2 and B2 (March 2010 - January 2011). These trends were similar regardless of the season and the HRT. The COD decreased from 170.38 ± 39.53 mg/L (post-pilot plant AD), to 106.69 ± 24.35 mg/L (post-HRAP train-A), and to 107.61 ± 33.19 mg/L (post-HRAP train-B) in autumn. The mean percentage removal efficiency was 33.28 % (HRAP train-A) and 31.86 % (HRAP train-B) in autumn. The COD decreased from 143.19 ± 16.08 mg/L (post-pilot plant AD), to 94.06 ± 7.55 mg/L (post-HRAP train-A), and to 99.07 ± 7.61 mg/L (post-HRAP train-B) in winter. The mean percentage removal efficiency was 34.35 % (HRAP train-A) and 30.49 % (HRAP train-B). The COD decreased from 155.65 ± 5.44 mg/L (post-pilot plant AD) to 85.80 ± 9.01 mg/L (post-HRAP train-A), and to 95.58 ± 15.33 mg/L (post-HRAP train-B) in spring. The mean percentage removal efficiency was 44.38 % (HRAP train-A) and 38.26 % (HRAP train-B). The COD decreased from 158.36 ± 18.14 mg/L (post-pilot plant AD), to 91.70 ± 14.97 mg/L (post-HRAP train-A), and to 115.71 mg/L ± 27.43 mg/L (post-HRAP train-B) in summer. The mean percentage removal efficiency was 43.43 % in HRAP train-A and 29.66 % in HRAP train-B.

Lower COD concentrations were obtained when a smaller pore size filter paper was used. This indicated the contribution of algal cells to the COD concentrations that were measured. The average percentage decrease in COD concentration in sampled filtered through 0.45 µm pore size filter paper versus 8 µm pore size filter paper, was 15.27 % in post-pilot plant AD samples, 5.99 % in the PFP, 13.22 % in HRAP A1, 18.99 % in HRAP A2, 26.99 % in HRAP B1 and 19.99 % in HRAP B2 (Figure 5.1.8.a).

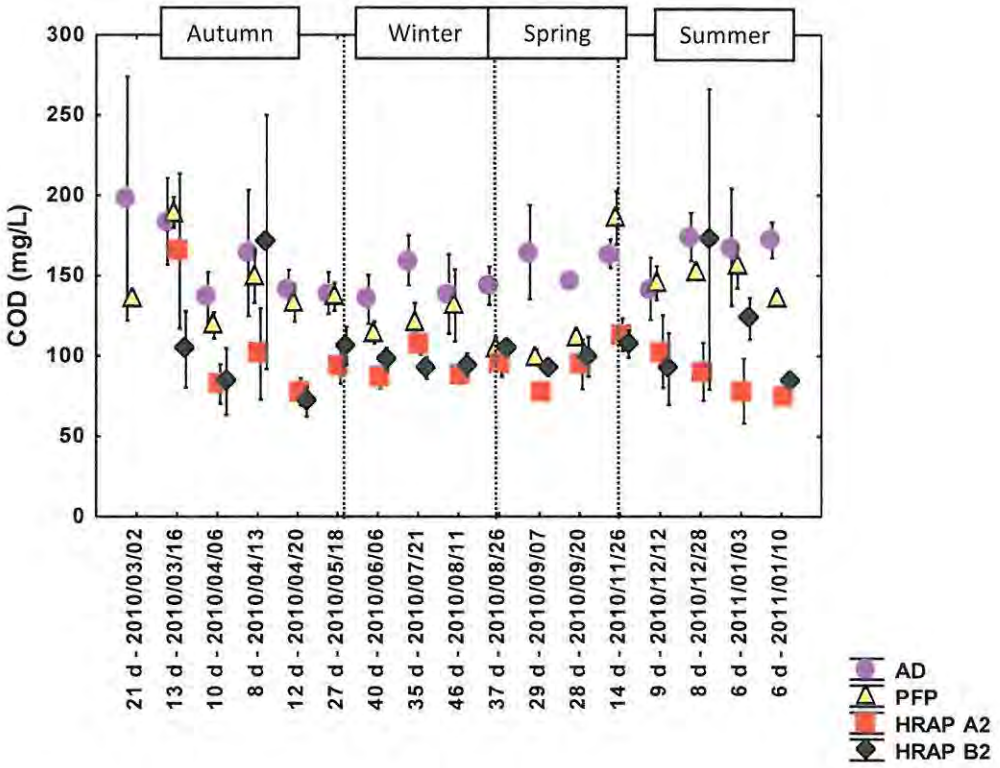

Figure 5.1.8.a: The mean (± standard error) monthly chemical oxygen demand (COD) (mg/L) in post-pilot plant anaerobically digested (AD) treated brewery effluent, in post-primary facultative pond (PFP) treated effluent and in post-high rate algal pond (HRAP) A2 and B2 effluent from 2 March 2010 until 16 January 2011. The length of the combined hydraulic retention time (HRT) in the PFP and HRAPs appears below the dates on the x-axis in days (d). Each date indicates the start of a new HRT.

5.1.9 Ammonia

The mean ammonia (NH_4-N) concentration decreased from 42.53 ± 1.38 mg/L (post-pilot plant AD) to an average of 1.70 ± 0.81 mg/L (post-HRAP effluent) over the duration of the optimization phase . The PFP was efficient at removing 7 % of the incoming NH_4-N concentration from the AD. Reductions of 95.84 % in HRAP train-A and 96.00 % in HRAP train-B of the incoming NH_4-N concentration was measured (Table 5.1.9.a, Figures 5.1.9.a, b and c).

Table 5.1.9.a: The ammonia concentration (mg/L) in post-anaerobically digested (AD) brewery effluent, in post-primary facultative pond (PFP) and in post-high rate algal pond (HRAP) treated effluent from 2 March 2010 until 16 January 2011. Data are presented as mean ± standard error, minimum, maximum and N-values.

System	Mean	Std.Err.	Min.	Max	N
Post-AD	42.53	1.38	13.40	82.00	100
Post-PFP	39.49	0.88	12.60	74.60	106
Post-HRAP A1	6.16	1.10	0.05	79.00	104
Post-HRAP A2	1.77	0.79	0.00	79.00	104
Post-HRAP B1	4.24	1.11	0.00	106.00	105
Post-HRAP B2	1.70	0.83	0.00	84.00	104

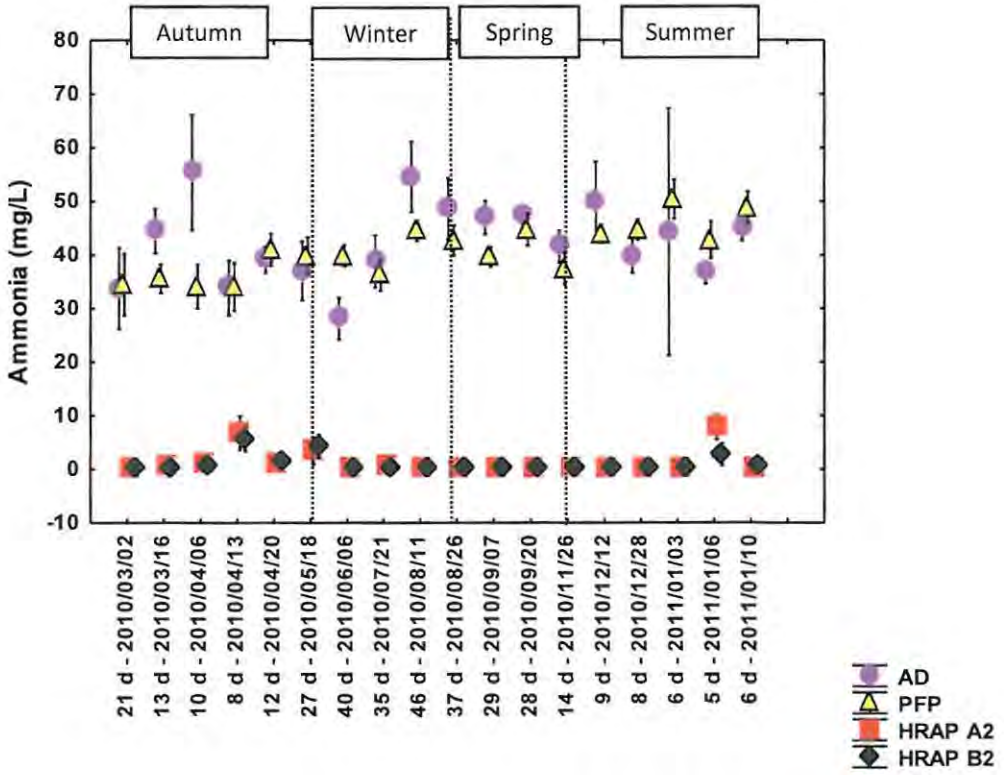

Figure 5.1.9.a: The mean (± standard error) monthly ammonia concentration in post-pilot plant anaerobically digested (AD) brewery effluent, in post-primary facultative pond (PFP) treated effluent and in post-high rate algal pond (HRAP) A2 and B2 treated effluent from 2 March 2010 until 16 January 2011. The length of the combined hydraulic retention time (HRT) in the PFP and HRAPs appears below the dates on the x-axis in days (d). Each date indicates the start of a new HRT.

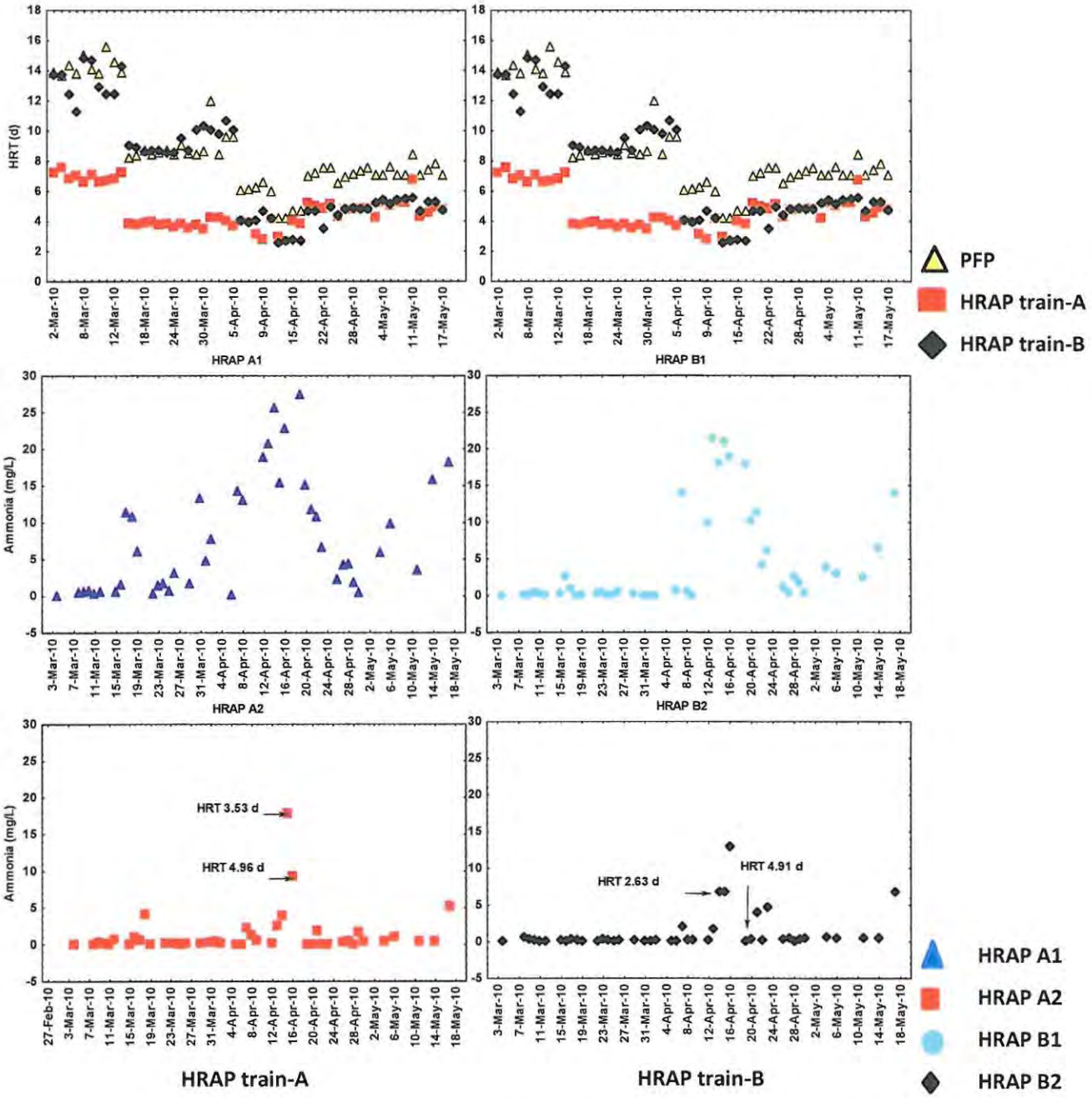

Figure 5.1.9.b: The response in ammonia concentration in post-high rate algal pond (HRAP) A1, A2, B1 & B2 effluent to shortened hydraulic retention time (HRT) in days (d) from the 2 March 2010 – 18 May 2010 (autumn optimization). Note the polishing effect that HRAP A2 and B2 had in removing excess NH_4-N from HRAP A1 and B1.

