Environmentally Sustainable Beneficiation of Brewery Effluent: Algal ponding, Constructed Wetland, Hydroponic Vegetables and Aquaculture

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EXECUTIVE SUMMARY

1. Introduction and rationale

The treatment of industrial effluent remains an economically and environmentally costly liability to the majority of industries today. Conventional methods of water treatment usually require high-tech equipment that is expensive to run and needs to be operated by a highly skilled workforce. Furthermore, conventional water treatment methods are usually centralised and operated by the local authority, which results in large volumes of effluent being translocated away from the primary water user so that treated water is not available for recovery and reuse.

This project aimed to develop a sequence of effluent treatment methods using existing technologies, such as algal ponding and constructed wetlands, to develop a unique, low cost, low-tech, environmentally sustainable industrial water treatment process. It also aimed to combine these technologies with the production of algae, vegetables and fish in such a way that the end result was not only treated industrial effluent, but also the production of recovered water available for reuse and/or used for producing valuable downstream products. The project's goal was to take industrial effluent and, using little more than the sun's energy and photosynthesis, turn it into clean water, valuable algae, fresh vegetables and fish.

2. The system and how it fits together

The pilot effluent treatment and beneficiation plant utilised effluent originating from the Ibhayi Brewery (SAB Ltd) in Port Elizabeth. It was first subject to anaerobic digestion in a commercial-scale anaerobic digester that is currently used to treat part of the brewery's effluent stream. Post-anaerobic digester effluent was drawn into the experimental system's holding tank, after which it was subjected to treatment in the primary facultative pond (Figure 1). Post-primary facultative pond effluent was split into two parallel streams (train-A and train-B), each leading to two paddlewheel-driven, D-ended raceways, i.e. two high rate algal ponding systems. Each high rate algal pond train consisted of two raceways with water gravity-fed from the first to the second using an up-stand overflow pipe (Figure 1). Algae were removed from the post-high rate algal pond effluent using conical, algal settling tanks (Figure 1). Effluent could be drawn from any stage in the algal treatment process (i.e. post-primary facultative pond or post-high rate algal pond) for further treatment in the constructed wetland (Figure 2). Similarly, treated effluent could be drawn from any point in the high rate algal pond or constructed wetland systems and used for experiments in either the hydroponic vegetable production system (Figures 1 and 3) or in the fish culture system (Figure 1). Different combinations of treatment and post-treatment effluent use were tested (Figure 4).

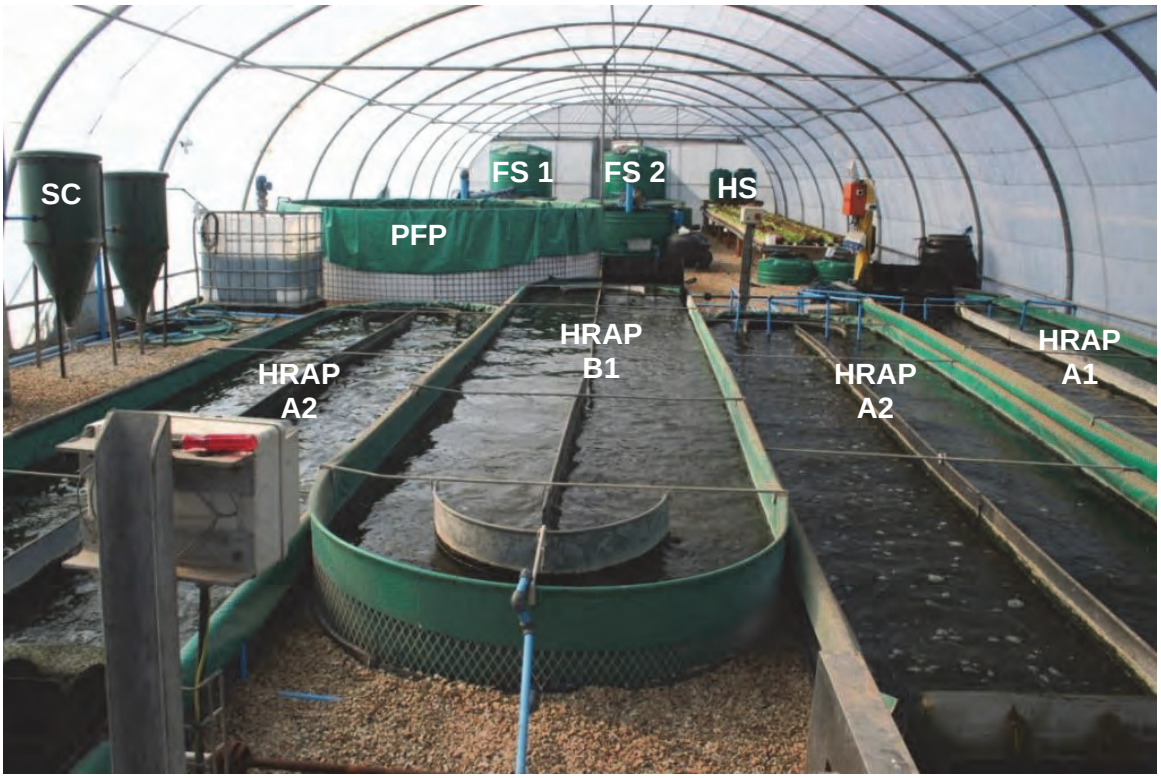

Figure 1 Brewery effluent flowed from the anaerobic digester into the primary facultative pond (PFP). The flow was then split into two trains of high rate algal ponds (i.e. HRAP train A and B), each with two ponds in series (i.e. HRAP 1 and 2). Algae were settled out in the settling cones (SC), and the remaining water was used in the hydroponic system (HS) and one of two aquaculture systems (FS 1 and 2). All the above were housed in a greenhouse tunnel (Photo: Rory Scheepers).

Figure 2 The constructed wetland used to treat brewery effluent subject to various pre-treatment processes (Photo: Rory Scheepers).

3. Algal ponding and constructed wetland: Baseline data collection

In the initial baseline studies it was demonstrated that the high rate algal pond/wetland system was a viable alternative to an activated sludge system, with a substantially lower environmental impact and lower operating costs than the more conventional method of treating effluent. The geographic footprint (i.e. the space) required to operate a full-scale high rate algal pond or constructed wetland system would be substantial, so optimising its performance was identified as a priority. The research that followed was thus aimed at increasing the flow of effluent through the systems without compromising the efficiency of nutrient removal, thus determining the minimum size of the physical footprint required to treat a given volume of effluent.

4. Optimising the performance of the high rate algal ponds

To optimise the rate of brewery effluent treatment in the high rate algal pond, the flow rate was progressively increased in one of the high rate algal pond trains, and this train's nutrient removal efficiency was compared to the second train in which flow rate was maintained at a rate known to efficiently remove nutrients. This experiment was repeated at different times of the year and in a heated and unheated high rate algal pond system.

In autumn it was possible to reduce the hydraulic retention time from 18.6 d to 3.8 d, and in summer to 2.5 d, i.e. a flow of 1400 L/d/high rate algal pond-train, without negatively affecting the rate of nutrient removal in the high rate algal pond system. The system's chemical oxygen demand decreased substantially when flow rate was increased; i.e. filtered chemical oxygen demand decreased from 140 mg/L post-anaerobic digester to 80 mg/L post-high rate algal pond (43% decrease). The lower chemical oxygen demand in the high rate algal pond at higher flow rates was probably due to the less dense algal biomass and the resultant increase in light penetration, and the photosynthetic efficiency of the algal cells in the system. Ammonia concentrations were lowered from around 30-60 mg/L post-anaerobic digester to close to 1 mg/L after the algal pond system at these faster flow rates. The system "crashed" (i.e. algal cells were washed out of the system at a faster rate than they were replaced and ammonia was not lowered to below the Department of Water Affairs general limit of 6 mg/L) when the hydraulic retention time in the high rate algal pond was lowered to 2.0 d in summer. Similar results were obtained in autumn.

In the winter and spring optimisation trials, however, it was initially not possible to decrease the hydraulic retention time to the same extent. There was evidence that the algal population was compromised at that time. When the optimisation experiment was repeated the following winter, after ensuring that the algal/bacterial community was not compromised, the hydraulic retention time was successfully reduced to 2.5 d, similar to that in autumn and summer. Although the algal cell cultures remained very thin (i.e. a low algal

biomass) with the increased hydraulic retention time in winter, these thin cultures remained highly efficient at removing nutrients from the effluent. In this final winter trial, NH_4-N was brought down from around 60 mg/L to about 3 mg/L and NO_3-N was also well within the Department of Water Affairs general limit of 15 mg/L. Similarly, chemical oxygen demand was lowered by around 30 to 50%, and in some cases it was close to the Department of Water Affairs general limit of 75 mg/L (filtered).

5. Optimising the performance of the constructed wetland

It became apparent during the baseline data collection trial that the full 60 m of the constructed wetland was probably not necessary for a final treatment stage at the flow rates used in that experiment (135±8.9 L/h). An analysis was subsequently done to determine the length of wetland needed to remove nutrients from post-high rate algal pond effluent, where effluent samples were taken after 13, 26, 39 and 52 m of gravel bed treatment and compared to the analysis at the inlet. The drop in pH (9.0 to 8.5) and ammonia (6 to 2 mg/L) levelled off within 13 m of the linear wetland and both parameters were well within the Department of Water Affairs general limits for the discharge of wastewater into a water resource. Nitrate continued to level off down the length of the raceway at a constant rate, with almost all the nitrate removed by the end of the raceway (10 mg/L at the inlet to 0.4 mg/L at the outlet). The rate at which chemical oxygen demand was lowered levelled off after about 26 m (i.e. after 34% reduction from 98 to 64 mg/L, filtered), at which point mean chemical oxygen demand levels were also within the Department of Water Affairs filtered general limit of 75 mg/L. It was concluded that the constructed wetland need not be more than half of its current length to treat post-high rate algal pond effluent at a rate of 135±8.9 L/h during daylight hours. Further work was required to (a) refine the required length of constructed wetland and (b) determine seasonal variation in the required hydraulic retention time in the constructed wetland.

The four channels in the constructed wetland were run in parallel in the trials that followed; i.e. four separate wetlands, each 15 m in length. The first of these trials was designed to determine the efficiency of the wetland in summer and spring, with effluent quality analyses carried out at the inlet and after 3, 6, 9 and 15 m. Almost all the ammonia was removed within the first three meters of the wetland, i.e. from around 1.0-1.7 mg/L to less than half that within three meters, and this was the same in spring and summer. Nitrate was lowered from 25-30 mg/L to well within the Department of Water Affairs general limit of 15 mg/L in spring. However, in summer nitrate was lowed in the first half of the constructed wetland but appeared to level off or increase after reaching the midway down its length, possibly due to nitrifying bacteria converting ammonia and nitrite into nitrate under warm summer conditions at a rate faster than the plants were able to remove it. Phosphate was lowered by approximately 50 % to around 22-35 mg/L, with most of this removal taking place in the first six meters of the constructed wetland. The pH was lowered from around 9.5-10 at the

inlet to between 7.5 and 8.5 within about six meters and this was the same in spring and summer. The filtered chemical oxygen demand was lowered to about 80 mg/L and salts increased in the system in both seasons due to evaporative losses. In summary, the majority of nutrient removal (particularly nitrogen and phosphate) occurred within the first 3-6 m of the wetland, and plant growth dropped off substantially down the length of the constructed wetland. It was hypothesised that the plants were probably nutrient-limited, and that an increase in nutrient load might improve the efficiency of the constructed wetland. The pH and possibly limited phosphate levels in the constructed wetland might have been responsible for the limited uptake of nitrate in summer, but this remains to be tested.

A trial was subsequently designed to determine if high rate algal pond pre-treatment was necessary prior to effluent treatment in the constructed wetland; i.e. to see if the constructed wetland efficiency might improve with the greater nutrient load in post-anaerobic digester effluent. It was found that the constructed wetland was able to treat brewery effluent drawn from the anaerobic digester (and subject to primary facultative pond treatment only) to a similar standard to effluent that was subject to high rate algal pond pre-treatment prior to the constructed wetland. This was found for all the water quality parameters measured. Furthermore, the increase in reed biomass was substantially greater in the treatment that received effluent directly from the anaerobic digester, i.e. when the high rate algal pond was removed from the treatment chain. These results suggest that it might be possible to cut the high rate algal pond system from the treatment process, without compromising the quality of the final effluent. However, it was not possible to remove the primary facultative pond from the system since the primary facultative pond was responsible for removing solid coagulants from the effluent that tended to clog the wetland. The footprint and cost of constructing a wetland required to treat equivalent volumes of water would need to be considered. Also, the longevity of the wetland should be taken into account. The organic load in a wetland is likely to build up slowly and parts of it may become anoxic with time (possibly over many years but maybe over a shorter period, depending on the organic load in the effluent), whereas a high rate algal pond can be cleaned and re-inoculated and brought back into full operation within days. Constructed wetland beds can, however, be replanted and revitalized.

6. Beneficiating brewery effluent

The project investigated the use of treated brewery effluent as a water source in aquaculture and hydroponic vegetable production (Figures 1 and 3). It also investigated the use of algae grown in the high rate algal ponds as a fish feed supplement, and the potential of harvesting energy in the form of methane from the anaerobic digester.

Brewery effluent can be used as a nutrient source for hydroponic lettuce (Figure 3) and tomato production. Plant growth in treated brewery effluent was not equivalent to the inorganic fertiliser control treatment, but production was increased significantly overall

when the pH of the treated brewery effluent was maintained between 6.0 and 6.5. The effluent-based solutions were able to provide all of the essential nutrients required for vegetative growth, flowering and fruiting suggesting that other vegetative, flower or fruit producing crops might be able to grow successfully in brewery effluent. The difference in growth was probably due to lower nitrogen levels, but this needs to be determined in future work.

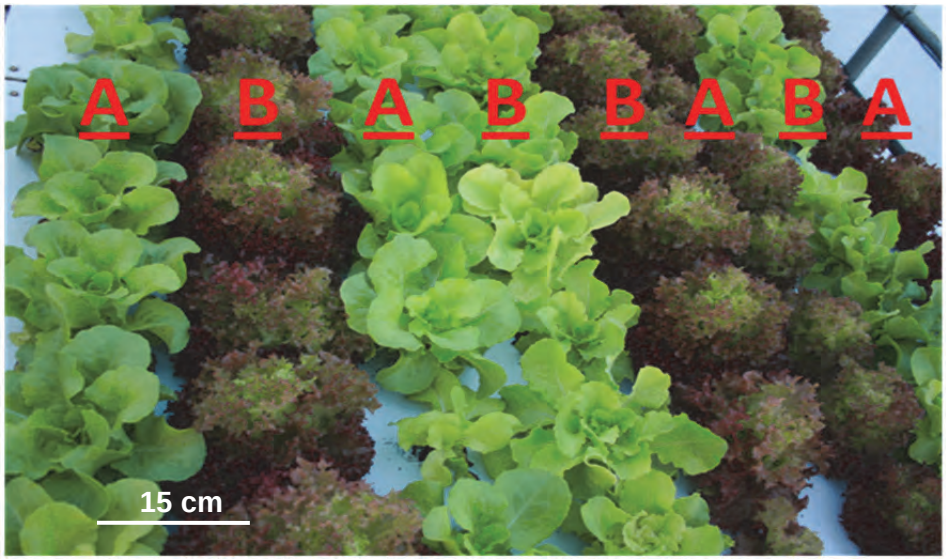

Figure 3 Lettuce grown in treated brewery effluent (channels marked A) and lettuce grown in municipal water using conventional inorganic fertiliser only (channels marked B) (Photo: Rory Scheepers).

The similarity of fish size, condition factor and reproductive output of adult and the growth of juvenile fish between treatments suggest that treated brewery effluent is a suitable water source for the culture of swordtail (*Xiphophorus helleri*). This conclusion was partly supported by the histological analysis, where fish in the treated effluent largely had healthier gill tissue, and were storing more energy as fat or glycogen in the liver. The liver tissue of these fish, however, showed evidence of environmental stress, so effluent might have negative effect on fish health over the course of their lives.

Algae harvested from the high rate algal pond brewery effluent treatment facility may be used as an effective protein replacement in various aquaculture feeds, such as Mozambique tilapia (*Oreochromis mossambicus*) and abalone (*Haliotis midae*). The degree of substitution was species and ingredient specific.

A desktop study was carried out to estimate the volume of methane (CH_4) that was produced in the brewery's anaerobic digester. Two methods were used to make this estimation: one was based on the chemical oxygen demand and other on the proportions of the different biogases produced in the anaerobic digester. Both methods estimated that the potential CH_4 harvest was in excess of a 1000 m^3 per day with an energy potential of

39.865 MJ/day. These estimates are based on a desk top study only and do not take all biological process into accounts and, as such, should be considered with this limitation in mind.

8. Concluding discussion

The high rate algal pond/wetland system is an environmentally sustainable method of treating brewery effluent that allows for the recovery of water and nutrients from the wastewater. It is a low-energy, low-maintenance system (both biologically and physically), driven mainly by gravity and the sun's energy. The only external energy inputs for the high rate algal pond system were two small (0.45 kW) motors that drove the paddlewheels. As such, the cost to build and operate the system could be recovered quickly and the potential exists to recover these costs even faster if the water and nutrients that are recovered are reused or sold.

The high rate algal pond and wetland system consistently brought most of the water quality parameters tested to within or close to the Department of Water Affairs general limits for the discharge of industrial effluent into a natural water resource. We also modelled these data to predict the success of this system under various conditions that might be applied to other industries (Appendix 1). Furthermore, the treatment/recovery process involved the production of downstream products such as algae; fish feed, fresh vegetables and fish. This program also saw the first attempt at optimising the use of industrial effluent as an inorganic source of fertiliser for hydroponic vegetable production.

Fish and vegetable production can take place using post-high rate algal pond water, or water that has been subjected to both high rate algal pond and constructed wetland treatment (Figure 4). Vegetable production can take place in post-anaerobic digester (i.e. without pre-treatment in the high rate algal pond or constructed wetland; Figure 4), provided it is subject to treatment in the primary facultative pond and provided that the pH of the medium is maintained between 6.0 and 6.5. The constructed wetland did not require pre-treatment in the high rate algal pond and operated more efficiently when high rate algal pond was not included in the treatment chain (Figure 4); however, it was not possible to exclude the primary facultative pond prior to treatment in the constructed wetland (Figure 4). The advantage of the wetland is that it is entirely self-sustaining but the disadvantages include difficult to clean/recharge, may clog up over time, and takes more time to commission, whereas the high rate algal pond can be inoculated and fully functional within days.

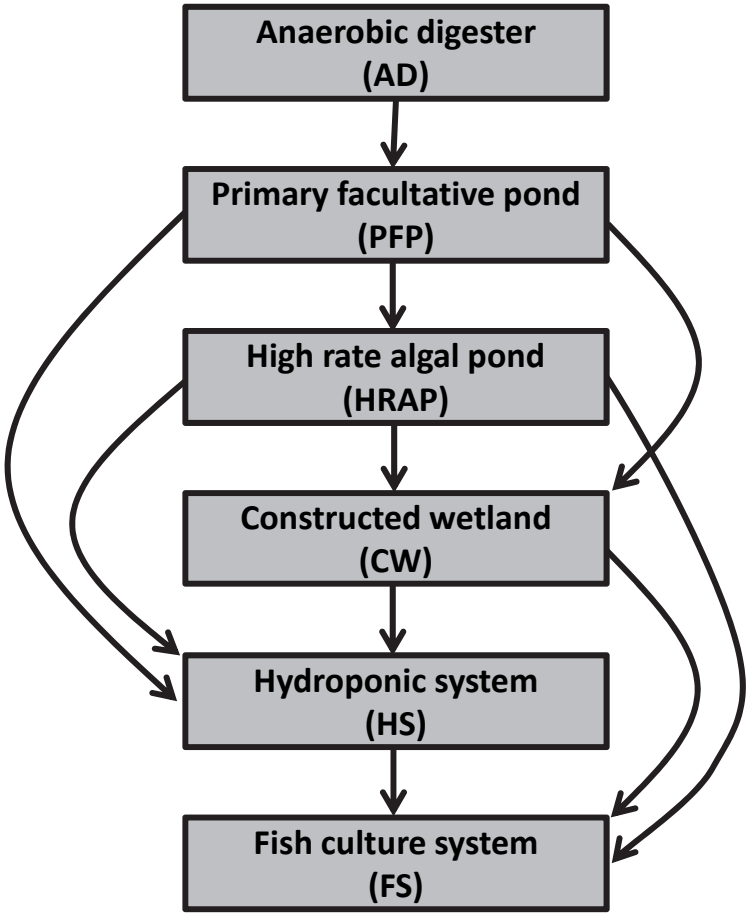

Figure 4 The primary facultative pond (PFP) was required to treat brewery effluent that came from the anaerobic digester (AD) prior to all other treatments and uses. Effluent did not have to be treated in the high rate algal ponds (HRAP) prior to treatment in the constructed wetland (CW). Vegetables were successfully produced in the hydroponic system (HP) in effluent drawn directly from the PFP and from the HRAP/CW. Fish culture in the aquaculture system (FS) needed effluent that had been treated in either the HRAP or the CW. Boxes not linked by an arrow suggest that the flow/use is not recommended

The downside of the high rate algal pond/constructed wetland system is that it takes up considerably more space than conventional methods of water treatment, for example activated sludge systems. The estimated area required to treat 1000 m^3 of post-anaerobic digester brewery effluent per day is probably around 1.4 to 2.0 ha. However, with improved efficiency and optimisation this footprint might be reduced further.

The programme has successfully demonstrated that industrial effluent, which is currently considered a costly liability by most industries, can be turned into a job-creating, income-generating stream, using simple technologies that have been available for years. It is just a matter of applying these technologies in a slightly different way.

Future work should consider:

- developing a better understanding of the dynamics within the algal ponds, the changes that take place to the algal/bacterial communities and the underlying mechanisms responsible for nutrient removal in the ponds;
- alternative methods of harvesting algae from the high rate algal ponds;
- adding value to the constructed wetland by growing crops instead of reeds;
- combining pH control with alternative methods of increasing the nitrogen that is available in the brewery effluent as a growth medium for hydroponically grown vegetables;
- determining the long-term (i.e. over the whole life cycle) effect of treated brewery effluent on the health of fish; and
- Scaling up the technologies tested here to pilot-commercial scale systems.

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SECTOR INVOLVEMENT

One of the aims of this project was to make the greater community aware of this work and its potential benefit to industry. In doing so, the intention was to introduce the concept that (a) industrial effluent can be treated using alternative, environmentally sustainable, low cost technologies, and (b) this process makes the water available for reuse on site or for use by downstream users in the immediate vicinity of the primary water user. This would (c) take pressure off the water treatment infrastructure currently used by the local authorities, and would (d) reduce the financial costs to the primary user by reducing their effluent treatment and primary water purchasing bill. Furthermore, (e) the treatment process is done by recovering nutrients locked in the effluent, and turning them into potential income-generating streams. These downstream effluent treatment/income-generating activities might not form part of the core-business of the primary water user, but (f) could be outsourced, with the potential of downstream industrial development and (g) job creation. Finally, the intention was to show that (h) it can be done. To do this, we presented the data and the conclusions that we were able to draw during the course of this project, to numerous groups within the sector.

In making the local authorities and industries aware of this work and its potential benefit, the second aim was to obtain "buy-in" and support from these role-players.

Rhodes University and SAB arranged a media launch designed to introduce this project to the public at large. The launch was hosted by the SAB Ibhayi Brewery on the 22 March 2011 and was attended by reporters from nine reporting agents, including newspaper, radio and television (Table 1), resulting in considerable media coverage (Table 2).

This work was presented to delegates from national, provincial and local government and industry from South Africa, as well as representatives from other countries in the region at the *Water Investment World Africa 2011* conference/workshop in Sandton, South Africa (25-28 October 2012). We also delivered three papers at international conferences, in Malawi, Italy and Egypt.

Numerous meetings were held with key role-players in industry and local, provincial and national government (Tables 3 and 4). There was consensus that the technologies had the potential to be successfully implemented and, based on the response by all the representatives at the meeting, the meeting achieved its goal of getting buy-in from the local and provincial government role-players.

Table 1 Attendance register at the Project Eden media launch held at the SAB Ibhayi Brewery) on 22 March 2011.

Media representatives	Company/Institution
Eleanor Seggie	Engineering News
Richard Caldiera	Waste Sewerage and Effluent Magazine (WASE Africa)
Phillip Hattingh	50/50
Ntokozo Mbuli	50/50
Lee-Ann Butler	The PE Herald
Reporter	Die Burger
Photographer	Die Burger
Mkhuseli Sizani	Daily Sun
Reporter	Bay FM
Tanya van Heerden	PE Express
Carmel Loggenberg	Algoa FM
Kholiswa Pemba	Algoa FM
Adrian English	SAB Central Office
Andre Fourie	SAB Central Office
Azure Janneker	SAB Central Office
Diarmaid de Burca	SAB Newlands Brewery
Dussie Padayachee	SAB Ibhayi Brewery
Geraldien De Villiers	SAB Newlands Brewery
Glynis Moodly	SAB Central Office
Sicelo Mabuza	SAB Ibhayi Brewery
Cliff Jones	Rhodes University
Martin Davies	Rhodes University

Table 2 A summary of the media coverage that resulted from the presentation that was delivered to the media at Ibhayi Brewery on 22 March 2011.

Media	Date/volume	Page number
Sunday Tribune	03-Apr-2011	12
Daily Sun (Port Elizabeth)	29-Mar-2011	11
Die Burger (Oos Kaap)	25-Mar-2011	8
Engineering News	15-Apr-2011	10
Port Elizabeth Express (North)	20-Apr-2011	5
Port Elizabeth Express (South)	20-Apr-2011	5
Port Elizabeth Express (Metro)	20-Apr-2011	5
Herald	23-Mar-2011	10
Simply Green	01-Mar-2011	12
Radio Algoa News	23-Mar-2011	Three broadcasts
50/50 (television documentary)	Not yet broadcast	
Water Sewage and Effluent	Vol 31 (5) – Sep 2011	38-46

Table 3 The list of representatives that attended the Eastern Cape branch of the Water Institute of Southern Africa (WISA) meeting/workshop on the 29 November 2011, hosted by SAB and Rhodes University.

Name	Organisation	Name	Organisation
P. Strassburg	Improchem	G. Foote	Nelson Mandela Metro
R. Blaauw	Nelson Mandela Metro	S. Mgudlwa	Nelson Mandela Metro
V. Slater	Sud-chemie	R. Shivambu	Nelson Mandela Metro
J. Roseveare	SAB	W.P. Mfebe	Nelson Mandela Metro
F. Karodia	Volkswagen SA	A. Mancotywa	Nelson Mandela Metro
P. Muller	Volkswagen SA	S. Thabethe	Nelson Mandela Metro
C. Bruntjies	Nelson Mandela Metro	O. Wentzel	
G. Mhlonyane	Nelson Mandela Metro	D. Zandberg	WISA
N. Mgadi	Nelson Mandela Metro	K. Ngesi	Nelson Mandela Metro
N. Sinyaya	Nelson Mandela Metro	S. Mabuza	SAB
V. Matwa		M. Davies	Rhodes University
L. Snyman	Nelson Mandela Metro	P. Britz	Rhodes University
B. Humani	Nelson Mandela Metro	C. Jones	Rhodes University
P. Matyolo	Nelson Mandela Metro	L. Crous	Rhodes University
F. Meltz	Nelson Mandela Metro	R. Scheepers	Rhodes University
P. Venter	SAB	H. Greeff	Sud-chemie
D. Brislin	Nelson Mandela Metro	M. Bekker	Volkswagen SA
D. Steyn	Nelson Mandela Metro	N. Oliphant	Nelson Mandela Metro

Table 4 A list of representatives that attended the stakeholder meeting hosted by SAB and Rhodes University at Ibhayi Brewery in Port Elizabeth on 1 December 2010.

Name	Organisation
Cliff Jones	Rhodes University
Martin Davies	Rhodes University
Peter Britz	Rhodes University
Rory Scheepers	Rhodes University
Andre Fourie	SAB Ltd (Director of sustainability)
Sicelo Mabuza	SAB Ltd
Andrew Lucas	Department of Water Affairs (Director, Easter Cape)
J. Jacobs	Department of Water Affairs (Eastern Cape)
Renald Nell	Department of Water Affairs (Eastern Cape)
A.M. Mancotywa	Nelson Mandela Metro
J.C. Kritzinger	Nelson Mandela Metro

Overall, the objective of making the sector aware of this project by engaging with local, regional and international role-players was achieved. The potential benefit of the technology developed in this project was demonstrated, and the concept was well-accepted by key role-players in industry, local, provincial and national government. The technology needs to be demonstrated on a commercial scale before further sector engagement is possible.

CONTENTS

Acronyms and Abbreviations

AD	Anaerobic digester
AS	Activated sludge
COD	Chemical oxygen demand (filtered)
CW	Constructed wetland
DIFS	Department of Ichthyology and Fisheries Science, Rhodes University
DWA	Department of Water Affairs
EBRU	Institute of Environmental Biotechnology Research Institute, Rhodes University
EC	Electrical conductivity
EQ	Equalisation tank
FS	Fish aquaculture system
FCR	Food conversion ratio
GSI	Gonadosomatic index
HRAP	High rate algal pond
HRT	Hydraulic retention time
HIS	Hepatosomatic index
PAS	Periodic acid Schiff histological slide stain protocol
PER	Protein efficiency ratio
PFP	Primary facultative pond
PVC	Polyvinylchloride
SAB	South African Breweries Ltd
SASSI	South African Sustainable Seafood Initiative
SC	Settling cone
TDS	Total dissolved solids
WWF	World Wildlife Fund

1. INTRODUCTION

The treatment of industrial effluent need not be an expensive process, but it remains an economically and environmentally costly liability to the majority of industries today. Conventional methods of water treatment usually require high-tech equipment that are expensive to run and need to be operated by a highly skilled workforce. Furthermore, conventional water treatment methods are usually centralised and operated by the local authority, which results in large volumes of effluent being translocated away from the primary water user so that treated water is not available for recovery and reuse. The overall aim of this project was to investigate the potential of using environmentally and economically sustainable technologies to treat and recover industrial effluent, using brewery effluent as the model.

Breweries produce a substantial effluent stream, rich in organic matter originating from the brewing process, which in most cases is sent to municipal sewage works after reduction of the chemical oxygen demand (COD) by means of anaerobic digestion. For example, South African Breweries Ltd (SAB) uses about 10.5-million m^3 of water per annum and approximately 70 % of this is discharged as wastewater. The SAB Ltd Ibhayi Brewery in Port Elizabeth (this project's study site) produced approximately 1800 m^3 effluent per day, which is disposed of through the municipal sewage works at a cost of R2-million/year to the brewery.

SABMiller Ltd has implemented a comprehensive set of sustainability objectives which include: working towards zero waste operations; making beer with less water (the group average is 4.56 L water per litre of beer); and reducing its energy and carbon footprint (which was 12.7 kg CO_2/hL beer). Recycling of the SAB Ltd effluent will contribute significantly to achieving these objectives, but environmentally sustainable "turnkey" effluent recycling systems are not available. SAB has thus entered into to a technology partnership with Rhodes University to develop knowledge to build systems which will treat brewery effluent and produce water and nutrient in a usable form. This project formed part of that programme.

Our programme included a multidisciplinary approach to generate the knowledge required for treating brewery effluent using (1) anaerobic bacterial digestion, (2) an algal ponding system and (3) hydroponic vegetable production to sequester the remaining nutrients from the effluent. Beneficiation included (4) growing hydroponic vegetables using nutrients in the effluent, (5) growing and harvesting algal biomass, (6) aquaculture of edible fish and high-value aquarium fish in the recovered water, and (7) production of fish feed containing the algal biomass grown in the treatment process. In addition to this, (8) the excess recovered water will be available for use in the brewery or for use in other downstream applications.

The unique aspect of this project was the sequencing and integration of the proposed effluent treatment and beneficiation technologies, which resulted in a novel approach to the way industrial effluent was processed and its constituents made available for reuse and

beneficiation. The challenge was to understand the dynamics of the parameters determining the rate processes and mass dynamics of the systems and how these change when the different processes were used together.

This project aimed to develop a sequence of effluent treatment methods using existing technologies, such as algal ponding and constructed wetlands (CW), to develop a unique, low cost, low-tech, environmentally sustainable industrial water treatment process. It also aimed to combine these technologies with the production of algae, vegetables and fish in such a way that the end result was not only treated industrial effluent, but also the production of recovered water available for reuse and valuable downstream products. The project's goal was to take industrial effluent and, using little more than the sun's energy and photosynthesis, turn it into clean water, valuable algae, fresh vegetables and fish.

The system that was used in this project is introduced in Chapter 2, together with an overview of our experimental approach. Baseline data of the high rate algal pond and CW are presented in Chapter 3 and this is followed by a series of trials where the performance of the high rate algal pond (HRAP) and CW were optimised (Chapters 4 and 5). Beneficiation of brewery effluent is the focus of the chapters that follow where we investigate the use of treated brewery effluent to grown vegetables hydroponically (Chapter 6) and produce fish (Chapter 7), and the use of effluent grown algae in fish and shellfish feed (Chapter 8) and the potential of harvesting methane from the treatment process (Chapter 9). The concluding chapters of this document deal summary of the human resources that were developed in this program (Chapter 10) and a summation of the research work including the potential of scaling up the technology to a commercial scale (Chapter 11). Appendix 1 includes an overall analysis of the data collected from the HRAP and CW over the full three years of this project. This section aimed to describe trends and to highlight environmental variables that influence the treatment process. The purpose of these analyses was to make these data available for future comparison with other work and to evaluate these system's potential for alternative applications in the future.

2. THE SYSTEM AND HOW IT FITS TOGETHER

The system included the brewery's existing anaerobic digester (AD) and a series of other treatment processes (Figure 2.1). These included a holding tank (Figure 2.2), a primary facultative pond (PFP; Figures 2.1 and 2.3), a splitter box (Figure 2.4) and two series of high rate algal ponding systems (HRAP; Figures 2.1, 2.5 and 2.6), algal settling cones (Figure 2.7), a constructed wetland (CW; Figures 2.1, 2.8 and 2.9), an aquaculture system (Figures 2.1, 2.11 and 2.12) and one of two hydroponic systems (Figures 2.1, 2.13, 2.14, 2.15 and 2.16). Different combination of effluent treatment and use were tested to determine sequences of effluent treatment/use (Figure 2.1).

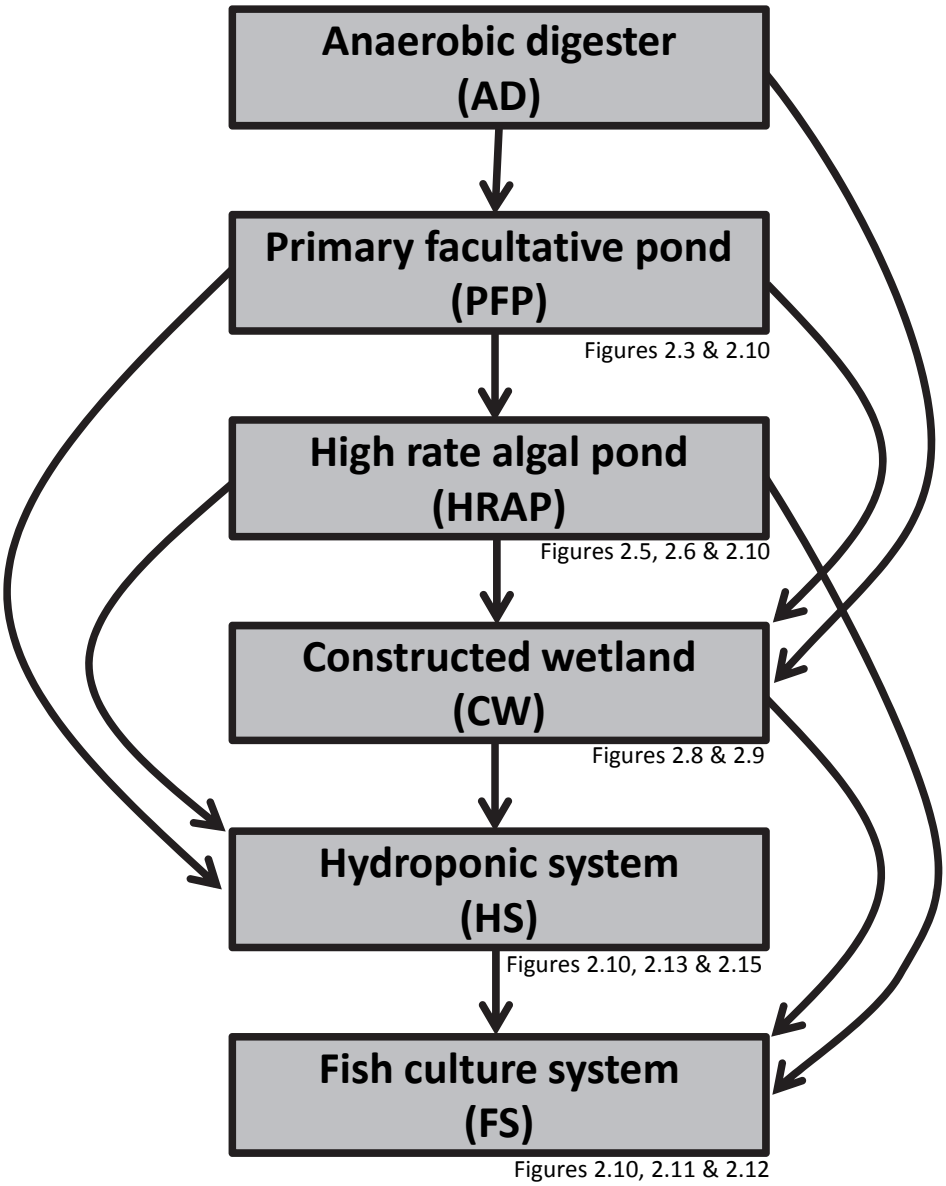


Figure 2.1 The sequence in which brewery effluent was treated or used by the different components that were tested in this project.

Effluent that had undergone a screening process was gravity fed from the Ibhayi AD into the project's 5000 L holding tank (Figure 2.2). The effluent was anaerobically digested by consortia of bacteria in the AD, which broke down complex organic molecules into smaller carbon dioxide (CO_2) and methane (CH_4). This lowered the effluent COD and re-mineralised nitrogen and phosphorous that was locked up in the organic-rich waste.

Figure 2.2 The 5000 L holding tank in which post-anaerobic digester (post-AD) effluent was held prior to entering the primary facultative pond (PFP) (Photo: Rory Scheepers).