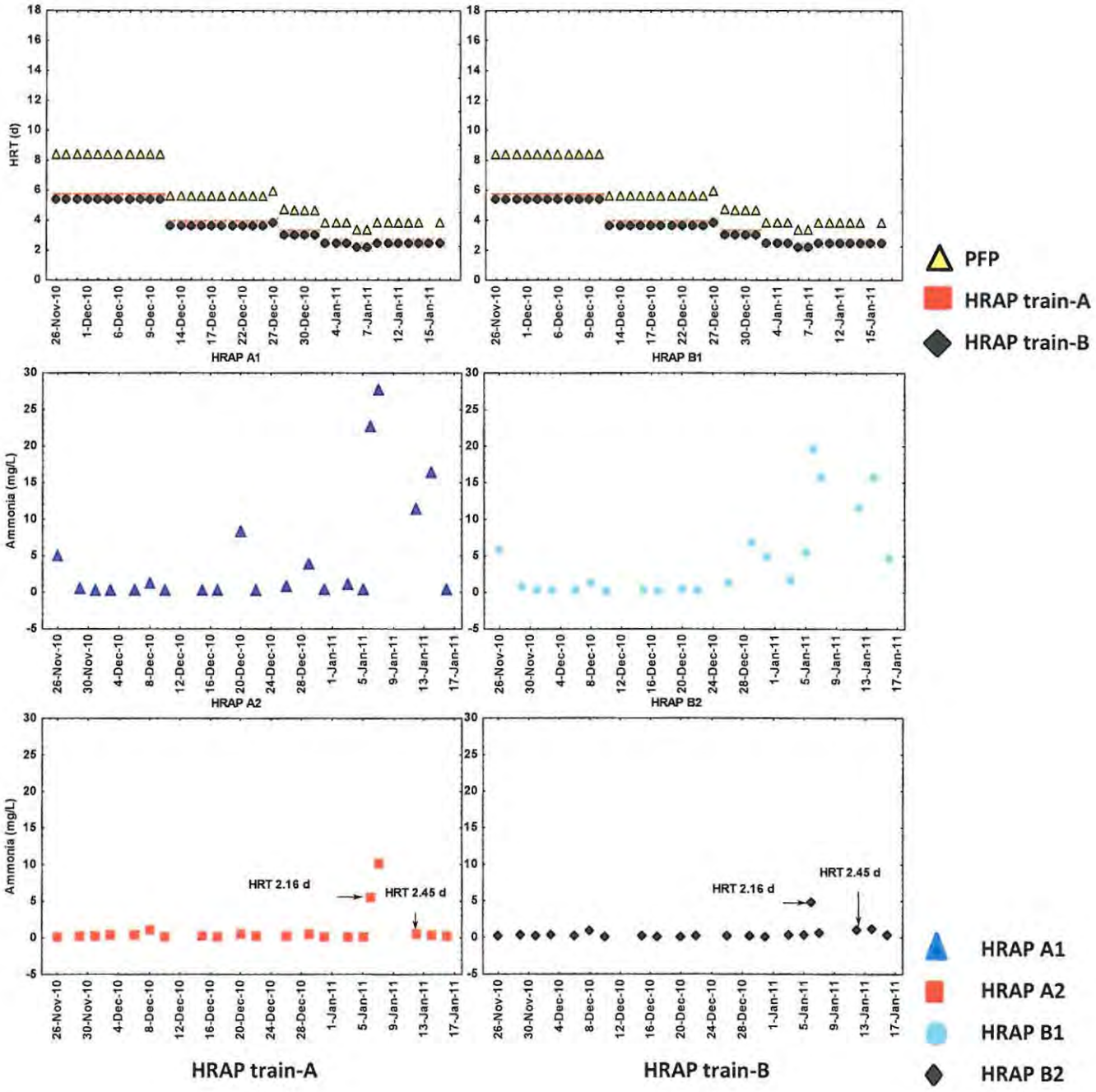

Figure 5.1.9.c: The response in ammonia concentration in post-high rate algal pond (HRAP) A1, A2, B1 & B2 effluent to shortened hydraulic retention time (HRT) in days (d) from the 26th of November 2010 until the 16th of January 2011 (summer optimization). Note the polishing effect that HRAP A2 and B2 had in removing excess NH_4-N from HRAP A1 and B1.

5.1.10 Nitrate

NO_3-N was constantly produced in the HRAPs. The NO_3-N concentration in post-pilot plant AD and PFP effluent remained below 5.00 mg/L for the entire period of data collection. It increased to between 5.00 and 25.00 mg/L in post-HRAP effluent. The mean NO_3-N concentration in post-HRAP effluent was 14.64 ± 0.89 mg/L during the optimization phase.

The NO_3-N concentration was below 15 mg/L in post-HRAP treated effluent in autumn (March – April 2010). It was between 15 and 25 mg/L in post-HRAP effluent in winter (May – August 2010). It fluctuated between 5.00 and 20.00 mg/L in post-HRAP effluent in summer (November 2010 - January 2011). (Figure 5.1.10.a, Table 5.1.10.a). There was a significant negative correlation between the NO_3-N concentration and algal productivity in HRAP A2, B1 and B2, suggesting that more NO_3-N was produced when algal productivity was low (Figure 5.1.10.b).

Table 5.1.10.a: The nitrate concentration (mg/L) in post-pilot anaerobically digested (AD) brewery effluent, in post-primary facultative pond (PFP) and in post-high rate algal pond (HRAP) A1, A2, B1 and B2 treated effluent from 2 March 2010 until 16 January 2011. Data are presented as mean ± standard error, minimum, maximum and N-values.

System	Mean	Std.Err.	Min.	Max	N
Post-AD	1.97	0.19	0.05	7.10	71
Post-PFP	1.82	0.17	0.03	7.70	72
Post-HRAP A1	16.20	1.06	1.10	32.30	72
Post-HRAP A2	13.82	0.77	0.05	26.70	70
Post-HRAP B1	16.16	0.98	0.17	34.40	72
Post-HRAP B2	12.37	0.76	0.05	26.50	69

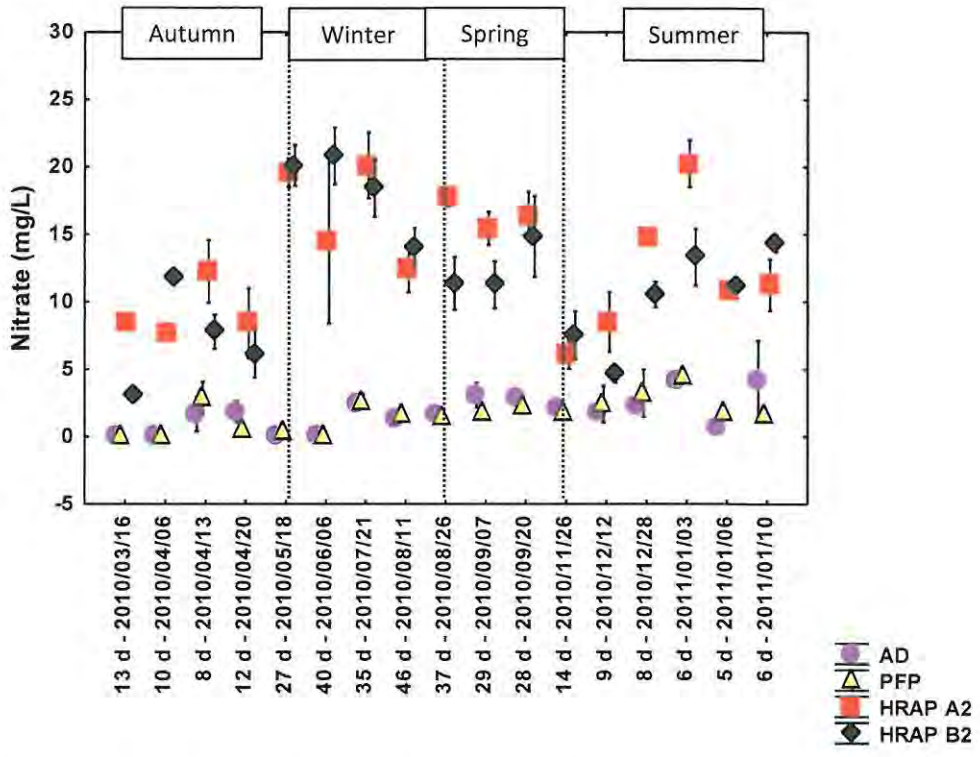

Figure 5.1.10.a: The mean (± standard error) monthly nitrate concentration in post-pilot plant anaerobically digested (AD) brewery effluent, in post-primary facultative pond (PFP) treated effluent and in post-high rate algal pond (HRAP) A2 and B2 treated effluent from 2 March 2010 until 16 January 2011. The length of the combined hydraulic retention time (HRT) in the PFP and HRAPs appears below the dates on the x-axis in days (d). Each date indicates the start of a new HRT.

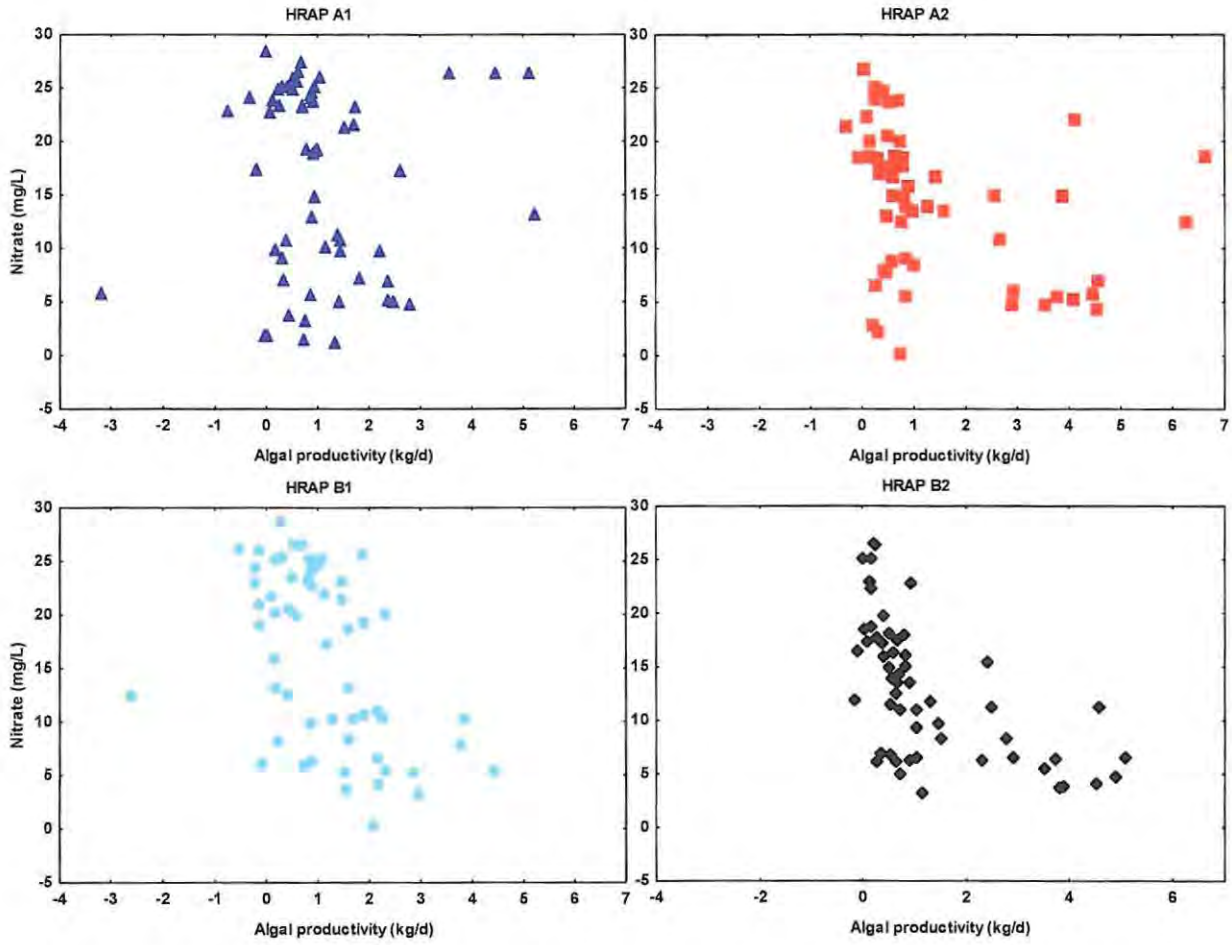

Figure 5.1.10.b: A multiple linear regression analysis of the relationship between the nitrate concentration and hydraulic retention time (HRT) in high rate algal pond (HRAP) A2, B1 & B2. HRAP A1: r = 0.02, R^2 = - 0.34, p = 0.900 and F = 0.01. HRAP A2: r = - 0.34, R^2 = 0.11, p = 0.009 and F = 7.32. HRAP B1: r = - 0.48, R^2 = 0.23, p < 0.0001 and F = 17.04. HRAP A2: r = - 0.59, R^2 = 0.35, p < 0.0001 and F = 29.86.

5.1.11 Nitrite

Minimal NO_2-N was produced in post-pilot plant AD and PFP effluent. The NO_2-N concentration was between 1.00 and 3.00 mg/L in the HRAP system when increasingly shorter HRTs were employed in autumn (March - April 2010). It remained below 1.00 mg/L in winter and spring when the HRT was lengthened (May - September 2010). The NO_2-N concentration in HRAP A2 and B2 was 8.00 mg/L (HRAP A2) and 10.00 mg/L (HRAP B2) when HRTs were incrementally reduced in summer (November 2010 - January 2011) (Figure 5.1.11.a).