The effluent was subsequently gravity fed from the top of the holding tank into the PFP where a broad spectrum of bacterial activity and opportunistic algal growth took place, further reducing the organic load of the effluent stream (Figure 2.3).

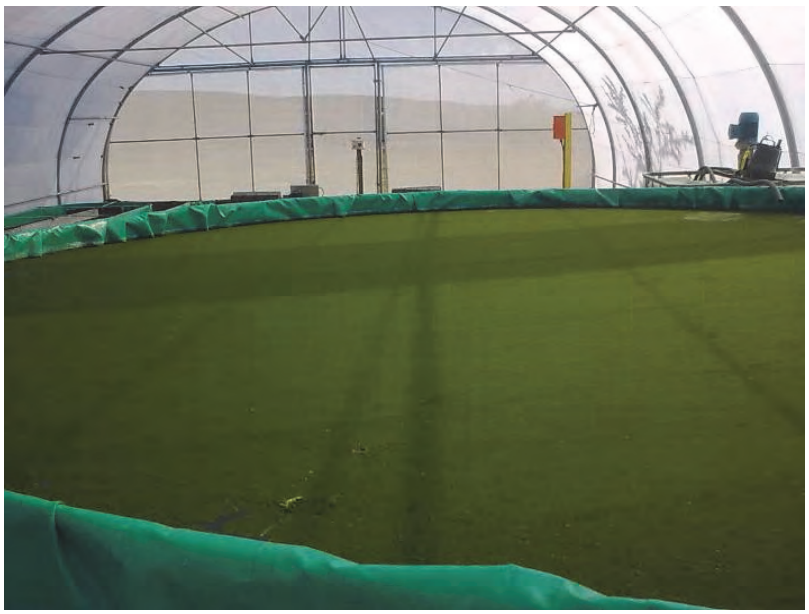


Figure 2.3 The primary facultative pond (PFP) with a dense layer of opportunistic algae growing on the surface (Photo: Rory Scheepers).

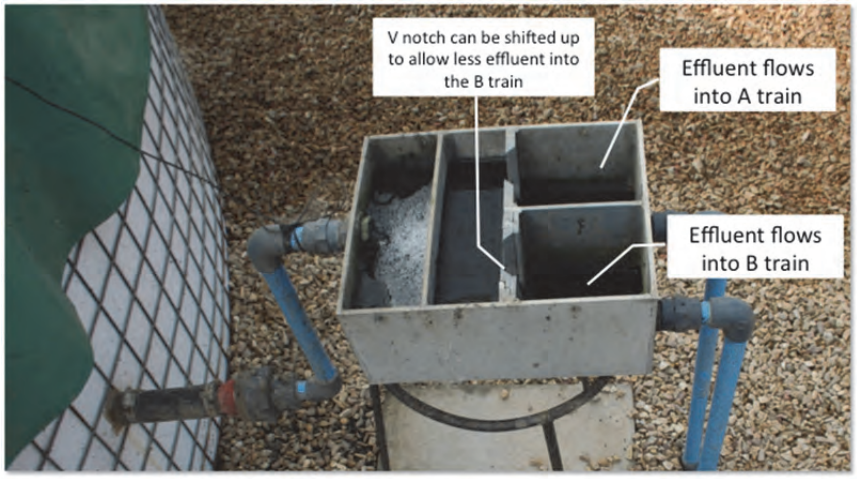


Figure 2.4 The splitter box that allowed us to adjust flow rates into the two trains of the high rate algal ponding system (HRAP) (Photo: Rory Scheepers).

The effluent stream left the PFP (Figure 2.3) into a splitter box where it was divided into two steams of equal flow (Figure 2.4), each of which passed into one of two series of HRAP's; i.e. train-A and train-B (Figure 2.5). These trains ran in parallel to each other and each train consisted of two D-ended ponds; effluent glowed by gravity from pond-A1 into pond-A2 and from pond-B1 into pond-B2, respectively (Figure 2.5). Each pond included a paddlewheel that was operated by a 0.45 kW motor (Figure 2.6).

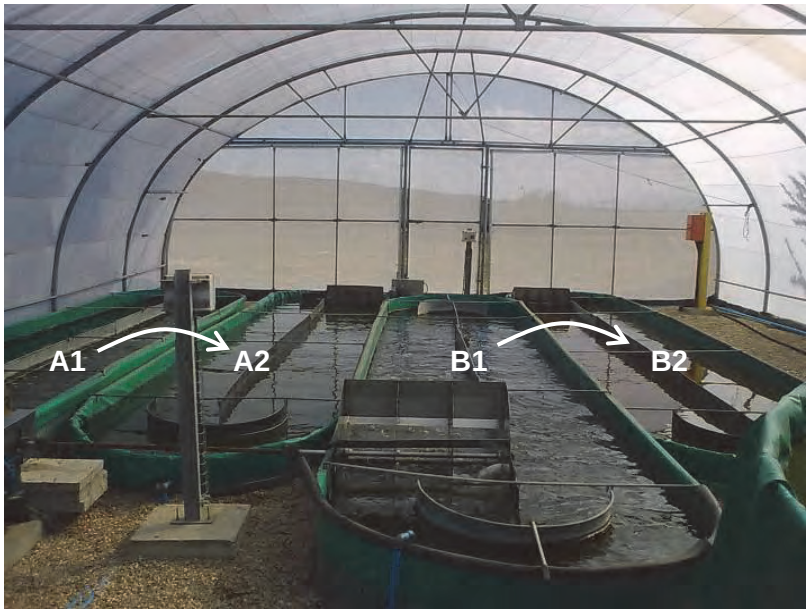


Figure 2.5 The high rate algal ponding system included two parallel systems, train-A and train-B. Each train consisted of two D-ended race-ways (i.e. raceway 1 and 2) connected to each other in series; the first raceway in each train had a volume of 3.5 m^3 (i.e. A1 and B1) and the second a volume of 1.74 m^3 (i.e. A2 and B2) (Photo: Rory Scheepers).

Figure 2.6 High rate algal ponds (HRAP) with a motor operated paddlewheel (Photo: Rory Scheepers).

The HRAP was where the main algal growth occurred and where the algal biomass was generated (Figures 2.5 and 2.6). Treated effluent leaving the HRAP gravity-fed into a sump tank, after which it was pumped into settling cones, which were used to remove a large portion of algal biomass from the effluent (Figure 2.7). The supernatant was then pumped into the CW (Figures 2.8 and 2.9), aquaculture or hydroponics systems. The PFP, HRAP, hydroponics and aquaculture systems were housed inside a greenhouse tunnel (Figure 2.10) and the CW was positioned outside (Figure 2.9).

Figure 2.7 The algal setting cones in which algae were separated from the effluent drawn from the high rate algal ponding (HRAP) system before the treated water was moved to either the constructed wetland (CW), hydroponic or aquaculture system (Photo: Rory Scheepers).

Figure 2.8 The constructed wetland (Photo: Rory Scheepers).

Figure 2.9 The constructed wetland is seen in the foreground with four parallel channels. The greenhouse tunnel that housed the algal ponding, hydroponics and aquaculture system is seen in the background (Photo: Rory Scheepers).

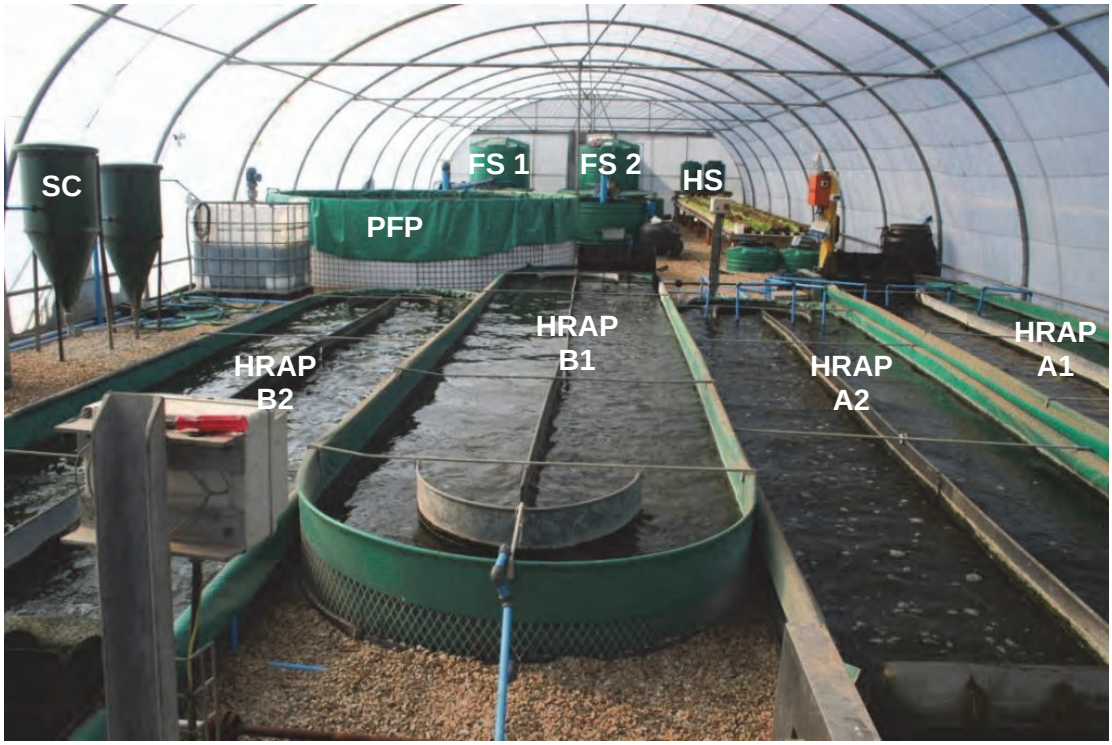


Figure 2.10 The high rate algal ponding system in the foreground (HRAP), the algal settling cones (SC) on the left, the primary facultative pond (PFP) behind the HRAP, the aquaculture filters (FS1 and 2) at the back and the hydroponic systems (HS) at the back on the right, all inside the greenhouse tunnel (Photo: Rory Scheepers).

The aquaculture system included 10 independent recirculating systems (Figures 2.11 and 2.12). Two of the systems included four tanks each and a common sump, and a filtration system. The remaining eight systems included a single tank only, each with its own air-uplift filtration system. These independent systems made it possible for us to test the effect of different water sources on the reproductive output, growth and health of fish.

Figure 2.11 The aquaculture system with settling tanks in the foreground, the mechanical filter at the front right, the fish grow-out tanks in the middle and the biological filters at the back (Photo: Rory Scheepers).

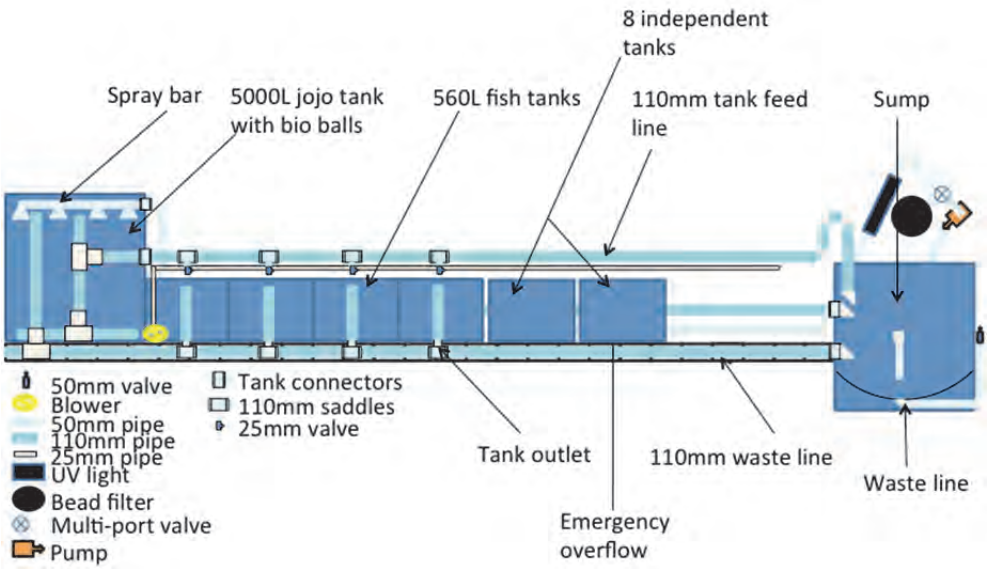


Figure 2.12 Section view of three of the aquaculture systems: the main system includes the spray bar filter tank, the sump and four of the eight 560 L fish tanks, while two of the eight independent tanks are shown in this diagram. Half of the systems were filled with water and the other with treated brewery effluent.

The hydroponic lettuce system was divided into two independent parts, each with its own sump and header tanks and each had four growing channels in which lettuce was grown (Figures 2.13 and 2.14). The hydroponic tomato system allowed for greater replication, with 30 independent systems each with its own pump, five-pot-growing channel and a sump (Figures 2.15 and 2.16).

Figure 2.13 the two hydroponic systems that run parallel to each other and that were used in all the hydroponic lettuce trials (Photo: Alistair Green).

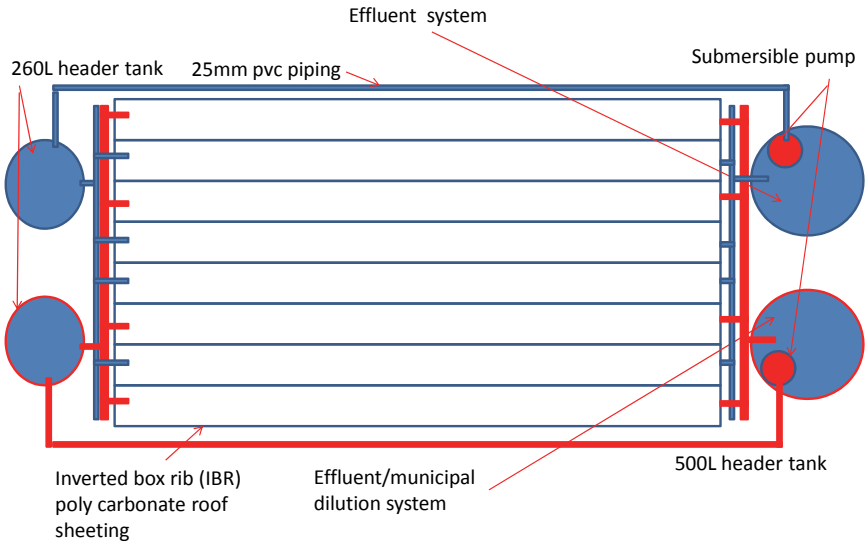
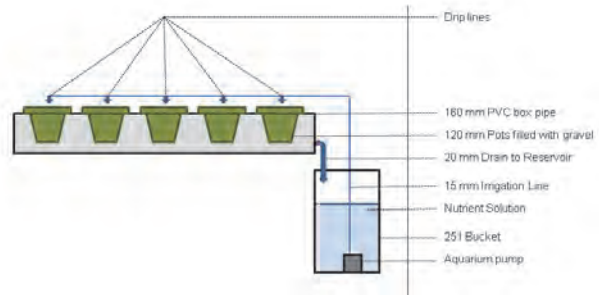


Figure 2.14 Aerial view of the hydroponics lettuce system showing the submersible pump in the sump on the left, eight parallel growing bays (four per system) and the header tanks on the left (one per system). The systems were each filled with different growth solution depending on the objectives of the experiment.

A

B



C

Figure 2.15 (A) The hydroponic system used for all tomato growth trials. (B) Each system consisted of a polyvinylchloride (PVC) growth channel with five extruded pot-holes; this picture was taken during the construction phase of the project. (C) Pot plants containing growth medium were suspended in the PRV channel (Figure 2.16); the growth solution drained by gravity from the pot plant into a sump (one sump for each group of five plants) and was circulated back to the plants using a small submersible pump (Photos: Sean Power).

Figure 2.16 The hydroponic system used for all tomato growth trials. Each system included five pot plants with a tomato plant in each (Photo: Sean Power).

Temperature, electrical conductivity (EC) and pH of the effluent post-AD, post-PFP, post-HRAP (i.e. post-A1 and -B1; post-A2 and -B2) at various positions in the CW and in the aquaculture and hydroponics systems were recorded using electronic meters. The COD (filtered to 8 µm) and ammonia, nitrite, nitrate and phosphate concentrations were monitored from the same positions in these systems, using a spectrophotometer and commercial test kits. These analyses were carried out in the site laboratory that was funded by this program (Figure 2.17). In addition, the concentration of the following elements were also determined from time to time: sodium; potassium; calcium; magnesium; iron; carbonate; bicarbonate; sulphur; boron; manganese; copper; zinc; phosphate; ammonia; nitrate; fluoride; total dissolved solids (TDS).

Figure 2.17 The air-conditioned, portable laboratory positioned close to the project's green-house tunnel at the research site at Ibhayi Brewery (SAB Ltd) in Port Elizabeth, with MSc students Anneke Cilliers (left) and Lara Crouse (right) (Photo: Rory Scheepers).

3. ALGAL PONDING AND CONSTRUCTED WETLAND: BASELINE DATA COLLECTION[1]

The performance of the high rate algal ponding (HRAP) system (Figure 3.1) was monitored for one year, from May 2009 to April 2010, to determine its efficiency in treating brewery effluent that had already been subject to pre-treatment in an anaerobic digester (AD). Post-HRAP effluent was subsequently passed through the constructed wetland (CW; Figure 3.1). The wetland was commissioned in March 2010 and these preliminary baseline data were collected up until the end of June 2012. The temperature, pH, filtered chemical oxygen demand (COD) and ammonia, nitrite, nitrate, phosphate and chloride concentrations were recorded at various positions in the HRAP and CW. The flow rate through the system was maintained at approximately 500 L/d and flowed through the system during the day only; it was only increased in one of the HRAP trains for a short period during the first HRAP optimisation trial (Section 4.1).

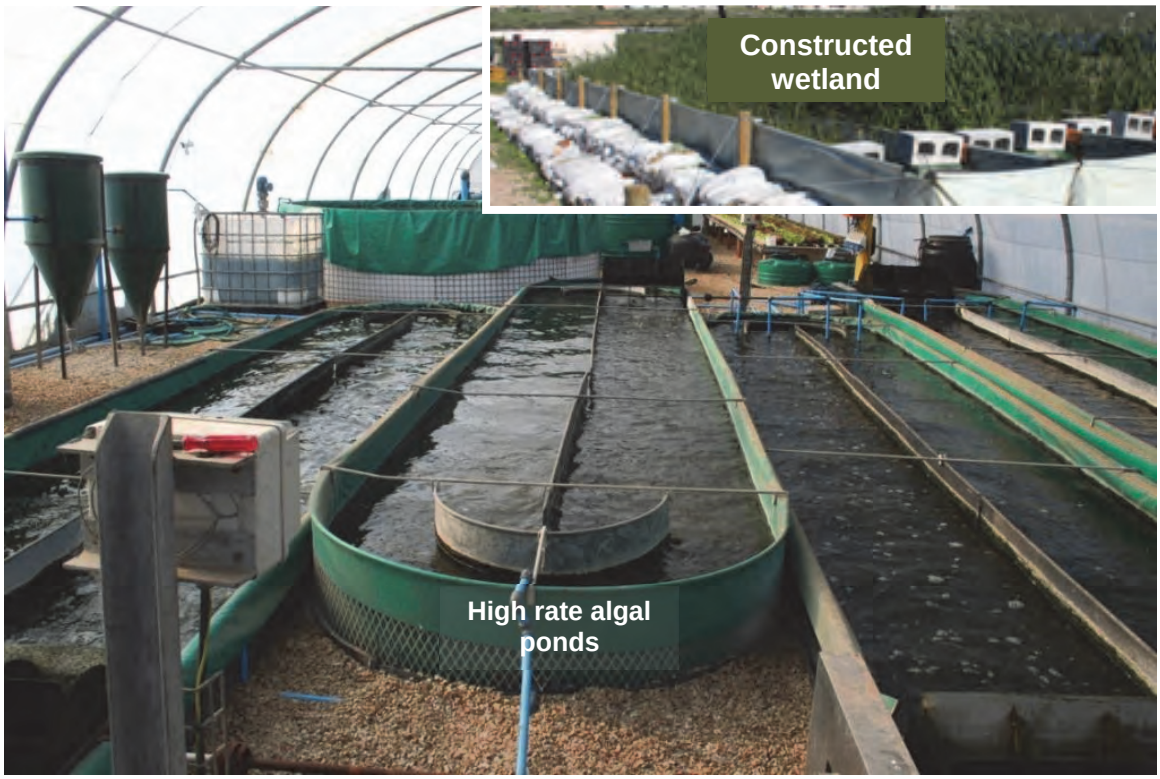


Figure 3.1 The high rate algal ponding system in the foreground (in the greenhouse tunnel), and the constructed wetland in the insert (situated outside the greenhouse) (Photos: Rory Scheepers).

[1] Parts of this section of the report were used in a report delivered to SAB, a co-funder of this section of the research.

3.1 Chemical oxygen demand

For most of the monitoring period, COD after treatment in the HRAP were similar to those of the effluent received from the anaerobic digester; however, once HRAP flow rates were optimised (April-June 2010; section 4.1) and the wetland added, COD levels were reduced to close to or below Department of Water Affairs (DWA) general limit (Figure 3.2).

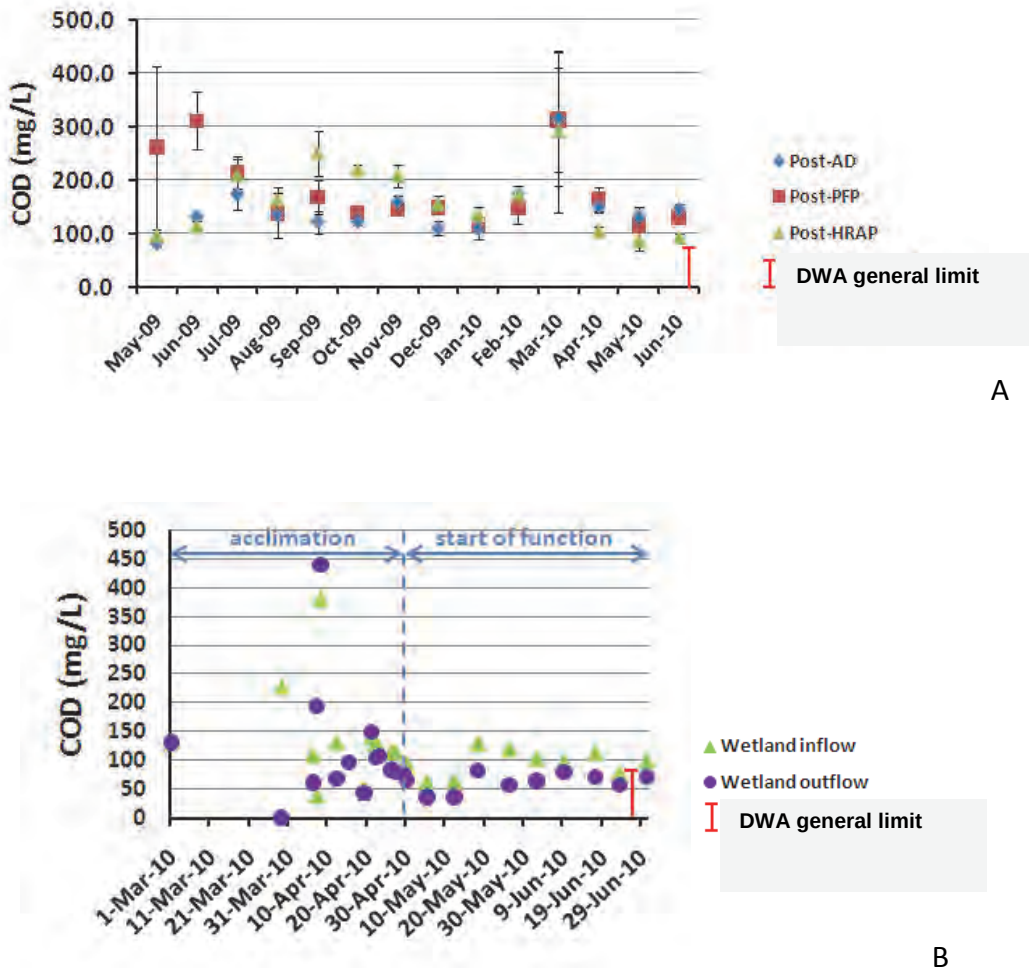


Figure 3.2 (A) Mean (± standard error) monthly filtered chemical oxygen demand (COD) of brewery effluent in the anaerobic digester (AD), the primary facultative pond (PFP), and post high rate algal ponding (post-HRAP) system from May 2009 to June 2010, and (B) COD in the wetland (raw data) from May 2009 to June 2010. AD – anaerobic digester; PFP – primary facultative pond; DWA – Department of Water Affairs. All samples filtered to 8 µm.

The high concentration of algal biomass under the slow effluent throughput regime (i.e. a hydraulic retention time, HRT, of about 18 d) was believed to be responsible for the persistently high COD levels. Once flow rates were increased and the rate of algal cell

density and turnover rate optimised (Section 4.1), COD levels decreased. Furthermore, as the DWA COD general limit excludes the algal cell contribution to COD (per DWA general limits), the COD levels post-HRAP with the algal fraction removed were largely within the DWA general limit of 75 mg/L.

During April 2010, COD coming out of the AD averaged around 140 mg/L, while COD post-HRAP was lowered to 80 mg/L (i.e. a 43 % decrease in COD), which was not far off the target <75 mg/L required by the DWA excluding algae. The CW was effective in reducing COD levels by a further 25% (Figure 3.2). These COD values were obtained after filtering the effluent to only 8 µm, and thus a fraction of smaller algal cells (<8 µm) contributed to the total COD recorded.

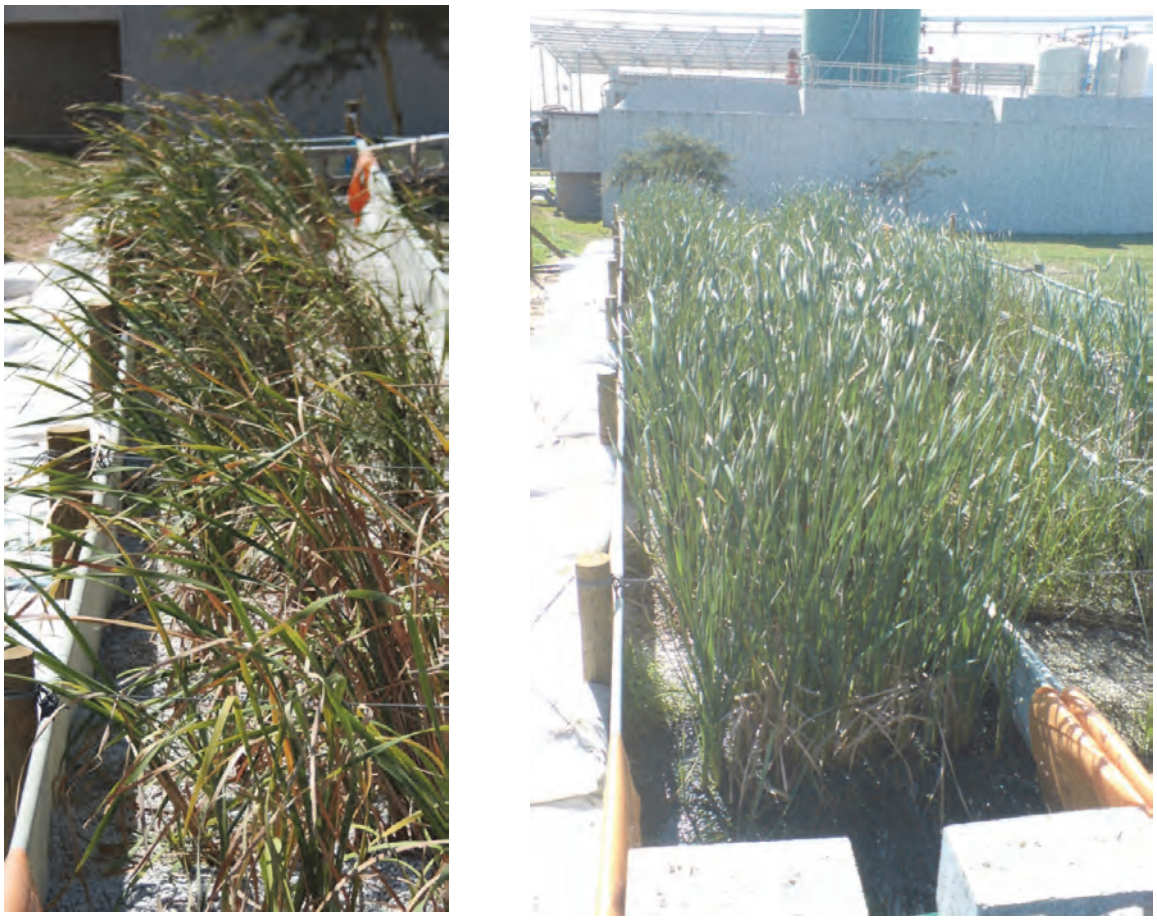


Figure 3.3 The wetland shortly after planting the reeds at the end of February 2010, and the same area approximately two months later (19 April 2010) (Photo: Rory Scheepers).

3.2 Ammonia, nitrite and nitrate

The HRAP was highly efficient at removing nitrogen from the effluent. Ammonia entered the system at between 30 and 60 mg/L and was reduced to 0.1-1.0 mg/L, even at the increased flow rates through the system during April 2010 (Figure 3.4A). The HRAP system was very resilient to shocks or changes in effluent quality, as well as seasonal effects of light and temperature. The post HRAP ammonia levels were consistently below the DWA general discharge limit of 6 mg/L indicating that the HRAP system achieved the required water quality objective for ammonia.

The wetland was effective in further reducing the post-HRAP ammonia levels (Figure 3.4B). When the flow rate optimisation trial was conducted in April, post-HRAP ammonia levels rose to 6 mg/L in mid-April (as the algal system reached its limit; see Figure 3.4B wetland in flow), but the wetland remained effective in reducing the levels to well within the DWA general limit.

For the bulk of the effluent monitoring period, nitrite and nitrate levels were negligible, as the algal cells directly assimilated the ammonia, and no nitrification was observed. During April 2010, however, when flow rates were increased through the HRAP, and algal cells began to thin out, measurable levels of nitrite and nitrate were observed as a result of bacterial activity converting the excess ammonia to nitrite and then nitrate. The April optimisation trial showed that the wetland was highly effective in lowering the post-HRAP levels of nitrite and nitrate (Figures 3.4C and 3.4D). The levels of nitrite and nitrate leaving the HRAP and wetland never exceeded the DWA limit of 10 mg/L. Overall, HRAP and wetland technologies can be considered super-efficient at nitrogen removal from brewery effluent.

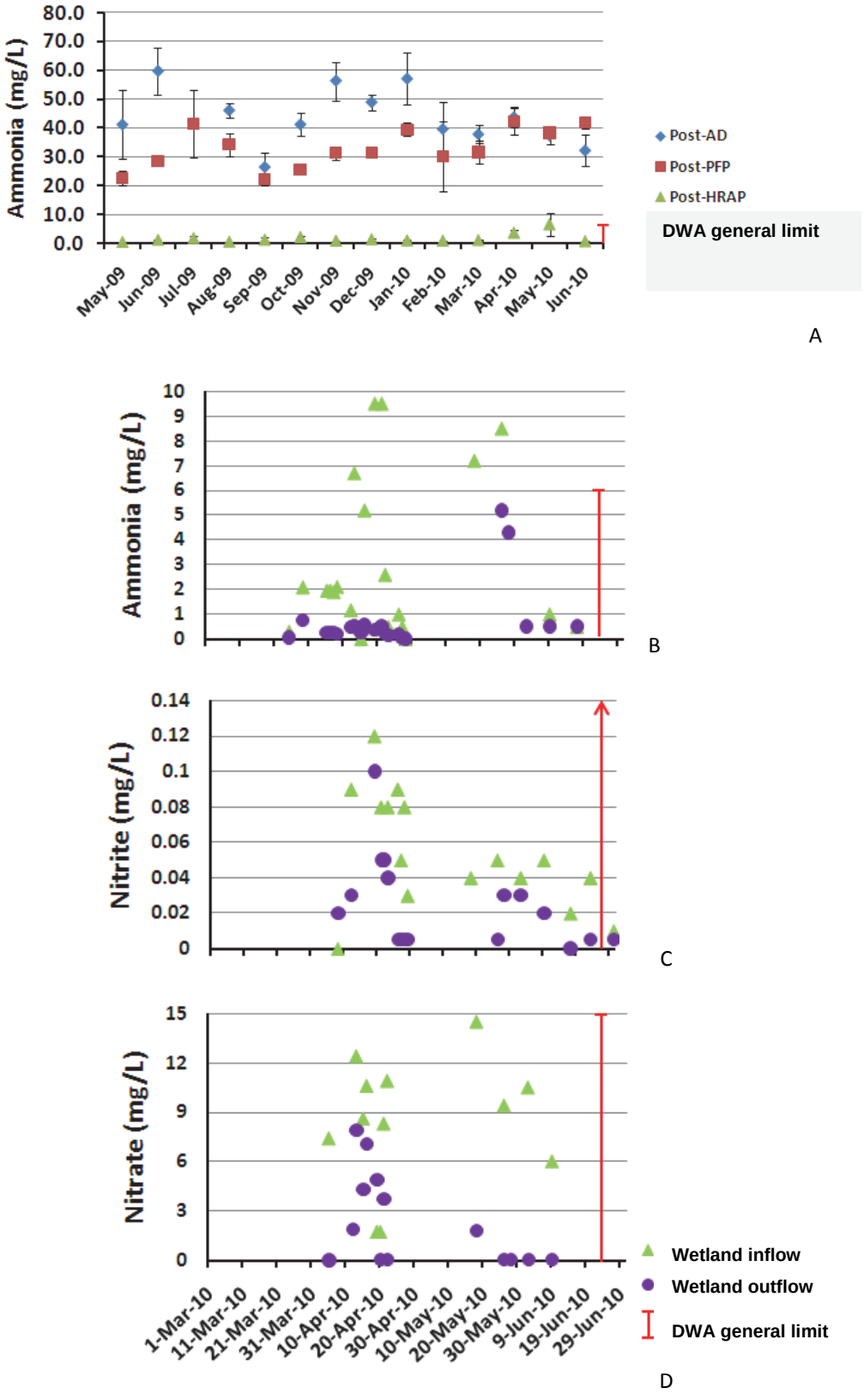


Figure 3.4 (A) Mean (± standard error) monthly ammonia concentration (mg/L) in treated brewery effluent post anaerobic digestion (post-AD), post primary facultative ponding (post-PFP) and post high rate algal ponding (post-HRAP) system. (B) Ammonia, (C) nitrite and (D) nitrate reduction in the constructed wetland. Compared with the Department of Water Affairs (DWA) general limit for effluent discharge into a natural water body.

3.3 Phosphate

Phosphate levels in the post-AD effluent were lowered significantly by the HRAP during the autumn and spring months, but very little was removed during the winter (Figure 3.5). From March 2010 onwards, the phosphate levels in the brewery effluent dropped to well below the DWA discharge general limit of 10 mg/L. The addition of the wetland in February 2010 further enhanced the capability of the system to remove phosphate; however, the efficiency of the wetland in phosphate removal cannot be quantified until phosphate is once again present in the effluent. Current phosphate levels are being reduced by both the HRAP and wetland and are below DWA discharge general limits. Despite investigations into the sources of phosphate in the effluent from the brewery, the reason for its disappearance remains unknown. The average efficiency of phosphate removal from domestic and industrial wastewater by constructed wetlands is 40% (Vymazal 2009), which means that, should phosphate rise to previous levels, the HRAP and wetland should bring phosphate to within DWA discharge standards.

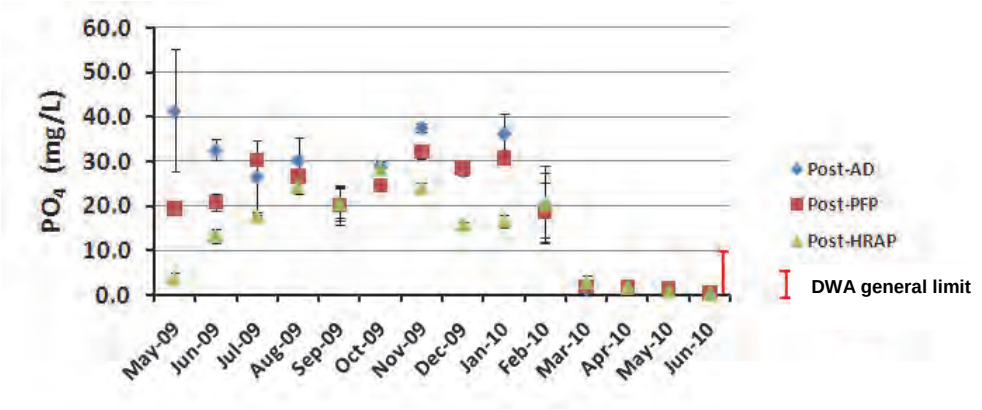
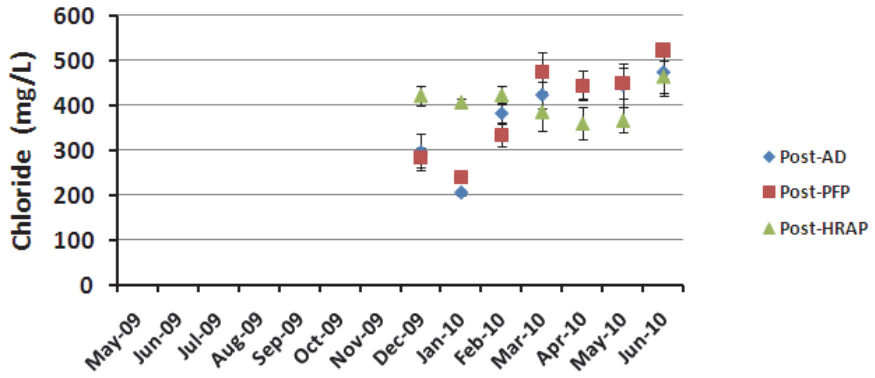


Figure 3.5 Mean (± standard error) phosphate concentration (mg/L) in treated brewery effluent post anaerobic digestion (post-AD), post primary facultative ponding (post-PFP) and post high rate algal ponding (post-HRAP) system compared with the Department of Water Affairs (DWA) general limit for effluent discharge into a natural water body.