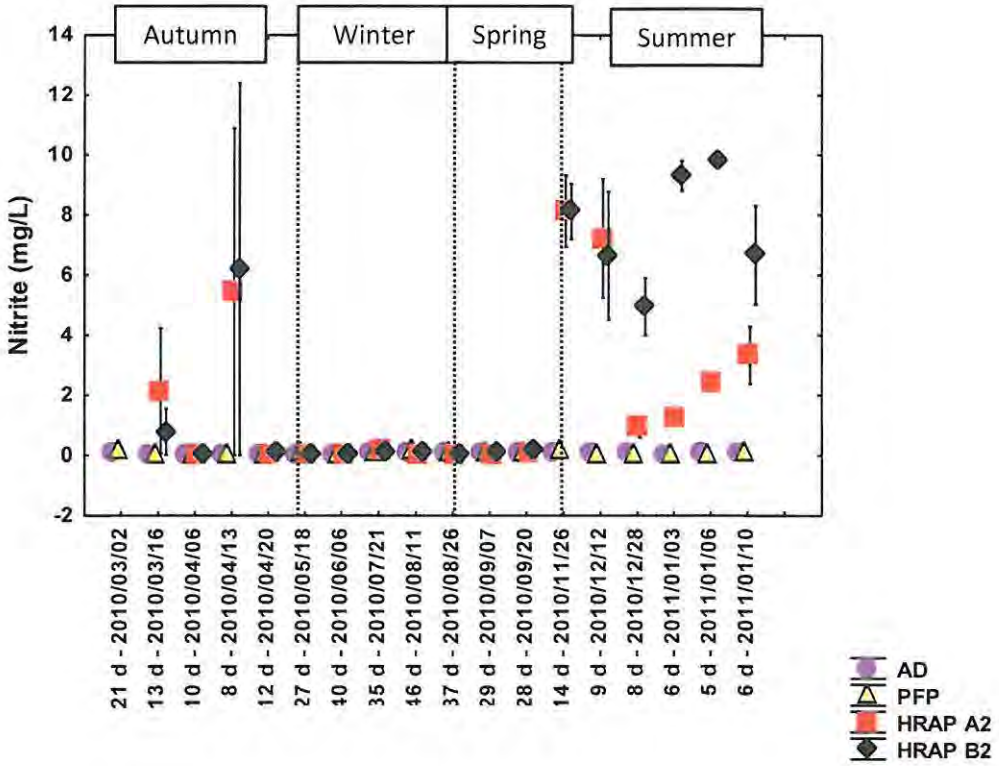

Figure 5.1.11.a: The mean (± standard error) monthly nitrite concentration in post-pilot plant anaerobically digested (AD) treated brewery effluent, in post-primary facultative pond (PFP) treated effluent, and in post-high rate algal pond HRAP A2 and B2 treated effluent from 2 March 2010 until 16 January 2011. The length of the combined hydraulic retention time (HRT) in the PFP and HRAPs appears below the dates on the x-axis in days (d). Each date indicates the start of a new HRT.

5.1.12 Phosphate

The presence of phosphate (PO_4-P) in the brewery effluent was erratic, and the removal of PO_4-P was inconsistent. No PO_4-P was detected in post-pilot plant AD, post-PFP or post-HRAP effluents from the 2nd of March until the 20th of July 2010. Measurable levels of PO_4-P in post-pilot plant AD effluent were observed for the first time on the 21st of July 2010, and increased to an average of 47 mg/L towards January 2011. PO_4-P was measured below 10 mg/L in post-HRAP effluent from July 2010 until January 2011, except for the 26th of November 2010, when the fresh algal culture was inoculated (19 mg/L) (Figure 5.1.12.a, Table 5.1.12.a).

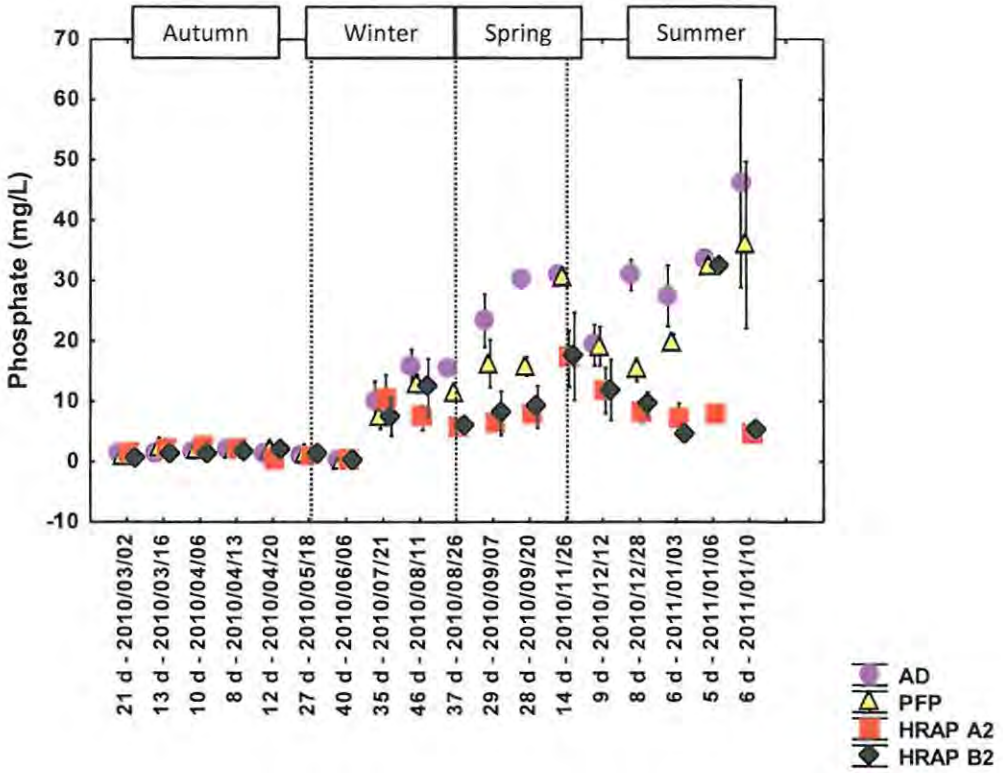

Figure 5.1.12.a: The mean (± standard error) monthly phosphate concentration in post-pilot plant anaerobically digested (AD) brewery effluent, in post-primary facultative pond (PFP) treated effluent and in post-high rate algal pond (HRAP) A2 & B2 treated effluent from 1 March 2010 until January 2011 with the hydraulic retention time (HRT) time intervals that were used. The length of the combined HRT in PFP and HRAPs appears below the dates on the x-axis in days (d). Each date indicates the start of a new HRT.

Table 5.1.12.a: The phosphate concentration (mg/L) in post-pilot plant anaerobically digested (AD) brewery effluent, in post-primary facultative pond (PFP) treated effluent and in post-high rate algal pond (HRAP) treated effluent from 2 March 2010 until 16 January 2011. Data are presented as mean ± standard error, minimum, maximum and N-values.

System	Mean	Std.Err.	Min.	Max	N
Post-AD	12.49	1.44	0.03	63.20	86
Post-PFP	9.50	1.12	0.03	62.30	93
Post-HRAP A1	6.45	0.86	0.03	33.00	91
Post-HRAP A2	5.23	0.68	0.03	30.00	90
Post-HRAP B1	5.78	0.68	0.00	34.00	91
Post-HRAP B2	5.53	0.86	0.03	32.50	89

5.1.13 Electrical conductivity and chloride

The HRAPs were ineffective at removing dissolved salts from the effluent. Concentrations in post-pilot plant AD, PFP and HRAP effluent followed the same trends. The electrical conductivity (EC) decreased from 3400 µS/cm to 2800 µS/cm in autumn as the medium became more diluted with the shorter HRTs. The EC reached a minimum in winter and remained between 2400 µS/cm and 2800 µS/cm in post-pilot plant AD, PFP and HRAP effluent. The EC in HRAP effluent was higher (2800 –

3500 µS/cm) than in post-AD and PFP effluent in spring (2400 – 2750 µS/cm) when longer HRTs were used. The EC in post-AD, PFP and HRAP effluent was below 3000 µS/cm in summer (Table 5.1.13.a, Figure 5.1.13.a).

Table 5.1.13.a: The electrical conductivity (µS/cm) in post-pilot plant anaerobically digested (AD) brewery effluent, in primary facultative pond (PFP) treated effluent and in high rate algal pond (HRAP) A1, A2, B1 and B2 treated effluent from 2 March 2010 until 16 January 2011. Data are presented as mean ± standard error, minimum, maximum and N-values.

System	Mean	Std.Err.	Min.	Max	N
Post-AD	2761.85	26.32	2056.00	3842.00	189
PFP	2761.50	19.87	1494.00	3373.00	187
HRAP A1	2695.11	19.40	1505.00	3575.00	190
HRAP A2	2867.51	24.53	1645.00	3980.00	181
HRAP B1	2703.54	22.51	1480.00	3399.00	190
HRAP B2	2 869.92	35.64	2 256.00	4 000.00	181

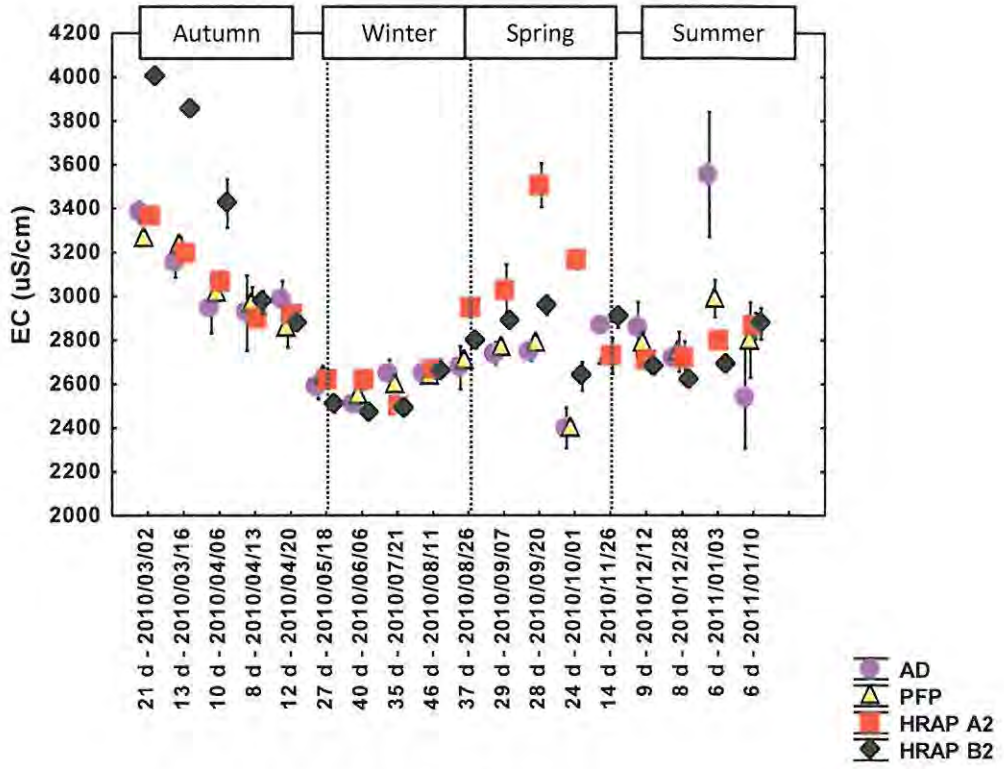

Figure 5.1.13.a: The mean (± standard error) electrical conductivity (EC, µS/cm) in post-pilot plant anaerobically digested (AD) brewery effluent, in primary facultative pond (PFP) treated effluent and in high rate algal pond (HRAP) A2 and B2 treated effluent from 1 March 2010 until January 2011. The length of the combined HRT in the PFP and HRAPs appears below the dates on the x-axis in days (d). Each date indicates the start of a new HRT.

5.1.14 Micronutrient analysis: 12/02/2010

One micronutrient analysis on the 12th of February 2010 indicated that calcium (Ca), carbonate (CO_3), carbonic acid (HCO_3) and sulphate (SO_4) were present in post-pilot plant AD, post-PFP and post-HRAP effluent (Table 5.1.14.a). EC, iron, zinc and fluoride concentrations exceeded the DWAF general limits for discharge into a natural water resource (Table 1, Appendix 1).

Table 5.1.14.a: A once-off micronutrient analysis of post-pilot plant anaerobically digested (AD) brewery effluent, post-primary facultative pond (PFP) effluent and post-high rate algal pond (HRAP) effluent. Bold values post-HRAP indicate nutrients that exceeded the Department of Environmental and Water Affairs' (DWAF) general limits for discharge into a natural water resource of 1998 (Table 1, Appendix 1, DWAF limit-right column of this table).

Parameter	Unit	AD	PFP	HRAP	DWAF limit
pH		8.10	8.20	9.80	9.50
EC	mS/m	306.00	288.00	**337.00**	70.00
Na		481.90	476.30	652.90	
K		13.30	12.30	17.50	
Ca		59.80	50.90	33.80	
Mg		13.10	10.70	12.00	
Fe		0.55	0.49	**0.31**	0.01
Cl		300.20	285.20	**375.30**	0.25 (chlorine)
CO_3		135.30	165.30	1142.30	
HCO_3		1191.90	1066.60	252.10	
SO_4	mg/L	27.00	24.00	22.00	
B		0.10	0.06	0.06	1.00
Mn		0.06	0.04	0.04	0.10
Cu		0.00	0.01	0.01	0.01
Zn		0.01	0.04	**0.11**	0.10
P		7.18	6.95	6.67	10.00
NH_4-N		28.98	24.72	2.46	6.00
NO_3-N		0.80	0.46	0.26	15.00
F		1.20	1.20	**1.10**	1.00
TDS		2300.00	2150.00	2510.00	

5.1.15 The effect of evaporation

Evaporation caused the effluent in the HRAPs to become more concentrated. The effluent was less concentrated during the optimization phase (shorter HRT) than during the baseline phase (longer HRT). This was reflected by a lower average EC in HRAP effluent of 2870 µS/cm during the optimization phase as well as lower COD, compared to a higher average EC of 3488 µS/cm in HRAP effluent during the baseline phase and higher COD. A large increase in the EC was observed in HRAP A2 during September-October 2010 when longer HRTs were used and temperatures were warmer. Evaporation probably influenced the COD more during the baseline phase (because of longer HRTs) than during the optimization phase (because of shorter HRTs) as evidenced by higher COD and EC in post-HRAP effluent during the baseline phase (Table 4.1.8.a, Table 5.1.15.a, Table 5.1.15.b).

Table 5.1.15.a: The effect of evaporation on the electrical conductivity (EC) and chemical oxygen demand (COD) in high rate algal pond (HRAP) A2 during the optimization phase (March 2010 – January 2011). The length of the combined hydraulic retention time (HRT) in the primary facultative pond (PFP) and high rate algal ponds (HRAP) appears in the first column in days (d). The dates indicate the duration the HRT was implemented for. A new date indicates the start of a new HRT that was used. The evaporation formula was used to calculate the values in Columns 4 - 9 (Chapter 3, Materials and Methods).

Total HRT and duration	EC PFP	EC HRAPA2	Volume V2	Volume lost per one litre (L)	Normalised volume (1 L + volume lost through evaporation)	% Increase in EC	COD Post-HRAP A2 (mg/L)	Normalised COD Post-HRAP A2
21 d - 2010/03/02	3267.70	3361.30	0.97	0.03	1.03	2.78		
13 d - 2010/03/16	3235.87	3199.33	1.01	-0.01	0.99	-1.14	165.33	167.24
10 d - 2010/04/06	3019.20	3070.40	0.98	0.02	1.02	1.67	82.50	81.15
8 d - 2010/04/13	2961.75	2901.25	1.02	-0.02	0.98	-2.09	101.20	103.36
12 d - 2010/04/20	2859.56	2913.75	0.98	0.02	1.02	1.86	77.73	76.31
27 d - 2010/05/18	2632.86	2616.36	1.01	-0.01	0.99	-0.63	94.33	94.93
40 d - 2010/06/06	2549.11	2615.57	0.97	0.03	1.03	2.54	86.33	84.19
35 d - 2010/07/21	2593.64	2497.07	1.04	-0.04	0.96	-3.87	106.67	110.96
46 d - 2010/08/11	2634.25	2665.25	0.99	0.01	1.01	1.16	88.00	86.99
37 d - 2010/08/26	2708.00	2944.57	0.92	0.08	1.08	8.03	95.00	87.94
29 d - 2010/09/07	2771.44	3023.89	0.92	0.08	1.08	8.35	77.00	71.07
28 d - 2010/09/20	2791.78	3504.67	0.80	0.20	1.20	20.34	94.60	78.61
24 d - 2010/10/01	2399.11	3166.56	0.76	0.24	1.24	24.24	113.00	90.96
14 d - 2010/11/26	2725.60	2726.10	1.00	0.00	1.00	0.02	102.50	102.48
9 d - 2010/12/12	2785.50	2711.40	1.03	-0.03	0.97	-2.73	90.00	92.53
8 d - 2010/12/28	2745.50	2715.00	1.01	-0.01	0.99	-1.12	78.00	78.89
6 d - 2011/01/03	2987.00	2800.67	1.07	-0.07	0.93	-6.65	75.00	80.35
5 d - 2011/01/06	2800.00	2863.00	0.98	0.02	1.02	2.20		

Table 5.1.15.b: The effect of evaporation on the electrical conductivity (EC) and chemical oxygen demand (COD) in high rate algal pond (HRAP) B2 during the optimization phase (March 2010 – January 2011). The length of the combined hydraulic retention time (HRT) in the primary facultative pond (PFP) and high rate algal ponds (HRAP) appears in the first column in days (d). The dates indicate the duration the HRT was implemented for. A new date indicates the start of a new HRT that was used. The evaporation formula was used to calculate the values in Columns 4 - 9 (Chapter 3, Materials and Methods).

Total HRT and duration	EC PFP	EC HRAP B2	Volume V2	Volume lost per one litre (L)	Normalised volume (1 L + volume lost through evaporation)	% Increase in EC	COD Post-HRAP B2 (mg/L)	Normalised COD Post-HRAP B2
21 d - 2010/03/02	3267.70	4000.00	0.82	0.18	1.18	18.31		
13 d - 2010/03/16	3235.87	3856.00	0.84	0.16	1.16	16.08	104.00	89.59
10 d - 2010/04/06	3019.20	3422.60	0.88	0.12	1.12	11.79	84.00	75.14
8 d - 2010/04/13	2961.75	2972.25	1.00	0.00	1.00	0.35	170.80	170.20
12 d - 2010/04/20	2859.56	2874.00	0.99	0.01	1.01	0.50	71.36	71.01
27 d - 2010/05/18	2632.86	2510.00	1.05	-0.05	0.95	-4.89	106.17	111.63
40 d - 2010/06/06	2549.11	2464.57	1.03	-0.03	0.97	-3.43	97.83	101.31
35 d - 2010/07/21	2593.64	2491.36	1.04	-0.04	0.96	-4.11	92.44	96.40
46 d - 2010/08/11	2634.25	2660.00	0.99	0.01	1.01	0.97	94.29	93.38
37 d - 2010/08/26	2708.00	2796.86	0.97	0.03	1.03	3.18	104.60	101.38
29 d - 2010/09/07	2771.44	2888.56	0.96	0.04	1.04	4.05	91.75	88.18
28 d - 2010/09/20	2791.78	2958.00	0.94	0.06	1.06	5.62	99.40	94.11
24 d - 2010/10/01	2399.11	2633.89	0.91	0.09	1.09	8.91	107.29	98.51
14 d - 2010/11/26	2725.60	2904.60	0.94	0.06	1.06	6.16	91.75	86.42
9 d - 2010/12/12	2785.50	2677.50	1.04	-0.04	0.96	-4.03	172.50	179.75
8 d - 2010/12/28	2745.50	2614.00	1.05	-0.05	0.95	-5.03	123.00	129.52
6 d - 2011/01/03	2987.00	2684.67	1.11	-0.11	0.89	-11.26	84.00	94.66
5 d - 2011/01/06	2800.00	2874.00	0.97	0.03	1.03	2.57		

5.2 Discussion

5.2.1 Managing algal productivity

The heating system that was installed in HRAP train-A (April 2010) did not have a significant effect on algal productivity or minimise the optimal HRT that could be employed in HRAPs. Seasonal light (hours of daylight) and temperature significantly increased algal productivity, but a small increase in temperature of 2.40 °C in winter did not increase productivity. Similar nutrient uptake efficiencies were recorded in heated and unheated HRAPs and NH_4-N concentrations that exceeded the DWAF limit of 6.00 mg/L started to occur at the same time in both HRAP trains. The temperature was only increased by 2.14 ± 1.38 °C in HRAP train-A, which raised it from an average of 18.78 ± 0.30 °C to an average of 21.07 ± 0.31 °C in HRAP train-A . Algal productivity generally increases with increased pond temperature until an optimum temperature is reached (Park *et al.* 2011). Above this temperature algal productivity will start to decline (Park *et al.* 2011). The optimal temperature for many algal species lies between 28 and 35 °C (Park *et al.* 2011). Algae can die off when temperatures are too low (< 13 °C) or too high (> 38 °C) (Golueke & Oswald 1959). When this happens the HRT can be increased to allow the algal culture to recover (Golueke & Oswald 1959). A greater temperature elevation to between 28 - 35 °C might have achieved improved algal productivity as this is the optimal temperature for most algal species (Park *et al.* 2011).

The seasons determined the optimal HRT that could be employed in the HRAPs. Results from this study indicated increased productivity with shorter HRTs during warmer seasons. HRT was significantly correlated with temperature. The optimal HRT determined for autumn was 4.30 d at a temperature of 20.53 °C in HRAP A2 (heated) and 18.96 °C in HRAP B2 (ambient). The optimal HRT for summer was 2.74 d at a temperature of 29.90 °C in HRAP A2 (heated) and 26.36 °C in HRAP B2 (ambient). The HRT that was used in winter fluctuated between 10 and 18 d in the HRAPs. Higher temperatures allow a shorter HRT and vice versa (Azov and Shelef 1982). Garcia *et al.* (2000) recommended a HRT of 4 d in spring and summer (22.9 – 27.3 °C), and 10 d in autumn and winter (11.8 - 21.7 °C) to produce an effluent with less than 15 mg/L total nitrogen in Spain. A HRT of 4.00 to 6.00 d can be used at temperatures between 26 and 27 °C (Golueke & Oswald 1959).

The optimal HRTs that were determined for autumn and summer were the shortest HRTs that could be used, and were based on keeping the NH_4-N concentration below the DWAF general limits for

discharge into a natural water resource of 6.00 mg/L (Table 1, Appendix 1). The optimal HRT for autumn (4.30 d) was shorter than the 10 d HRT that has been suggested, and the optimal HRT for summer (2.74 d) was also shorter than the suggested 4 d HRT in Spain (Garcia *et al.* 2000). Future HRAP operations at iBhayi brewery can use slightly longer HRTs than the optimal HRTs that were determined for autumn and summer in this study, based on guidelines provided by other authors and to prevent placing stress on the HRAP system. The HRTs in winter were not based on keeping the NH_4-N concentrations within the DWAF general limits for discharge into a natural water resource of 1998 (Table 1, Appendix 1), but aimed to lower NO_3-N concentration to acceptable limits. The long HRTs that were tested during winter did not succeed in producing an effluent with less NO_3-N. Future HRAP operations could test shorter HRTs than the 10 – 18 d that were used in winter, as NO_3-N concentration is more likely to decrease at shorter HRTs (Craggs *et al.* 2004), provided low ambient temperatures do not constrain algal growth. In summer, light limitation in dense algal cultures could be addressed by shortening the HRT to increase algal productivity and promote nutrient removal. However, in winter, temperatures recorded here (14 to 18 °C) may have played a major role in influencing algal productivity and subsequently, nutrient removal. Therefore, when determining the optimal HRT in winter, both the HRT and ambient temperature should be considered when aiming to achieve optimal nutrient removal success.

The optimal HRT was shorter in summer than in autumn. HRAP optimization fluctuates seasonally (Azov and Shelef 1982). High algal yields concomitant with short HRT can be expected in summer and lower algal yields concomitant with a longer HRT in winter (Azov and Shelef 1982). A shorter HRT in warmer temperatures implies that the space that will be required to treat effluent in summer could be one eighth of the space required to treat effluent in winter (Azov and Shelef 1982). Results from this study were consistent with those of Azov & Shelef (1982), and indicated high algal productivity with a short HRT in summer, and low productivity with a longer HRT in winter. Based on the optimal HRTs that were determined for autumn and summer in this study, 1.1 m^3 was treated in autumn at a running time of 10 h/d (HRT 4.30 d), and 2.0 m^3/d in summer at a running time of 12 h/d (HRT 2.74 d) in one HRAP train that consisted of two ponds in series (volume 5.46 m^3, surface area 30 m^2). The daily effluent production in the brewery was 1041 m^3/d during 2010 (Mabuza, *pers. comm.*, engineering manager, iBhayi brewery, Port Elizabeth, SAB Ltd., SABMiller, November 2010). Therefore, to treat the full volume of effluent that the brewery produced would require an area of 3.00 ha in autumn and 1.60 ha in summer (Table 5.2.1.b). The optimal HRT and areal requirements for mid-winter need to be determined by feeding the HRAP system with increased volumes until NH_4-N concentration that exceeds the DWAF limit of 6.00 mg/L are observed. The optimal HRT for winter at this stage is inconclusive.

Table 5.2.1.b: The area (hectares - ha) required to treat different volumes (%) of the total volume effluent that was produced by the brewery per day. The optimal hydraulic retention time (HRT) that was determined in autumn and summer without carbon dioxide addition was used to calculate the area in ha.

	100%	50%	30%	15%
Volume effluent (m^3/d)	1041.00	520.50	312.31	156.15
Space (ha) needed in autumn	3.00	1.42	0.85	0.43
Space (ha) needed in summer	1.60	0.78	0.45	0.23

The HRT determined the in-situ algal biomass concentration. The algal biomass concentration became more diluted with a shorter HRT in autumn and summer, and denser with a longer HRT in winter and spring. The algal biomass concentration can affect algal productivity due to the shading effect that cells have on each other (Azov & Shelef 1982). Cells in a more diluted culture can utilize light more efficiently, which facilitates improved nutrient removal from effluent (Azov & Shelef 1982). Photosynthesis increases with increased light intensity until maximum productivity is attained at the light saturation point (Richmond 2000, Park *et al.* 2011). Beyond this point, photoinhibition can occur (Richmond 2000, Park *et al.* 2011). The algal biomass concentration can influence algal productivity more than seasonal solar radiation fluctuations due to the shading effect that cells have on each other (Azov & Shelef 1982). The HRT controls the shading effect by determining the concentration of the algal culture. Algal productivity (*Spirulina* sp.) has been reported to decline due to a shortage of light (Phang *et al.* 2000). In this study, a shorter HRT diluted the culture and increased the amount of light available, which caused a 33 % increase in algal productivity. The rate of nutrient addition in the form of brewery effluent (as determined by the HRT) therefore played an important role in the amount of light that cells were exposed to. Low algal productivity during winter might have been attributed to a dense algal culture in which cells were exposed to insufficient light to photosynthesize optimally at a longer HRT. NH_4-N concentrations that exceeded the DWAF limit of 6.00 mg/L in autumn and summer might have been partly due to cells receiving too much light and becoming photoinhibited when the algal culture became increasingly diluted with a short HRT. This might have influenced algal productivity and subsequent nutrient removal. A balance was sought to adjust the HRT so that a thin algal culture could be maintained to receive sufficient light to allow optimum photosynthesis and productivity, and to subsequently improve nutrient uptake, but to not become too dilute so as to lose its ability to remove nutrient from the wastewater efficiently (Azov & Shelef 1982, Park *et al.* 2011). The effects of temperature also need to be taken into account in such a scenario.