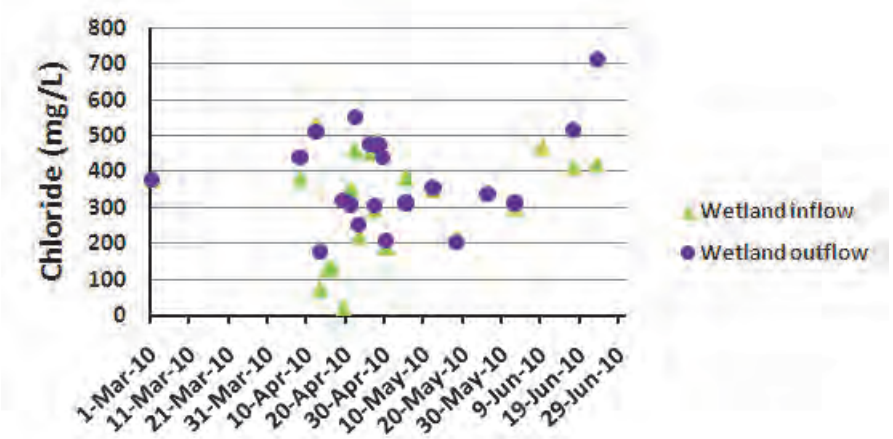
3.4 Chloride and electrical conductivity

The HRAP process and wetland were not effective at lowering either chloride or conductivity, which is an indicator of total dissolved salts in the effluent. The chloride concentration and conductivity either remained unchanged, or increased through the HRAP and wetland due to evaporation – particularly during the summer months (Figures 3.6 and 3.7). During the winter months, and during the period of increased flow rate (April 2010), chloride and conductivity levels were effectively unchanged through the HRAP/wetland system.

The recorded conductivity levels of around 2000-3000 µS/cm (Figure 3.7) thus exceed DWA general limits for effluent discharge into a water resource. As Ibhayi effluent discharges into a saline ecosystem, the Swartkops River estuary, it would pose no environmental risk and an authorisation could be obtained from DWA to discharge this slightly saline effluent, which is equivalent to a total dissolved solids (TDS) range of 1200-1800 ppm. Salt levels in most open estuaries that are subject to tidal fluctuations reach up to 35 g/L (i.e. 35000 mg/L) so the Ibhayi chloride levels (200-500 mg/L) would be minimal by comparison.



A



B

Figure 3.6 (A) Chloride concentration (mg/L) in treated brewery effluent post anaerobic digestion (post-AD), post primary facultative ponding (post-PFP) and post integrated algal ponding system (post-HRAP), and (B) chloride concentration in the wetland (raw data plotted, with the bi-weekly mean ± standard error in the summary table).

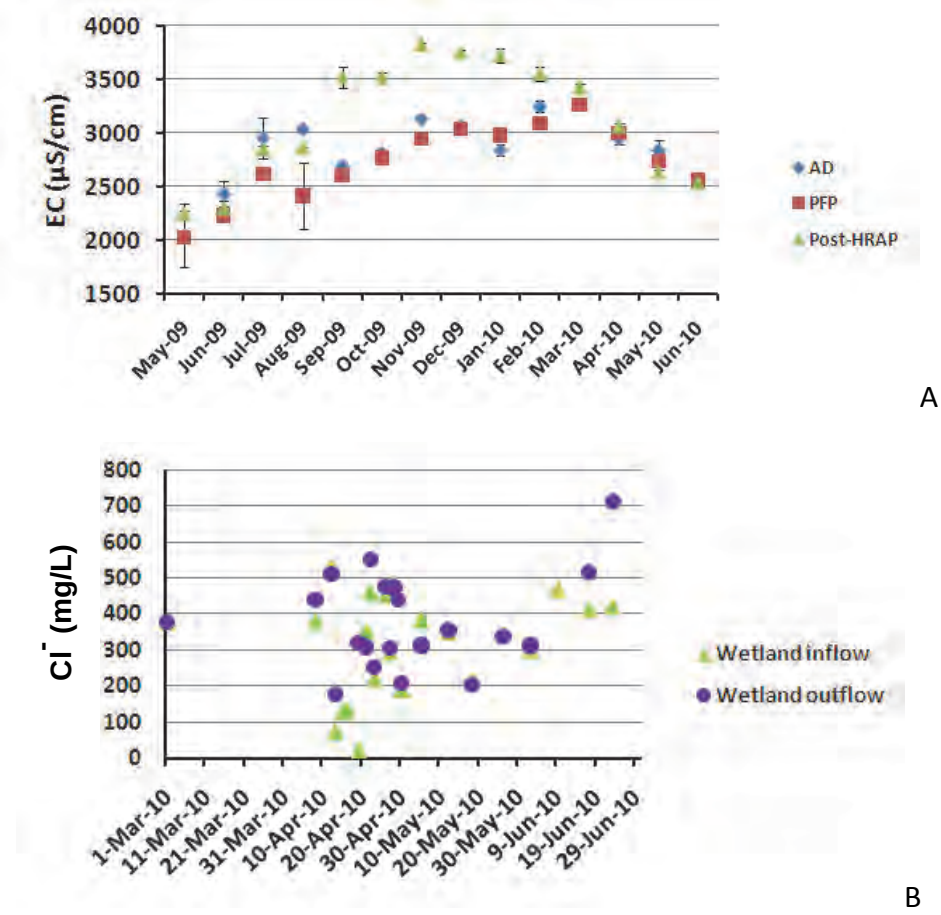


Figure 3.7 (A) Electrical conductivity (EC) of treated brewery effluent in the anaerobic digester (AD), primary facultative pond (PFP), post high rate algal ponding system (post-HRAP) and (B) in the wetland.

3.5 Effluent pH

The HRAP system increased the post-anaerobic digester pH from 7.4-8.0 to around 9.5 due to the photosynthetic activity of the algae (Figure 3.8A). The wetland was effective in reducing the pH to between 8.3 and 9 (Figure 3.8B); however, its efficiency at reducing pH has increased as the wetland has matured (Figure 3.8B). It is thus possible that the wetland might further reduce pH as the wetland matures. The DWA discharge standard for pH is 5.5-9.5, so post-HRAP pH is largely within these limits and post-wetland effluent is already well within the limits.

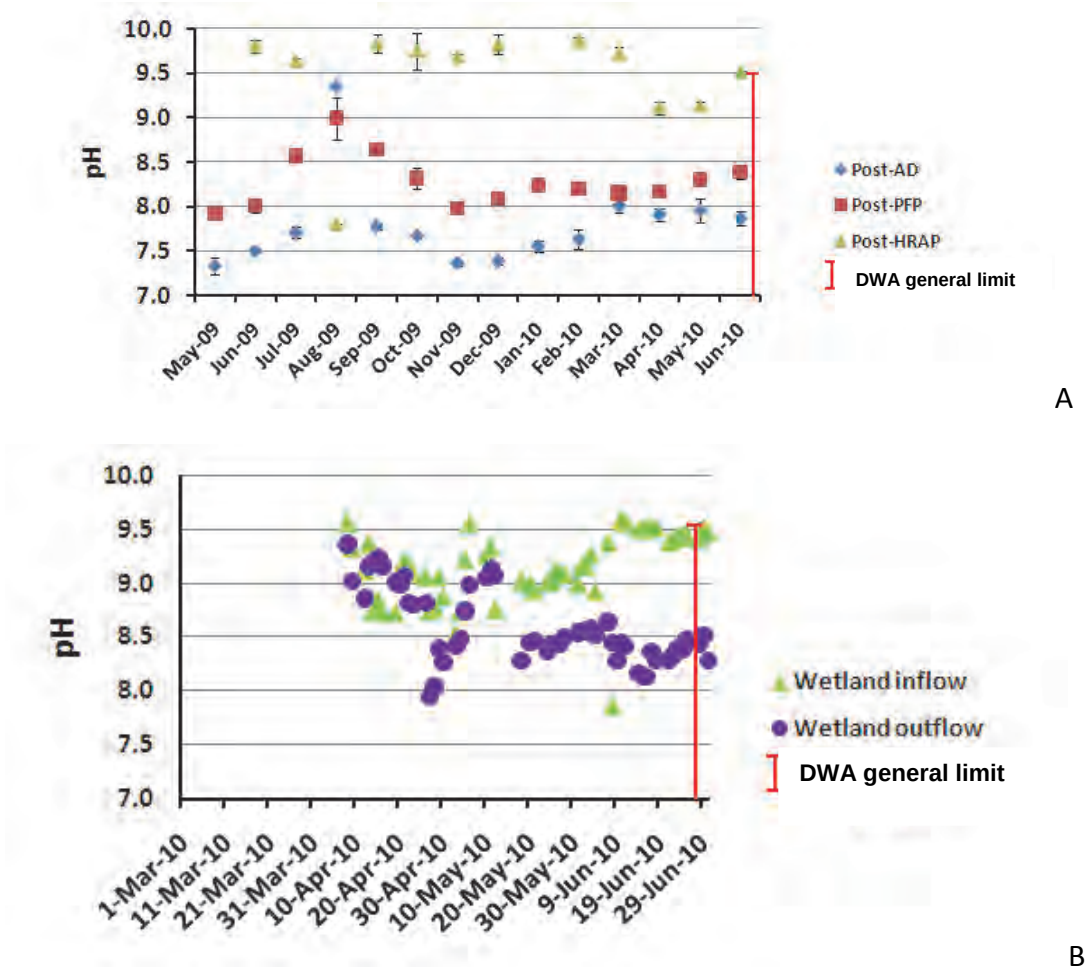


Figure 3.8 (A) The pH of treated brewery effluent post anaerobic digestion (post-AD), post primary facultative ponding (post-PFP) and post high rate algal ponding system (post-HRAP), and (B) the pH entering and leaving the wetland compared with the Department of Water Affairs (DWA) general limit for discharge effluent into a natural water body.

4. OPTIMISING THE PERFORMANCE OF THE HIGH RATE ALGAL PONDS

Base line data presented in Chapter 3 lead to the suggestion that the high rate algal pond (HRAP) was a suitable method of treating brewery effluent. However, these systems require a large geographical "foot print", so their use is limited to industries with sufficient space. Furthermore, their efficiency is also linked to temperature and day length so the change in efficiency and the space required to treat a given volume of effluent is likely to vary at different times of year. The overall aim of this section was to determine the optimal hydraulic retention (HRT) of brewery effluent in the HRAP at different times of year.

4.1 Preliminary optimisation of flow rate through the high rate algal ponds

During April 2010, the flow rate through the HRAP system was incrementally increased to determine the maximum effluent volume that it could treat. To achieve this, the flow rate was increased in one of the HRAP systems, while the flow in the second system remained unchanged to act as a "control" for comparison. The system temperature was approximately 25°C during this period with sunny, hot weather.

Table 4.1.1 Mean rate (L/h) that brewery effluent flowed into the primary facultative pond (PFP) and into two high rate algal pond (HRAP) systems. The flow rate of effluent into one HRAP system remained unchanged at ~50 L/d; with the exception that effluent was allowed to flow through that system for 12 rather than 8 h/d (i.e. control treated 410 to 620 L/d). The flow into the second HRAP was ~780 L/d for two weeks, and was thereafter increased to ~1400 L/d and then to ~2000 L/d.

	Flow rate (L/h)	Effluent treated (L/d)	Effluent retention time (d)
PFP (pre-optimisation)	116	931	18.6
PFP (1/3-15/3)	148	1182	14.6
PFP (17/3-19/3)	248	1982	8.7
HRAP-control (1/3-15/3)	51	411	13.3
HRAP-control (17/3-19/3)	51	620	8.8
HRAP-780L/d (1/3-15/3)	98	784	6.9
HRAP-1400L/d (17/3-19/3)	118	1414	3.8
HRAP-2000L/d	170	2000	System crashed 2.7

The increased flow rates (Table 4.1.1) reduced the retention time in the primary facultative pond (PFP) from 18 days to 9 days, while the retention time in the HRAP was reduced from 13.3 days to 3.8 days (Table 4.1.1). The system was pushed to 2000 L/d but it crashed and was unable to maintain low ammonia levels at this flow (Figure 4.1.1), since the algal/bacterial cells were washed out faster than they could reproduce. The optimum flow rate for the HRAP system, under this set of environmental conditions was thus 1400 L/d which is equivalent to a retention time of 3.8 days. The total time taken to treat brewery

effluent through the PFP and HRAP under these current conditions was thus reduced from 31 to 13 days (Table 4.1.1 and Figure 4.1.1).

Ammonia concentration was recorded as an indicator to evaluate the success of the HRAP at increased flow rates, and was compared to that of the control HRAP. Ammonia remained well below the maximum concentration for effluent discharge into a river system, even when the flow rate was increased from ~450 L/d to ~780 and then to ~1400 L/day, but the system became less efficient when increased to ~2000 L/day (Figure 4.1.1).

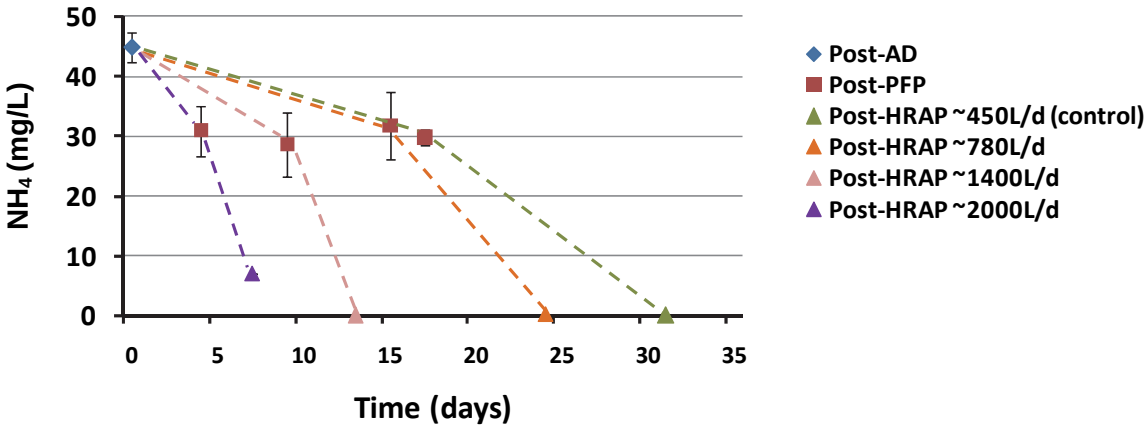


Figure 4.1.1 Mean ammonia concentration recorded in post-anaerobic digester (AD) brewery effluent, post-primary facultative pond (PFP) and post-high rate algal ponds (HRAP) through which brewery effluent flowed at either 450 to 620 L/d (i.e. control flow of ~50 L/h) or at flow rates of firstly ~780 L/d, rising to 1400 L/d and then to ~2000 L/d.

A seasonal variation in this "optimised" flow rate will occur. The trial was conducted in autumn with day lengths already shortening, but the weather was sunny and hot producing pond temperatures of 25°C. Higher productivity could thus be expected in summer, with literature values indicating that a HRAP retention time of two days is achievable. Winter HRAP productivity will be lower due to lower temperatures and shorter day lengths. Thus heating the HRAP ponds would be required in order to minimise the HRAP pond area required in winter.

4.2 Optimisation of high rate algal pond flow rate in different seasons

The overall aim of this trial was to describe the seasonal variation in the efficiency with which the HRAP system removed nutrients from brewery effluent and, in so doing, determine its efficiency at turning the effluent into water and algae suitable for use in other industries, i.e. beneficiation. The flow rate of post-AD brewery effluent into the primary facultative pond (PFP) and subsequently through the HRAP system was progressively increased at different times of the year. All water quality parameters were recorded. The

Department of Water Affairs (DWA) general limits for discharge into a natural water source for nitrogen (i.e. ammonia, nitrite and nitrate) were used as governors, and flow rate was only increased if nitrogen in the treated effluent remained within the DWA general limits. This was 6 mg/L for ammonia and 15 mg/L for nitrate and nitrite. Train-A of the HRAP system was heated and train-B remained at the ambient temperature of the greenhouse tunnel in spring and summer (Figure 4.2.2E and F).

The first optimisation trial was run in autumn of 2010 and has been previously reported on. The HRT of the HRAP was reduced to about 3.5 days in autumn with ammonia remaining well within the DWA general limits and no nitrite or nitrate recorded.

The trial was repeated in the winter of 2010, during which time the HRT remained between 12 and 20 days (Figure 4.2.1A). The system remained efficient at reducing ammonia from as high as 80 mg/L post-AD to close to zero post-HRAP in both the A- and B-train (Figure 4.2.1E). The nitrate levels increased from around 1 mg/L post-AD to between 7 and 20 mg/L post-HRAP (Figure 4.2.1K), which is why the flow rate could not be further increased (i.e. nitrate increased above the DWA general limit of 15 mg/L). The conversion of ammonia to nitrate indicated the presence of nitrifying bacteria in the HRAP system with less algal biomass to remove nitrate relative to the autumn run, which is supported by the low algal productivity (Figure 4.2.2A). Phosphate was present and was reduced in the HRAP system to below the DWA general limit of 10 mg/L post-HRAP (Figure 4.2.1N) and filtered chemical oxygen demand (COD) was also reduced to around the DWA general limit of 75 mg/L (Figure 4.2.2G). At the time, the reduced efficiency of the system was attributed to lower water temperatures and shorter days associated with winter.

Very similar trends were recorded in spring (Figures 4.2.1F, I, L, O and S), even though temperature and day length had increased. This suggested that the reduced efficiency that was recorded might not have been due to reduced temperature and day length alone. A subsequent analysis of the algal community found that it lacked the diversity it had originally had, and that there was a build-up of detritus in the ponds. The reduced efficiency of the HRAP during spring and possibly during winter might have been confounded by the structure and age of the algal community, which was not given the opportunity to recover after the “wash-out” (i.e. algal cell removed from the system faster than the rate of cell generation) during the autumn optimisation experiment followed immediately by winter.

The system was subsequently drained, cleaned and re-inoculated with algae taken from the HRAP system from the Environmental Biotechnology Research Institute (EBRU, Rhodes University, Grahamstown) during October and November 2010. It was possible to reduce the HRT to about 2.5 days in summer without compromising the efficiency of ammonia removal; however, when HRT was reduced to two days ammonia and nitrate in the post-HRAP effluent increased above the DWA general limit of 6 mg/L and 15 mg/L respectively (Figure 4.2.1G and M).

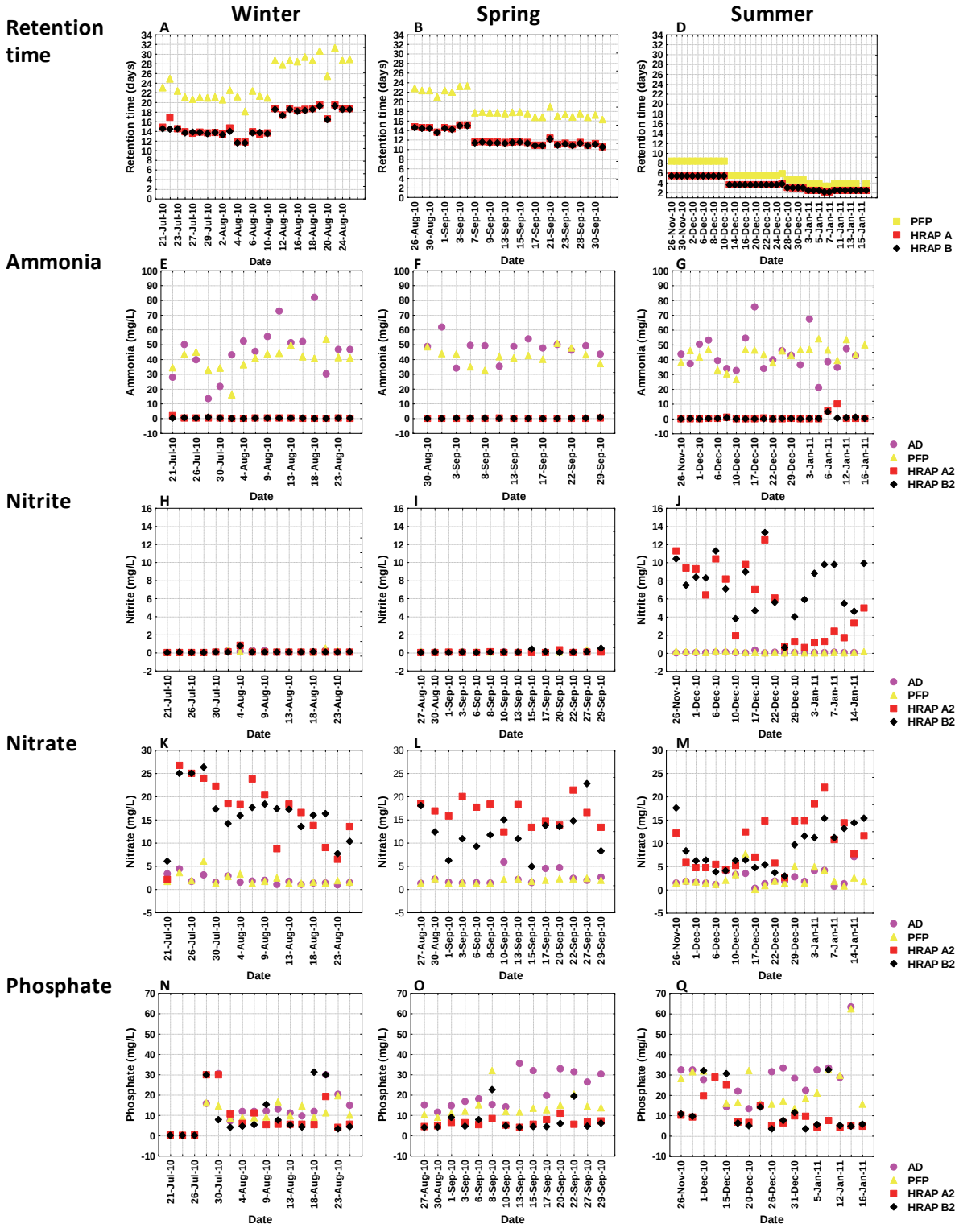


Figure 4.2.1 The hydraulic retention time, ammonia (NH_4-N), nitrite (NO_2-N), nitrate (NO_3-N) and phosphate (PO_4-P) recorded at different times of the year in the anaerobic digester (AD), primary facultative pond (PFP) and two parallel trains of high rate algal ponds (HRAP).

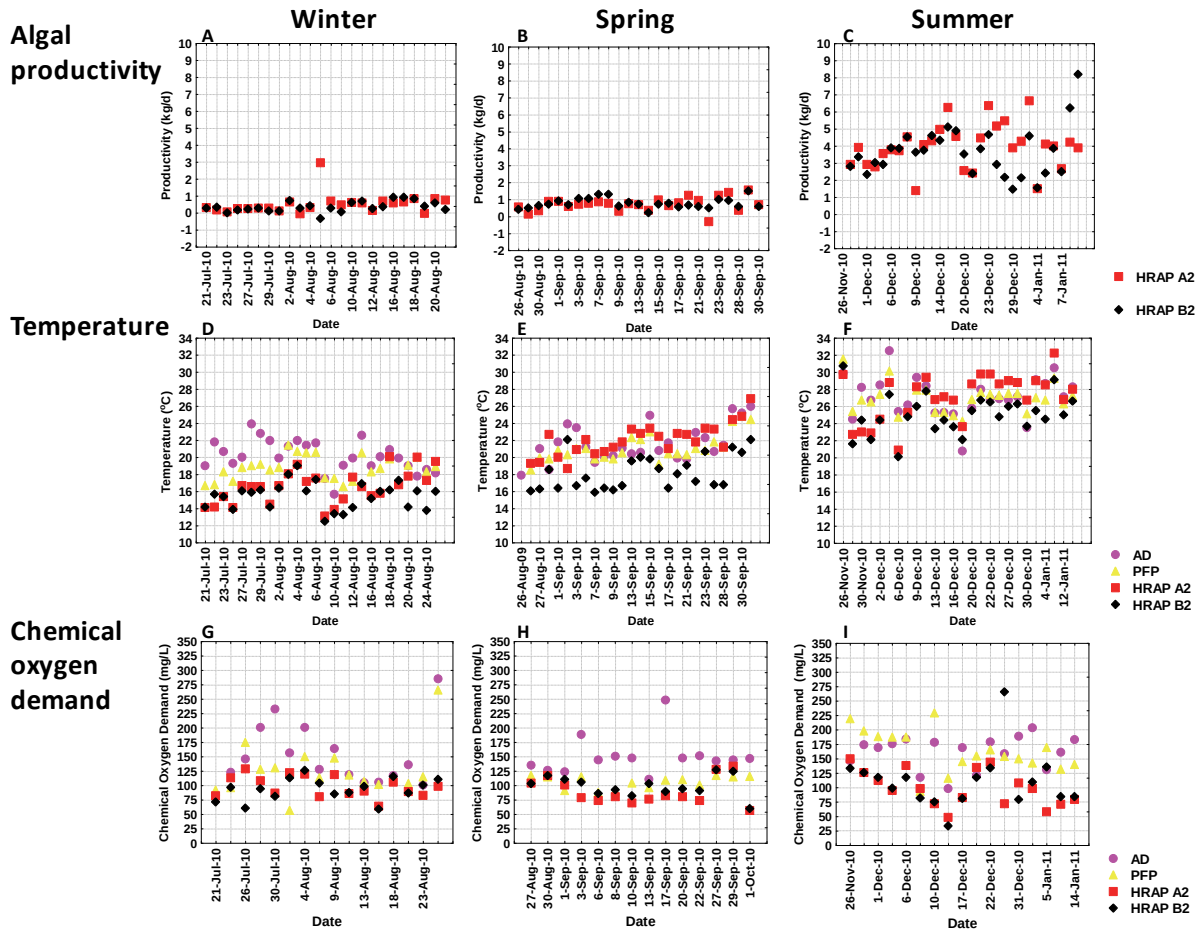


Figure 4.2.2 The temperature and filtered chemical oxygen demand (COD) recorded at different times of the year in the anaerobic digester (AD), primary facultative pond (PFP) and two parallel trains of high rate algal ponds (HRAP) and algal productivity recorded in the HRAP over the same period.

Similar values were recorded in both the heated and unheated HRAP trains. However, nitrate in the un-heated train-A was about half that of the heated train-A on many occasions during spring (Figure 4.2.1L) and summer (Figure 4.2.1M).

The HRT of the HRAP system was reduced to 2.5 days under the conditions experienced in the summer of 2010, without compromising the nitrogen removal efficiency. The efficiency of the HRAP in spring and possibly in winter might be improved during future work if the algal community in the system at that time of year is monitored and manipulated to ensure a community structure suitable for maximum nutrient uptake. Further optimisation is suggested for the cooler times of year to determine if it is possible to reduce HRT and thus reduce the footprint needed to treat large volumes of brewery effluent using HRAP.

4.3 Further optimisation of the high rate algal pond flow rate in winter

In the past winter trial (Section 4.2) it was shown that the optimal flow of effluent through the HRAP was between 1500 and 2000 L/day. During the winter of 2011 the aim was to determine if this was accurate and if optimum flow rates varied seasonally.

The flow rate through both HRAP trains was set at 500 L/d/train at the start of the trial (Figure 4.3.1). This rate was progressively increased in both systems for three weeks until both were running at 1000 L/d (Figure 4.3.1). The B-train was subsequently maintained at 1000 L/d for the rest of the trial and acted as a control. The flow rate through the A-train was increased progressively until the DWA general limit for ammonia (i.e. 6 mg/L) could not be maintained in the post-HRAP effluent.

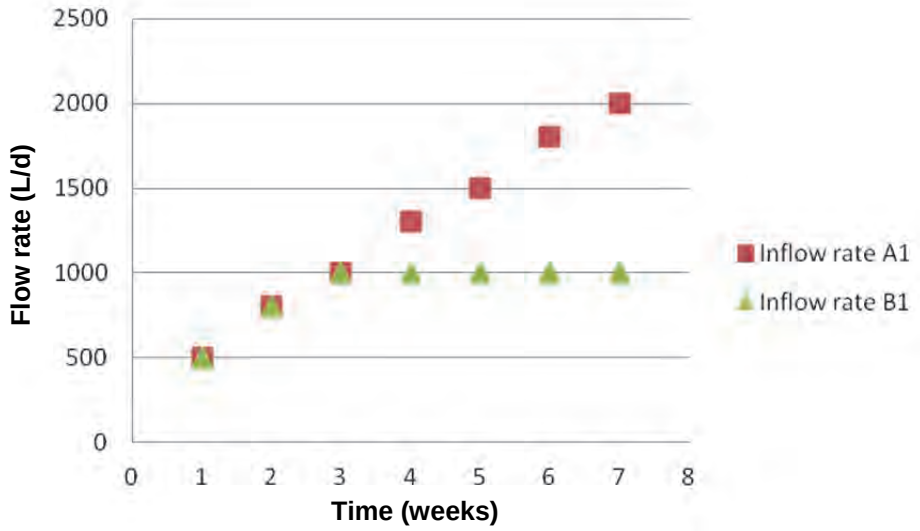


Figure 4.3.1 Flow rate of effluent water during the winter trial of 2011 (26 July to 14 September) at the post anaerobic digester (AD) and inflows to the high rate algal ponds (HRAP) A1 and B1 system.

Both systems remained efficient at lowering ammonia from as high as 80 mg/L post-AD to close to zero post-HRAP in both the A- and B-trains (Figure 4.3.2). The nitrate levels increased from around 1 mg/L post-AD to between 7 and 20 mg/L post-HRAP in 2010 and this was slightly higher in 2011 (Figure 4.3.3). The reason for the termination of the 2010 trial was because nitrate increased above the DWA general limit of 15 mg/L in post HRAP water. The winter 2011 trial was terminated when ammonia concentration exceeding the 6 mg/L general limit; however nitrate was higher. The conversion of ammonia to nitrate in both trials indicated the presence of nitrifying bacteria in the HRAP system. This meant that there was less algal biomass to remove nitrate, which was supported by the low algal productivity. Phosphate was present and was reduced in the HRAP system to below the DWA general limit of 10 mg/L post-HRAP in 2010 and this low level was not attained in 2011 (Figure 4.3.4). The COD was also reduced to around the DWA general limit of 75 mg/L during winter 2010 and in 2011 the DWA general limit was not attained (Figure 4.3.5). Reduced

efficiency of the system is attributed to lower water temperatures and shorter days associated with winter. Chloride increased in both systems (Figure 4.3.6).

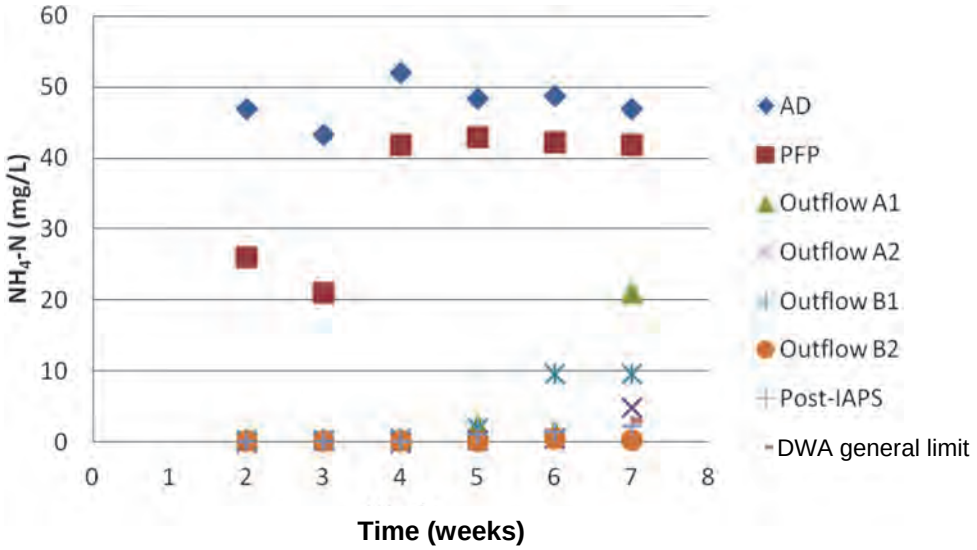


Figure 4.3.2 Ammonia (NH_4-N) concentrations during the winter trial of 2011 (26 July to 14 September) at the various sampling points, post anaerobic digester (AD), post primary facultative pond (PFP), outflows of high rate algal ponds (HRAP) A1, A2, B1, B2, post HRAP, post equalization tank (EQ) compared to the Department of Water Affairs (DWA) general limit.

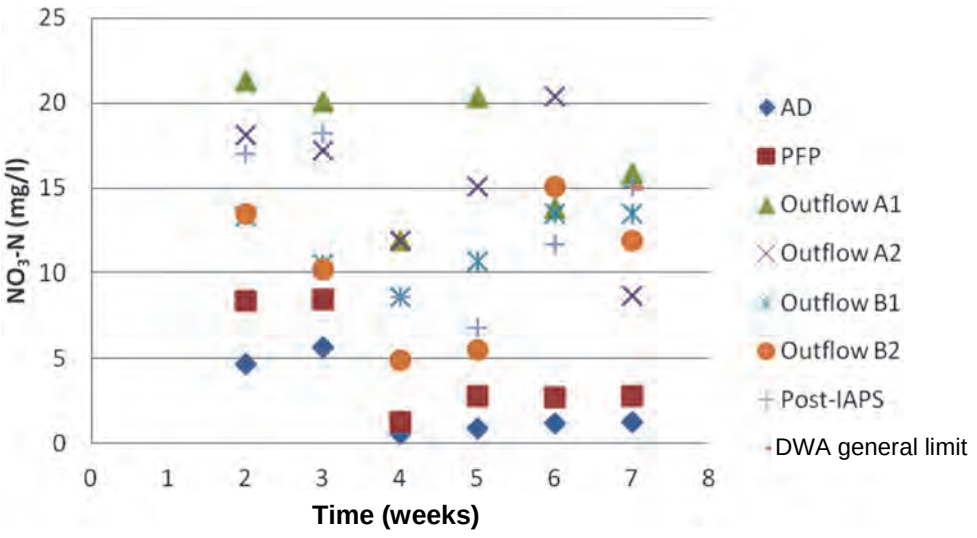


Figure 4.3.3 Nitrate (NO_3–N) concentrations during the winter trial of 2011 (26 July to 14 September) at the various sampling points, post anaerobic digester (AD), post primary facultative pond (PFP), outflows of high rate algal ponds (HRAP) A1, A2, B1, B2, post HRAP, post equalization tank (EQ) compared to the Department of Water Affairs (DWA) general limit.

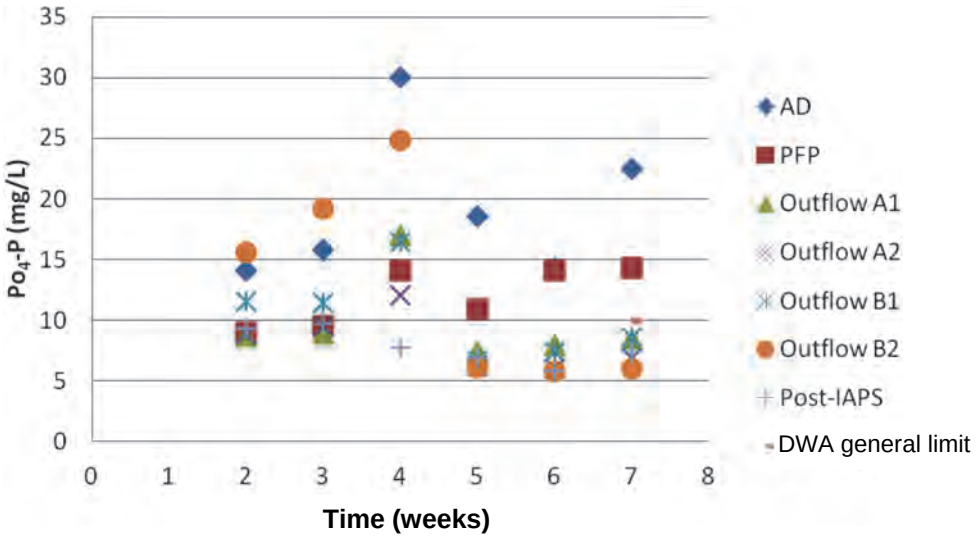


Figure 4.3.4 Phosphate (PO_4-P) concentrations during the winter trial of 2011 (26 July to 14 September) at the various sampling points, post anaerobic digester (AD), post primary facultative pond (PFP), outflows of high rate algal ponds (HRAP) A1, A2, B1, B2, post HRAP, post equalization tank (EQ) compared to the Department of Water Affairs (DWA) general limit..

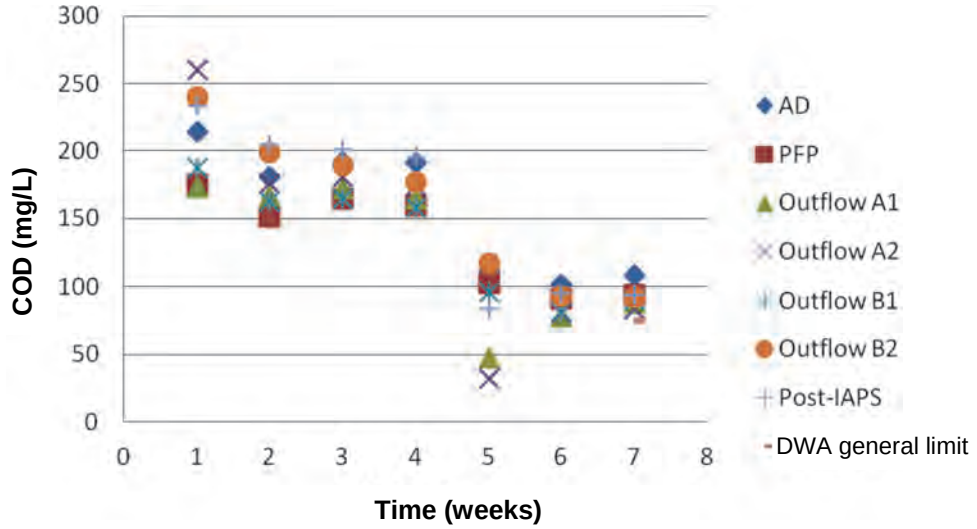


Figure 4.3.5 Filtered chemical oxygen demand (COD) concentrations during the winter trial of 2011 (26 July to 14 September) at the various sampling points, post anaerobic digester (AD), post primary facultative pond (PFP), outflows of high rate algal ponds (HRAP) A1, A2, B1, B2 and post HRAP compared to the Department of Water Affairs (DWA) general limit..