Although inorganic and organic carbon was not measured during this study, the literature suggests that carbon dynamics played a role in the results that were obtained. Carbon is the principal component in algal cells (Knud-Hansen 1998), and constitutes around 48.9 % of the biomass in algal

species *Chlorella vulgaris* (Minowa & Sawayama 1999). Algae incorporate dissolved inorganic carbon (DIC) into biomass (Knud-Hansen 1998). The four main forms of DIC generally present in algal ponds are CO_2, bicarbonate (HCO_3^-), carbonic acid (H_2CO_3) and carbonate (CO_3^{2-}) (Knud-Hansen 1998, Goldman & Shapiro 1973). The growth rate of algae is controlled by the total inorganic carbon concentration (C_T). $C_T = CO_2 + H_2CO_3 + HCO_3^- + CO_3^{2-}$ (Goldman & Shapiro). Carbonic anhydrase (CA) is the enzyme which catalyses the interconversion of CO_2 and H_2O to HCO_3^- and H^+ protons and vice versa (Tzuzuki & Miyachi 1989). CA activity has been reported in most algae on the cell surface and in the chloroplasts. Its location within the cell and working mechanisms differ from species to species (Tzuzuki & Miyachi 1989). CA on the cell surface is important for the conversion of HCO_3^- into CO_2, which is subsequently absorbed by algae. Algae in a high CO_2 environment (6 %) assimilate only CO_2, whilst algae in a low CO_2 environment use both HCO_3^- and CO_2 (Tzuzuki & Miyachi 1989). The enzyme Ribulose-1,5-bisphosphate carboxylase oxygenase (RuBisCO) then converts CO_2 molecules into energy rich molecules such as glucose. The concentration of HCO_3^- ions is pH-dependent, and HCO_3^- ions are dominant at a pH of around 7.00 (Knud-Hansen 1998, Tzuzuki & Miyachi 1989). The synthesis of the CA enzyme is induced when the CO_2 concentration is low, and stops when the CO_2 concentration is raised during algal growth. CA may therefore also inhibit photosynthesis at high CO_2 concentrations since the transformation of CO_2 into HCO_3^- is accompanied by the formation of H^+. When the CO_2 concentration reaches a certain point, the pH in the stroma of the chloroplast will be lowered, and this will hamper CO_2 fixation. The pH in the stroma of isolated chloroplast from spinach leaves can actively fix carbon at a pH of around 8.00 (Hogetsu & Miyachi 1989). In addition to a low CO_2 concentration, light is essential for the induction of CA in *Chlorella* cells (Tzuzuki & Miyachi 1989). The carbonic anhydrase catalysed utilization of bicarbonate as a photosynthetic carbon source can cause an increase in pH (Goldman & Shapiro).

Carbon dynamics contributes to the pH of the medium as well as algal productivity (Knud-Hansen 1998). The three main routes that soluble CO_2 can enter the HRAPs are through: 1) the dissolution of CO_2 into surface water; 2) equilibrium reactions of dissolved CO_2; and 3) the decomposition of organic matter in HRAPs (Knud-Hansen 1998). The dissolution of CO_2 in water is sparing and forms carbonic acid (H_2CO_3), a weak acid that partially dissociates to HCO_3^- and H^+ (pKa_t = 6.30). This process can only form CO_3^{2-} if the solution in which it dissolves is strongly alkaline (pKa_2 = 10.33). This means that at a pH of above 11.00, most of the carbon in solution will be unsuitable for algal uptake. An increase in pH can cause the solubility of CO_2 to increase, as the CO_2 reacts directly with OH^- ions in solution to form HCO_3^-, and enable more atmospheric CO_2 to enter HRAP surface waters (Knud-Hansen 1998). When aquatic concentrations reach saturation levels, CO_2 is likely to return into the atmosphere (Knud-Hansen). The pH influenced the forms that DIC occurred in, and the

subsequent concentrations of free CO_2 and HCO_3^- ions that were available for assimilation into algal biomass. Carbon limitation is likely to occur at higher pH, which is why a pH closer to neutral is more conducive to improved algal productivity. Algal productivity was lower during winter, perhaps partly due to the increasing alkalinity that was observed in the culture medium (Figure 5.1.6.a). The pH increased from 9.30 (18 May 2010) to between 10.00 and 10.50 (7 September 2010) post-HRAP A2 and B2 by the end of winter at a constantly long HRT. Utilizable forms of DIC could have diminished during winter, which might account for the low algal productivity which was observed, along with other factors such as low temperature and shorter day length. The increase in pH might have been caused by the accumulation of OH^- ions because of a lower dilution rate. The pH at the end of summer was 10.00 post-HRAP A2 and B2, which was the same as during winter. Algal productivity remained high despite the high pH, which means that other factors such as temperature and light probably facilitated higher productivity during that time. The pH steadily decreased during optimization periods in autumn and summer. This decrease might be attributed to fewer OH^- ions that were present due to a more rapid dilution rate. The pH can also be lowered through organic decomposition that produces CO_2, or through the production of organic acids (Knud-Hansen 1998, Table 5.2.1.c).

Table 5.2.1.c: The distribution (%) of the three different forms of dissolved organic carbon at varying pH and a temperature of 25 °C (Knud-Hansen 1998).

pH	CO_2	HCO_3^-	CO_3^{2-}
5.00	95.70	4.30	0.00
6.00	69.20	30.80	0.00
7.00	18.30	81.60	0.00
8.00	2.20	97.40	0.50
9.00	0.20	95.30	4.50
10.00	0.00	68.10	31.00
11.00	0.00	17.60	82.40

The addition of carbon in the form of CO_2 could have enhanced algal productivity and also ensured that no carbon limitation would occur (Park *et al.* 2011). CO_2 availability in HRAPs primarily depends on the heterotrophic oxidation of organic compounds by bacteria (Park *et al.* 2011). The aerobic heterotrophic bacteria that oxidise organic matter in HRAPs generally have an optimum pH of 8.30. Above this pH, bacterial activity becomes increasingly inhibited. Domestic sewage typically contains insufficient carbon to completely support complete nitrogen removal (Park *et al.* 2011). The carbon-nitrogen ratio in wastewater is 3:7 compared to 6:15 in algal biomass (Park *et al.* 2011). When organic carbon in wastewater feed exceeds a biological oxygen demand (BOD) of 300 mg/L, carbon limitation is unlikely to occur (Park *et al.* 2011). BOD was not measured in this study, so it was

unknown whether carbon limitation occurred and what the effect of CO_2 addition on algal productivity in HRAPs might be.

pH influences nitrogen removal through the volatilization of NH_4-N into NH_3-N gas (Park *et al.* 2011). The amount of NH_4-N that can be removed in a HRAP system through NH_4-N volatilization increases as the pH increases above 9.50 (Idelovitch & Michail 1981, Dekker 2002, Park *et al.* 2011). In this study, NH_4-N volatilization was probably the main method for nitrogen removal due to the high pH that prevailed in HRAPs during winter and the low algal productivities reported. As the pH decreased with shortened HRTs in summer and autumn, nitrogen removal through higher algal productivities probably became more important (Park *et al.* 2011). The pH therefore not only influenced algal productivity, but also nitrogen removal efficiency in HRAPs. Garcia *et al.* (2000) used NH_4-N volatilization at a high pH as the main strategy for nitrogen removal from effluent, whilst Park *et al.* (2011) used increased NH_4-N uptake through algal assimilation at high algal productivities, short HRT and CO_2 addition, as the main method for nitrogen removal. A shorter HRT will reduce the area required to treat brewery effluent, which is why the short HRTs and the addition of CO_2 would be a feasible option to test in future studies.

PO_4-P precipitation is another process that depends on the pH in HRAPs (Dekker 2002). PO_4-P can precipitate with calcium, unchelated ferric iron and magnesium (Dekker 2002). A calcium concentration of more than 50.00 mg/L and a pH higher than 7.50 are necessary to facilitate precipitation (Dekker 2002, Powell *et al.* 2008, Park *et al.* 2011). A calcium concentration of 59.80 mg/L was measured in post-pilot plant AD effluent, and 33.80 mg/L was present in post-HRAP effluent at a pH higher than 7.50 (February 2010). Phosphate precipitation with calcium might therefore have occurred, as the calcium concentration was higher than 50.00 mg/L in post-pilot plant AD effluent at one time. Precipitates generally become more soluble as the pH is reduced at night, which means that more soluble PO_4-P will probably be found at night when the pH is low during respiration, and will precipitate again during the day as the pH rises (Knud-Hansen 1998). Dekker (2002) reported that as much as 80 % PO_4-P precipitated in HRAPs. Calcium was not measured throughout the study and it was therefore not possible to draw any firm conclusions regarding the precipitation of PO_4-P in HRAPs or other PO_4-P removal mechanisms. An increase in pH from 9.00 to 10.50 in HRAP A2 and B2 during winter and spring suggested that precipitation could have occurred if these elements were present.

High DO concentrations influence algal productivity (Park *et al.* 2011). The percentage DO saturation in this study ranged between 100 % and 150 % in HRAPs A2 and B2. DO saturation of 200 – 300 %

has reportedly reduced algal photosynthetic activity by 17 - 25 % (Park *et al.* 2011). The bacterial oxidation of NH_4-N firstly into NO_2-N and then into NO_3-N through nitrification, is one process that uses the DO produced by algae (Knud-Hansen 1998). Soluble PO_4-P can adsorb to calcium carbonate ($CaCO_3$), iron (Fe^{2+}) and aluminium (Al^{3+}) oxides. This happens more readily when a high DO concentration is present, and increases as the pH increases above 7.50 (Dekker 2002). One dry weight unit of algal growth can produce one and a half as much DO (Oswald 2003). HRAP effluent that is discharged into the environment will not lead to the depletion of oxygen in receiving water due to the high DO concentration, which might improve the health of aquatic ecosystems (Wells 2005). More research is required to find out how to make use of the large amount of oxygen that is produced in HRAPs (Oswald 2003).

The species composition of the algal culture and the presence of grazers and algal detritus influences algal productivity (Johnson 2010, Park *et al.* 2011). There was a build-up of detritus and the formation of aggregates of algae, bacteria, fungi and grazers by October 2010, after the HRAPs had been operational for 17 months under different HRTs. There were few algae species of the genera *Chlorella*, which is generally an indicator of a healthy population (Johnson 2010). The presence of these aggregates and zooplanktonic grazers (protozoa and Ostracods) supported the decision to revitalise the algae community in HRAPs by re-inoculating them with an actively growing algae community from EBRU on the 16th of November 2010. The combined effects of re-inoculation, high temperatures, long daylengths and short HRTs, were that algal productivities were the highest recorded for 2010.

Species control in HRAPs is desirable but not an easy task (Johnson 2010, Park *et al.* 2011). The reason why species control is desirable is that certain genera have qualities that make their propagation easier to manage and qualities for improved nutrient removal from an effluent treatment perspective (Park *et al.* 2011). Desirable attributes can include high productivity, tolerance to seasonal and diurnal variation in outdoor growing conditions, and species that form aggregates and therefore settle more readily through gravitation. This facilitates more cost-effective harvesting methods (Park *et al.* 2011). There are usually one or two dominant algal species in HRAPs. The dominant species in HRAPs at Project Eden was most probably *Pediastrum*, as these were the dominant species in the HRAPs at Project Eden, which were used to inoculate ponds at Project Eden (Johnson 2010). The dominant species can change in a transition period when algal productivity is low (Johnson 2010). Contamination from native species might therefore have occurred during the baseline phase and the winter of 2010 when algal productivity was low. The organic load in effluent is one of the main factors that influence the population composition in HRAPs. In one study, a BOD

of 250 mg/L resulted in the dominance of *Scenedesmus* whilst a BOD of 60 mg/L resulted in the dominance of *Miractinium* (Azov *et al.* 1980). Selective biomass circulation with the aim of increasing the population of easily harvestable algae, nutrient limitation and HRT control are methods that could potentially be employed to achieve at least partial species control (Benemann *et al.* 1977, Park *et al.* 2011).

Infestation of zooplankton grazers can have devastating effects on an algal culture (Johnson 2010). Rotifers and cladocerans have reduced algal populations by 90 % in two days (Johnson 2010, Park *et al.* 2011). The growth of zooplankton can be controlled by short HRTs (Benemann *et al.* 1977, Johnson 2010) and leads to their wash-out. The long HRTs that were used during the baseline phase and during the winter of 2010 may have created suitable conditions for a change in the dominant species, allowed the establishment of native species and the infestation of grazers. Shorter HRT operating procedures in the future might be used to manage the contamination of HRAPs with non-desirable species and zooplankton.

To conclude this section, it is recommended that warm temperatures, a short HRT, a pH of 8.00 and CO_2 addition could improve algal productivity (Figure 5.2.1.a). A shorter HRT would allow more light to reach cells resulting in improved algal productivity. A shorter HRT and a more diluted algal culture can also reduce the pH with the resulting decreased NH_4-N volatilization and PO_4-P precipitation, leading to higher uptake of these elements by algae. CO_2 addition lowers the pH and increases algal productivity by supplying algae with more DIC to utilise during photosynthesis. Although a lower pH might reduce nutrient removal processes such as NH_4-N volatilization and PO_4-P precipitation, it has been shown that this reduction in treatment can be offset by increased algal productivity and subsequent nutrient assimilation into biomass (Park *et al.* 2011). Species control remains a challenge. Monocultures in HRAPs can be maintained for three months before they become contaminated with native species or zooplankton (Park *et al.* 2011). Selective recycling of a portion of the harvested algae and maintaining a short HRT are currently the most practical ways of managing this problem (Johnson 2010, Park *et al.* 2011).

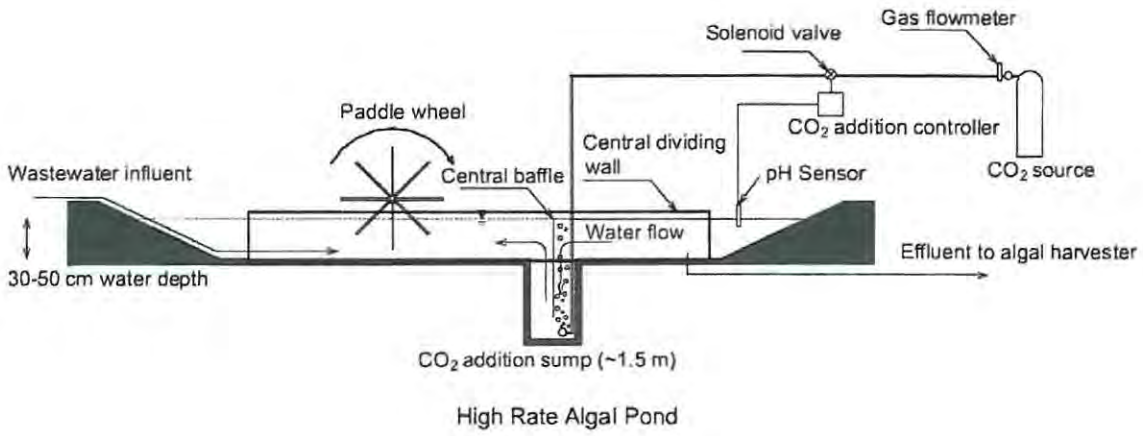

Figure 5.2.1.a: A high rate algal pond with carbon dioxide (CO_2) addition to enhance algal productivity (Park *et al.* 2011).

5.2.2 Effluent quality

The system was unable to produce an effluent that met the DWAF general limits for discharge into a natural water resource of 75 mg/L for COD (Table 1, Appendix 1). The mean COD in post-pilot plant AD effluent was reduced from 152.33 ± 4.85 mg/L to 95.00 ± 3.75 mg/L (HRAP A2), and to 100.82 ± 5.93 mg/L (HRAP B2). The HRAPs at EBRU were also unable to meet the DWAF general limits for discharge into a natural water resource for COD (Wells 2005). COD removal efficiencies in this study varied between 30.49 % and 44.38 % in the HRAPs. Wells (2005) reported 43 % COD removal in HRAPs and algal settling ponds, whilst El Hamouri *et al.* (1995) reported 31 % removal and Oswald (1990) 53 % removal. COD removal was better during the optimization phase with shorter HRTs (95-100 mg/L COD post-HRAP) than during the baseline phase (171.21 ± 7.99 mg/L COD post-HRAP). Insoluble organic content can cause an increase in the COD of HRAPs (Wells 2005). The medium was more diluted at a shorter HRT during the optimization phase, and therefore contained less organic matter. CODs were lower when algal biomass was removed from effluent (Wells 2005). Similarly, COD readings in all the system components were lower when a smaller pore size filter paper (0.45 µm versus 8 µm) was used to filter COD samples prior to sampling.

The HRAPs were successful at removing NH_4-N from effluent with all the HRTs that were tested. It was possible to produce an effluent that met the DWAF general limits for discharge into a natural water resource for NH_4-N of 6.00 mg/L (Table 1, Appendix 1). Incoming inorganic nitrogen decreased from a mean of 42.53 ± 1.38 mg/L in post-pilot plant AD effluent, to 1.70 ± 0.81 mg/L in post-HRAP effluent. The only exceptions occurred when minimum HRTs were tested. At this point NH_4-N concentration started to exceed the DWAF general limits for discharge into a natural water resource for NH_4-N of 6.00 mg/L (Table 1, Appendix 1). Results from this study indicated increased

productivity with shorter HRT in warmer temperatures, which decreased the pH and probably resulted in less NH_4-N volatilization which led to its appearance in post-HRAP treated effluent as NH_4-N due to saturation in algal uptake. The processes that could have facilitated the transformation and/or removal of nitrogen in HRAP effluent were:

1) NH_4-N volatilization at a high pH (Idelovitch & Michail 1981, Garcia *et al.* 2000, Dekker 2002);
2) the assimilation of NH_4-N into algal biomass (Park *et al.* 2011, Craggs *et al.* 2011). The transformation of NH_4-N into an unknown pool of soluble proteins occurs as a consequence of this reaction (Laubscher, *pers. comm.,* senior researcher at the Institute of Environmental Biotechnology (EBRU), Rhodes University, Grahamstown, June 2011).;
3) the conversion of NH_4-N into NO_2-N and NO_3-N by aerobic bacteria (Dekker 2002, Garcia *et al.* 2000); and
4)

All four of these methods were acknowledged as possible means for the transformation and removal of nitrogen. Environmental conditions in the HRAPs determined the nitrogen removal efficiency as well as the transformation of nitrogen into its different forms (Knud-Hansen 1998). NH_4-N volatilization was the main method of nitrogen removal at a high pH and a HRT between 3.00 and 10.00 d (Garcia *et al.* 2000). Forty seven per cent NH_4-N was removed through NH_4-N volatilization in a HRAP with a longer HRT, whilst 32 % was removed in an identical HRAP with a shorter HRT from an initial NH_4-N concentration of 51.00 ± 14.20 mg/L in the influent (Garcia *et al.* 2000). More NH_4-N volatilization occurred in the HRAP with a longer HRT (Garcia *et al.* 2000). Algal uptake was the second most important mechanism for nitrogen removal (Garcia *et al.* 2000). Algal uptake becomes more important as the pH is lowered and algal productivity increases at a short HRT (Park *et al.* 2011). CO_2 addition can cause more NH_4-N to be assimilated into algal biomass (Park *et al.* 2011). The addition of carbon in the form of CO_2 improves algal productivity, and also ensures that no carbon limitation would occur, which could otherwise inhibit the removal of NH_4-N through algal uptake at a low pH (Park *et al.* 2011). Algae can take up NH_4-N, NO_3-N and NH_3-N gas during their growth and reproduction. NH_4-N is generally the first choice of nitrogen to be taken up algae, as the incorporation of NO_3-N requires more metabolic energy and enzymatic activity (Knud-Hansen 1998).

NO_3-N concentrations post-HRAP generally remained below the DWAF general limits for discharge into a natural water resource of 15 mg/L for NO_3-N (Table 1, Appendix 1) in autumn and summer, but sometimes exceeded the limit in winter and spring when concentrations went up to 25 mg/L NO_3-N. Nitrification reactions occur when NH_4-N is still available after algal uptake and NH_4-N volatilization have occurred (Garcia *et al.* 2000). Nitrification accounted for 33 % of the total Kjeldahl

nitrogen removal (De Godos *et al.* 2009). NO_2-N concentrations were recorded post-HRAP when the HRT was short. NO_2-N concentrations between 1.00and 3.00mg/L were observed in autumn (April 2010) and up to 10 mg/L NO_2-N in summer (November 2010 – January 2011). Oxidised nitrogen concentrations of 12 mg/L occurred at a HRT between 3.00 and 10 d (Garcia *et al.* 2000). The NO_3-N concentration was 15 mg/L in a HRAP that was used to treat wine lees effluent (Dekker 2002). NO_3-N concentrations between 33 mg/L and 46 mg/L NO_3-N were recorded in HRAPs treating swine effluent in Spain (De Godos *et al.* 2009). The NO_3-N concentration was below the DWAF general limits for discharge into a natural water resource of 15 mg/L in HRAPs treating sewage, although NO_3-N removal was inconsistent (Wells 2005). Similarly, NO_3-N removal in this study was inconsistent and below 15 mg/L in autumn and summer with shorter HRTs. The increase in HRT during winter did not succeed in lowering the NO_3-N concentration in post-HRAP effluent to within the DWAF general limits for discharge into a natural water resource (Table 1, Appendix 1). Nitrification occurs during the winter when algal uptake of NH_4-N generally decreases, and a lower daytime pH reduces the amount of NH_4-N volatilization that occurs (Craggs *et al.* 2004). Nitrification is inhibited for the rest of the year, either due to a high pH in HRAP effluent, or because it is prevented due to a lack of substrate (Craggs *et al.* 2004). NO_3-N and NO_2-N concentrations possibly exceeded the DWAF general limits for discharge into a natural water resource (Table 1, Appendix 1) in winter due to a high HRT and lower pH. A shorter HRT can induce lower concentrations of these compounds by encouraging algal uptake of NH_4-N (De Godos *et al.* 2009). NO_3-N and NO_2-N concentrations above 100 mg/L can occur when the algal culture gets old or is grazed (Neba & Rose 2004). Nitrification can therefore be managed by maintaining a healthy algal culture and by operating HRAPs at a short HRT. If high nitrification persistently occurs, a denitrification unit can assist in removing excess NO_3-N from the effluent (Neba & Rose 2004, Chapter 2, Section 2.2.4)

The HRAPs were effective at removing PO_4-P from the effluent to within the DWAF general limits for discharge into a natural water resource for PO_4-P of 10 mg/L (Table 1, Appendix 1), with the exception of November 2010, when the fresh culture was inoculated. PO_4-P was absent from post-pilot plant AD effluent from the 2nd of March 2010 until the 20th of July, when it reappeared. From this date onwards the PO_4-P concentration in post-pilot plant AD effluent steadily increased from 10 mg/L (July 2010), to 47 mg/L (January 2011). Once PO_4-P reappeared in the effluent, its removal in the HRAPs was fairly consistent to below the DWAF general limits for discharge into a natural water resource for PO_4-P of 10 mg/L (Table 1, Appendix 1). The maximum PO_4-P concentration post-HRAP was 20 mg/L when the HRAPs were re-inoculated with a new algal culture. HRAPs have reportedly removed 30 % of the incoming PO_4-P concentration (Dekker 2002). Although HRAPs were efficient at removing 26 % of the incoming PO_4-P, it was still not able to produce and effluent that met the

DWAF general limits for discharge into a natural water resource (Table 1, Appendix 1) of 10 mg/L (Wells 2005). El Hamouri *et al.* (1995) reported 38 % PO_4-P removal whilst Cromar *et al.* (1996) reported 34 % removal. PO_4-P could have been removed from HRAPs through adsorption, precipitation and biological uptake (Knud-Hansen 1998). The amount of PO_4-P that precipitated with calcium, magnesium or unchelated iron probably decreased as the pH decreased when the HRT was shortened. Shorter HRT caused an increase in algal productivity, which possibly caused the biological uptake of PO_4-P by algae to become a more important mechanism for PO_4-P removal (Dekker 2002, Park *et al.* 2011). The amount of PO_4-P that is removed through precipitation can decrease with CO_2 addition by lowering the pH (Park *et al.* 2011). This decrease can be offset by increased algal productivity and assimilation into algal biomass (Park *et al.* 2011). PO_4-P removal in the HRAPs was generally good, but could potentially be improved by CO_2 addition and a short HRT (Park *et al.* 2011). Due to the erratic nature of the presence of PO_4-P in post-pilot plant AD effluent, further research is required to determine the PO_4-P removal efficiency at a constant short HRT. The exact mechanisms of PO_4-P removal could not be determined. Future research needs to measure calcium, magnesium and iron to determine the mechanisms of PO_4-P removal in the HRAPs at iBhayi brewery.

The disappearance of PO_4-P from post-pilot plant AD effluent for four months could not be explained. Phosphoric acid was being used as a cleaning detergent in the brewery from January – December 2010, so PO_4-P should theoretically have been present in the post-pilot plant AD samples (Viljoen, *pers. comm.,* brewing master, SAB Ltd. iBhayi brewery, SABMiller, March 2011). Complete PO_4-P precipitation in the iBhayi brewery AD could possibly explain the absence of PO_4-P in post-pilot plant AD effluent. However, complete PO_4-P - calcium precipitation could not have occurred as the pH in the iBhayi brewery AD was too low to allow it. PO_4-P reappeared in post-pilot plant AD and HRAP effluent after four months. If complete precipitation did occur, it would have continued to occur for the whole course of the optimization phase. A white precipitate blocked the pipes that connected the iBhayi brewery AD to the pilot plant AD in October 2010. This precipitate was removed manually and might have been a PO_4-P - calcium precipitate, although further analyses would be necessary to determine its composition. A hard substrate was found on the sides and floors of HRAPs when cleaned in October 2010. This precipitate could have been a PO_4-P precipitate, although further analysis would be necessary to determine its composition. A more practical explanation for the disappearance of PO_4-P from post-pilot plant AD effluent is that the PO_4-P sampling tablets might have been old or damaged, and did therefore not test efficiently for PO_4-P. An interesting observation was that algae were able to grow without PO_4-P during the four months when it was absent from post-pilot plant effluent, as productivity increased at the time (autumn optimization). The typical composition of an algal cell is $C_{106}H_{181}O_{45}N_{16}P$ (Park *et al.* 2011). The ratio

of N:P can vary from 4:1 to almost 40:1, depending on the algal species and nutrient availability in the culture (Park *et al.* 2011). Even though PO_4-P was absent from the HRAP during autumn, algal productivity increased with the shortened HRT. The algal species in HRAPs were therefore either able to grow with very little PO_4-P requirements, or PO_4-P was present in the effluent and was not effectively measured with the sampling procedure that was used.

Evaporation with a long HRT caused the concentration of dissolved salts and COD to increase. HRAP effluent was more concentrated during the baseline phase when a long HRT was used than during the optimization phase when shorter HRTs were used and the medium was more diluted. The result of water loss was that the medium became more concentrated, which subsequently had an effect on the COD. Shorter HRTs could assist in producing an effluent with lower COD because of being more diluted. The HRAPs did not remove dissolved salts from the effluent as their growth does not require dissolved salts. Apart from salinity reduction HRAPs can produce a quality effluent to compliance with surface water discharge standards (Dekker 2002).