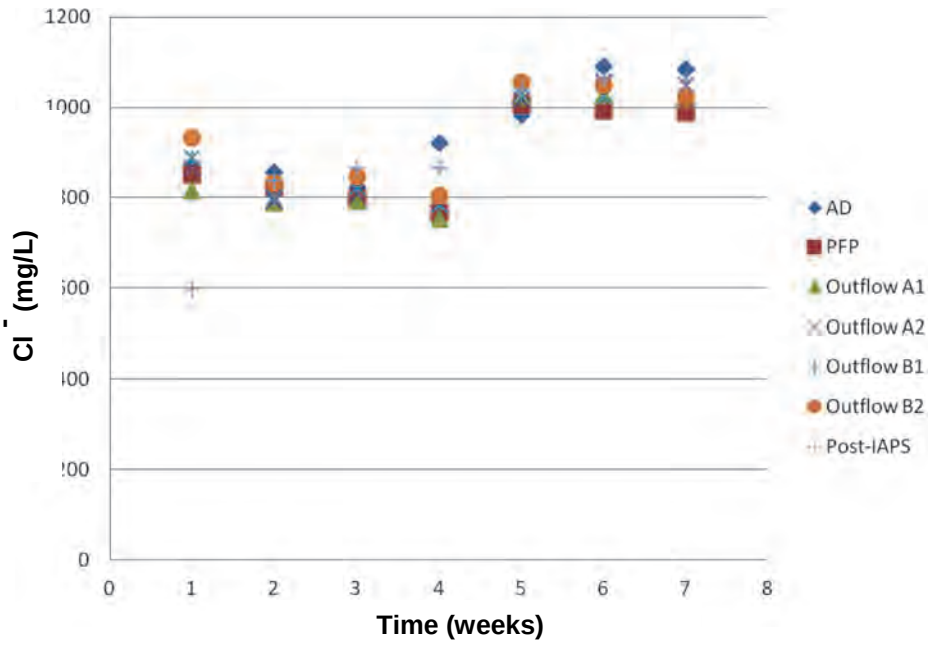


Figure 4.3.6 Chloride (Cl^-) concentrations during the winter trial of 2011 (26 July to 14 September) at the various sampling points, post anaerobic digester (AD), post primary facultative pond (PFP), outflows of high rate algal ponds (HRAP) A1, A2, B1, B2, post HRAP, post equalization tank (EQ) compared to the Department of Water Affairs (DWA) general limit.

The first optimisation trial was run in the autumn of 2010. The hydraulic retention time (HRT) of the HRAP was reduced to about 3.5 days at that time, with ammonia remaining well within the DWA general limits and no nitrite or nitrate being recorded. In winter 2011, the HRT in the HRAP was also about 3.0 days. The HRT of autumn and winter could not match that of the summer months where HRT was reduced to 2.5 days in the HRAP, without compromising the nitrogen removal efficiency. The efficiency of the HRAP in spring and possibly in winter might be improved during future work if the algal community in the system at that time of year is monitored and possibly manipulated to ensure a community structure suitable for maximum nutrient uptake. The effect that algal community structure has on HRAP efficiency should be investigated in future research.

4.4 Optimising algal productivity in the high rate algal ponds [2]

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 4.4.1).

Algal productivity was positively correlated with warmer water temperatures (Figure 4.4.2) and negatively correlated with shorter HRTs (Figure 4.4.3) in all four algal ponds. The

[2] This section comes from MSc student Anneke Cilliers's thesis and Cilliers et al. 2013 in review.

average algal productivity during the optimization phase was approximately 1.09 kg/d in all four HRAPs (Table 4.4.1).

Flow rate, and subsequently HRT, influenced algal productivity in the warmer months. The optimal HRT determined for autumn was 4.30 d at a temperature of 18.96 - 20.53°C. The optimal HRT for summer was 2.74 d at a temperature of 26.36 - 29.90°C.

Algal productivity generally increases with increased pond temperature until an optimum temperature is reached, above which 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). Productivity in the current study increased with increased temperature but the temperatures were not in the optimal range proposed by Park et al. (2011).

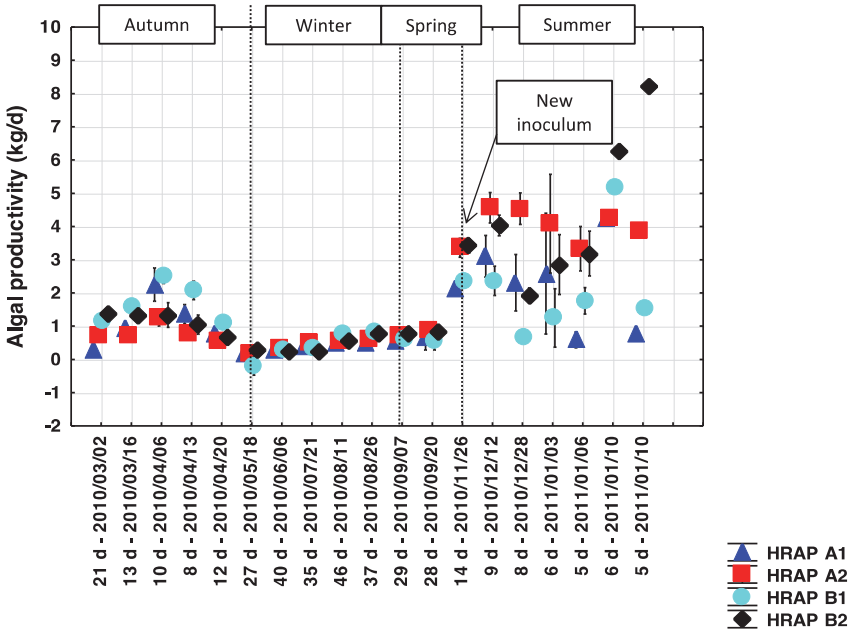


Figure 4.4.1 The mean (± standard error) algal productivity (kg/d) in high rate algal pond (HRAP) A1, A2, B1 & B2. The length of the combined hydraulic retention time (HRT) in the primary facultative pond (PFP) and HRAPs appears below the dates on the x-axis in days (d). Each date indicates the start of a new HRT (Cilliers 2012; Cilliers et al. 2013 in review).

Table 4.4.1 The mean (± standard error), minimum and maximum algal productivity (kg/d) in high rate algal ponds (HRAP) A1, A2, B1 and B2 from January 2010 to January 2011 (Cilliers 2012; Cilliers et al. 2013 in review).

System	Mean	Std.Err.	Min.	Max	N
Post-HRAP A1	0.90	0.09	-3.17	6.42	166
Post-HRAP A2	1.19	0.12	-0.29	6.64	161
Post-HRAP B1	1.02	0.09	-2.60	5.17	167
Post-HRAP B2	1.23	0.11	-0.33	8.20	161

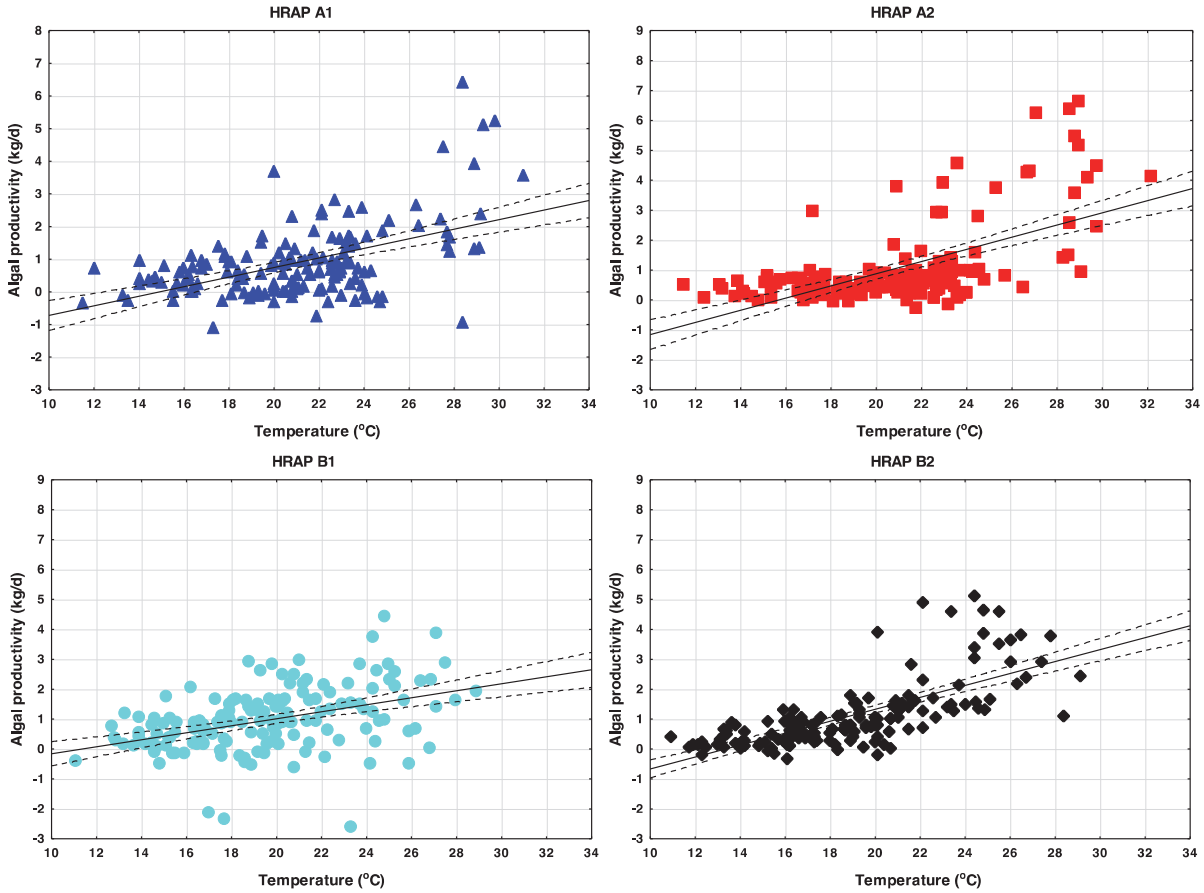


Figure 4.4.2 A multiple linear regression analysis of the relationship between algal productivity and water temperature in high rate algal pond (HRAP) A1, A2, B1 and B2. HRAP A1: r = 0.51, R2 = 0.21, p < 0.0001, F = 40.15. HRAP A2: r = 0.61, R2 = 0.37, p < 0.0001 and F = 88.06. HRAP B1: r = 0.42, R2 = 0.18, p < 0.0001 and F = 34.26. HRAP B2: r = 0.72, R2 = 0.51, p < 0.0001 and F = 156.94 (Cilliers 2012).

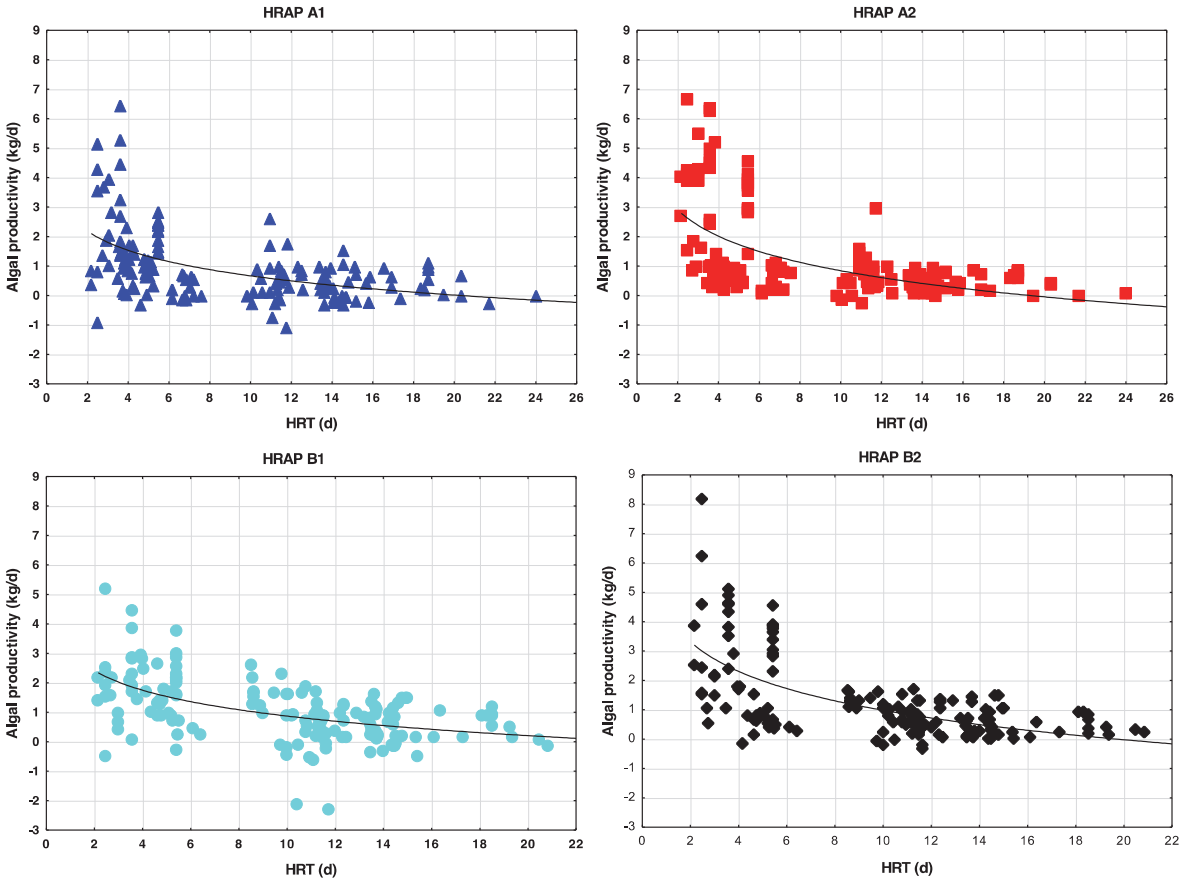


Figure 4.4.3 A multiple linear regression analysis of the relationship between algal productivity and hydraulic retention time (HRT) in high rate algal pond (HRAP) A1, A2, B1 and B2. HRAP A1: r = -0.46, R2 = 0.27, p < 0.0001, F = 44.34. HRAP A2: r = -0.49, R2 = 0.30, p < 0.0001 and F = 50.59. HRAP B1: r = -0.52, R2 = 0.26, p < 0.0001 and F = 59.38. HRAP B2: r = 0.58, R2 = 0.37, p < 0.0001 and F = 79.36 (Cilliers 2012).

A shorter HRT resulted in more dilute algal culture, yet higher algal productivity in the long-term trial. The increased algal productivity under these conditions was probably due to increased exposure to sunlight that resulted from reduced shading in the more dilute cultures (Azov & Shelef 1982; Ogbonna and Tanaka 1996).

With higher algal productivity, a smaller area would be needed for an HRAP to treat a given volume of effluent. The addition of CO_2 might increase algal productivity and minimise further the area that would be needed to treat effluent (Park et al. 2011). This should be tested in future studies.

The species composition of the algal culture and the presence of grazers and algal detritus influences algal productivity (Johnson 2010; Park et al. 2011). Future work should take algal species and community composition into consideration.

Increased flow rates and reduced HRT could potentially address the problem of high COD, reduce the space needed to treat a given volume of brewery effluent and increase the volume of algae that can be produced as a downstream product of brewery effluent treatment.

4.5 Scaling up to a commercial-scale high rate algal ponding system

Two exercises were carried out to estimate the area needed to treat 1000 m^3 of post-AD) brewery effluent per day, based on the mean treatment rates that were obtained by the experimental-scale HRAP system. These exercises assumed a direct relationship between treatment rate, surface area and volume of the experimental and full commercial-scale system. In reality, scaled up figures are not always directly proportional to experimental-scale figures; the assumption that the figures are directly proportional still needs to be verified in a full commercial-scale system. The exercise was done here for illustrative purposes only at this time. The first exercise assumed an experimental-scale treatment rate of 1.47 m^3/d and a mean HRAP depth of 0.2 m (i.e. the actual depth of the experimental system), and estimated an area of 2.0 ha would be needed to lower the ammonia concentration and chemical oxygen demand of 1000 m^3/d of post-AD brewery effluent to 1 mg/L and 170 mg/L, respectively (Table 4.5.1, Exercise 1). The second assumed the same parameters as the first, only the commercial scale pond depth was assumed to be 0.3 m (Table 4.5.1). This saw the required area of the HRAP systems lowered to 1.4 ha.

The area needed to treat 1000 m^3 of post-AD brewery effluent per day in a commercial-scale HRAP is likely to lie somewhere between 1.4 and 2.0 ha. However, this has not been tested on a commercial scale and the limitations of the assumptions used to scale-up these data have already been mentioned.

Table 4.5.1 An estimate of the area required to treat 1000 m^3/d of post-AD brewery effluent in an HRAP system based on experimental efficiencies that treated 1.4 m^3/d and commercial-scale ponds with different water depths.

Exercise 1

	Pond dimensions		
Inputs	Pond 1	Pond 2	
Pond width (m)	1.7	1.7	
Pond length (excl. ends) (m)	6.8	7.1	
Area of pond (m^2)	14.77	15.17	29.94
Pond depth (m)	0.28	0.12	
Pond volume (m^3)	4.14	1.82	5.96
Output water specs			
HRAP Temperature (°C)		18	°C
Post-HRAP ammonia (mg/L)		1.0	mg/L
Post-HRAP COD (mg/L)		170	mg/L
Mean volume of effluent treated in exp. system			
Volume treated in exp. system (m3/c		1.470	m3/d
Ratio of AREA to inflow		20.367	
Area needed to achieve these specs		**1000**	**m3/d**
Area required:		20367	m^2
Assumed mean pond depth		0.20	m
		2.0	**ha**

Exercise 2

	Pond dimensions		
Inputs	Pond 1	Pond 2	
Pond width (m)	1.7	1.7	
Pond length (excl. ends) (m)	6.8	7.1	
Area of pond (m^2)	14.77	15.17	29.94
Pond depth (m)	0.28	0.12	
Pond volume (m^3)	4.14	1.82	5.96
Output water specs			
HRAP Temperature (°C)		18	°C
Post-HRAP ammonia (mg/L)		1.0	mg/L
Post-HRAP COD (mg/L)		170	mg/L
Mean volume of effluent treated in exp. system			
Volume treated in exp. system (m3/d		1.470	m3/d
Ratio of **VOLUME** to inflow		4.052	
Area needed to achieve these specs		**1000**	**m3/d**
Volume needed		4052	m^3
Assumed pond depth		0.30	m
Area required:		13506	m^2
		1.4	**ha**

5. OPTIMISING THE PERFORMANCE OF THE CONSTRUCTED WETLAND

The constructed wetland (CW) removed most nutrients from brewery effluent to within the Department of Water Affairs (DWA) general limits for discharge into a natural water body (Chapter 3). Like the high rate algal ponds (HRAP), these systems require a large geographical "foot print", so their use is also limited to industries with sufficient space and their operations needs to be optimised to best use this space. The overall aim of this section was to determine the length of CW needed to remove nutrients from brewery effluent and to determine when/where the CW should be included in the suite of technologies tested in this program, i.e. as a polishing or a primary treatment process.

5.1 Optimising constructed wetland length

The full length of the CW was probably not necessary for a final treatment stage at the flow rates used in that experiment (135±8.9 L/h, during daylight hours only; Section 3). An analysis was subsequently done to determine the optimum length of wetland needed to remove nutrients from post-HRAP effluent at this flow rate.

The four channels of the CW were run in series – effectively creating a single wetland with a gravel bed that was 52 m in length – to determine the optimum length of the CW needed to remove nutrients from the brewery effluent at a flow rate of 135±8.9 L/h (n = 40; during day light hours).

The change in pH and ammonia levelled off somewhere between 0 and 13 m of the linear wetland, i.e. within one quarter of the total length of the wetland's gravel bed (Figures 5.1.1A) with both parameters well within the DWA general limit for the discharge of wastewater into a water resource. Nitrate continued to level off down the length of the raceway, with almost all the nitrate removed by the end of the raceway (Figure 5.1.1B); however, nitrate levels were within the DWA general limit at all times. The change in filtered chemical oxygen demand (COD) levelled off after about 26 m, i.e. halfway down the length of the wetland (Figure 5.1.1C), at which point mean COD levels were also within the DWA general limit.

In summary, the wetland need not be more than half of its current length to treat effluent at a rate of 135±8.9 L/h during daylight hours.

Wetland length (m)	Mean Ammonia (mg/L) ± standard error	n	Reduction (%)
0	6.3 ± 2.58	5	0
13	3.0 ± 2.48	4	53
26	2.8 ± 1.96	4	56
39	2.3 ± 1.64	4	64
52	2.2 ± 1.05	5	65

A

Wetland length (m)	Mean Nitrate (mg/L) ± standard error	n	Reduction (%)
0	10.1 ± 1.75	4	0
13	7.1 ± 1.12	4	30
26	3.7 ± 1.26	4	63
39	1.4 ± 0.80	4	86
52	0.4 ± 0.35	5	96

B

Wetland length (m)	Mean COD (mg/L) ± standard error	n	Reduction (%)
0	98.1 ± 9.88	7	0
13	83.0 ± 4.95	7	15
26	64.4 ± 5.69	7	34
39	78.7 ± 15.18	7	20
52	61.1 ± 7.23	7	38

C

Figure 5.1.1 The reduction of (A) ammonia, (B) nitrate and (C) chemical oxygen demand (COD – filtered to 8 µm; i.e. not all algae removed from sample) down the length of a wetland at a flow rate of 135±8.9 L/h.

5.2 The treatment of brewery effluent in the constructed wetland without pre-treatment in the high rate algal pond

The CW was included as a finishing or polishing process to further remove nutrients from brewery effluent that was first pre-treated in the anaerobic digester (AD) and then in the HRAP system (Chapter 3; Section 5.1). Post-AD effluent is high in nutrients, and algae in the HRAP assimilate these nutrients into algal biomass. However, it was hypothesised that it might be possible to adequately treat brewery effluent in a CW without initially reducing the nutrient load in the HRAP. The aim of this trial was thus to determine if the CW could adequately remove nutrients from post-AD effluent that had not undergone pre-treatment in the HRAP; and the objective was to compare the efficiency of that wetland to a similar wetland fed with effluent that had undergone HRAP pre-treatment.

Post-HRAP effluent was drained from one of the wetland beds and was replaced with post-AD effluent that was drawn from the primary facultative pond (PFP), i.e. before HRAP treatment, and was subsequently fed from the same source at 200 L/day every weekday (i.e. “post-AD” treatment). A second wetland bed was used as a control, referred to as the “post-HRAP” treatment, which was fed with post-HRAP effluent at the same rate as the first bed.

Water quality parameters were measured from well points positioned at intervals (0, 3, 6, 9 and 15 m) down the length of each 15 m wetland bed. By pumping a known volume of water every weekday into each bed, it was possible to track the water from the inlet, through the bed to the outlet using plug flow reactor modelling.

The reeds in both beds were cropped to a height of 10-15 cm one week before the start of the trial.

The plants in the wetland that received water directly from the PFP (i.e. post-AD treatment) increased in biomass at a faster rate than those in the wetland that received effluent from the HRAP (Figure 5.2.1), due to the higher nutrient load in post-AD effluent. The reeds in the post-AD treatment appeared healthy and able to cope with post-AD effluent, whereas algae in the HRAP removed sufficient nutrient from the effluent to retard reed growth in the wetland that received post-HRAP effluent (Figure 5.2.1).

The filtered COD entering the post-HRAP effluent was uncharacteristically high – as high as the COD entering the post-AD wetland, both at around 190 mg/L (Figure 5.2.2). As such, the comparison here of the post-HRAP and post-AD treatments should be made in relation to the two treatments only, rather than focusing on the absolute values that were recorded. The efficiency of COD removal was similar for both treatments as the wetlands that received post-AD and post-HRAP effluent both lowered COD by approximately 30 % from about 190 to 135 mg/L (Figure 5.2.2).

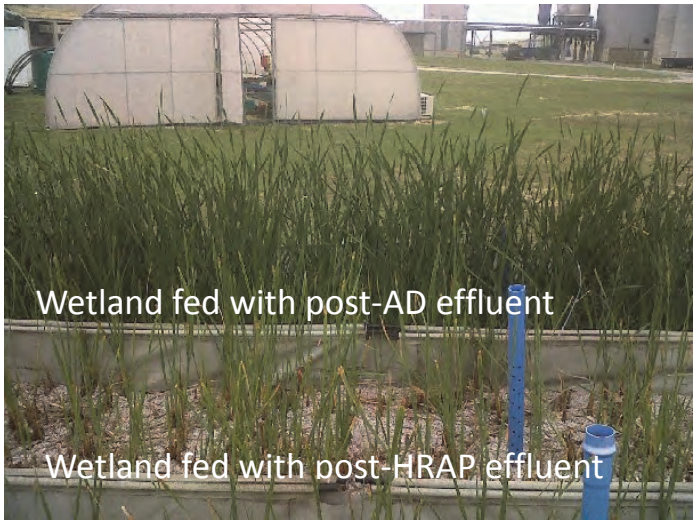


Figure 5.2.1 Reed growth in two constructed wetlands; one fed with effluent from the anaerobic digester (AD) via the primary facultative pond, and one fed from effluent that was first treated in the high rate algal ponding (HRAP) system. The picture was taken on 15 April 2011 (Photo: Lara Crous).

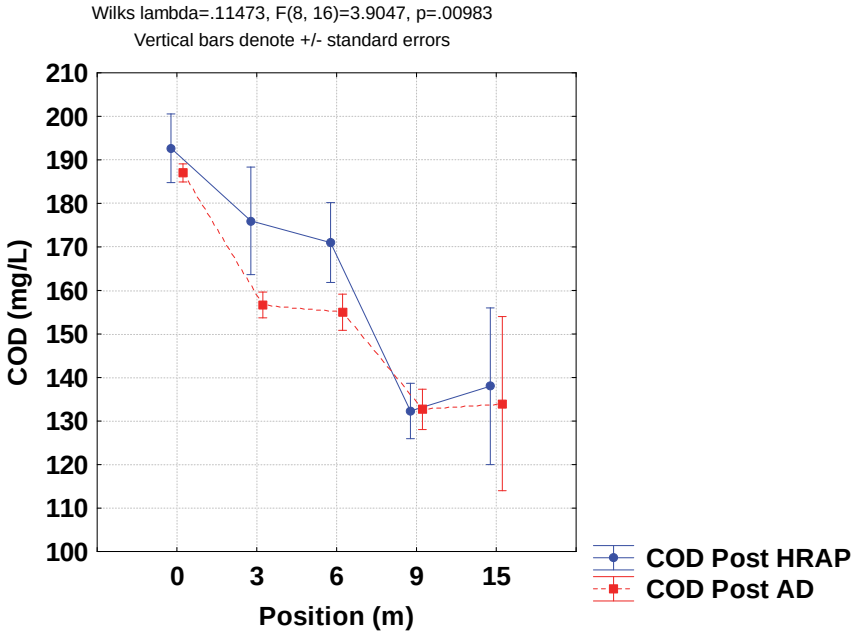


Figure 5.2.2 Mean (± standard error) filtered chemical oxygen demand (COD) of brewery effluent as it moved through a 15 m wetland. The inlet of the wetland, i.e. 0 m, was fed by effluent that had been subjected to either anaerobic digestion (AD), followed by primary facultative pond (PFP) and high rate algal pond (HRAP) treatment prior to the wetland (i.e. "Post HRAP" treatment), or to AD and PFP only (i.e. "Post AD" treatment, where HRAP was excluded).

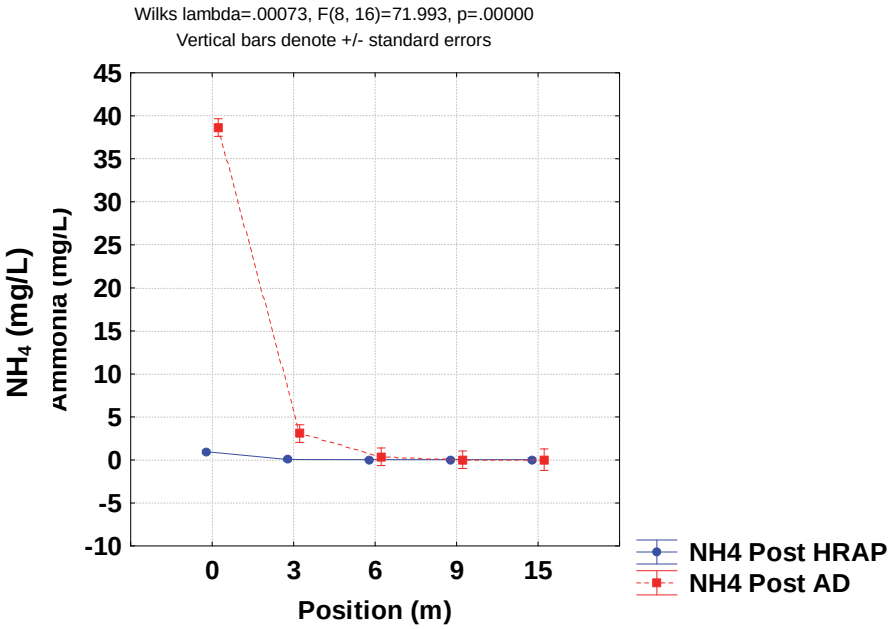


Figure 5.2.3 Mean (± standard error) ammonia concentration of brewery effluent as it moved through a 15 m wetland. The inlet of the wetland, i.e. 0 m, was fed by effluent that had been subjected to either anaerobic digestion (AD), followed by primary facultative pond (PFP) and high rate algal pond (HRAP) treatment prior to the wetland (i.e. "Post HRAP" treatment) or to AD and PFP only (i.e. "Post AD" treatment, where HRAP was excluded).

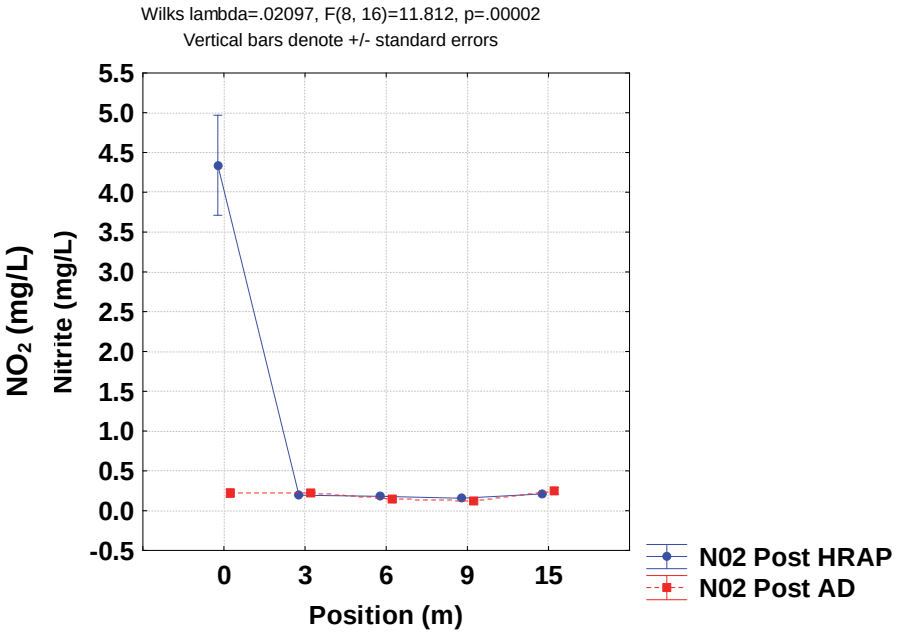


Figure 5.2.4 Mean (± standard error) nitrite concentration of brewery effluent as it moved through a 15 m wetland. The inlet of the wetland, i.e. 0 m, was fed by effluent that had been subjected to either anaerobic digestion (AD), followed by primary facultative pond (PFP) and high rate algal pond (HRAP) treatment prior to the wetland (i.e. "Post HRAP" treatment) or to AD and PFP only (i.e. "Post AD" treatment, where HRAP was excluded).

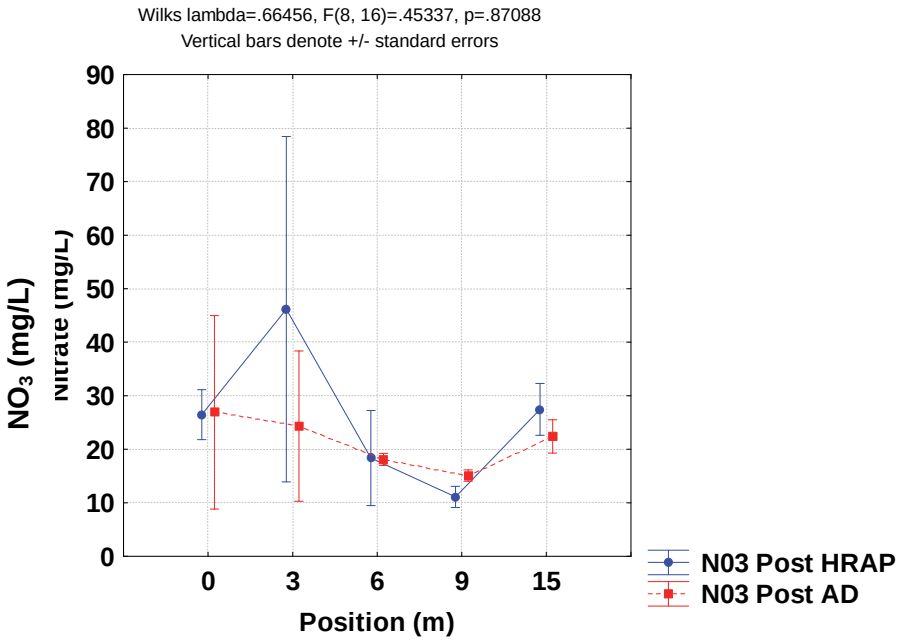


Figure 5.2.5 Mean (± standard error) nitrate concentration of brewery effluent as it moved through a 15 m wetland. The inlet of the wetland, i.e. 0 m, was fed by effluent that had been subjected to either anaerobic digestion (AD), followed by primary facultative pond (PFP) and high rate algal pond (HRAP) treatment prior to the wetland (i.e. "Post HRAP" treatment) or to AD and PFP only (i.e. "Post AD" treatment, where HRAP was excluded).

The wetlands were efficient at removing nitrogen from the effluent and there is no evidence that this efficiency was reduced when the HRAP was removed from the treatment process (Figures 5.2.3 to 5.2.5). There was substantially more ammonia in the post-AD effluent than the post-HRAP effluent (Figure 5.2.3). However, the wetland was efficient at removing ammonia from the post-AD effluent and brought it to within the DWA general limit of 6 mg/L within the first three meters of the wetland (Figure 5.2.3). The post-HRAP wetland removed what little ammonia was available after the algae had removed ammonia in the HRAP ponds (Figure 5.2.3). The presence of nitrite entering the post-HRAP wetland was a result of bacterial activity in the HRAP where ammonia was converted to nitrite (Figure 5.2.4). The wetland removed most nitrite in the effluent within the first three meters (Figure 5.2.4). The presence of nitrate entering the post-AD treatment is probably due to the conversion of ammonia to nitrate in the PFP (note: post-AD effluent was drawn from the PFP and not directly from the AD). Nitrate removal in the wetland was similar for both treatments and was brought within the DWA general limit after nine meters of treatment (Figure 5.2.5). The reason for the increase of nitrate in the sump (i.e. 15 m) requires further investigation (Figure 5.2.5).

Final phosphate concentration was similar for both wetlands, irrespective of differences in the phosphate level in the influent of both systems (Figure 5.2.6). The high phosphate levels of the influent are a result of increased phosphate entering the system. The phosphate concentrations in the effluent at the end of the wetland were not brought to within the DWA general limit of 10 mg/L; this was the same for both wetlands in this experiment. Alternative sustainable methods of precipitating phosphate from the system, which could be used in combination with the technologies being tested here, should be investigated. For example, stoichiometric coupling to microbial growth or enhanced storage in the biomass could be investigated. Other possible processes might involve the addition of certain chemicals such as lime, alum and ferric chloride. These chemicals act on the phosphorus and form a precipitate with low solubility, which can then be removed from the systems by sedimentation, filtration or flotation, for example.

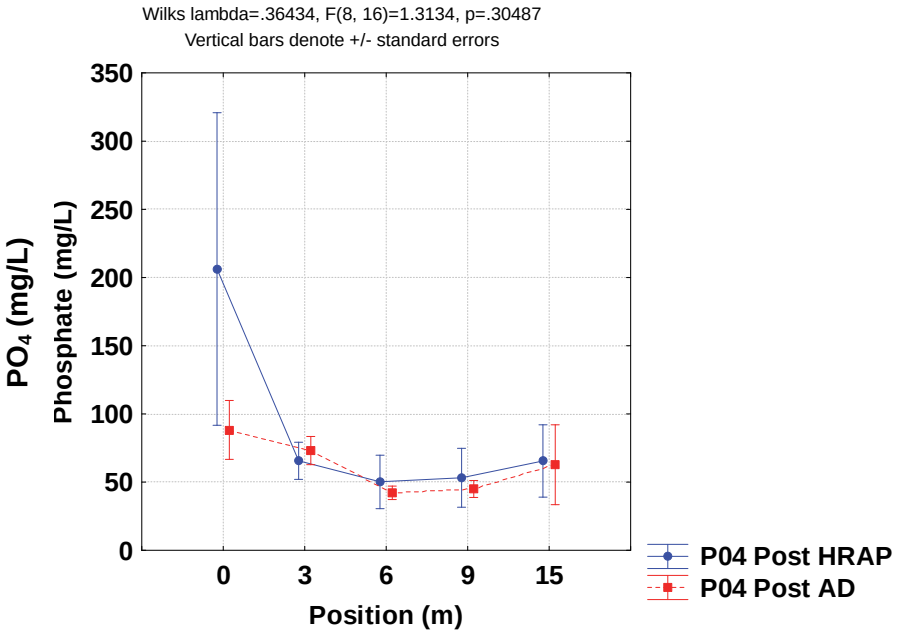


Figure 5.2.6 Mean (± standard error) phosphate concentration of brewery effluent as it moved through a 15 m wetland. The inlet of the wetland, i.e. 0 m, was fed by effluent that had been subjected to either anaerobic digestion (AD), followed by primary facultative pond (PFP) and high rate algal pond (HRAP) treatment prior to the wetland (i.e. "Post HRAP" treatment), or to AD and PFP only (i.e. "Post AD" treatment, where HRAP was excluded).