To conclude, the HRAPs were effective at maintaining an effluent that met the DWAF general limits for discharge into a natural water resource of 1998 (Table 1, Appendix 1) for nitrogen and PO_4-P at most times, although the ranges exceeded the limits during abnormal intervals such as when the maximum dilution rate had been reached, or when a new culture was inoculated into the HRAPs. Even though the COD removal efficiency post-HRAP improved with shorter HRTs during the optimization phase, it did not meet the DWAF general limits for discharge into a natural water resource for COD of 75 mg/L to allow the discharge of treated effluent into a natural water resource (Table 1, Appendix 1). The pH post-HRAP was generally close to the permissible maximum limit of 9.50, and occasionally a little higher (9.70). If algal cells were harvested from post-HRAP effluent, the pH could be reduced to within the range of 5.50 to 9.50. A shorter HRT could also produce an effluent with a lower pH. EC and Cl^- concentrations post-HRAP was significantly higher (2860 µS/cm) than the allowed discharge standard of 700 µS/cm. The HRAPs were therefore not effective at removing dissolved salts because algal growth did not assimilate it (Table 5.2.2.a).

Table 5.2.2.a: The quality of effluent produced in high rate algal ponds (HRAP) A2 and B2 from 2 March 2010 until 16 January 2011 compared to the Department of Water Affairs and Forestry's (DWAF) general limits for discharge into a natural water resource of 1998 (Table 1, Appendix 1, DWAF limit - right-hand column of this table).

Parameter	HRAP A			HRAP B2			DWAF limit
	Mean	Std. Err.	N	Mean	Std. Err.	N	
Temperature (°C)	21.07	0.31	185	18.78	0.30	185	
pH	9.68	0.04	182	9.84	0.04	182	5.5 - 9.5
Dissolved oxygen (mg/L)	10.26	0.19	145	11.88	0.82	144	
COD (mg/L)	95.00	3.75	82	100.82	5.93	82	75.00
Ammonia ionised and un-ionised (mg/L)	1.77	0.79	104	1.70	0.83	104	6.00
Nitrite (mg/L)	1.72	0.39	77	2.18	0.43	77	15.00
Nitrate (mg/L)	13.82	0.77	70	12.37	0.76	69	15.00
Ortho-phosphate as phosphorous (mg/L)	5.23	0.68	90	5.53	0.86	89	10.00
Chloride (mg/L)	546.53	15.77	80	542.97	15.38	79	
Electrical conductivity (µS/cm)	2867.51	24.53	181	2869.92	35.64	181	700.00
Algal productivity (kg/d)	1.19	0.12	161	0.11	1.23	161	

5.2.3 Design of the integrated system

Anaerobic digestion was a necessary primary treatment method to break down macro-molecules into micro-molecules that could be assimilated by algae in HRAPs through aerobic degradation processes (Figure 5.2.3.a, Angelidaki & Sanders 2004, Chapter 2, Section 2.2). The primary function of anaerobic digestion was COD removal (Wells 2005). The COD in brewery effluent typically varies between 2000 mg/L and 6000 mg/L (Simate *et al.* 2011). This measurement was decreased an average of 152.33 mg/L in post-pilot plant AD effluent.

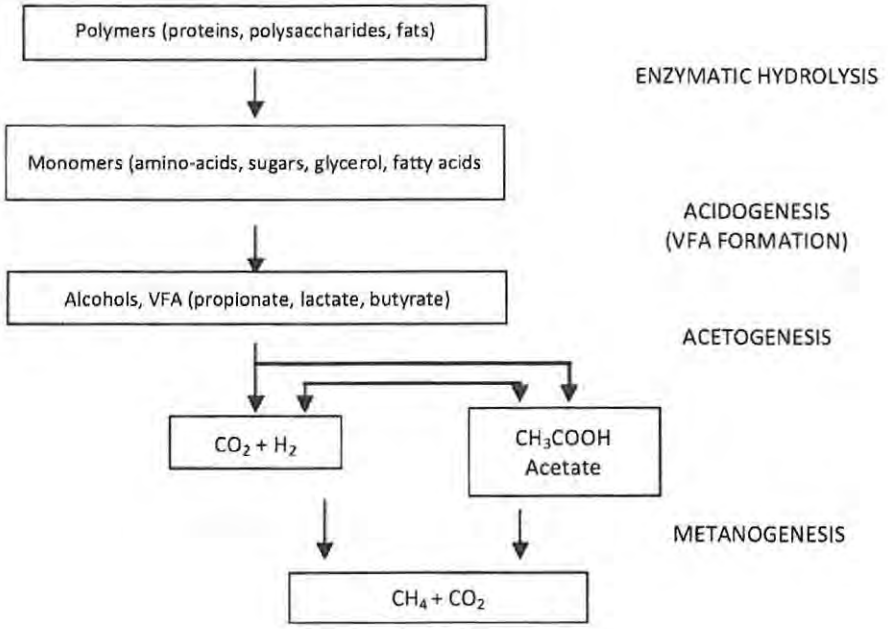

Figure 5.2.3.a: The anaerobic degradation of organic material (Wells 2005).

The primary facultative pond (PFP) was a necessary component in the integrated system. The most important function of the PFP at Project Eden was the sedimentation of organic material. Solids settled to the bottom of the PFP to form sludge (Wells 2005). The total suspended solids (TSS), COD and BOD were lower in HRAPs than in primary effluent because of particulate organic sedimentation in the PFP (Garcia *et al.* 2000, Wells 2005). A three day experiment was conducted to test the effect of excluding the PFP from the system (7 - 9 July 2010). Effluent was fed directly from the pilot plant AD into the HRAP system through an outlet pipe that lead directly into the splitter box post-PFP from the pilot plant AD. The result was that the splitter box and outlet pipes into HRAPs became blocked with microbial granules from the AD. The PFP therefore played an important role from a process perspective. Nutrient concentrations in the PFP were lower than in the incoming post-AD effluent, probably due to the sedimentation of solids. The mean COD removal in the PFP was 11.41 % of the incoming COD in pilot plant AD effluent during the optimization phase. The mean NH_4-N removal was 7.15 %, and mean PO_4-P removal was 24 % in the PFP (Table 5.2.3.a). The PFP therefore assisted in some nutrient removal. A PFP can also assist in the removal of metals when algae are recirculated into the PFP from HRAPs (Oswald 2003). Metals bind onto algal cells and settle to the bottom of the PFP (Oswald 2003). This concept can be applied when the algae from HRAPs are destined to become feedstock or fertilizer, and in an effluent that contains metals (Oswald 2003).

Aerobic and anaerobic functions can be combined by including a fermentation pit at the bottom of a PFP (Wells 2005). A fermentation pit can ensure the complete fermentation of solids and eliminate sludge removal over a period of 20 to 30 years (Wells 2005). Since an anaerobic digester was included in the integrated system at Project Eden, it will probably not be necessary to include a fermentation pit in future PFP designs, as the anaerobic degradation of macro-molecules and subsequent COD removal function was already incorporated in the iBhayi brewery AD. The term facultative implies that water conditions are aerobic near the surface and anaerobic near the bottom (Green *et al.* 1996). Algal growth in the top layers of a PFP enables the entrapment and oxidation of odour causing compounds (Wells 2005). Algal growth in the top layers of the PFP did occur at Project Eden, but was absent a lot of the time. CO_2 facilitates the growth of algae in the top layers of a PFP (Wells 2005). Anaerobic fermentation in the bottom layers of a PFP typically produces the CO_2 that supports algal growth in top layers (Wells 2005). It might be possible that the CO_2 concentrations were insufficient to support the growth of algae because the PFP was too shallow for anaerobic fermentation to occur (Wells 2005). Low DO and NO_3-N/NO_2-N concentrations, however, indicated that conditions in the PFP were mainly anaerobic.

The second (terminal) ponds in each HRAP train played an important role in nutrient removal. The mean NH_4-N concentration decreased from 5.95 ± 1.10 mg/L (HRAP A1) and 4.27 mg/L ± 1.11 mg/L (HRAP B1), to 1.27 mg/L ± 0.79 mg/L (HRAP A2) and 0.98 ± 0.83 mg/L (HRAP B2). The operation of HRAPs in series had little effect in further removal of COD. In this study, the algal biomass concentration was markedly higher in HRAP A2 and B2 than HRAP A1 and B1 respectively, in summer, but less so in the cooler months (Figure 5.1.4.a). The mean COD was also higher in HRAP A2 and B2 compared to their respective upstream ponds (HRAP A1 and B1) (Table 5.2.3.a). This suggests that there is a link between algal biomass concentration and COD in the HRAPs. Thus, maintaining shorter HRTs should lower the COD in the HRAPs through reducing the accumulation of algal biomass and also through diluting the effluent. The effects of higher algal productivities stimulated through CO_2 addition should be tested with shorter HRTs, to see whether this will lower COD in the HRAPs (Craggs *et al.* 2011).

Table 5.2.3.a: A summary of effluent treatment in an integrated system that consisted of an anaerobic digester (AD), primary facultative pond (PFP) and high rate algal ponds (HRAP) A1, A2, B1 and B2 for chemical oxygen demand (COD), ammonia (NH_4-N), nitrate (NO_3-N) and phosphate (PO_4-P) removal. All values represent mg/L.

	COD	NH_4-N	NO_3-N	PO_4-P
Post-AD	157.07	42.51	1.79	16.18
Post-PFP	136.58	40.81	1.73	12.60
Post-HRAP A1	86.82	5.95	15.16	8.52
Post-HRAP A2	95.45	1.27	13.22	5.65
Post-HRAP B1	93.40	4.27	15.00	6.74
Post-HRAP B2	105.95	0.98	11.83	7.32

In summary, anaerobic digestion was found necessary to reduce the organic loading in brewery effluent through remineralisation. Integration of an algal culture step significantly reduced the nutrient loading in the effluent generated through the anaerobic digestion process, although further reduction in COD was less successful. The PFP was found to serve an important process step in PO_4-P removal and in settling organic particulates leaving the AD. The HRAPs significantly reduced NH_4-N and PO_4-P in effluent in the warmer months when HRTs were shorter, but were less successful in reducing NO_3-N in the colder winter months when HRTs were longer. The second ponds of the HRAPs series (A2 and B2) served as polishing facilities, where further NH_4-N and PO_4-P removal took place. Higher algal biomass in the terminal HRAP ponds could be linked to unsatisfactory reductions in COD. The cost estimations for erecting a full-scale HRAP system for the treatment of brewery effluent (1041 m^3/d) is provided in Table 5.2.3.b (courtesy of Peter Britz and SAB iBhayi brewery).

5.2.4 Future research

Future data collection that could contribute further to our understanding of HRAP systems in the treatment of brewery effluent include:

1) The determination of total and soluble COD.
2) Batch experiments to determine the relative importance of phenomena such as ammonia volatilization and phosphate precipitation, under the conditions prevalent in the HRAPs.
3) Sample ponds on a more frequent basis, examine samples microscopically, take photographs and identify species. It is the algal population that is responsible for the nutrient removal, so additional information on which were the dominant species and whether these changed as a function of media composition or season would have added significant value.
4) A material balance approach can provide valuable insight, particularly in terms of explaining the trends in nitrogen and phosphorous removal. A generic, elemental formula, for algal biomass is presented in the literature review. Combining this with the algal productivity data

could allow the determination of a theoretical value for how much nitrogen and phosphorous would have had to be assimilated in order to support the measured algal productivity.

5) Light measurement to study the influence of light
6) By using thermodynamic constants, calculate whether calcium phosphate would precipitate.

Chapter six
Conclusion

The two-year pilot phase of Project Eden at iBhayi brewery demonstrated that it was possible to treat effluent with an integrated anaerobic digester (AD)/primary facultative pond (PFP)/high rate algal pond (HRAP)/wetland system. The chemical oxygen demand concentration was consistently lowered during the optimization phase, although not to within the 75 mg/L range as stipulated in the Department of Water Affairs and Forestry's (DWAF) general limits for discharge into a natural water resource of 1998 (Table 1, Appendix 1). Nitrogen and phosphate removal was lowered to within the DWAF range, with exceptions during unusual incidents such as when the HRAP system was pushed with increased volumes of effluent until the NH_4-N concentration increased to above 6.00 mg/L, or during the re-inoculation of the ponds, when the PO_4-P concentration was initially above 10.00 mg/L. The electrical conductivity (EC) concentration was consistently higher than the allowed DWAF limit, as algae do not assimilate salts.

Potential benefits of the HRAP system apart from its use as a tertiary wastewater treatment system are that it sequesters carbon dioxide (CO_2), and that it produces algae that could be used in the generation of methane (CH_4)-rich biogas through anaerobic digestion, other biofuels, animal feeds, fertilizers and pharmaceutical products. HRAPs represent a cost-effective and low-maintenance technology to treat brewery effluent after it has been anaerobically digested.

The most important limitations of the HRAP system were:

1) that it did not succeed in reducing the COD to within the DWAF general limits for discharge into a natural water resource of 1998 (Table 1, Appendix 1);
2) that a large surface area would be required for a commercial HRAP plant;
3) that the algal cultures' vitality degraded over time with a subsequent reduction nutrient in removal efficiency; and
4) that it did not remove dissolved salts from the effluent.

There are ways in which these limitations could be mitigated, and, given the sound foundation that was laid by the pilot study, options to improve HRAP performance could be considered.

Ways to lower the COD measured in HRAP treated effluent exist. The choice of method will depend on what the treated effluent will be used for after it has been treated (Simate *et al.* 2011).

7) A shorter HRT might exclude some of the effects of evaporation that raises COD and dissolved salt concentrations and improve algal productivity (Craggs *et al.* 2011).
8) CO_2-addition could increase algal productivity and minimise the area that would be required to treat effluent (Park *et al.* 2011).

The presence of algae contributed to higher COD concentrations. HRAP treated effluent that contains algae will most likely not have a detrimental impact on the environment. The presence of algae in effluent can be beneficial to some receiving waters and in agricultural irrigation (Green *et al.* 1995). Algae can serve as an additional food source for benthic organisms (Craggs *et al.* 2004). The discharge of algal cells in effluent that has been properly treated may increase the productivity of fish and certain invertebrate species (Craggs *et al.* 2004, Wells 2005). Although the DWAF general limits for discharge into a natural water resource (Table 1, Appendix 1) stipulate that the COD in treated effluent should be less than 75 mg/L, HRAP treated effluent with a COD higher than 75 mg/L due to the presence of algal cells should still be allowed to be discharged into a natural water resource, or to be used for irrigation.

Flexibility on behalf of regulatory bodies should allow higher COD concentrations in HRAP treated effluent for the purposes of recharge or irrigation to enable the re-use of HRAP treated effluent. A written exemption would be required from the Department of Water and Environmental Affairs for the use of HRAP treated effluent according to the National Water Act, Act 36 of 1998, Section 21 (e): Irrigation of any land with waste or water containing waste generated through any industrial activity or by a waterwork; and/or Section 21 (f): Discharge of waste or water containing waste into a water resource through a pipe, canal, sewer or other conduit; and disposing in any manner of water which contains waste from, or which has been heated in, any industrial or power generation process. The DWAF Guidelines for the Utilisation and Disposal of Wastewater Sludge (Volume 2: Requirements for the agricultural use of wastewater sludge), Department of Water Affairs and Forestry (March 2006) provides guidance on the agricultural use of sludge.

By using the by-products from wastewater grown algae, it could potentially reduce the costs of HRAP systems. The applications for wastewater grown algae in the agricultural and energy sectors could make it economically feasible to invest in the harvesting of algae with inexpensive methods such as electrolytic flocculation or simple gravitational settling, as the by-products could generate additional income or save on costs otherwise. The majority of algae could be removed from effluent in this way. The small percentage of cells that are left in the effluent that cause an increase in the COD concentration of effluent, should not be a reason to discredit the use of HRAP systems in wastewater treatment, as nitrogen and phosphate concentrations are lowered to within DWAF

standards under normal conditions when the system is not being pushed with increased volumes of effluent, or re-inoculated.

One of the main conclusions that was drawn from the optimization phase of the study, was that the HRT was the main factor that influenced algal productivity in the warmer months. A shorter HRT caused higher algal productivity. With higher algal productivity, a smaller area is needed to treat a given volume of water using HRAP, because of a faster treatment rate. CO_2-addition could increase algal productivity and minimise the area that would be required to treat effluent (Park *et al.* 2011). A shorter HRT causes the medium to become more diluted, and that the effect of algal cells on COD is likely to be minimised. Even though the space requirements for integrated HRAP systems is more than those of conventional activated sludge (AS) systems, the benefits are those of reduced construction and operational costs, carbon fixation and the production of secondary products that could be used in the generation of CH_4 –rich biogas, biodiesel, animal feedstock or fertilizer (Park *et al.* 2011). HRAP systems require approximately 50 times more land area than AS systems, although this does not take into account the land needed for AS sludge disposal (Park *et al.* 2011). The construction costs, on the other hand, are less than half, and operational costs less than one fifth of those of AS systems (Park *et al.* 2011). The effect of CO_2 addition on algal productivity and a short HRT could be tested as means to reduce the space requirements for the treatment of brewery effluent in a commercial HRAP system. This would address two problems: the problem of a high COD concentration, as well as the problem of space.

The importance of a healthy algal culture was highlighted earlier. Excessive NO_3-N and NO_2-N concentrations can occur in grazed or old cultures (Neba & Rose 2004), which can hamper total nitrogen removal from effluent and subsequently lead to eutrophication in receiving water bodies. It would therefore be beneficial from a commercial point of view to monitor the species composition in HRAPs and to ensure that a healthy culture is permanently maintained that can be used to re-inoculate the ponds when it becomes necessary. A short HRT can assist in the prevention of contamination from native species and/or grazers (Johnson 2010, Park *et al.* 2011).

An option of how the problem of insufficient dissolved salt removal in HRAPs could be mitigated is to discharge saline HRAP treated effluent into a saline estuary if it meets the DWAF general limits for discharge into a natural water resource of 1998 (Table 1, Appendix 1) for the remaining constituents in effluent, apart from the electrical conductivity (EC) and COD. The DWAF regional manager stated that an exemption with regard to meeting the limit for dissolved salt discharge could be issued to iBhayi, as dissolved salt disposal into a saline estuary is unlikely to have a negative environmental impact (Britz, *pers. comm.*, Department of Ichthyology and Fisheries Science, Rhodes University, 15

August 2011). Discharging treated saline effluent into a saline estuary is one way to manage the brewery's treated effluent and to lower the cost of sending effluent to the municipal sewer. The problem with discharging effluent into an estuary is that valuable freshwater will be lost that could have been re-used in local production processes or irrigation. This will create the subsequent necessity to purchase additional freshwater at a high cost. The water that supplies the Nelson Mandela Metropolitan Municipality comes from surrounding catchment areas, but is also transported from the Orange River (Cloete *et al.* 2010). The cost of purchasing water from the municipality is probably going to increase in the coming years. The re-use of water would create improved economic and environmental resilience.

Project Eden has demonstrated that HRAP systems can produce an effluent that meets the DWAF general limits for discharge into a natural water resource of 1998 (Table 1, Appendix 1) for nitrogen and phosphate, which are the main nutrients that lead to eutrophication, as well as other micro pollutants. If construction and Research and Development capital investment is less than the cost of purchasing and treating water with an AS system for the next twenty years, it could justify further investment into the development of an integrated HRAP effluent treatment system that is able to produce water suitable for re-use in the production of beer, cleaning-in-production and for irrigation.

The development of an integrated effluent treatment system that is able to recycle effluent 100 % into drinking water would enable SABMiller to realize its sustainable water management goals. Wastewater can be recycled and re-used with the combined benefit of the production of valuable secondary products with economic and social value. This refers to the beneficiation of wastewater. Integrated systems work on the principle that the waste product of one process is applied as the fuel that drives the next. Complex dissolved organics can be broken down into simple dissolved organics that can subsequently be used by algae, fish, macrophytes or vegetables as the nutrient that supports their growth, and so the beneficiation of wastewater is realized. Dissolved salts in brewery effluent can be used to generate electricity in microbial fuel cells (Feng *et al.* 2008, Wang *et al.* 2008, Riley *et al.* 2010), and the CH_4-rich biogas produced during anaerobic digestion can be used as a renewable energy source. The beneficiation of wastewater could assist in realizing SAB Ltd.'s goal of sustainable water management (Appendix 2).

Sustainable water management requires a shift in the traditional management mindset and creative solutions. It also requires flexibility on the behalf of government regulatory bodies. Partnership between government, industries, communities and non-governmental organizations are needed to facilitate the transition. In the greater scheme of things, multi-stakeholder partnerships can combine

more resources to assist in the implementation of sustainable water management. Such partnerships can spread the responsibility evenly amongst the players involved in its management, and create a learning platform that can serve as an example for similar initiatives to draw from in the future.

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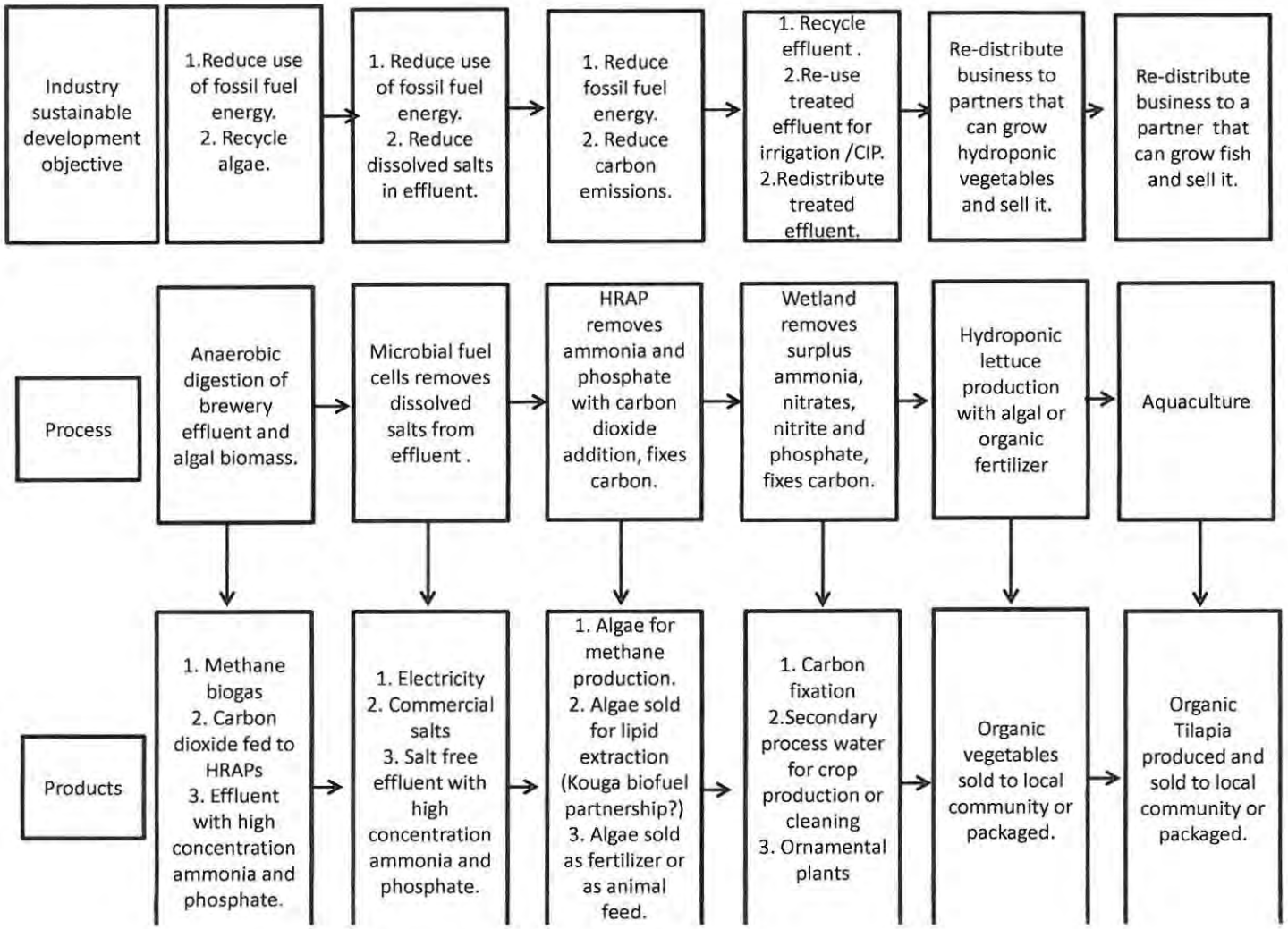
Appendix 1

Table 1: The Department of Water and Environmental Affairs general limits for discharge into a natural water resource. *Revision of the general authorizations in terms of Section 39 of the National Water Act, 1998: The Department of Water Affairs and Forestry's general limits for discharge into a water resource*, 26 March 2004, National Gazette No. 26187).

Substances/Parameter	General limit	Special limit
Faecal Coliforms (per 100 mL)	1000.00	0.00
Chemical Oxygen Demand (mg/L)	75.00 (i)	30.00 (i)
pH	5.50 - 9.50	5.50 - 7.50
Ammonia (ionised and un-ionised) as Nitrogen (mg/L)	6.00	2.00
Nitrate/Nitrate as Nitrogen (mg/L)	15.00	1.50
Chlorine as Free Chlorine (mg/L)	0.25	0.00
Suspended Solids (mg/L)	25.00	10.00
Electrical Conductivity (µS/m)	700 µS/m above intake to a maximum of 1500 µS/m	500 µS/m above background receiving water, to a maximum of 1000 µS/m
Ortho-Phosphate as phosphorous (mg/L)	10.00	1.00 (median) and 2.50 (maximum)
Fluoride (mg/L)	1.00	0.01
Soap, oil or grease (mg/L)	2.50	0.00
Dissolved orsenic (mg/L)	0.02	0.02
Dissolved Cadmium (mg/L)	0.01	0.00
Dissolved Chromium (mg/L)	0.05	0.02
Dissolved Copper (mg/L)	0.01	0.00
Dissolved Cyanide (mg/L)	0.02	0.01
Dissolved Iron (mg/L)	0.30	0.30
Dissolved Lead (mg/L)	0.01	0.01
Dissolved Manganese (mg/L)	0.10	0.10
Mercury and its compounds (mg/L)	0.01	0.00
Dissolved Selenium (mg/L)	0.02	0.02
Dissolved Zinc (mg/L)	0.10	0.04
Boron (mg/L)	1.00	0.50

(i) After removal of algae

Appendix 2


Industry sustainable development objective
1.Reduce use of fossil fuel energy. 2. Recycle algae.
1. Reduce use of fossil fuel energy. 2. Reduce dissolved salts in effluent.
1. Reduce fossil fuel energy. 2. Reduce carbon emissions.
1. Recycle effluent . 2.Re-use treated effluent for irrigation /CIP. 2.Redistribute treated effluent.
Re-distribute business to partners that can grow hydroponic vegetables and sell it.
Re-distribute business to a partner that can grow fish and sell it.
Process
Anaerobic digestion of brewery effluent and algal biomass.
Microbial fuel cells removes dissolved salts from effluent .
HRAP removes ammonia and phosphate with carbon dioxide addition, fixes carbon.
Wetland removes surplus ammonia, nitrates, nitrite and phosphate, fixes carbon.
Hydroponic lettuce production with algal or organic fertilizer
Aquaculture
Products
1. Methane biogas 2. Carbon dioxide fed to HRAPs 3. Effluent with high concentration ammonia and phosphate.
1. Electricity 2. Commercial salts 3. Salt free effluent with high concentration ammonia and phosphate.
1. Algae for methane production. 2. Algae sold for lipid extraction (Kouga biofuel partnership?) 3. Algae sold as fertilizer or as animal feed.
1. Carbon fixation 2.Secondary process water for crop production or cleaning 3. Ornamental plants
Organic vegetables sold to local community or packaged.
Organic Tilapia produced and sold to local community or packaged.