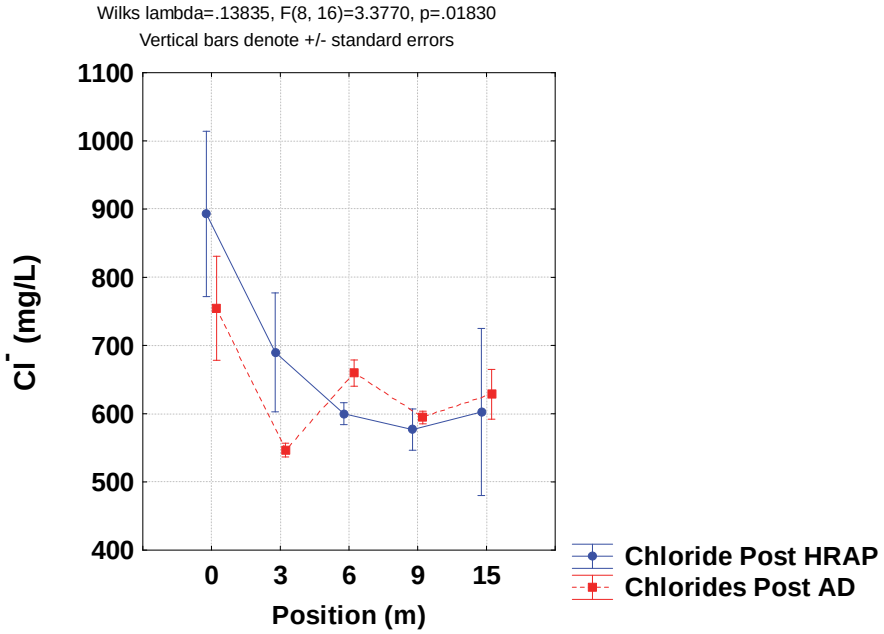


Figure 5.2.7 Mean (± standard error) chloride concentration of brewery effluent as it moved through a 15 m wetland. The inlet of the wetland, i.e. 0 m, was fed by effluent that had been subjected to either anaerobic digestion (AD), followed by primary facultative pond (PFP) and high rate algal pond (HRAP) treatment prior to the wetland (i.e. "Post HRAP" treatment), or to AD and PFP only (i.e. "Post AD" treatment, where HRAP was excluded).

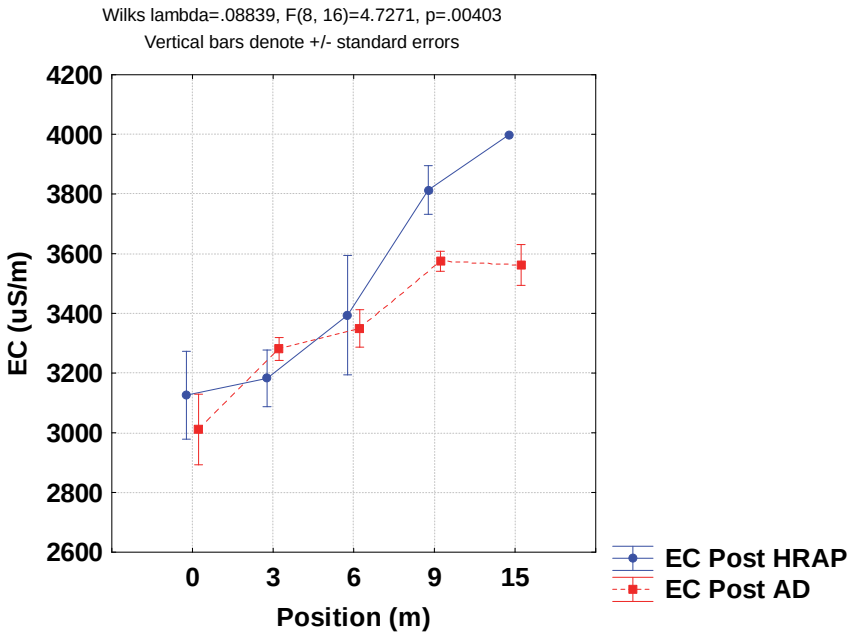


Figure 5.2.8 Mean (± standard error) electrical conductivity (EC) of brewery effluent as it moved through a 15 m wetland. The inlet of the wetland, i.e. 0 m, was fed by effluent that had been subjected to either anaerobic digestion (AD), followed by primary facultative pond (PFP) and high rate algal pond (HRAP) treatment prior to the wetland (i.e. "Post HRAP" treatment), or to AD and PFP only (i.e. "Post AD" treatment, where HRAP was excluded).

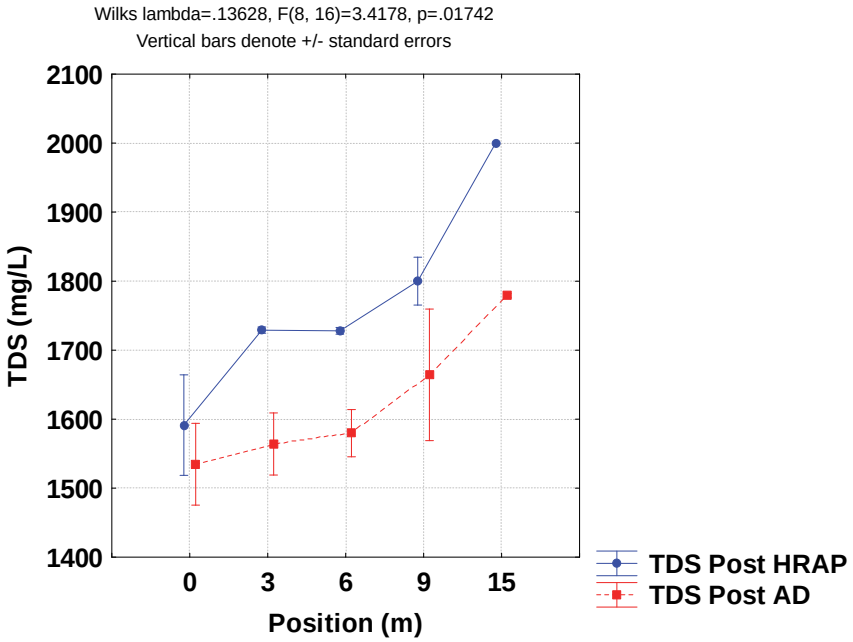


Figure 5.2.9 Mean (± standard error) total dissolved solid (TDS) concentration of brewery effluent as it moved through a 15 m wetland. The inlet of the wetland, i.e. 0 m, was fed by effluent that had been subjected to either anaerobic digestion (AD), followed by primary facultative pond (PFP) and high rate algal pond (HRAP) treatment prior to the wetland (i.e. "Post HRAP" treatment), or to AD and PFP only (i.e. "Post AD" treatment, where HRAP was excluded).

The wetland lowered the chloride concentration by an average of about 27% and this trend was similar for both the wetlands (Figure 5.2.7), and is not consistent with previous data, probably due to large volumes of rain at the time of the experiments. Total dissolved solids (TDS) and electrical conductivity (EC) increased down the length of the wetland, which is consistent with past results (Figures 5.2.8 and 5.2.9); however, the increase in the post-AD treatment was not as high as the increase in the wetland that received effluent from the HRAP, even though the starting EC and TDS were similar in both treatments (Figure 5.2.7). Again, the rain might have influenced the absolute results here.

The wetland continued to reduce pH down its length and the rate of pH lowering was similar for both wetlands (Figure 5.2.10). The difference in the final pH between treatments is probably due to the starting difference. The pH of effluent from the HRAP is higher on average due to the photosynthetic activity of the algae. All pH values remained within in the DWA general limit of between pH 5.5 and 9.5 (Figure 5.2.10).

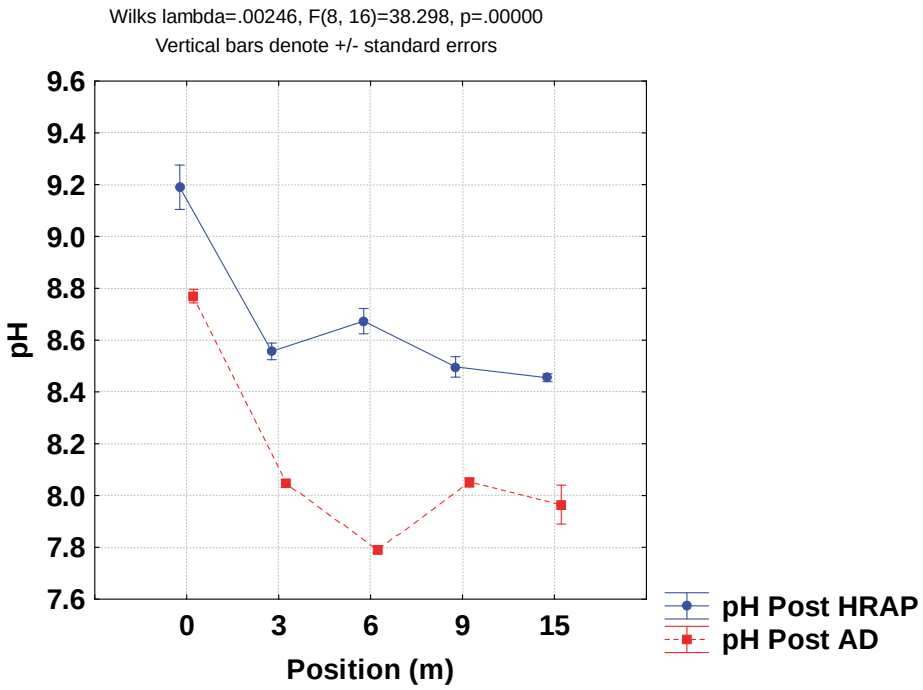


Figure 5.2.10 The pH (± standard error) of brewery effluent as it moved through a 15 m wetland. The inlet of the wetland, i.e. 0 m, was fed by effluent that had been subjected to either anaerobic digestion (AD), followed by primary facultative pond (PFP) and high rate algal pond (HRAP) treatment prior to the wetland (i.e. "Post HRAP" treatment), or to AD and PFP only (i.e. "Post AD" treatment, where HRAP was excluded).

Brewery effluent that had not undergone HRAP pre-treatment was successfully treated in the CW, to a similar standard of effluent that had undergone HRAP pre-treatment. These results suggest that it might be possible to cut the HRAP system from the treatment process,

without compromising the quality of the final effluent. However, the footprint and cost of constructing a wetland required to treat equivalent volumes of water would need to be considered. Also, the longevity of the wetland should be taken into account. The organic load in a wetland is likely to slowly build up and parts of it may become anoxic with time (possibly over many years but maybe over a shorter period, depending on the organic load in the effluent), whereas an HRAP pond can be cleaned and re-inoculated and brought back into full operation within days. Wetland beds can, however, be replanted and revitalised.

6. BENEFICIATION OF BREWERY EFFLUENT – HYDROPONICS

Brewery effluent consists of water and nutrients; however, neither the water nor the nutrients are available for re-use in the form of raw brewery effluent. The overall aim of the hydroponic experiments was to develop protocols to use treated brewery effluent as (a) a source of water and (b) a source of nutrient. These protocols needed to be developed for different times of year. Preliminary experiments were carried out using lettuce and more in depth studies were subsequently carried out using tomatoes. Growth rates of vegetables grown using treated effluent as a water and/or nutrient source were compared to those grown using more conventional inorganic fertilizer and fresh water as a reference.

6.1 Preliminary experiments – growing lettuce in treated brewery effluent[3]

The growth of lettuce in 100 % post-high rate algal pond (HRAP) was compared to lettuce grown in municipal water and inorganic fertiliser only, i.e. the control treatment (Trial 1). The same methods used in Trial 1 were used in Trials 2 and 3, with the exception that the treated brewery effluent was diluted with municipal water in Trial 2 and the effluent diluted with municipal water was supplemented with inorganic fertilizer in Trial 3.

The electrical conductivity (EC) of the water in the treated brewery effluent system ranged from 2700 to more than 4000 µS/cm over the period of this trial, which took place in mid-summer. It was concluded that lettuce cannot be grown in full strength brewery effluent at high temperatures since the rate of evaporation and transpiration increased at elevated temperatures, leaving behind concentrated nutrient solution. The lettuce did not respond well and showed signs of physiological stress. Municipal treatment lettuce grew eight times faster than effluent treatment lettuce, for both the frilly red and tudela varieties (4.88±0.14 and 0.66±0.03 g/day and 8.90±0.15 and 1.05±0.02 g/day, respectively; Table 6.1.1).

High temperatures were experienced in March 2010, at which time trials were run to test the effect of regular dilution of treated brewery effluent as a growth medium for hydroponic lettuce production (Trial 2). The increased salt concentration as a result of increased evaporation was assumed to be the reason for reduced growth observed earlier. The aim of this experiment was thus to develop a protocol that regulated salt concentrations that were previously accumulating as a result of increased evaporation at these temperatures. The health of the seedlings improved, compared to those grown previously in undiluted effluent, and the treated brewery effluent lettuce grew better than in the previous trial. Inorganic/municipal-water lettuce grew at only twice the rate of equivalents grown in brewery effluent (1.48 ± 0.15g v 0.8 ± 0.11 g/day for frilly red lettuce and 0.74 ± 0.58g v 0.31 ± 0.11g for tudela lettuce; Table 6.1.1). Furthermore, with dilution, there was no evidence of physiological stress (i.e. wilting and yellowing). However, the growth rate of the seedlings in brewery effluent was still considerably lower than that of the

[3] This section of the report was used in a report delivered to SAB, a co-funder of this section of the research

seedlings grown in municipal water and inorganic fertilizer (i.e. the control). It was established that the plants in the diluted effluent treatment in Trial 2 showed symptoms consistent with nutrient deficiency.

Table 6.1.1 Mean (± standard error) daily weight gain (i.e. growth rate) of lettuce grown from Jan-Feb 2010 (30 days), in March 2010 (18 days) and from Apr-May 2010 (43 days) in either treated brewery effluent (Effluent) or using a conventional water source and organic fertiliser (Municipal).

	Effluent	Municipal	Supporting Statistics	
			Sig. diff.	P-value
Growth Trial 1 (Jan-Feb 2010)				
Growth rate (g day^{-1}) "frilly red"	0.66 ± 0.03	4.88 ± 0.14	yes	<0.0001
Growth rate (g day^{-1}) "tudela"	1.05 ± 0.02	8.90 ± 0.15	yes	<0.0001
Growth Trial 2 (Mar 2010)				
Growth rate (g day^{-1}) "frilly red"	0.80 ± 0.11	1.48 ± 0.15	yes	0.00097
Growth rate (g day^{-1}) "tudela"	0.31 ± 0.11	0.74 ± 0.58	yes	0.00105
Growth Trial 3 (Apr-May 2010)				
Growth rate (g day^{-1}) "frilly red"	0.79 ± 0.10	1.70 ± 0.16	yes	<0.0001
Growth rate (g day^{-1}) "butter lettuce"	0.02 ± 0.03	0.70 ± 0.07	yes	<0.0001

A fertilizer that was specifically formulated to compensate for the nutritional deficiencies in the treated brewery effluent was formulated. This was used to supplement the nutrients in the brewery effluent treatment in Trial 3. In addition to the supplementation of nutrients, frequent water changes were necessary as the plants began to show stress (wilting and yellowing of the leaves) early in the grow-out phase. Physiological stress was noticed within the first two weeks, even when the conductivity had not reached the upper limits of 4000 µS/cm, indicating that more dilution was required at the high temperatures experienced. Again, it was confirmed that the amount of water available for the uptake and fixing of nutrients by the plants was probably limiting.

The plants showed signs of recovery and grew to a harvestable size in the same time period as those in the inorganic/municipal water treatment in Trial 3 (Figure 6.1.1). However, the frilly red lettuce in municipal treatment had a significantly faster growth rate than the same variety of lettuce grown in the brewery effluent treatment at the time of harvest (1.7 ± 0.16 and 0.79 ± 0.1 g/day respectively; Table 6.1.1). The comparison of the butter lettuce in the municipal water and treated brewery effluent showed a similar trend in this trial (0.7 ± 0.07 and 0.02 ± 0.03 g/day respectively; Table 6.1.1). Although the final mean growth rate of the crop grown in brewery effluent was less than that grown using inorganic fertiliser, the fast recovery of the latter treatment after the initial lag (which probably accounts for the difference in growth rate), demonstrated that we were on the right track in developing a suitable production method. This is well illustrated in Figure 6.1.1, which illustrates the similarity of the lettuces leading up to the harvest.

Figure 6.1.1 Lettuce grown in treated brewery effluent supplemented with inorganic fertiliser (channels marked A) and lettuce grown in municipal water using conventional inorganic fertiliser (channels marked B) (Photo: Rory Scheepers).

Lettuce production under the conditions recorded in Trial 3 required a water change twice weekly and additional nutrient supplementation for the entire growth period. A dilution of 150 L of municipal water to 250 L post-IAPS effluent, i.e. a ratio of 3:5, with the addition of inorganic fertiliser supplementation, was required.

Treated brewery effluent can be used to grow lettuce hydroponically, but when temperatures are hot and evaporative water loss is high, the effluent must be diluted. Furthermore, the nutrient levels in the brewery effluent change seasonally, because the treated brewery effluent was poor in nutrients due to (a) the lack of phosphate coming out of the brewery and (b) the efficiency of nutrient uptake by algae in the summer months. This, combined with initially inadequate dilution, probably accounted for the initial poor growth in Trial 3. Further optimisation of lettuce growth using treated brewery effluent, which would be aimed particularly at optimising nutrient availability to the plants, is needed.

Trial 4 investigated cutting out the addition of an inorganic fertiliser by increasing the frequency that effluent was added to the hydroponic system. The top-up regimes were as follows: (1) The first system was topped up with post-HRAP effluent every seven days and (2) the second system was topped up with post-HRAP effluent every 14 days. The conductivity in the systems was difficult to maintain at the recommended 2000 µs/cm as the conductivity in post-HRAP water is characteristically upwards of 3000 µs/cm. For this

reason, the top-up schedule was based on how much water had evaporated and transpired.

The lettuce grown in the weekly top-up treatment increased more in overall size compared with those in the fortnightly treatment. Lettuce looked healthier and attained a heavier plant weight, with an overall mean after 44 days of 94.64 ± 19.43 g and 57.69 ± 12.95 g for lettuce in the seven-day and fourteen-day top-up regimes, respectively. It was concluded that nutrients in the brewery effluent were available to the plants and that a more frequent top-up regime resulted in better growth.

Trial 5 compared the growth of lettuce grown in a system with unlimited access to nutrients from brewery effluent (i.e. where addition of brewery effluent was not restricted by EC, which is an indirect measure of nutrient availability in the hydroponic industry) to a control treatment where EC was maintained within a range that is currently used in inorganic hydroponic lettuce production. The first system was topped up with post-HRAP effluent every Monday, Wednesday and Friday for the duration of the trial, irrespective of the EC of the hydroponic growth medium, and (2) the second system was also topped up with post-HRAP effluent, but it was diluted with municipal water to maintain EC between 2000 and 3000 µs/cm.

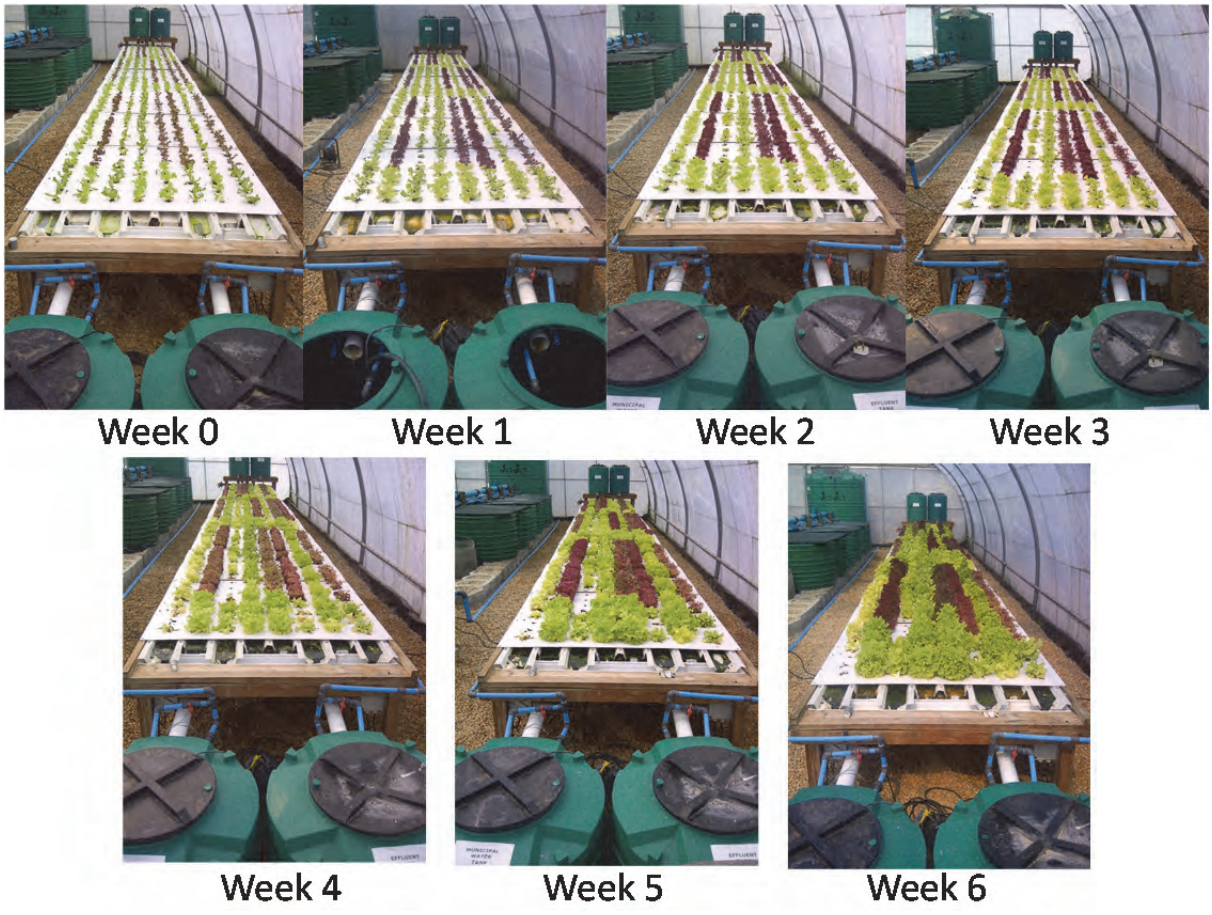


Figure 6.1.2 Hydroponic lettuce growth in treated effluent and municipal diluted effluent over the period of the trial under tunnel conditions (Photos: Rory Scheepers).

After 40 days, the lettuce grow in the effluent top-up treatment had significantly lower mean root and leaf weight gains compared to those grown in the treatment where effluent was diluted (Student t-test; t=4.93, p=0.000028; t=4.1, p=0.0002, respectively). Lettuce heads (i.e. leaves) were an average of 32 % heavier in the treatment where EC was maintained between 2000 and 3000 µs/cm. Overall, the lettuce grew well in this trial and it was again concluded that brewery effluent can be used as a nutrient source for hydroponic vegetable production (Figure 6.1.2). However, the reason for the poorer growth in the undiluted treatment remains unclear: it might have been due to increased salt concentrations or that the nutrients in the effluent were not available to the plants. This requires further research.

6.2 Can pH manipulation increase nutrient availability in brewery effluent hydroponics?

The results of preliminary hydroponic trials using treated brewery effluent demonstrated two important points. Firstly, water that has been treated in the HRAP ponds can be used as a hydroponic nutrient solution. Secondly, the nutrient in the post-HRAP water was not always available to the plants.

In traditional hydroponic systems the nutrient solution is tailored to suit the specific crop that is produced. Some nutrient solutions are even adjusted according to the different stages of plant growth since each stage has different nutrient requirements (e.g. vegetative growth, flowering or fruiting). This level of micro-management is probably not feasible in the case of the brewery effluent. The effluent stream is a complex, dynamic system. We are not able to control the upstream processes; however, the HRAP has been shown to be extremely stable, producing a consistent effluent irrespective of upstream fluctuations. Nonetheless, we must understand the subtle changes in this effluent and, if necessary, develop technologies/processes to simply and effectively manage the chemical fluxes of the effluent stream. We need to determine how well the effluent nutrients will support the vegetative growth, flowering and fruiting of crops and to determine the kind of effluent treatment or management that will turn treated brewery effluent into the most valuable nutrient solution for hydroponic vegetable production.

This section of work seeks to shed light on how the post-AD water treatment influences the water chemistry of the effluent and how these processes then influence nutrient availability and plant productivity of valuable crops. The two main areas of focus will be the changes in the nutrient balance of the effluent stream and how pH can act as a governing factor in nutrient availability.

The nutrient solutions used in the treatments were municipal water with added commercial hydroponic nutrients (i.e. control), post-primary facultative pond (PFP) water and post-HRAP water.

A multi-factor experiment was designed to determine if plant growth was influenced by different nutrient sources (i.e. conventional inorganic fertilizer, post-PFP effluent and post-HRAP effluent; factor 1; Table 6.2.1), with and without pH adjustment (factor 2; Table 6.2.1). The treatments were each replicated five times; i.e. five growth channel systems per treatment (Figure 2.13 and Figure 2.15) resulting in 25 plants per treatment.

Table 6.2.1 The six treatments (i.e. T1 to T6) that formed a multi-factor experiment where three different nutrient solutions (factor 1) were be tested with and without pH adjustment (factor 2).

Nutrient solution	pH not adjusted	pH adjusted to 6.0 to 6.5
Commercial (control)	T1	T2
Post-PFP effluent	T3	T4
Post-HRAP effluent	T5	T6

Tomato plants grew larger and produced more fruit when grown using inorganic fertiliser (i.e. control) compared with post-PFP and post-HRAP brewery effluent (Figures 6.2.1 to 6.2.4). There was largely no difference in development between plants grown in post-PFP and post-HRAP growth medium (Figures 6.2.1 to 6.2.4), with the exception that post-PFP plants were taller than post-HRAP plants when pH was maintained between 6.0 and 6.5 (Figure 6.2.3). However, in all instances development was increased significantly for both the post-PFP and post-HRAP plants when pH was maintained between 6.0 and 6.5 (Figures 6.2.1 and 6.2.2).

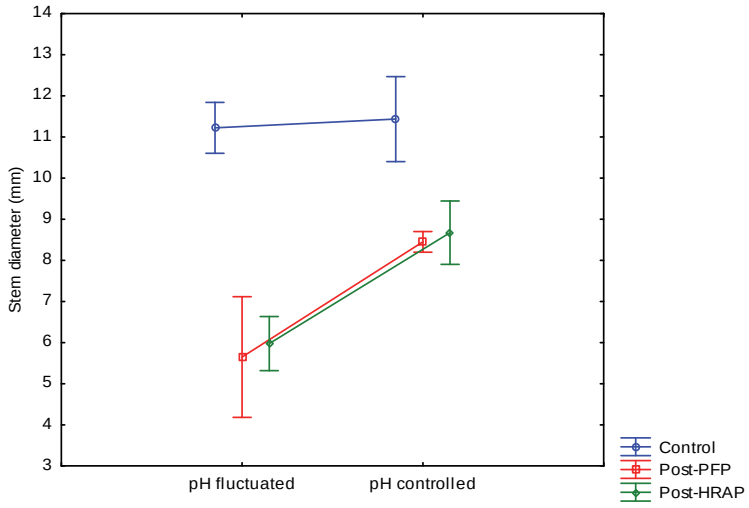


Figure 6.2.1 Mean (± 95 % confidence interval) stem diameter of tomato plants grown in either inorganic fertiliser (control), post-primary facultative pond (post-PFP) or post-high rate algal pond (post-HRAP) brewery effluent, with and without pH adjustment (multi-factor ANOVA: $F_{(2,24)}$=10.59, p=0.005).

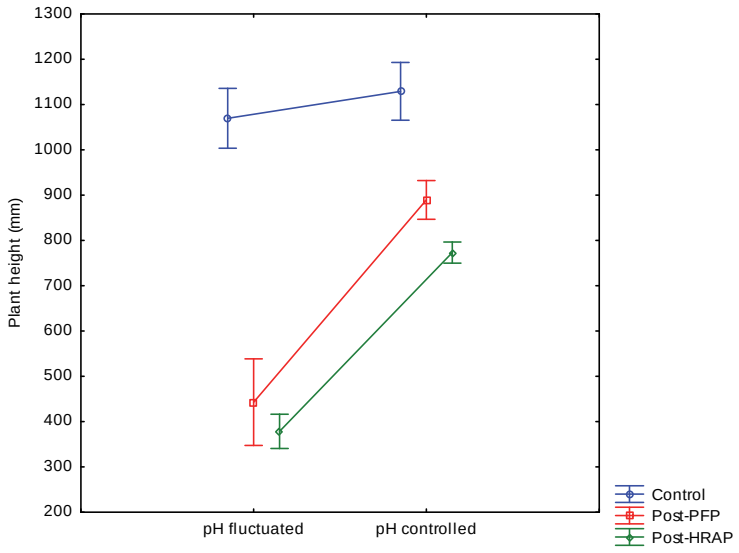


Figure 6.2.2 Mean (± 95 % confidence interval) total height of tomato plants grown in either inorganic fertiliser (control), post-primary facultative pond (post-PFP) or post-high rate algal pond (post-HRAP) brewery effluent, with and without pH adjustment (multi-factor ANOVA: $F_{(2,24)}$=47.78, p<0.00001).

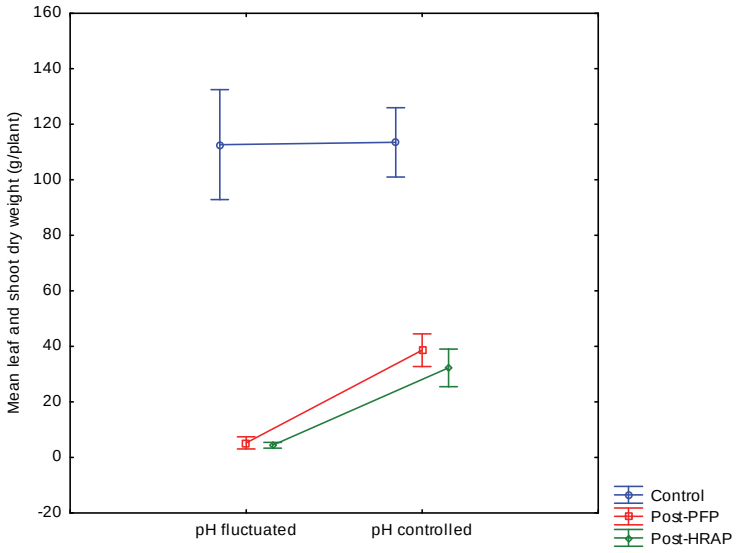


Figure 6.2.3 Mean (± 95 % confidence interval) dry shoot and stem weight of tomato plants grown in either inorganic fertiliser (control), post-primary facultative pond (post-PFP) or post-high rate algal pond (post-HRAP) brewery effluent, with and without pH adjustment (multi-factor ANOVA: $F_{(2,24)}$=11.06, p=0.00039).

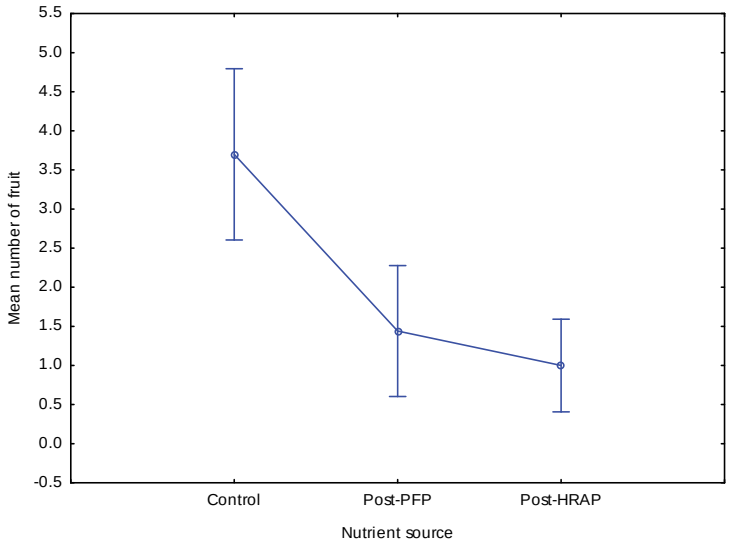


Figure 6.2.4 Mean (± 95 % confidence interval) number of fruit harvested from tomato plants grown in either inorganic fertiliser (control), post-primary facultative pond (post-PFP) or post-high rate algal pond (post-HRAP) brewery effluent (ANOVA: $F_{(2,24)}$=21.0, p=0.0001).

The difference in growth between the inorganic fertiliser treatment and treated brewery effluent was probably due to differences in nutrient concentrations. Phosphorus was not limited, since it remained present in the growth medium, with less variation when the effluent was subject to pre-treatment in the HRAP (Figure 6.2.5). Nitrate remained abundant in the inorganic fertiliser treatment, while it was nearly always depleted in the pH corrected brewery effluent treatments, so low nitrogen levels were probably responsible for the poorer vegetative growth in the treated brewery effluent plants (Figure 6.2.6). While ammonium was removed from all treatments at a similar rate, the rate of nitrate removal was significantly increased in the post-PFP treatment, but only when pH was maintained between 6.0 and 6.5 (Figure 6.2.6). The rate of nitrogen (ammonium, nitrite and nitrate) removal was significantly improved in the pH corrected treatments. This suggests that pH control, and not the addition of nutritional phosphorus derived from the phosphoric acid used to balance the pH, was responsible for the difference in the uptake of nitrogen in these plants and the overall improvement in plant growth and development.

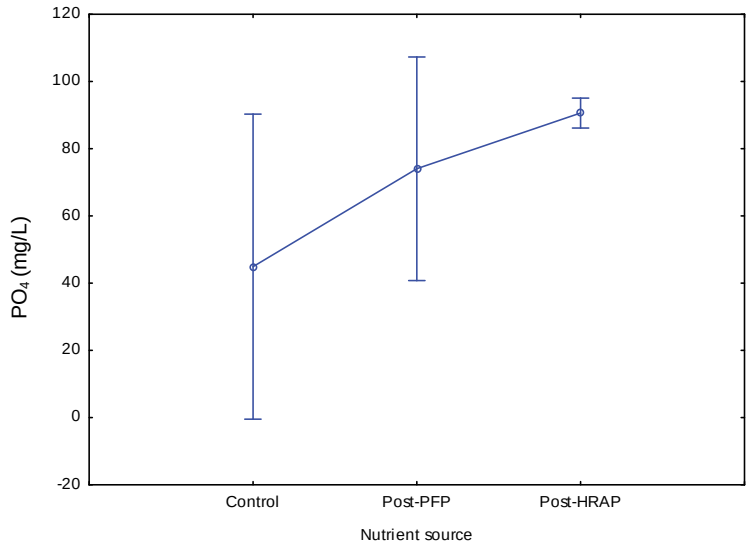


Figure 6.2.5 Mean (± 95 % standard error) phosphate (PO_4) concentration of either inorganic fertiliser (control), post-primary facultative pond (post-PFP) or post-high rate algal pond (post-HRAP) brewery effluent growth media used to grow tomato plants; data collected in the last week of a 49-day trial (ANOVA: $F_{(2,6)}$=8.43, p=0.018).

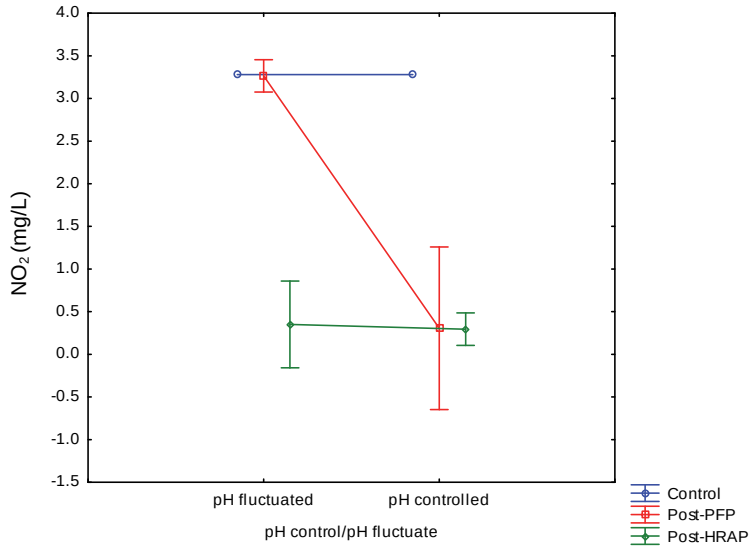


Figure 6.2.6 Mean (± 95 % standard error) nitrate (NO_2) concentration of either inorganic fertiliser (control), post-primary facultative pond (post-PFP) or post-high rate algal pond (post-HRAP) brewery effluent growth media, subject to pH control or no pH control, and used to grow tomato plants; data collected in the last week of a 49-day trial (ANOVA: $F_{(2,6)}$=1120.8, p<0.0001).

In conclusion, brewery effluent can be used as a nutrient source for hydroponic tomato production. Plant growth in treated brewery effluent was not equivalent to the inorganic fertiliser control treatment, but production was increased significantly overall when the pH of the treated brewery effluent was maintained between 6.0 and 6.5. The effluent based solutions were able to provide all of the essential nutrients required for growth, flowering and fruiting suggesting that other flower or fruit producing crops might be able to grow successfully in brewery effluent. The difference in growth was probably due to lower nitrogen levels, so future work should look at combining pH control with alternative methods of increasing the nitrogen that is available in the brewery effluent growth medium.

7. BENEFICIATION OF BREWERY EFFLUENT – AQUACULTURE

The high rate algal pond (HRAP) and constructed wetland (CW) produced a treated effluent that was either within or close to the Department of Water Affairs (DWA) general limits for the discharge of industrial effluents into natural water bodies; i.e. an effluent that is likely to have minimal or no negative effects on the plants and animals that live in rivers. The aim of this work was to establish if HRAP/CW could be used to treat brewery effluent to a standard suitable for fish culture. The growth, reproductive output and health of swordtail (*Xiphophorus helleri*), a live-bearing fish that is farmed for the aquarium fish trade, produced in treated brewery effluent, were compared to those of fish produced in control systems using a conventional water source.

7.1 Health, growth and reproduction of swordtail in treated brewery effluent[4]

Fish were stocked into one of two treatments: (1) five systems were filled and maintained with post-HRAP effluent and (2) five systems were filled and maintained with water from the municipal main water supply (Figures 2.11 and 2.12). The health and reproductive output of the broodstock held in the two systems and the growth of the juvenile fish produced in the two systems were compared.

Table 7.1.1 Reproductive output of swordtail kept in either treated brewery effluent using high rate algal ponds and a constructed wetland or a conventional water source (control) between 11 October 2010 and 17 January 2011.

	Mean no. of offspring/brood	Mean no. of offspring/tank	Total no. of broods	Total no. offspring
Control	34.7 ± 2.9	305 ± 33.4	44	1525
Effluent	42.7 ± 4.8	324 ± 68.9	38	1622
Statistical test	Student t-test	Mann-Whitney U-test	Chi-square	Chi-square
p-value	0.14	0.83	0.51	0.08
t-value	-1.47			
z-statistic		0.21		
Chi-square			0.44	2.99

[4] This section of the report was used in a report delivered to SAB, a co-funder of this section of the research, and will form part of Rory Scheepers' MSc thesis.

Broodstock grown in the two systems were similar in size and overall condition. There was no significant difference in the number of juvenile fish harvested between treatments (i.e. and average that ranged from 305 to 324 juveniles/tank, p=0.83; Table 7.1.1), with no differences in brood size (34.7 to 42.7 juveniles/female; p=0.14; Table 7.1.1) or in the number of broods produced in the two systems (44 to 38 broods/tank: p=51; Table 7.1.1). Similarly, there were no significant differences in mean weight (1.30±0.12 g; t=-0.43, p=0.68), standard length (44.59±0.97 mm; t=0.20, p=0.85), and weight gained (0.48±0.15 g; t=0.36, p=0.73) between juveniles grown in the effluent and control treatments (Table 7.1.2).

Table 7.1.2 The mean (standard deviation) initial length and condition factor and the final weight and length of juvenile swordtail grown in treated brewery effluent (Student t-test; p<0.05).

	Effluent	Control	t-value
Initial standard length (mm)	15.77 ± 0.82	14.87 ± 0.64	-1.93
Initial condition factor	0.0017 ± 0.0002	0.0017 ± 0.0002	0.33
Final weight (g)	1.32 ± 0.06	1.28 ± 0.18	-0.43
Final standard length (mm)	44.52 ± 0.51	44.65 ± 1.43	0.20
Weight gained (g)	0.46 ± 0.11	0.49 ± 0.18	0.36

A qualitative and semi-quantitative analysis of the gill and liver tissue found abnormalities as common features between treatments. Effluent fish showed better gill architecture, fewer hyperplasia cases and blood congestion (Figure 7.1.1). Similar trends were seen for gill lamellae fusion, lamellar aneurysms, lamellar disorganization and epithelial detachment and ruptures. Control fish showed better liver architecture, fewer intravascular haemolysis cases, more centrally located hepatocyte nuclei, less hepatocyte degeneration and ceroid pigment. Effluent kept fish livers showed more evidence of fat and glycogen storage (Figure 7.1.2), less melanine in bile ducts, and centrally located nuclei. Similar melano-macrophage centres sizes and very few lipid droplet zones were seen in both treatments.

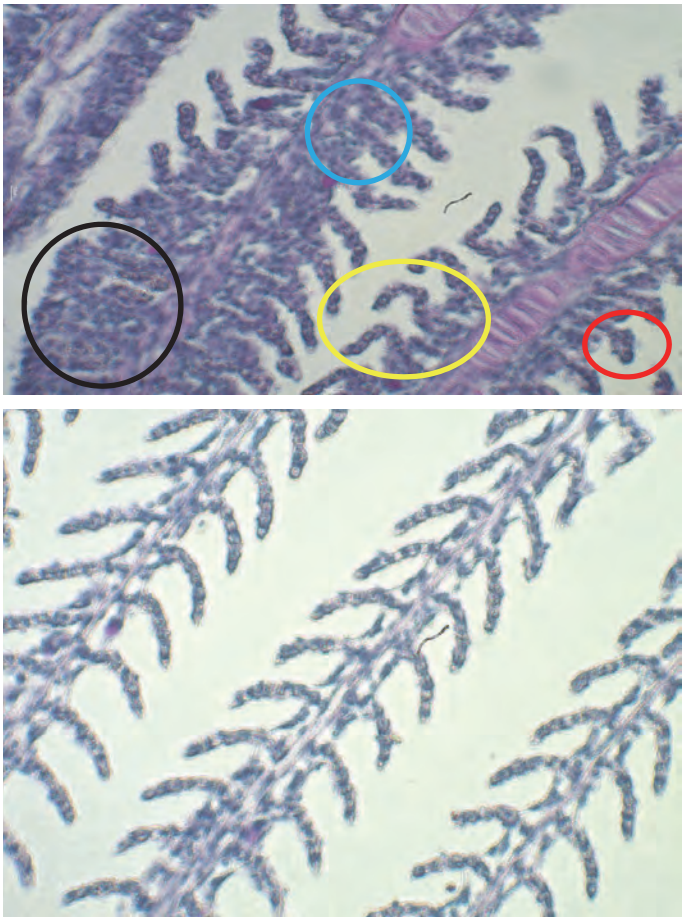


Figure 7.1.1 (A) Gill tissue from a female fish held in the control treatment showing lamellar fusion (black circle), lamellar disorganization (yellow circle), hyperplasia (blue circle) and lamellar aneurysm (red circle), compared with (B) healthy gill tissue (PAS stain; magnification: x 40) (Photos: Rory Scheepers).

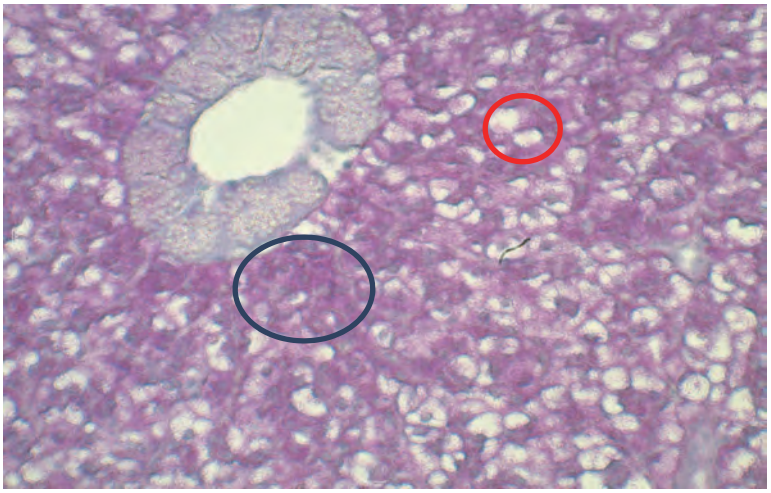


Figure 7.1.2 Liver tissue from a female that was held in the control treatment, showing glycogen storage (blue circle) and small amounts of lipid storage (red circle) (PAS stain; magnification: x 40) (Photo: Rory Scheepers).

The similarity of fish size, condition factor, reproductive output and growth of juvenile fish between treatments suggests that treated brewery effluent is a suitable water source for the culture of swordtail. This conclusion was partly supported by the histological analysis, where fish in the treated effluent largely had healthier gill tissue, and were storing more energy as fat or glycogen in the liver. The liver tissue of these fish, however, showed evidence of environmental stress, so effluent might have a negative effect on fish health over its entire life span, which would need to be established during future research.

Fish mortality did not differ significantly between treatments during this trial. However, there was a single incident, prior to the experiment, when all the adult swordtails in the effluent treatment only were killed. The juvenile swordtails were not affected and the tilapia *Oreochromis mossambicus* that were being held in the system also survived. This fish kill followed an unscheduled dumping of wort in the brewery. It is likely that the kill could have been prevented if mitigating measures had been taken to ensure that the spill did not filter through to the fish system after the unscheduled dump. The residence time in the anaerobic digester and algal ponds, i.e. the treatment processes that precede the fish culture system, allows for sufficient time to implement such measures.

The similarity of fish size, condition (i.e. health) and reproductive output between swordtail cultured in the treated brewery effluent compared to those cultured in the municipal treatment, shows that treated brewery effluent can be used to culture fish without negative effects. This work provides evidence that treated brewery effluent is a suitable water source for the culture of fish, provided mitigating measures are adopted to ensure that fish health is not affected by the unscheduled upstream dumping of brewery waste.

8. BENEFICIATION OF BREWERY EFFLUENT – AQUACULTURE FEED

One of the biggest problems facing aquaculture and fish nutrition in general is that feed accounts more than half of the variable operating cost and therefore improving feed efficiency in industrial production has a high priority. Alternative protein sources that are locally available, and that satisfy the nutritional requirements of the animal, need to be sourced. Unicellular algae have been found to be a suitable protein source for a range of aquatic animals, including abalone (Ismail et al. 2009; Shipton and Britz 2011) and tilapia (El-Hindawy et al. 2006; Badwy et al. 2008), provided the nutritional requirements of the animals are met. The aim of this investigation was to determine if unicellular algae, harvested from the high rate algal ponding (HRAP) system that forms part of the brewery wastewater treatment facility, is a suitable protein source for juvenile abalone *Halotis midae* and juvenile and adult Mozambique tilapia *Oreochromis mossambicus*.

8.1 Effluent-grown algae in abalone diets[5]

Two experiments were carried out simultaneously where either the soya (Experiment 1) or fishmeal (Experiment 2) portion of the protein source was substituted with algal protein. First, the amino acid profile of soya and fishmeal protein sources were compared with those of the unicellular algae and the soft tissue of abalone (Table 8.1.1). These data were used to formulate isonitrogenous diets (32.8% protein), where only the protein source varied. Protein substitution ranged from 0% (control) to a maximum of 75% in Experiment 1 and 50% in Experiment 2 (Table 8.1.2). The diets were fed to replicate baskets of abalone under farm-like conditions at Department of Ichthyology and Fisheries Science Port Alfred laboratory for 75 days.

Figure 8.1.1 Abalone *Haliotis midae* is a marine gastropod that is farmed in South Africa and that naturally feeds on macro and microalgae. The industry uses formulated feeds, some of which include algae as an ingredient (Photo: Devin Ayres).

[5] This section is a summary of Morgan Brand's Honours thesis.

Table 8.1.1 Proximal analysis of feed ingredients (g/kg) included in the diets of abalone, as well as that of the soft tissue of abalone (g of protein/kg of protein). Bold font indicates essential amino acids (Potts 1998, Sales and Britz 2001, 2003).

Component (g/kg)	Soft tissue 10 - 20 mm	Fish Meal	Soybean meal	Fresh algae
Crude Protein	446.7	705.9	478.5	354.5
Crude Fat	24	91.6	14.3	22.1
Ash	119.6	130	64.6	238.9
Amino Acid Profile				
Arginine	85.29	63.75	83.39	48.52
Histidine	19.70	21.67	29.68	13.82
Isoleucine	43.88	49.16	54.13	38.03
Leucine	74.77	78.91	86.10	80.26
Lysine	67.16	83.16	71.68	43.02
Phenylalanine	42.09	40.80	56.64	47.76
Methionine	22.83	32.16	10.87	19.33
Threonine	53.95	44.48	41.80	45.42
Valine	49.92	56.38	59.77	66.61
Tryptophan	8.06			
Aspartic acid	113.72	108.23	132.92	90.25
Serine	55.29	39.38	49.74	33.66
Glutamic acid	159.84	161.35	219.44	94.31
Proline	56.64	48.73	66.25	43.08
Glycine	90.22	63.18	46.60	71.38
Alanine	62.91	64.88	47.02	88.84
Tyrosine		28.47	30.09	28.72

There were no significant differences in length or weight gain among abalone in the treatments of Experiment 1 (i.e. soya replacement), with an overall mean (± standard deviation) of 1.82±0.19 mm.month^{-1} and 0.4±0.04 g.month^{-1} (Table 8.1.3; ANOVA; p=0.054 p=0.36 respectively). There was however, a general increase in food conversion ratio (FCR; Figure 8.1.2), protein efficiency ratio (PER; Figure 8.1.3) and feed consumption (Figure 8.1.4) and a drop in specific growth rate (SGR; Figure 8.1.5) as the level of algal inclusion increased, with significant differences between 75 % protein substitution compared to the control (ANOVA; $p<0.05$).

Table 8.1.2 The percentages of protein used in the experimental diets as well as the total contribution that the dietary ingredients made to the total protein content.

	Diet					
Experiment 1	**1**	**2**	**3**	**4**		
Experiment 2				**4**	**5**	**6**
Proportion of soya protein replacment (%) with algal protein	25	50	75	0		
Proportion of fishmeal protein replacment (%) with algal protein				0	25	50
Fishmeal (%)	61.4	61.4	61.4	61.4	50.2	42.4
Soya (%)	23.2	19.5	16.8	28.7	28.7	28.7
Algae (%)	5.8	9.7	12.6	0	12.5	21.2
Protein from other (%)	9.5	9.3	9.2	9.8	8.5	7.6
Total (%)	100	100	100	100	100	100

Table 8.1.3 Mean length (L), mean length gain per month, mean weight (Wt), mean weight gain per month, and mean condition factor (CF) for *H. midae* fed one of five experimental diets over a 75 days. Values represent the mean triplicate groups of diets 1 – 4 with varying percentage of soya protein substitution. No significant differences were found (ANOVA, $p<0.05$).

Diet	**1**	**2**	**3**	**4**	***p-value***
Proportion of soya protein replacment (%) with aglal protein	25	50	75	0	
Mean Initial L (mm)	19.49 ± 0.60	19.42 ± 0.61	19.42 ± 0.59	19.19 ± 0.61	0.51
Mean Final L (mm)	23.90 ± 0.90	24.22 ± 1.16	23.69 ± 0.68	24.44 ± 0.91	0.77
Mean L gain per month (mm)	1.76 ± 0.11	1.71 ± 0.24	1.71 ± 0.08	2.10 ± 0.17	0.54
Mean Initial Wt (g)	1.04 ± 0.12	1.10 ± 0.13	1.02 ± 0.14	0.97 ± 0.12	0.66
Mean Final Wt (g)	2.04 ± 0.30	2.08 ± 0.28	1.89 ± 0.20	2.08 ± 0.27	0.77
Mean Wt gain per month (g)	0.40 ± 0.08	0.39 ± 0.06	0.35 ± 0.03	0.44 ± 0.06	0.36
Mean Initial CF	0.81 ± 0.05	0.80 ± 0.04	0.78 ± 0.02	0.79 ± 0.01	0.93
Mean Final CF	0.81 ± 0.05	0.80 ± 0.04	0.78 ± 0.02	0.79 ± 0.01	0.65

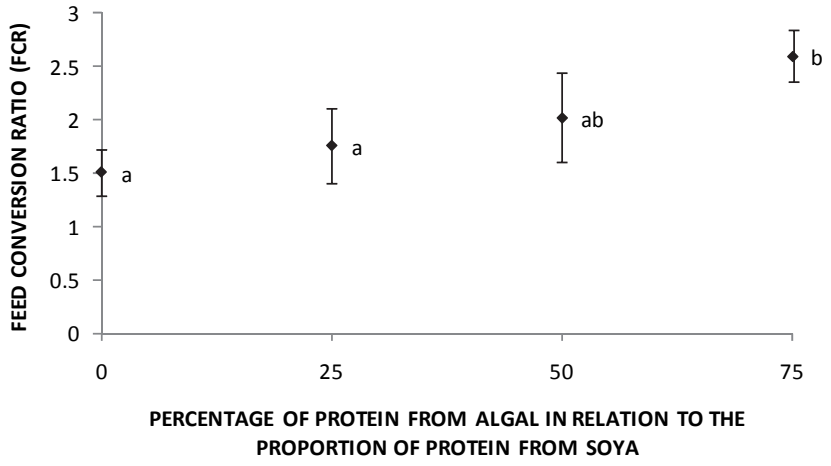


Figure 8.1.2 The mean (± standard deviation) feed conversion ratio of abalone fed diets where soya protein was replaced with graded levels of algal protein, for 75 days. Values with the same letters are not significantly different (ANOVA; $F_{(3, 8)}=6.49$; $p=0.02$).

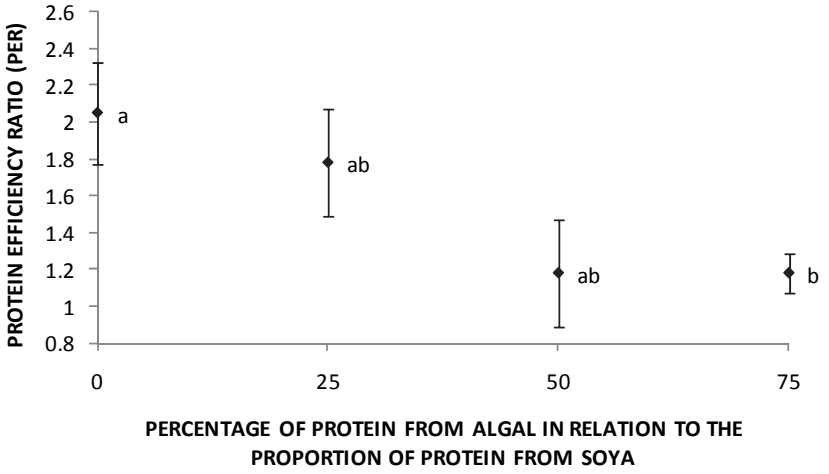


Figure 8.1.3 The mean (± standard deviation) protein efficiency ratio of abalone fed diets where soya protein was replaced with graded levels of algal protein, for 75 days. Values with the same letters are not significantly different (ANOVA; $F_{(3, 8)}$=5.14; p=0.03).

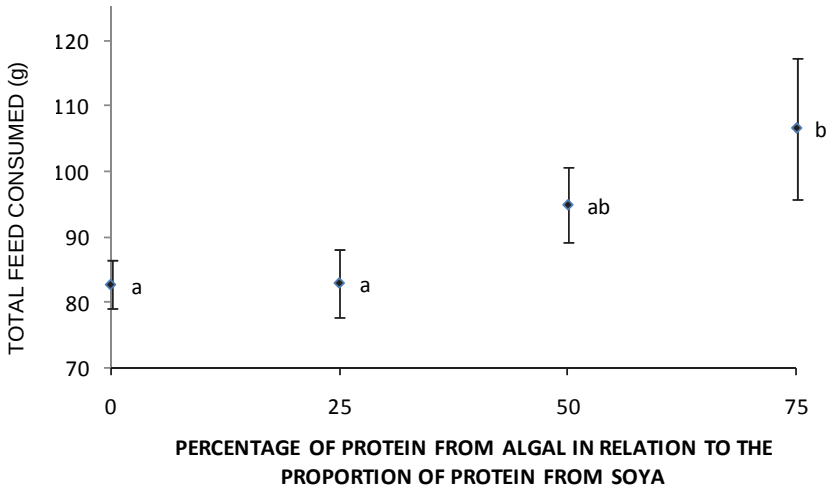


Figure 8.1.4 The mean (± standard deviation) weight of feed consumed by abalone fed diets where soya protein was replaced with graded levels of algal protein, for 75 days. Values with the same letters are not significantly different (ANOVA; $F_{(3, 8)}$=8.79; p=0.01).

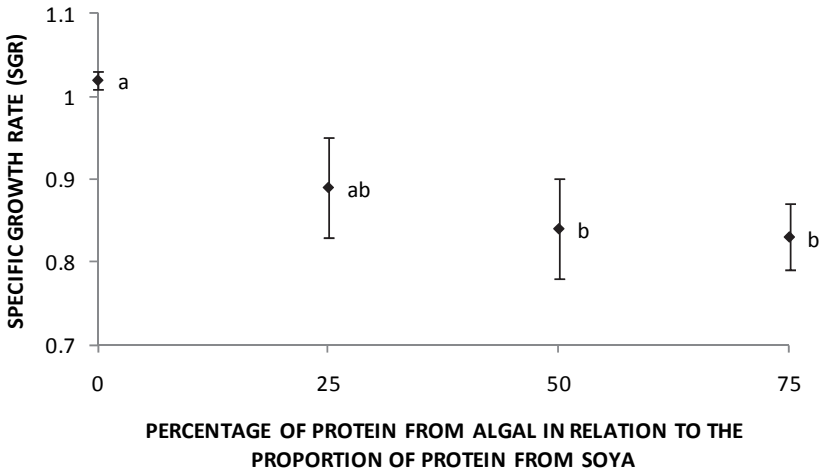


Figure 8.1.5 The mean (± standard deviation) specific growth rates of abalone fed diets where soya protein was replaced with graded levels of algal protein, for 75 days. Values with the same letters are not significantly different (ANOVA; $F_{(3, 8)}$=6.56; p=0.02).

In Experiment 2 (i.e. fishmeal replacement) there were no significant differences in final length, final weight, final CF between treatments (Table 8.1.4; ANOVA, p<0.05). However, when 50 % of the protein from fishmeal was substituted with protein from algal there were significantly reduced mean length gain (1.24±0.16 mm.month^{-1}; ANOVA; $F_{(2, 6)}$ =9.66; p=0.01) and mean weight gain (0.28±0.01 g.month^{-1}; ANOVA; $F_{(2, 6)}$ =13.28; p=0.01) when compared to the control (2.1±0.17 mm.month^{-}1; 0.44±0.06 g.month^{-1}) (Table 8.1.4). Similarly, SGR dropped (Figure 8.1.6) and FCR (Figure 8.1.7) and PER (Figure 8.1.8) increased significantly with increasing substitution.

Table 8.1.4 Mean length (L), mean length gain per month, mean body weight (Wt), mean weight gain per month, and mean condition factor (CF) for *H. midae* fed one of five experimental diets over a 75 day period. Values represent the mean triplicate groups of diets 4 – 6 with varying percentage of protein substitution. Values in the same row with the same letters are not significantly different (p = 0.05) and where no letters are present no significant differences were found.

Diet	**4**	**5**	**6**	***p-value***
Proportion of fishmeal protein replacment (%) with aglal protein	0	25	50	
Mean Initial L (mm)	19.19 ± 0.61	19.69 ± 0.62	19.77 ± 0.29	0.40
Mean Final L (mm)	24.44 ± 0.91	24.23 ± 0.94	23.33 ± 0.92	0.28
Mean L gain per month (mm)	2.10 ± 0.17 ^{c}	1.82 ± 0.23 ^{cd}	1.42 ± 0.16 ^{d}	0.01
Mean Initial Wt (g)	0.97 ± 0.12	1.06 ± 0.13	1.08 ± 0.07	0.49
Mean Final Wt (g)	2.08 ± 0.27	2.06 ± 0.19	1.79 ± 0.06	0.20
Mean Wt gain per month (g)	0.44 ± 0.06 ^{c}	0.40 ± 0.04 ^{c}	0.28 ± 0.01 ^{d}	0.01
Mean Initial CF	0.79 ± 0.01	0.79 ± 0.01	0.77 ± 0.02	0.99
Mean Final CF	0.79 ± 0.01	0.79 ± 0.01	0.77 ± 0.02	0.10

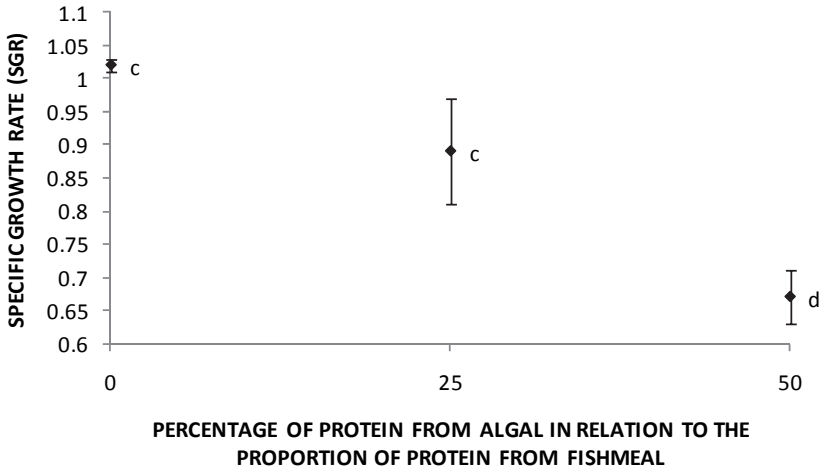


Figure 8.1.6 The mean (± standard deviation) specific growth rate of abalone fed diets where fishmeal protein was replaced with graded levels of algal protein, for 75 days. Values with the same letters are not significantly different (ANOVA; $F_{(2, 6)}$ =33.44; p<0.001).

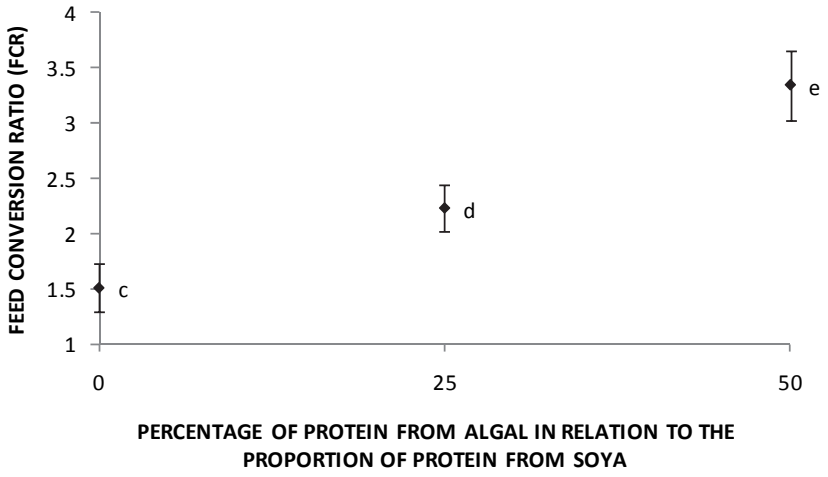


Figure 8.1.7 The mean (± standard deviation) feed conversion ratio of abalone fed diets where fishmeal protein was replaced with graded levels of algal protein, for 75 days. Values with the same letters are not significantly different (ANOVA; $F_{(2, 6)}$ =41.27; p<0.001).

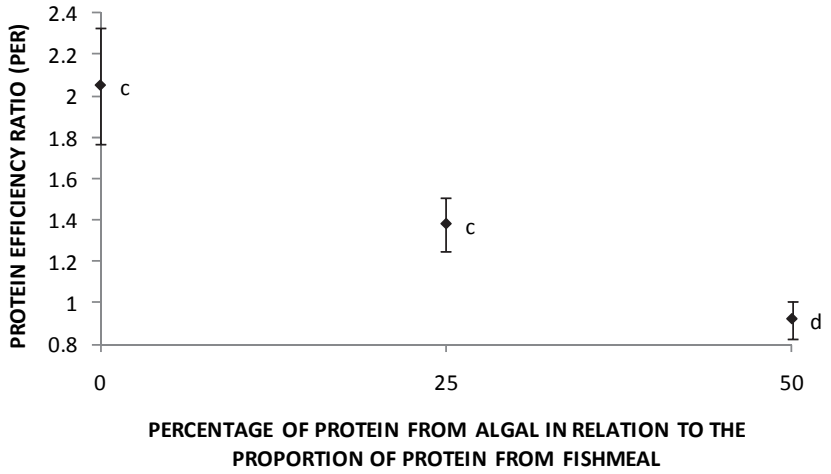


Figure 8.1.8 The mean (± standard deviation) protein efficiency ratio of abalone fed diets where fishmeal protein was replaced with graded levels of algal protein, for 75 days. Values with the same letters are not significantly different (ANOVA; $F_{(2, 6)}$ =28.83; p<0.001).

Without exception, there was a trend of decreasing growth and increasing feed conversion ratio and protein efficiency ratio among juvenile abalone *H. midae*, with increasing levels of brewery-effluent-grown microalgae in the diet. However, up to 25% of either the soya or fishmeal protein in the diet can be replaced by effluent-grown algae as an alternative protein source, without significantly reducing juvenile abalone growth; but the FCR at this level was significantly increased when fishmeal was substituted.

In conclusion, microalgae grown in brewery effluent can be used as a dietary substitute up to 25% of the soybean meal protein source in juvenile abalone diets with no significant effects; however, these algae are not a suitable substitute for dietary fishmeal due to the increased FCR when fishmeal protein was replaced.

8.2 Effluent-grown algae in juvenile Mozambique tilapia diets[6]

Fishmeal is conventionally a large component of commercial fish feeds, but is expensive and often short in supply. In recent years, a vast amount of research has been aimed towards identifying alternative protein sources with potential use in fish feeds and algae may be one of these sources. The SAB plant in Port Elizabeth has invested in an Integrated Algal Ponding System (IAPS), which removes unwanted nutrients from effluent water while producing single celled algae in large. This study investigates the use of these effluent-grown algae (35.45% protein) as a protein source in the diets for Mozambique tilapia, *Oreochromis mossambicus*.

The algae met the minimum amino acid requirements of lysine (3.78%) and methionine (0.99%), which are required for efficient fish growth (Table 8.2.1). Fish meal protein and soya protein portions of the diet were replaced with algal protein at substitution levels of 25%, 50% and 75% (Table 8.2.2). A control diet contained no algae, and a commercial tilapia starter feed provided a reference diet for the experimental diets. The eight treatments were each replicated three times (i.e. fed to the fish in three randomly selected tanks). Feed was made available to fish in the same quantities throughout the experiment.

Table 8.2.1 The essential amino acid content of the algal protein source (g/100 g), their percentage of the total protein present and the minimum amino acid requirements of young *O. mossambicus* (%).

Amino Acid	g/100 g	Percent (%)	Requirements (%) [a]
Protein (N x 6.25)	35.446	100.00	-
Alanine	3.149	8.88	-
Ammonia	2.484	7.01	-
Arginine	1.720	4.85	2.82
Aspartic	3.199	9.02	-
Cysteic Acid	0.431	1.22	-
Glutamic	3.343	9.43	-
Glycine	2.530	7.14	-
Histidine	0.490	1.38	1.05
Iso leucine	1.348	3.80	2.01
Leucine	2.845	8.03	3.40
Lysine	1.525	4.30	3.78
Methionine Sul	0.685	1.93	0.99
Phenylalanine	1.693	4.78	2.50
Proline	1.527	4.31	-
Serine	1.193	3.37	-
Threonine	1.610	4.54	2.93
Tyrosine	1.018	2.87	-
Valine	2.361	6.66	2.20
Protein recovered from listed amino acids	33.151	93.53	-

[6] This section is a summary of Gareth Nicholson's Honours thesis.

Table 8.2.2 Protein composition of the diets fed to the Mozambique tilapia.

		Replacement of soya with Algae			Replacement of fishmeal with Algae		
Diet	**Control**	**S25**	**S50**	**S75**	**F25**	**F50**	**F75**
Protein from algae (%)	0.0	13.8	23.1	29.9	5.1	8.6	11.2
Protein from soya (%)	68.1	55.1	46.2	39.8	68.0	68.0	68.0
% algae relative to soya	0.0	25.0	50.0	75.0	-	-	-
Protein from fishmeal (%)	24.8	24.7	24.7	24.7	20.4	17.2	14.9
% algae relative to fishmeal	0.0	-	-	-	25.0	50.0	75.0
Protein from maize meal (%)	7.2	6.4	5.9	5.6	6.6	6.2	6.0

After a six-week experimental period, growth and health indices were calculated for each diet and compared.

No significant differences were observed between diets ($P > 0.05$) for any of the tested variables (Tables 8.2.3 and 8.2.4); however, with the replacement of fishmeal protein, decreasing trends in growth, food conversion ratio (FCR) and protein efficiency ratio (PER) were observed. These trends would likely have become significant if feeding trials were run over a longer period of time.

Table 8.2.3 Mean growth and health indices of Mozambique tilapia fed the control diet (Con, with no replacement) and diets in which 25 % (S25), 50 % (S50) and 75 % (S75) of the soya meal protein had been replaced with algal protein. Values in the same row represented by the letter are not significantly different (ANOVA, $p < 0.05$).

	Diet				**Pooled mean ± standard error**	**Commercial diet**
	Con	**S25**	**S50**	**S75**		
Percentage weight gain (%)	418.71 [a]	409.91 [a]	430.81 [a]	414.85 [a]	418.57 ± 18.71	413.07 [a]
Daily mean weight gain (g)	0.07 [a]	0.07 [a]	0.08 [a]	0.07 [a]	0.07 ± 0.00	0.07 [a]
Specific growth rate (SGR)	3.91 [a]	3.83 [a]	3.97 [a]	3.89 [a]	3.90 ± 0.09	3.89 [a]
Condition factor before (x 100)	0.17 [a]	0.16 [a]	0.17 [a]	0.17 [a]	0.17 ± 0.00	0.15 [a]
Condition factor after (x100)	0.13 [a]	0.19 [a]	0.14 [a]	0.11 [a]	0.14 ± 0.02	0.19 [a]
Food conversion ratio (FCR)	1.57 [a]	1.74 [a]	1.47 [a]	1.53 [a]	1.58 ± 0.09	1.60 [a]
Protein efficiency ratio (PER)	1.98 [a]	1.87 [a]	2.07 [a]	1.99 [a]	1.98 ± 0.08	1.91 [a]
Hepatosomatic index (HSI)	1.51 [a]	1.51 [a]	1.93 [a]	1.61 [a]	1.34 ± 0.32	1.23 [a]
Gonadosomatic index (GSI)	0.8 [a]	1.56 [a]	1.42 [a]	1.52 [a]	1.64 ± 0.11	0.96 [a]

Table 8.2.4 Mean growth and health indices of Mozambique tilapia fed the control diet (Con) and diets in which 25% (F25), 50% (F50) and 75% (F75) of the fishmeal protein had been replaced with algal protein. Values in the same row represented by the symbol [a] are not significantly different (ANOVA, $p < 0.05$).

	Diet				Pooled mean ±	Comercial
	Con	F25	F50	F75	standard error	diet
Percentage weight gain (%)	418.71 [a]	430.03 [a]	394.27 [a]	359.44 [a]	400.61 ± 14.40	413.07 [a]
Daily mean weight gain (g)	0.07 [a]	0.08 [a]	0.07 [a]	0.06 [a]	0.07 ± 0.00	0.07 [a]
Specific growth rate (SGR)	3.91 [a]	3.96 [a]	3.8 [a]	3.62 [a]	3.82 ± 0.07	3.89 [a]
Condition factor before (x 100)	0.17 [a]	0.18 [a]	0.16 [a]	0.17 [a]	0.17 ± 0.00	0.15 [a]
Condition factor after (x100)	0.13 [a]	0.14 [a]	0.13 [a]	0.22 [a]	0.16 ± 0.02	0.19 [a]
Food conversion ratio (FCR)	1.57 [a]	1.63 [a]	1.66 [a]	1.90 [a]	1.69 ± 0.07	1.60 [a]
Protein efficiency ratio (PER)	1.98 [a]	1.87 [a]	1.85 [a]	1.63 [a]	1.83 ± 0.07	1.91 [a]
Hepatosomatic index (HSI)	1.51 [a]	2.05 [a]	1.73 [a]	2.11 [a]	1.07 ± 0.16	1.23 [a]
Gonadosomatic index (GSI)	0.8 [a]	1.41 [a]	0.88 [a]	1.16 [a]	1.85 ± 0.13	0.96 [a]

In conclusion, protein from brewery effluent-grown algae can be used to effectively replace up to 75% of the fishmeal protein in the diet of Mozambique tilapia. The nutritional value of the algae is perhaps equivalent to that of soybean meal for this fish. Future work should look at increasing the substitution level above 75% of the protein source.

8.3 Effluent-grown algae in broodstock Mozambique tilapia diets

Little information is known about how the replacement of dietary fishmeal with alternative protein/lipid sources influences reproductive output. Mozambique tilapia, *Oreochromis mossambicus,* is omnivorous and therefore utilises proteins obtained from plants, insects and fish. Commercial tilapia broodstock diets still use fishmeal and soya as the main protein sources. Both are becoming increasingly expensive dietary ingredients. Furthermore, fishmeal originates from capture fisheries and is a limited resource. Consumers are placing the aquaculture industry under increasing pressure to reduce the volume of fishmeal used in the production of farmed fish, making aquaculture products more environmentally sustainable. Similarly, soya was traditionally a human food crop, but with the advent of biofuels and an increased demand for soya, the aquaculture industry is looking at alternatives. An opportunity exists to use the algae grown in brewery effluent treatment as an alternative to fishmeal or soya in the diets of broodstock Mozambique tilapia. This study addresses the hypothesis that algae replacement will not have an effect on reproductive output because algae form part of their natural diet. The objectives of this research project were to compare fecundity, relative fecundity, gonadosomatic index (GSI), maturity stages and size of eggs in *O. mossambicus* fed on diets containing brewery effluent-grown algae.

Algae (28.1% protein; Table 8.3.1) were collected from the high rate algal ponds (HRAP) that form part of an experimental brewery effluent treatment plant at Ibhayi Brewery in Port Elizabeth. These algae were included as a protein source in two diets. They were prepared with substitution levels of algae for fishmeal on an equivalent protein basis, where 40% and 80% of the fishmeal protein were replaced with algae (Table 8.3.1). A control diet contained a protein formulation (35% protein), with fishmeal accounting for 53.2 % and soya for 40% of the protein fraction. With the exception of fishmeal substitution, all other aspects remained constant between treatments. Adult female *O. mossambicus* (39.8g ± 30.06) were fed three percent of their body weight per day for 11 weeks. The three treatments were each replicated five times (i.e. five independent tanks per treatment). The health and reproductive output of fish fed the different diets were compared.

Table 8.3.1 Proximate composition of the algal protein source and experimental diets on a dry basis (mean ± SD). The control diet contained no algae; F40 and F80 diets where 40 % and 80 % of the fishmeal protein portion was replaced with protein from algae.

Content	Algae	Control	F40	F80
Protein (%)	28.1 ± 0.12	37.9 ± 0.20	36.8 ± 1.49	35.3 ± 0.19
Moisture (%)	12.2 ± 0.21	7.40 ± 0.09	9.50 ± 3.29	9.02 ± 0.10
Fat (%)	0.53 ± 0.04	10.7 ± 0.01	9.30 ± 0.12	7.80 ± 0.50
Ash (%)	38.4 ± 0.21	6.80 ± 0.03	11.8 ± 1.72	16.2 ± 0.07
Gross energy (MJ/kg)	13.3 ± 0.13	20.6 ± 0.07	19.7 ± 0.63	18.8 ± 0.03

There was no significant difference in the size of the fish among treatments at the start and end of the study ($p > 0.05$; Table 8.3.2). Mean fish length at the end of the trial ranged from 152 to 158 mm with a condition factor of 1.8 to 1.9 (Table 8.3.2). Similarly, the hepatosomatic index (HSI) remained similar among treatments with an overall mean of 2.6 % ± 0.56 ranging from 1.2% to 4.4% ($F_{(2, 12)} = 0.3$; $p = 0.7$; Table 8.3.2).

There was no significant difference in the gonad length and gonad width among female tilapia fed the different diets ($F_{(2, 12)} = 1.2$; $p = 0.3$ and $F_{(2, 12)} = 1.02$; $p = 0.4$, respectively; Table 8.3.2). Gonad length ranged from 20.0 to 68.1 mm, while gonad width ranged from 1.3 to 12.3 mm (Table 8.3.2). There were also no significant differences in egg length or width between females fed the different diets, and these ranged from 269.4 to 4014.78 µm and 168.4 to 3167.9 µm ($F_{(2, 12)} = 2.4$; $p = 0.13$ and $F_{(2, 12)} = 3.4$; $p = 0.06$, respectively; Table 8.3.2). However, egg volume differed significantly between diets, with eggs from females fed the 80 % substitution diet being significantly larger than those from the 40% substitution diet ($F_{(2, 12)} = 4.8$; $p = 0.028$; Figure 8.3.1).

Table 8.3.2 Reproductive and growth performance of female *O. mossambicus* reared on experimental diets (mean ± SD). The control diet contained no algae; F40 and F80 diets where 40 % and 80 % of the fishmeal protein portion was replaced with protein from algae. Values with the same superscript in each row are not significantly different from each other (ANOVA, $p > 0.05$). GSI = gonadosomatic index; CF = condition factor; DWG = daily weight gain.

	Control	**F40**	**F80**
Reproduction			
Egg length (µm)	2049.3 ± 335.9^{a}	1916.4 ± 288.57^{a}	2277.8 ± 220.8^{a}
Egg width (µm)	1579.5 ± 437.5^{a}	1393.7 ± 413.3^{a}	1679.1 ± 473.3^{a}
Egg volume (mm³)	3.3 ± 2.3^{ab}	2.4 ± 1.8^{a}	4.01 ± 2.76^{b}
GSI (%)	3.2 ± 1.49^{a}	2.5 ± 1.16^{a}	3.8 ± 1.19^{a}
Fecundity (total eggs fish^{-1})	814.5 ± 477.5^{a}	894.1 ± 568.9^{a}	697.9 ± 322.6^{a}
Relative fecundity (egg g^{-1})	9.9 ± 3.1^{a}	10.9 ± 2.7^{a}	10.5 ± 3.9^{a}
Gonad length (mm)	36.9 ± 11.5^{a}	37.1 ± 10.4^{a}	36.3 ± 5.4^{a}
Gonad width (mm)	6.7 ± 2.4^{a}	7.1 ± 2.6^{a}	8.3 ± 1.9^{a}
Growth			
CF	1.8 ± 0.13^{a}	1.9 ± 0.17^{a}	1.9 ± 0.16^{a}
Initial weight (g)	39.7 ± 19.9^{a}	50.3 ± 47.8^{a}	32.2 ± 14.8^{a}
Final weight (g)	78.3 ± 37.96^{a}	84.4 ± 52.49^{a}	71.3 ± 31.03^{a}
Initial length (mm)	126.3 ± 16.6^{a}	131.9 ± 36.6^{a}	117.9 ± 16.7^{a}
Final length (mm)	156.1 ± 22.7^{a}	158.8 ± 33.1^{a}	151.8 ± 19.2^{a}
DWG gain (g fish^{-1} day $^{-1}$)	0.5 ± 0.3^{a}	0.4 ± 0.2^{a}	0.5 ± 0.4^{a}

The frequency distribution of the maturity stages varied among dietary treatment with a tendency for most fish to obtain a maturity stage of 3 in the F80 treatment (Contingency analysis, $\chi^2 = 98.6$; $\chi^2_{0.05, 6} = 12.6$; Figure 8.3.2).The gonadosomatic index (GSI) did not differ significantly among treatments, ranging from 0.3 to 6.6% ($F_{(2, 12)} = 2.7$; $p = 0.1$; Table 8.3.2). There were also no significant differences in fecundity or relative fecundity among treatments ($F_{(2, 12)} = 2.2$; $p = 0.2$ and $F_{(2, 12)} = 0.1$; $p = 0.9$; Table 8.3.2). There was a significant relationship between fecundity and fish mass ($y = 67.1 + 9.4*x$; $R^2 = 0.68$; $p < 0.00001$), as well as fecundity and fish length ($y = -1562.7 + 15.2*x$; $R^2 = 0.69$; $p < 0.00001$). The number of eggs produced increased as a function of fish length and mass (Figures 2.3 and 2.4), and there was a tendency to increased variation in fecundity for heavier fish (Figure 8.3.3).

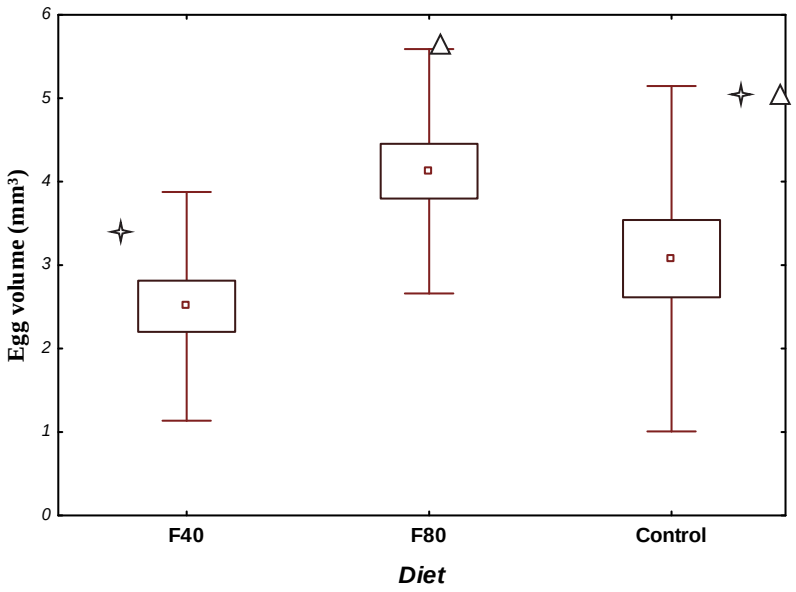


Figure 8.3.1 The mean egg volume (mm^3) for each of the control diet containing no algae, and F40 and F80 diets where 40% and 80% of the fishmeal protein portion is replaced with protein from algae respectively. The box represents the 0.25 and 0.75 quartiles; the mean is the point in the box and the whiskers represent the standard deviation above and below the mean. Means with same symbol above each box were not significantly different from each other ($p<0.05$).

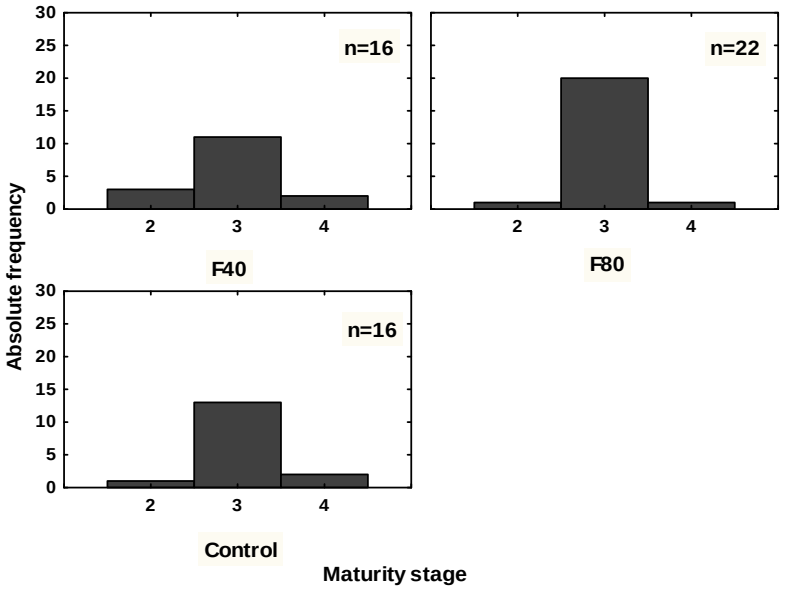


Figure 8.3.2 The maturity stages of female tilapia fed either a control diet containing no algae, or F40 and F80 diets where 40% and 80% of the fishmeal protein portion is replaced with protein from algae respectively. Stage 2 is active, stage 3 is ripe and stage 4 is spent. There were no immature fish at stage 1.

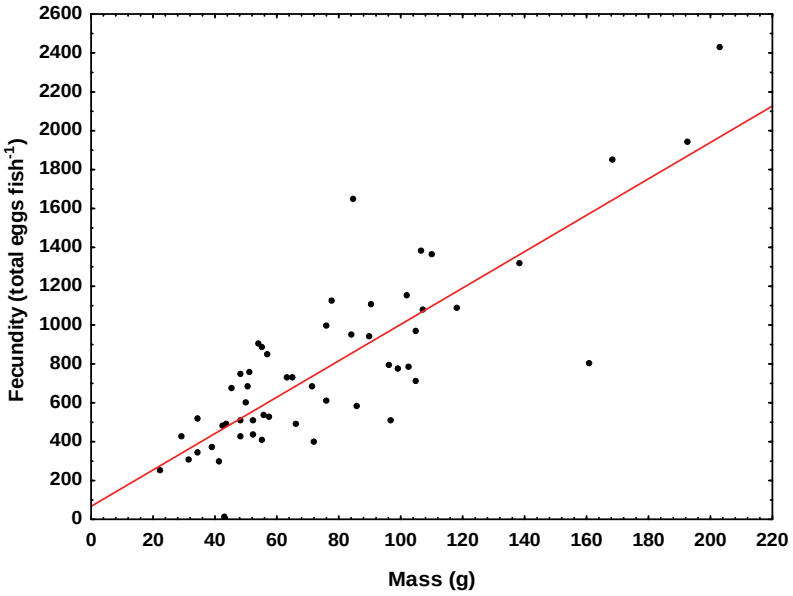


Figure 8.3.3 The fecundity (total eggs fish^{-1}) as a function of fish mass (g) for *O. mossambicus*. The trend line fitted to the plot indicates a significant regression ($y = 67.1 + 9.4^* x$; $R^2 = 0.68$; $p < 0.00001$).

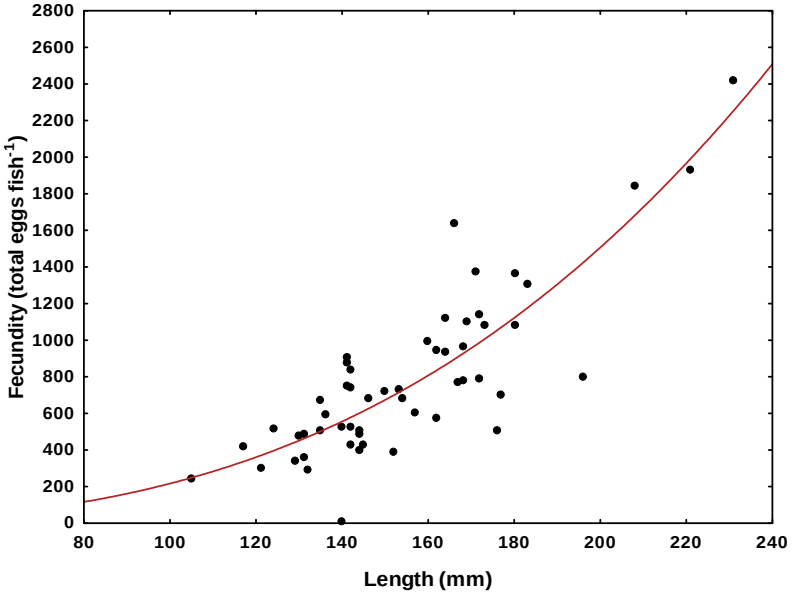


Figure 8.3.4 The relationship between fecundity (total eggs fish^{-1}) (Y) and fish length (mm) (X) of *O. mossambicus* and line of best fit according to the model $y = aX^b$; $r = 0.8$; $p < 0.00001$; where $a = 0.0005$ and $b = 2.8$.

Table 8.3.3 Water quality (mean ± SD) for each of the diets - the control diet contained no algae; F40 and F80 diets where 40 % and 80 % of the fishmeal protein portion was replaced with protein from algae. Values with the same superscript in each row are not significantly different from each other (ANOVA, $p > 0.05$).

Water quality parameter	**Control**	**F40**	**F80**
Temperature (°C)	27.3 ± 1.10^a	26.0 ± 1.90^a	26.3 ± 1.50^a
pH	8.40 ± 0.40^a	8.50 ± 0.52^a	8.50 ± 0.36^a
O_2 Saturation (%)	74.3 ± 5.60^a	74.5 ± 4.20^a	76.3 ± 5.09^a
apr	5.60 ± 0.40^a	6.10 ± 0.50^a	5.90 ± 0.51^a
Conductivity (µS/cm)	5.70 ± 4.90^a	4.30 ± 1.60^a	3.90 ± 1.90^a
Salinity (ppt)	0.03 ± 0.01^a	0.02 ± 0.05^a	0.02 ± 0.04^a

There were no significant differences among treatments for any of the water quality variables (ANOVA, $p > 0.05$; Table 8.3.3). The water temperature in the system ranged from 23.3 to 29.1°C with means for the different treatments that ranged from approximately 26 to 27°C (Table 8.3.3). The pH ranged from 8.4 to 8.5, mean oxygen saturation ranged from 74.3 to 76.3%, electrical conductivity ranged from 3.9 to 5.7 $\mu S.cm^{-1}$, and the work was carried out in fresh water with mean salinity of 0.2 to 0.3 ppt (Table 8.3.3).

Brewery effluent-grown algae can be used as a protein source in the diets for broodstock Mozambique tilapia, *Oreochromis mossambicus*, without negatively affecting reproductive output. Although there were no significant differences among diets for most of the reproductive variables, egg volume was influenced by dietary composition. The larger egg volume in the 80% replacement diet compared to the 40% replacement suggests that the fish may have invested more energy into gametes when fed the high-algae diet. Egg length (mm) and egg width (mm) showed no difference between diets suggesting that the shape of the ellipsoid eggs might have varied among treatments.

Mozambique tilapia invest considerable energy in ensuring that a limited number of young are likely to survive (Noakes and Balon 1982); egg volume might be a good way of indexing this investment in future studies. Winemiller and Rose (1992) and Winemiller (2005) recognize three reproductive strategies: (1) opportunistic (characterised by a short generation time, high reproductive effort, small body size, low batch fecundity, and low investment per offspring), (2) periodic (i.e. a long generation time, moderate reproductive effort, large body size, high batch fecundity, and low investment per offspring), and (3) equilibrium (i.e. a moderate to long generation time, low reproductive effort, variable body size, low batch fecundity, and high investment per offspring). Their model defines a set of

life history 'trade-offs' and it has been suggested by Coward and Bromage (1999) that total egg volume was a more appropriate index of egg production and of total reproductive output since it takes the egg size and egg number into consideration. In this study, egg volume was the only reproductive variable that was significantly influenced by diet, suggesting that it may be the most responsive variable related to reproductive output. However, while a difference was found between treatments for egg volume there was still no evidence of a reduction in total reproductive output.

Dietary protein has been shown to influence seed production in some tilapia species (Santiago et al. 1985; Wee and Tuan 1988; De Silva and Radampola 1990), while there is conflicting evidence that reproduction remains unaffected by protein level (Cisse´ 1988; Gunasekera et al. 1996). In this study, the protein level remained constant between treatments; only the protein source changed and we did not test for the availability of protein in effluent-grown algae. The similarity in reproductive output here either provides evidence that the reproductive output of this fish is independent of dietary protein, or it demonstrates that the nutrients offered by the algae were available to the fish.

The worldwide decline of ocean fisheries stocks has been mirrored by growth in the aquaculture industry; however the environmental sustainability of aquaculture has been questioned (Naylor et al. 2000). It takes about 1.9 kg of wild fish to produce 1 kg of farmed salmon, and for flounder, sole, cod, sea bass and tuna it takes more than 5 kg of wild fish to produced 1 kg of farmed fish (Naylor et al. 2000). The World Wildlife Fund for Nature's (WWF) Southern African Sustainable Seafood Initiative (SASSI) promotes awareness that is aimed at reducing the amount of fishmeal used by the aquaculture industry. Although tilapia farming already uses considerably less fishmeal than these carnivorous species, this study contributes to the environmental sustainability of tilapia farming, since less fishmeal in the diet results in more farmed fish per unit of wild-caught fish that was reduced to fishmeal. The future of aqua-feeds could largely depend upon lower-grade raw materials (Costa-Pierce et al. 2011), such as those tested in the current study.

Alternatives to fishmeal are required by the aquaculture industry, and effluent-grown algae appear to be a viable alternative. Since the algal replacement did not reduce the reproductive output of tilapia, this dietary ingredient could be used as an alternative in tilapia feeds. Effluent-grown algae could be used as a feed ingredient at 80 % replacement of the fishmeal protein portion to reduce feed costs and improve environmental sustainability without reducing the reproductive potential of *O. mossambicus*. Long-term studies under farm conditions need to be carried out.

9. BENEFICIATION OF BREWERY EFFLUENT – HARVESTING ENERGY (DESKTOP STUDY)

Anaerobic digestion (AD) is "a biological conversion process without external electron acceptor such as oxygen as in aerobic processes or nitrate/sulphate as in anoxic processes" (Angelidaki & Sanders (2004). A consortium of chemoheterotrophic, non-methanogenic bacteria and methanogenic bacteria convert the organic carbon in a feedstock into methane, organic carbon in its most reduced state, and carbon dioxide in its most oxidised state (Speece 1983, Angelidaki & Sanders 2004). Methane is a widely recognised and used fuel source. For example, methane catered for 20% of the energy requirements of the United States in about 2001 (Chynoweth et al. 2001). The AD process thus combines wastewater treatment with the production of a highly valuable fuel source.

An understanding of the optimum conditions under which AD occurs is crucial in maintaining the effective and efficient breakdown of carbonaceous matter. A number of factors can affect the metabolism of the microalgae and hence the production of biogas (Sialve et al. 2009). Firstly the volume of effluent being fed through the system will cause the hydraulic retention time to vary which will result in a more or less complete digestion of the organic carbon compounds in the effluent (Laubscher *pers. comm*. 2012). This is important because the intended purpose of the anaerobic digestion system will dictate the required level of efficiency. By their nature, water treatment plants focus on water quality objectives and not methane production (Laubscher *pers. comm*. 2012). Therefore the emphasis on the economic efficiency of the water treatment may result in incomplete digestion of the organic material and methane production below the theoretical potential of the effluent (Laubscher *pers. comm*. 2012). Methane production can also be severely limited by the availability of nutrients in the effluent and temperatures or pH levels outside of the optimum range and concentrations of volatile fatty acids or ammonia (Kryvoruchko et al. 2009). Management of the conditions of the effluent stream and AD system allow for the optimisation of the bacterial activity and methane production potential of a given AD.

The aim of this report is to present a desktop study that highlights the potential of harvesting methane from anaerobic digesters used to treat brewery effluent, and to identify any other sources of potential energy that might be captured from a brewery.

The on-site wastewater treatment plant at Ibhayi Brewery is managed by an independent wastewater treatment engineering company, which treats the effluent produced by the brewery before it is either sent to the municipal wastewater system or reused on site. The wastewater treatment plant at Ibhayi Brewery consists of a mechanical screen filter, equalisation tank, an AD, activated sludge (AS), clarifier, polishing filters (sand, activated carbon, reverse osmosis, micro-filtration) and a chlorine dosing system. Of the total volume of brewery effluent treated in the AD about 35% is discharged into the municipal system and about 64% receives treatment in the AS system. About 16% of the original effluent volume is recycled through the on-site filters and reused at the brewery (Mabuza 2010, as cited by Cilliers 2012). According to data recorded by the independent engineering

company, between January 2010 and March 2012, the AD received an average daily effluent volume of 1191 m³ at an average rate of 50 m³/h. Potential might exist to harvest methane from the AD at Ibhayi Brewery.

9.1 Methane production estimates – Flare stack biogas analysis

The first method of estimating the methane generation potential at a local brewery was based on extrapolating limited gas composition analysis data and combined biogas volumes. The volume of biogas produced by the AD was constantly monitored, although the chemical composition of the gas was not. The average volume of gas produced between January 2010 and March 2012 was 1560 Nm³ per day, at an average rate of 64.65 Nm³/h. In 2008 an additional independent engineering company conducted an analysis of the biogas produced in the AD system at the brewery. It drew six samples of gas from the gas duct leading to the flare stack on the 30th of October 2008 (Table 9.1.1). Five samples were taken on the 25th of November 2008 to determine the concentration of H_2S in the AD gas (Table 9.1.2).

Table 9.1.1 Composition of gas samples taken from an anaerobic digester used to treat brewery effluent (CMCE 2008).

		13h33	13h35	13h37	13h45	13h48	14h50
		Test 1	Test 2	Test 3	Test 4	Test 5	Test 6
CO_2	%	24.19	24.13	14.51	24.11	24.51	21.97
CH_4	%	72.80	72.94	42.00	72.97	72.04	64.74
O_2	%	1.12	1.16	9.74	1.14	1.19	3.27
N_2	%	1.88	1.77	33.75	1.78	2.25	10.02

Table 9.1.2 Composition of gas samples taken from the anaerobic digester used to treat brewery effluent (CMCE 2008).

Time		**16h50 – 17h00**				
H_2S	**mg/m³**	455	922	527	586	30

It was stated in the engineering report that the results from the third and fifth test (Table 9.1.1) “should be interpreted with care” (CMCE 2008). Evidently the sample containers leaked and the samples might have been contaminated which would explain the variation in those results. As such, the mean composition profile was determined, excluding tests three and five (Table 9.1.3).

The estimated volume of gasses produced by the anaerobic digester of a local brewery were extrapolated using Table 9.1.3 and the records that were made available by the brewery and the independent consultants and are presented in Table 9.1.4. These figures are a rough estimate only, since the process of methane production can be limited or fluctuate according to a number of factors, including those described earlier.

Table 9.1.3 Mean composition of the gas produced by an anaerobic digester that was used to treat brewery effluent.

Compound	Average Concentration
CO_2	23.78%
CH_4	71.28%
O_2	1.57%
N_2	3.54%
Average H_2S Concentration	
H_2S	633.5 mg/m³

Table 9.1.4 Estimated volume of the different gasses produced by an anaerobic digester that is used to treat brewery effluent.

Compound	Rate of production (Nm³/h)	Rate of production (Nm³/d)
CO_2	15.37	371.11
CH_4	46.08	1112.35
O_2	1.01	24.44
N_2	2.29	5.24

9.2 Methane potential of the effluent based on chemical oxygen demand

Angelidaki & Sanders (2004) describe an alternative method for estimating the methane potential of waste based on chemical oxygen demand (COD). The Angelidaki & Sanders (2004) method was used to estimate the amount of methane generated in an AD used to treat brewery effluent, using COD measurements of the effluent entering and leaving the AD. From the independent engineer’s data it was establish that the average reduction in COD was 2.53 g per litre of effluent.

The following equations were used to determine the methane production from the previous two years (Angelidaki & Sanders 2004):

$$B\mathrm{o,th} = \frac{\left(\frac{n}{2} + \frac{a}{8} - \frac{b}{4}\right) 22.4}{\left(n + \frac{a}{4} - \frac{b}{2}\right) 32} \left(STP \frac{lCH_4}{g\text{–}COD}\right)$$

where $B_{o.th}$ refers to Buswell's equation of potential methane yield as used by Angelidaki & Sanders (2004), *n* stands for the number of carbon atoms, *a* stands for the number of hydrogen atoms and *b* stands for the number of oxygen atoms in the material being digested. Angelidaki & Sanders (2004) show the "theoretical characteristics of typical substrate components" which include carbohydrates, protein, lipids, ethanol, acetate and propionate and the potential CH_4 yield for each substrate. *STP* stands for standard temperature (0° C) and pressure (1 atmosphere) which is the unit used to compare gas densities and volumes, *l*CH_4 stands for litres of methane produced and *g*-COD stands for the reduction in grams of COD in the effluent after treatment in the AD. The formula provides us with a theoretical estimation of the volume of methane gas produced, at the standard temperature and pressure, per gram of chemical oxygen demand consumed in the AD.

We thus estimate a CH_4 yield of 0.35 STP l/g-COD. Based on this we can estimate the average daily yield of CH_4:

$$STP \frac{0.35\ lCH_4}{g - COD} * l/d$$

$$= \frac{0.35 l\mathrm{CH}^4}{\mathrm{gCOD}} * 2.53\mathrm{gCOD}/l\ \mathrm{effluent}$$

$$= \frac{0.0886\ l\mathrm{CH}^4}{1{,}191{,}000}$$

$$= 1{,}054{,}630\ l\mathrm{CH}_4/\mathrm{day}$$

Therefore the average daily yield of CH_4, using the COD data and the method by Angelidaki & Sanders 2004), was approximately 1,055 m³/d. Based on an energy density of 0.0378 mega joules per litre we can estimate that the energy potential of the anaerobic digester is around 39.865 MJ/day.

Both the biogas and COD-based analysis show that there is a daily average of over 1000 m³ CH_4 generated by the AD in that brewery. There is opportunity to exploit this stable and prolific source of methane.

It must be acknowledged, however, that there are limitations when extrapolating theoretically derived data into real situations. The equations and methods used to derive the estimates presented here have not taken all variables that could potentially influence the biological process into account. As such, the estimates presented here should be considered with this limitation in mind.

10. HUMAN RESOURCE DEVELOPMENT

Together with industry/THRIP funding we have been able to run SEVEN student programmes (four MSc and three Honours students) so far.

10.1 Current students

1. Rory Scheepers (**MSc student** – by thesis) investigated the use of treated brewery effluent as a medium to produce swordtail. He is scheduled to complete his degree during 2013.
2. Sean Power (**MSc student** – by thesis) is investigating the use of treated brewery effluent as a medium to produce hydroponically grown vegetables. He is currently running trials for this project. The data collected in these trials will be used in the preparation of his MSc thesis.
3. Lara Crous (**MSc student** – by thesis) investigated the use of constructed wetlands to treat brewery effluent and is focussed on nutrient removal. She is scheduled to complete her degree in 2012. Note that Ms Crous is no longer working fulltime on her thesis, but has taken a fulltime job. She is currently completing the write-up of her thesis part-time.

10.2 Graduated students

4. Anneke Cilliers (**MSc student** – by thesis) investigated the use of high rate algal ponds to treat brewery effluent, with a focus on seasonal nutrient removal and algal productivity. Her thesis has been successfully examined and she **graduated in April 2012**.
5. Devin Ayres (**BSc Honours** student – course work and thesis) investigated the use of effluent-grown algae as an alternative dietary protein source for *Oreochromis mossambicus* (Peters 1852): Effect on reproductive output. This research programme formed a substantial part of the student's degree (about 30% of the overall mark, and more than 40% of course work time was dedicated to this project). He has already passed (with distinction) and **graduated in April 2012**.
6. Morgan Brand (**BSc Honours student** – course work and thesis) investigated the use of brewery effluent-grown algae as an ingredient in the diets of abalone *Haliotis midae*. This research programme formed a substantial part of the student's degree (about 30% of the overall mark, and more than 40% of course work time was dedicated to this project). He **graduated in 2011**.

7. Gareth Nicholson (**BSc Honours** student – course work and thesis) investigated the use of brewery effluent-grown algae as an ingredient in the diets of tilapia *Oreochromis mossambicus*. This research programme formed a substantial part of the student's degree (about 30 % of the overall mark, and more than 40% of course work time was dedicated to this project). He **graduated in 2011**.

11. CONCLUSION

The high rate algal pond (HRAP) and constructed wetland (CW) system is an environmentally sustainable method of treating brewery effluent that allows for the recovery of water and nutrients from the wastewater. It is a low-energy, low maintenance system (both biologically and physically), driven mainly by gravity and the sun's energy. The only external energy inputs for the HRAP system were two small (0.45 kW) motors that drove the paddlewheels. As such, the cost to build and operate the system could be recovered quickly and the potential exists to recover these costs even faster if the water and nutrients that are recovered are reused or sold.

The HRAP and wetland system consistently brought most water quality parameters tested here to within or close to the Department of Water Affairs general limits for the discharge of industrial effluent into a natural water resource. We also developed a model that made it possible to predict the success of this system under various conditions that might be applied to other industries. Furthermore, the treatment/recovery process involved the production of downstream products such as algae; fish feed, fresh vegetables and fish. This program also saw the first attempt at optimising the use of industrial effluent as an inorganic source of fertiliser for hydroponic vegetable production.

Fish and vegetable production can take place using post-HRAP water, or water that has been subject to both HRAP and CW treatment (Figure 11.1). The CW did not require pre-treatment in the HRAP and operated more efficiently when HRAP was not included in the treatment chain (Figure 11.1); however, it was not possible to exclude the primary facultative pond (PFP) prior to treatment in the CW (Figure 11.1). The advantage of the wetland is that it is entirely self-sustaining but is difficult to clean/recharge, may clog up over time and takes more time to commission, whereas the HRAP can be inoculated and fully functional within days.

The downside of the HRAP/CW system is that it takes up considerably more space than conventional methods of water treatment, such as activated sludge systems, for example. The estimated area required to treat 1000 m^3 of post-anaerobic-digester brewery effluent per day is probably around 1.4 to 2.0 ha. However, with improved efficiency and optimisation this footprint might be further reduced.

The program has successfully demonstrated that industrial effluent, which is currently considered a costly liability by most industries, can be turned into a job-creating, income-generating stream, using simple technologies that have been available for years. It is just a matter of applying these technologies in a slightly different way.

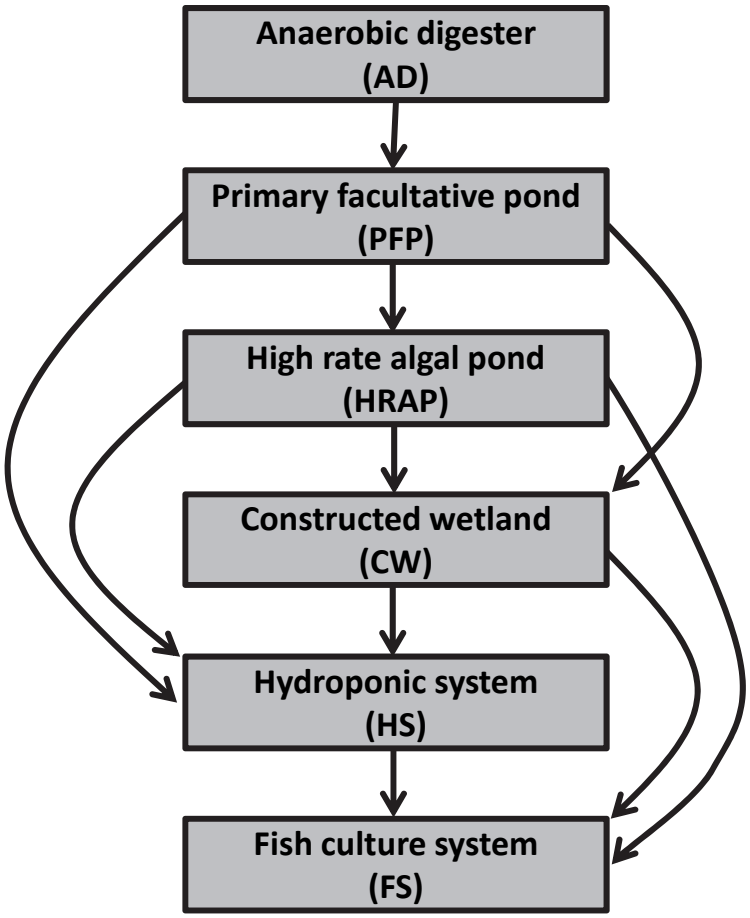


Figure 11.1 The primary facultative pond (PFP) was required to treat brewery effluent that came from the anaerobic digester (AD) prior to all other treatments and uses. Effluent did not have to be treated in the high rate algal ponds (HRAP) prior to treatment in the constructed wetland (CW). Vegetables were successfully produced in the hydroponic system (HP) in effluent drawn directly from the PFP and from the HRAP/CW. Fish culture in the aquaculture system (FS) needed effluent that had been treated in either the HRAP or the CW. Boxes not linked by an arrow suggest that the flow/use is not recommended

Future work should consider:

- developing a better understanding of the dynamics within the algal ponds, the changes that take place to the algal/bacterial communities and the underlying mechanisms responsible for nutrient removal in the ponds;
- alternative methods of harvesting algae from the high rate algal ponds;
- adding value to the constructed wetland by growing crops instead of reeds;
- combining pH control with alternative methods of increasing the nitrogen that is available in the brewery effluent as a growth medium for hydroponically grown vegetables;

- determining the long-term effect (i.e. over the whole life cycle) of treated brewery effluent on the health of fish; and
- Scaling up the technologies tested here to pilot-commercial scale systems.

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APPENDIX 1: MODELLING THE DATA – DEVELOPING A PREDICTIVE TOOL

1 - An overall analysis of the program's high rate algal pond data set

This chapter covers statistical tests on all data that had been collected during the three years of project. Various approaches to least-squares regression analysis and non-linear regression models were used, all with the aim of describing trends and of highlighting how environmental variables and effluent system management parameters influenced the composition of the effluent.

More than 100,000 measurements and data were made available, and correlation analyses were performed on all combinations of water quality variables and system management data, such as flow rate, retention time, daylight hours, the number of days the system was in operation, and other experimental changes that were made during the operation of the treatment systems.

As the size of the data set increases, a wide range of environmental conditions and their effect on treatment efficacy can be analysed. Screening tests for correlations between all variables led to the selection of only those variable pairs or combinations of dependent variables that could be used for predictive modelling and correlation analyses. Thus, not all possible correlations and regression models will be presented here. In some cases, however, the lack of significant relationships, which will be presented using the relevant statistics in tables, can lead to meaningful and interesting conclusions.

The usefulness of statistical analysis for such a large data set needs to be discussed. For example, in many instances, correlations between variables are significant, i.e., the tests suggest a relationship between data based on the fact that the null hypothesis, which states that the coefficient of correlation is significantly different from zero, was rejected. Importantly, a significant correlation between variables, even if highly significant, does not necessarily mean that values for the dependent variable can be predicted accurately as a function of the independent factor. Correlation does not always constitute causation.

The most likely reason is that the change in one variable is a function of more than one factor having an influence on its variability. Therefore, based on the results of simple regression methods, variables should be selected for multiple regression analysis. Presenting the outcome of multiple regression models in graphs is very difficult, if not impossible. Here, the distribution of residuals should be seen as an important support for the validity of the chosen models.

In addition, least-squares regression analyses and correlation tests of a very large data set are more likely to suggest a significant correlation between variables than analyses of small data sets. Thus, highly significant relationships between variables or differences between treatments do not always have to be *meaningful*. Here, the use of statistics must be combined with judgments and interpretations using experience and the knowledge gained during the process of analysing such a data set. In short, this approach to data analysis

involves the application of several steps with increasing complexity. Initially, data will be graphed and simple analyses will be run to detect relationships between variables and trends. This will be followed by simple regression analyses, which in turn provides a foundation for multiple regression tests.

Similar to the concept of a “meta-analysis”, where information from many sources is pooled into a large data set in order to detect trends, there are advantages and disadvantages to this approach. The advantages are that trends can be detected and highlighted that may provide the basis for future studies. This approach also helps to understand relationships between variables. A potential disadvantage is that the effects of multiple independent variables on a measured outcome may be difficult to present.

The following section comprises twelve graphs and tables. Analysis of variance was used to compare means of water quality variables between treatment systems and seasons. Thus, these two independent variables were used as main effects. Since measurements were taken from the same systems repeatedly, the statistical error caused by pseudo-replication needed to be taken into account. Thus, in this first step of data analysis, average values for systems and seasons were used from the three years of data collection.

The percentage reduction in water quality variables was calculated using the post-anaerobic digester (AD) values as reference points. Values above zero represented a decrease in the concentration of a water quality variable, while negative values represented an increase, except for pH where lower values meant higher H^+-Ion concentrations. Here, high values represent an increase.

Results

Chemical oxygen demand (COD mg/L; Figure 1.1)

The filtered chemical oxygen demand (COD) can be used as a measure of effluent quality. It is a “sum parameter” which is influenced by a wide range of chemical compounds. Thus, it provides an indication of the capacity of the effluent to demand oxygen from the environment.

There were significant differences in average values between treatment systems, but not between seasons (Figure 1.1). Using the average COD measurements at the AD-effluent as a reference value, COD was significantly reduced by 32 % at the outflow of A1 and A2, and by 24 % at the outflow of B1 and B2.

The small increase of 11 % in COD values between the first and the second tank in series from an average of 109 mg/L to 121 mg/L and the absence of a significant difference between these values and the post-AD values, suggests that the additional effluent treatment (A2, B2) did not contribute significantly to the treatment success.

Ammonia (total ammonia mg/L; Figure 1.2)

There was no seasonal influence on the average concentration of ammonia at the outflow of the treatment systems. By the end of the treatment process (post-A2, post-B2), ammonia values had been reduced by 97 % relative to the post-PFP values. Average ammonia concentrations did not differ between post-high rate algal pond (HRAP) and the A and B trains (see ANOVA table, Figure 1.2). The HRAP treatment achieved a reduction of ammonia by 94 %. Although the additional reduction of the ammonia concentration of a further 3 % to 97 % at the outflow of the final treatment systems may seem marginal and non-significant, this small difference in concentrations has applications. For fish culture to be successful, ammonia concentrations must be very low, so that a reduction from, for example, 4 mg/L to <1 mg/L, is more meaningful to beneficiation by aquaculture than the initial drop from 48.2 to 39.7 mg/L.

Thus, the effects of environmental conditions on ammonia levels need to be analysed in more detail in order to estimate how water quality and management of treatment systems may influence ammonia levels at low concentrations. This will be done using regression analysis.

Nitrite (NO_2^- mg/L; Figure 1.3)

The effluent treatment methods did not have an effect on the nitrite concentration (Figure 1.3, ANOVA table). The data, however, highlight the possibility of large spikes in concentrations in the post-A and post-B effluent during the summer months. These increases coincided with high ammonia levels (Figure 1.2). Across all treatment systems nitrite concentrations averaged 1.2 mg/L NO_2^-. While nitrite is poisonous to fish, the consistently high concentration of chloride in this effluent is likely to act antagonistically and reduce the harmful effect of nitrite. Thus, nitrite removal is not likely to be a main criterion to judge effluent treatment success for beneficiation by aquaculture, using the methods tested here.

Nitrate (NO_3^- mg/L; Figure 1.4)

The concentration of nitrate increased as a function of effluent treatment method. This is likely to be the result of nitrification, i.e., the oxidation of ammonia to nitrite through autotrophic bacteria. Nitrate concentrations at the effluent of systems A1 and B1 were significantly higher than post-AD and post-PFP values (see ANOVA table, Figure 1.4). However, at average concentrations of 44 mg/L NO_3^-, levels that may be harmful to fish were probably not reached.

Phosphate (P mg/L; Figure 1.5)

While the concentration of this nutrient in the post-PFP effluent was not significantly lower than the post-AD values, the HRAP treatment and trains A and B significantly reduced the values by 40 %, with a reduction of 27 % through the HRAP-treatment alone. There were no seasonal influences on the concentration of phosphate (ANOVA table, Figure 1.5).

Orthophosphate (PO_4^{3-} mg/L; Figure 1.6)

In this study, variability of PO_4^{3-} concentrations within treatment variation was larger than variation between treatments. As there were no differences in concentration between treatment methods (Figure 1.6), we estimate that orthophosphate levels could be reduced by 44 % relative to post-AD levels.

Chloride (Cl^- mg/L; Figure 1.7)

The concentration of chloride changed seasonally with high values in spring and summer. In particular, the average chloride concentration during spring was lower than the values in autumn and winter (see ANOVA table, Figure 1.7). The average concentration of chloride at the outflow of the PFP system was 7 % lower than post-AD values, but the subsequent use of treatment systems from HRAP to trains A and B increased the chloride values by 17 % above the post-AD concentrations.

Water temperature (°C; Figure 1.8)

Temperature fluctuations and significant differences between seasons are more likely to be confounded by changes in system management, for example, retention time, than by the design of the effluent treatment. Only average winter temperatures (measured in the morning) were lower than the average summer and autumn temperature. Considering that temperature is likely to influence biochemical reactions, it is more important to estimate the effect of water temperature on the success of effluent treatment than it is to understand temperature as a function of treatment system design. Thus, both water temperature and ambient temperature (see analysis of wetland systems) will be used as predictors in multiple regression models to predict water quality.

pH (Figures 1.9 and 1.10)

The pH values varied significantly as a function of both “system type” and “season”. As there was no significant interaction between these two main effects, a multiple range test (Tukey’s post-hoc HSD test) could be performed to compare average pH values separately between systems and seasons.

Differences between systems are complex, yet there was some consistency. For example, for both treatment trains A and B, the second train had significantly higher pH values than the first train in both morning and evening measurements. The lowest pH values were recorded for the Post-AD and Post-PFP effluent (Figures 1.9 and 1.10).

The effect of season on pH depended on the time of day pH measurements were taken. While pH during summer was higher than in autumn for measurements taken in the morning (Figure 1.9), pH in the afternoon was higher in spring than in autumn and summer (Figure 1.10).

The interpretation of these results should take into account light conditions and system depth, as photosynthesis by the suspended algae in the high-rate algal ponds is the most likely reason for fluctuations in pH.

Thus, some factors influencing pH can be controlled through effluent system management and design, and other variables, which influence pH, fluctuate seasonally. As with the other water quality variables, there is therefore a need to test not only the effect of pH on water quality, but also a combination of conditions. This will be accomplished through multiple regression analysis.

Conductivity (µs/cm; Figure 1.11)

Between-treatment variability of conductivity was smaller than within-treatment variability. Thus, the effluent treatment method could not be identified as a factor influencing conductivity. There were, however, differences in average conductivity values between seasons. Spring and summer values were higher than values in winter. Overall, conductivity values after treatment showed similar trends for the A and B trains. Conductivity increased by 6.7 % relative to the Post-AD values.

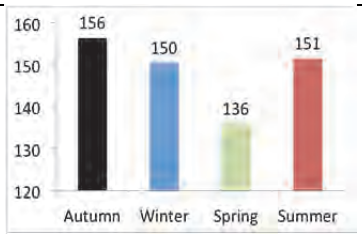
Dissolved oxygen (O_2 mg/L; Figure 1.12)

Effluent treatment design influenced the concentration of oxygen. Average values for the Post-AD, Post-PFP and Post-HRAP were similar at an average of 2.9 mg/L for all three systems, while the conditions in the A and B trains resulted in a significant increase in oxygen concentration, frequently to values above 100 % saturation. The average

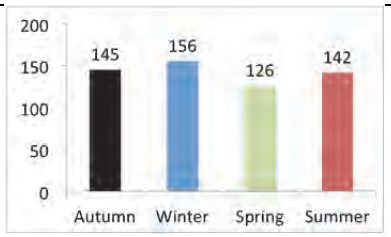
concentration in these systems was 10.7 mg/L O_2. Thus, similar to the changes in pH, oxygen values differed between systems.

Conclusions

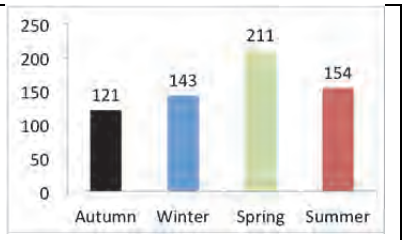
Although the main effects “system” and “season” had significant effects on water quality in the effluent, the graphs and analyses suggest that in many cases more than one factor must have influenced water quality. It is not clear from these tests whether relationships between variables are linear. Thus, the following section will apply simple and multiple regression modelling to detect relationships between variables and trends.



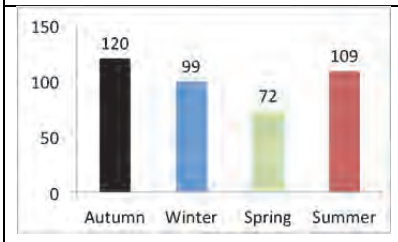
Post-AD



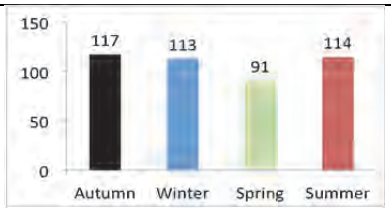
Post-PFP



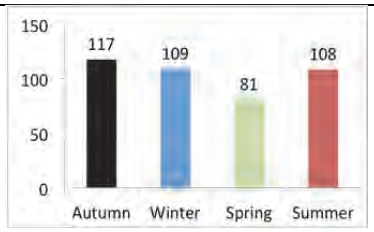
Post-HRAP



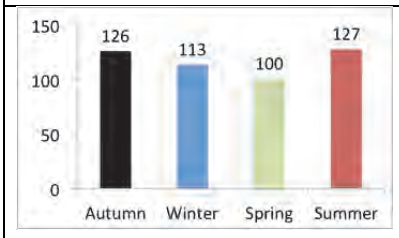
Post-A1



Post-A2

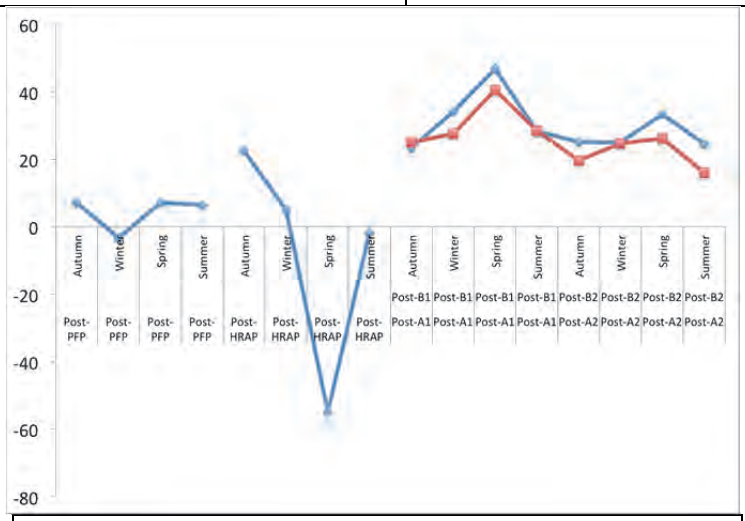


Post-B1



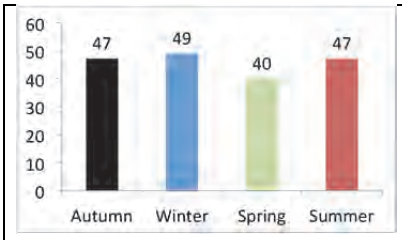
Post-B2

Increase / reduction of COD as percentage of Post-AD levels. Positive levels represent reduction of COD, and negative values represent an increase in COD concentrations. The red line shows the average values for the B-Train.

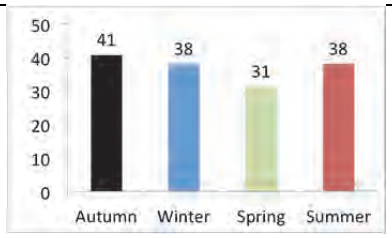


ANOVA table; values are averages; different letters suggest significant differences between treatment means.		
Post-AD	150.8	A
Post-PFP	143.8	A B
Post-HRAP	142.3	A B
Post-A1	107.5	B
Post-A2	117.3	A B
Post-B1	110.3	B
Post-B2	123.9	A B
System: F = 4.02, p = 0.002; Season: F = 0.996, p = 0.40 System x season: F = 0.48, p = 0.96		

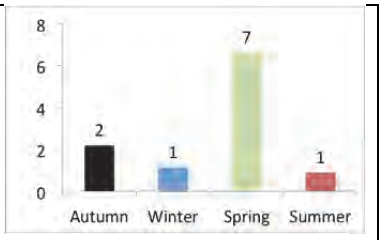
Figure 1.1 Average COD concentration (mg/L) for systems and seasons and the effect of treatment method on the increase or reduction of COD.



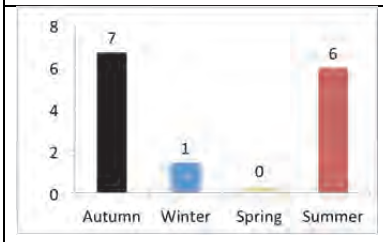
Post-AD



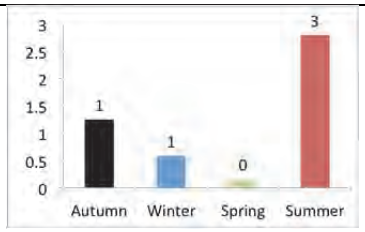
Post-PFP



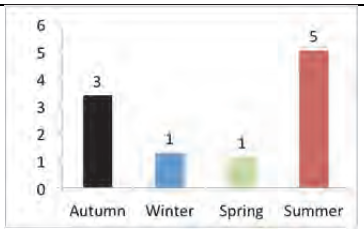
Post-HRAP



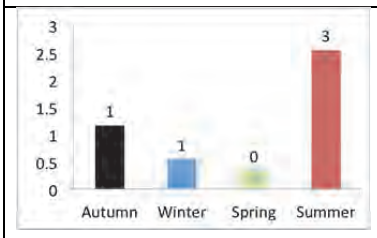
Post-A1



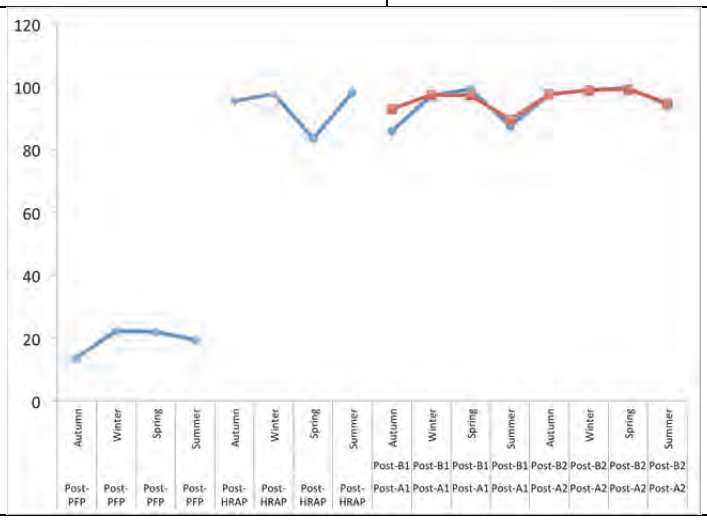
Post-A2



Post-B1



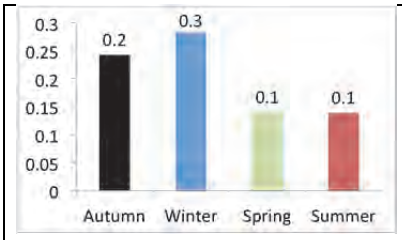
Post-B2



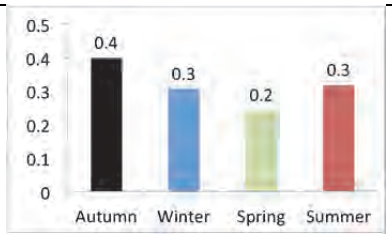
Reduction of ammonia as percentage of Post-AD levels. Positive levels represent removal of ammonia. The red line shows the average values for the B-Train.

ANOVA table; values are averages; different letters suggest significant differences between treatment means.		
Post-AD	48.2	A
Post-PFP	39.7	B
Post-HRAP	2.17	C
Post-A1	4.27	C
Post-A2	1.29	C
Post-B1	2.86	C
Post-B2	1.24	C
System: F = 107.2, p < 0.00001; Season: F = 0.34, p = 0.34 System x season: F = 0.32, p = 0.99		

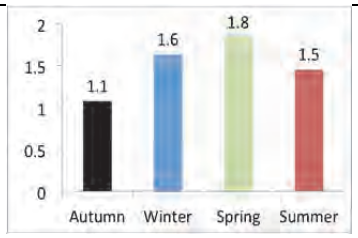
Figure 1.2 Average ammonia concentration (mg/L) for systems and seasons and the effect of treatment method on the percentage reduction of ammonia in the effluent.



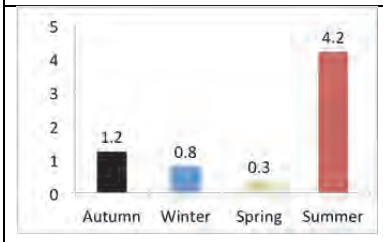
Post-AD



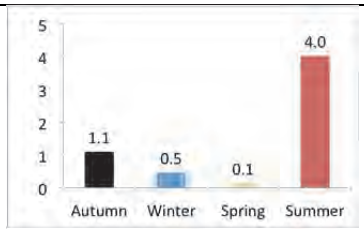
Post-PFP



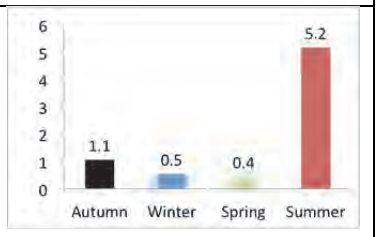
Post-HRAP



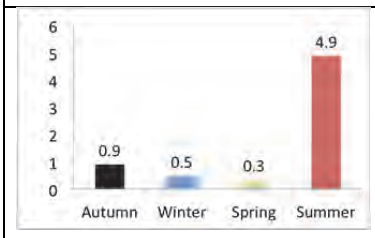
Post-A1



Post-A2

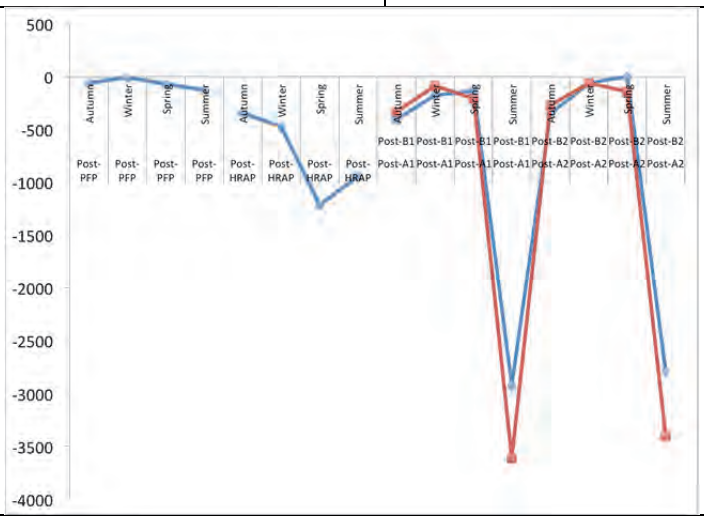


Post-B1



Post-B2

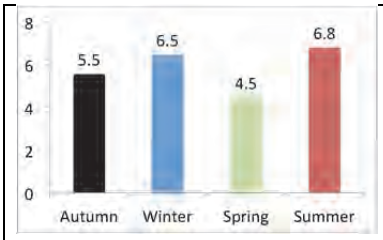
Change of nitrite concentration in the effluent as percentage of Post-AD levels. Positive levels represent removal of nitrite, and negative values represent an increase in nitrite concentration. The red line shows the average values for the B-Train.



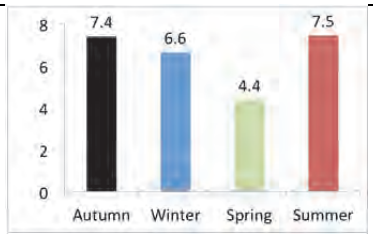
ANOVA table; values are averages; different letters suggest significant differences between treatment means.

Autumn	0.84	A
Winter	0.98	A
Spring	0.63	A
Summer	2.49	B
System: F = 2.18, p = 0.06; Season: F = 7.9, p = 0.0002 System x season: F = 0.98, p = 0.49		

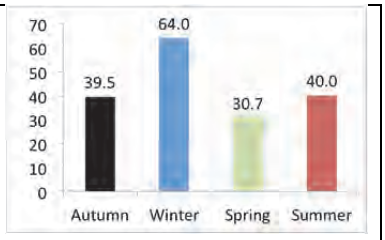
Figure 1.3 Average nitrite concentration (mg/L) for systems and seasons and the effect of treatment method on the percentage reduction of nitrite in the effluent.



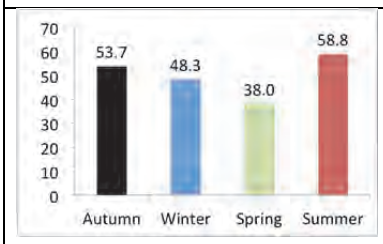
Post-AD



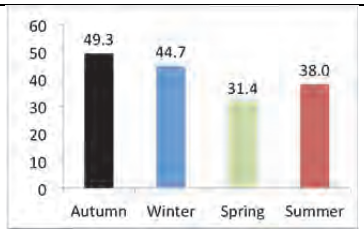
Post-PFP



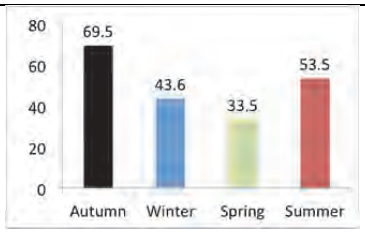
Post-HRAP



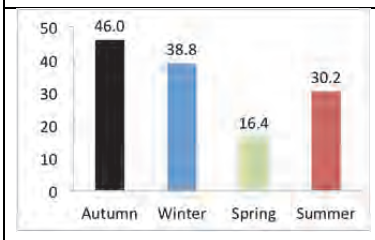
Post-A1



Post-A2

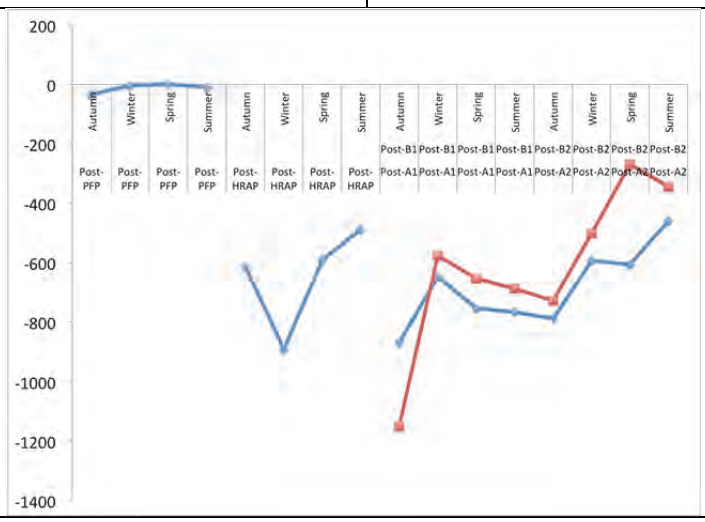


Post-B1



Post-B2

Change of nitrate concentration in the effluent as percentage of Post-AD levels. Positive levels represent removal of nitrite, and negative values represent an increase in nitrite concentration. The red line shows the average values for the B-Train.

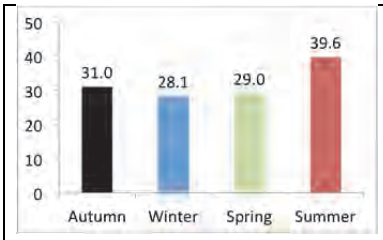


ANOVA table; values are averages; different letters suggest significant differences between treatment means.

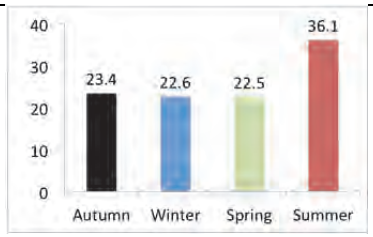
Post-AD	6.90	B C
Post-PFP	8.29	B
Post-HRAP	45.6	A B C
Post-A1	58.8	A
Post-A2	50.3	A C
Post-B1	58.5	A
Post-B2	39.4	A B C

System: F = 4.67, p < 0.0007; Season: F = 0.83, p = 0.47
System x season: F = 0.15, p = 0.99

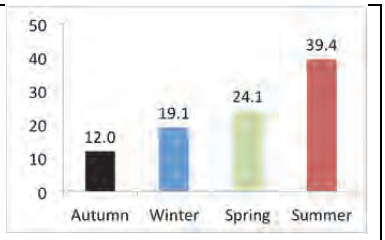
Figure 1.4 Average nitrate concentration (mg/L) for systems and seasons and the effect of treatment method on the percentage reduction of ammonia in the effluent.



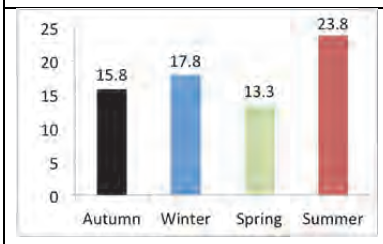
Post-AD



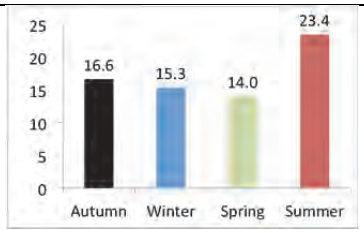
Post-PFP



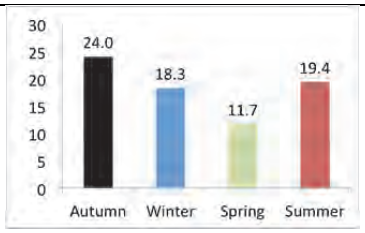
Post-HRAP



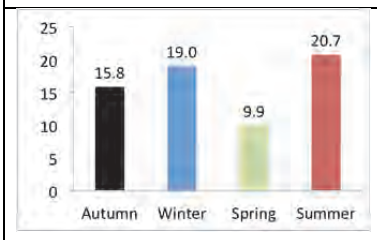
Post-A1



Post-A2

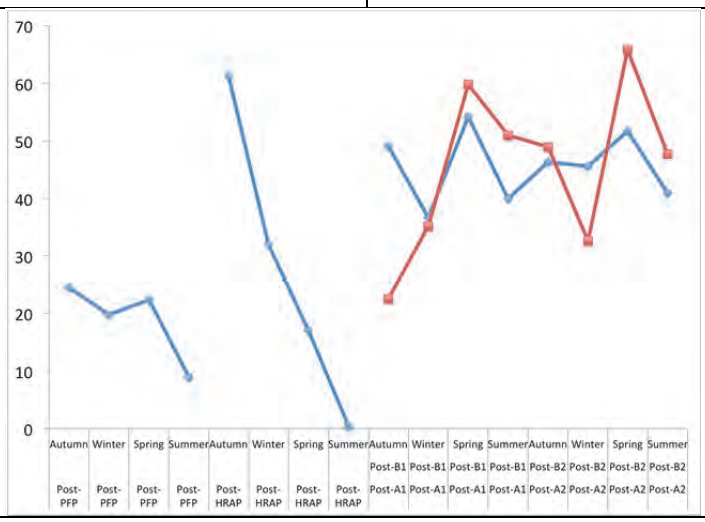


Post-B1



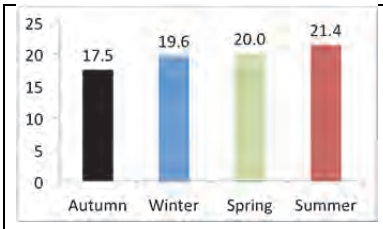
Post-B2

Reduction of phosphate as percentage of Post-AD levels. Positive levels represent removal of phosphorous. The red line shows the average values for the B-Train.

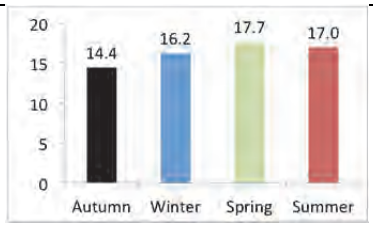


ANOVA table; values are averages; different letters suggest significant differences between treatment means.		
Post-AD	20.2	C
Post-PFP	16.4	B C
Post-HRAP	9.63	A
Post-A1	11.5	A B
Post-A2	11.9	A B
Post-B1	9.8	A
Post-B2	10.0	A B
System: F = 8.2, p < 0.0006 Season: F = 2.67, p = 0.08 System x season: F = 0.47, p = 0.93		

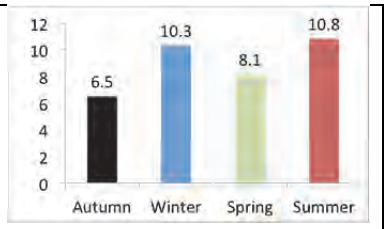
Figure 1.5 Change of phosphate concentration (mg/L) in the effluent as percentage of Post-AD levels. Positive levels represent removal of phosphorous.



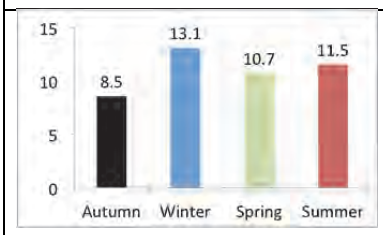
Post-AD



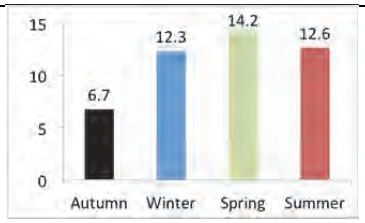
Post-PFP



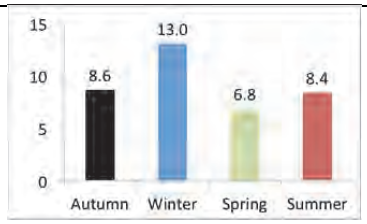
Post-HRAP



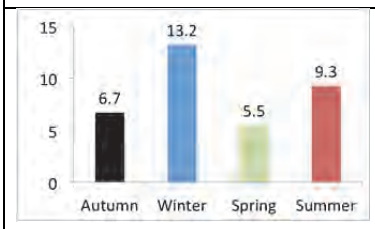
Post-A1



Post-A2

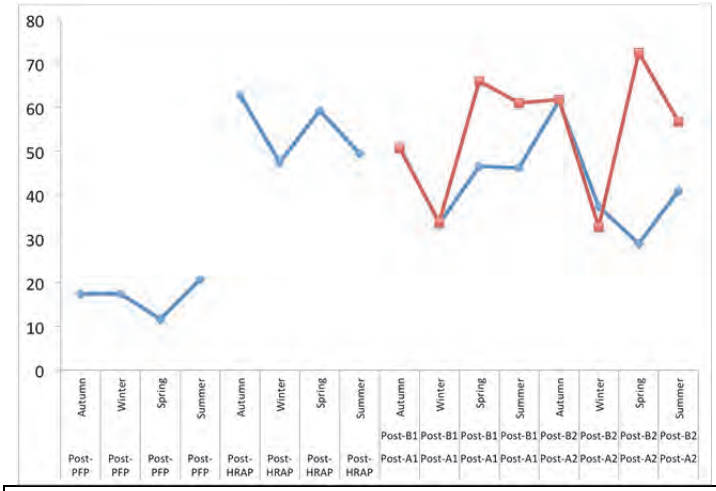


Post-B1



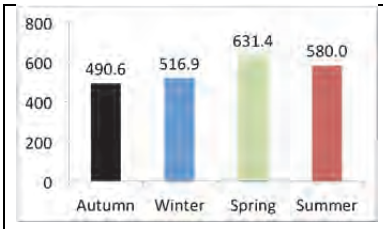
Post-B2

Reduction of PO_4^{3-} as percentage of Post-AD levels. Positive levels represent removal of phosphate. The red line shows the average values for the B-Train.

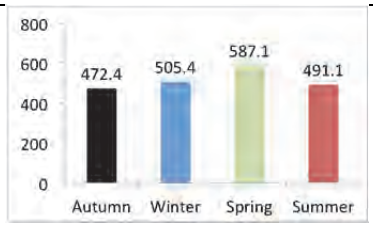


ANOVA statistics to test the effect of system and season on Orthophosphate concentrations
System: F = 0.92, p < 0.48 Season: F = 0.46, p = 0.71 System x season: F = 0.24, p = 0.99

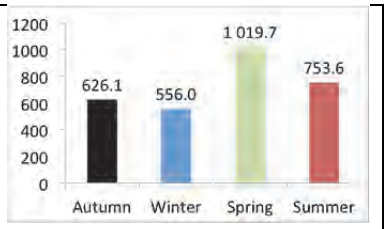
Figure 1.6 Change of PO_4^{3-} (mg/L) in the effluent as percentage of Post-AD levels. Positive levels represent percentage removal of phosphate.



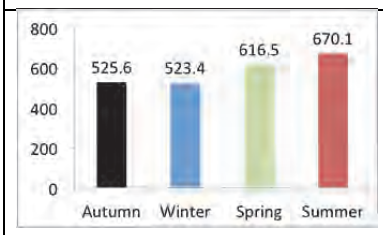
Post-AD



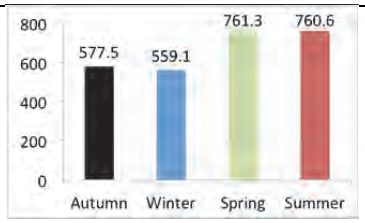
Post-PFP



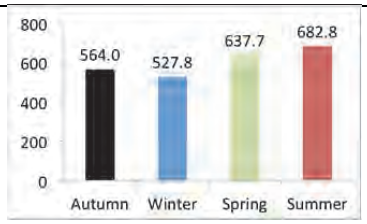
Post-HRAP



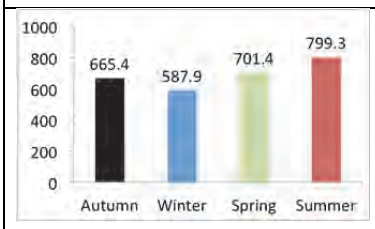
Post-A1



Post-A2

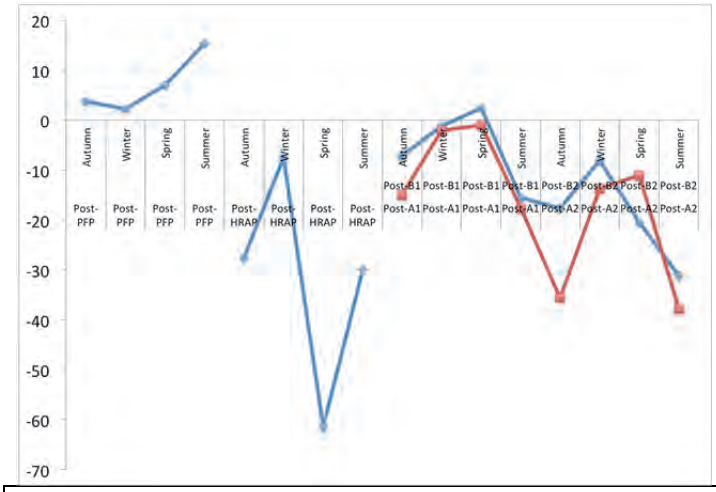


Post-B1



Post-B2

Change of chloride concentration in the effluent as percentage of Post-AD levels. Positive levels represent removal of chloride, and negative values represent an increase in chloride concentration. The red line shows the average values for the B-Train.

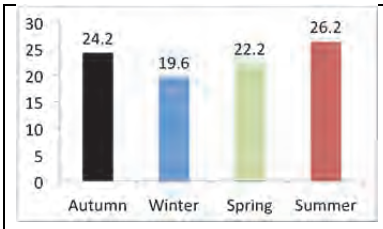


ANOVA table; values are averages; different letters suggest significant differences between treatment means.

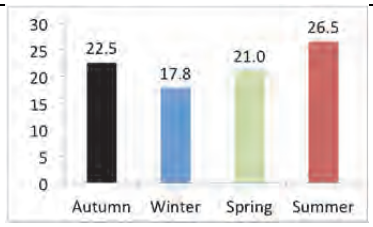
Autumn	554.7	A
Winter	535.7	A
Spring	787.3	B
Summer	724.3	A B

System: F = 0.99, p = 0.43
Season: F = 5.16, p < 0.003
System x season: F = 0.104, p = 0.99

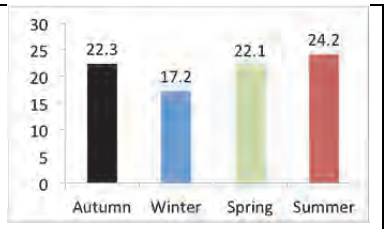
Figure 1.7 Change of chloride concentration (mg/L) in the effluent as percentage of Post-AD levels.



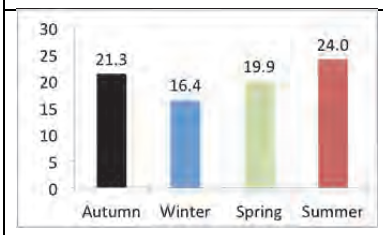
Post-AD



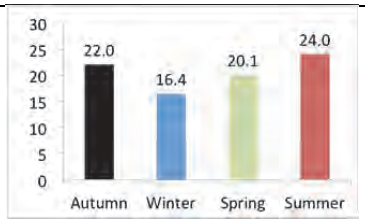
Post-PFP



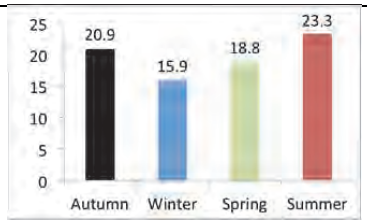
Post-HRAP



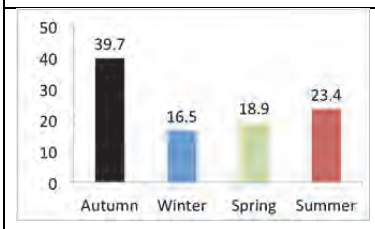
Post-A1



Post-A2

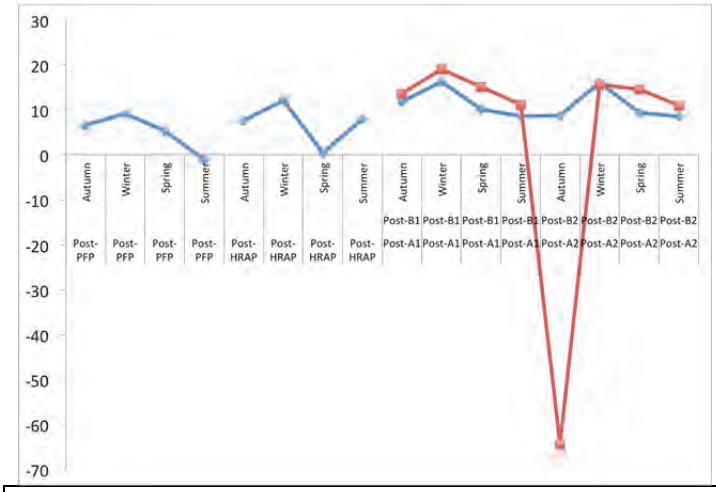


Post-B1



Post-B2

Change of average temperature (°C) in the effluent as percentage of Post-AD levels. The red line shows the average values for the B-Train.

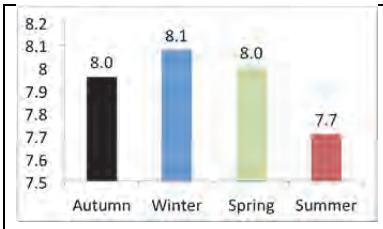


ANOVA table; values are averages; different letters suggest significant differences between treatment means.

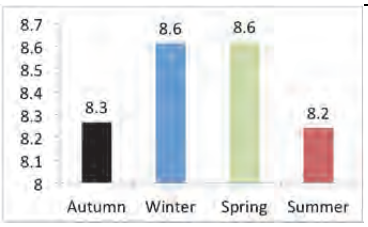
Autumn	23.7	A
Winter	17.1	B
Spring	20.4	A B
Summer	25.2	A

System: F = 0.69, p = 0.65
Season: F = 8.23, p = 0.00003
System x season: F = 0.665, p = 0.83

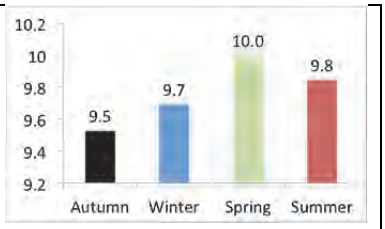
Figure 1.8 Change of average temperature (°C) in the effluent as percentage of Post-AD levels.



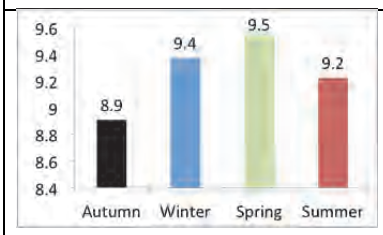
Post-AD



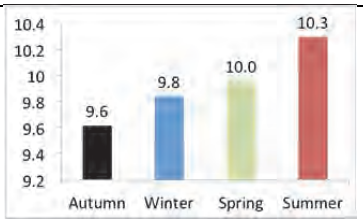
Post-PFP



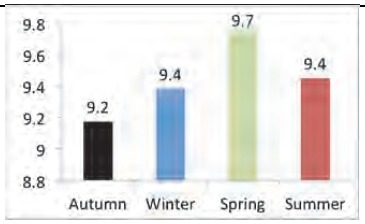
Post-HRAP



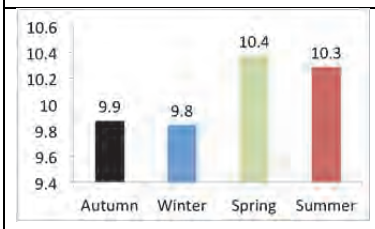
Post-A1



Post-A2

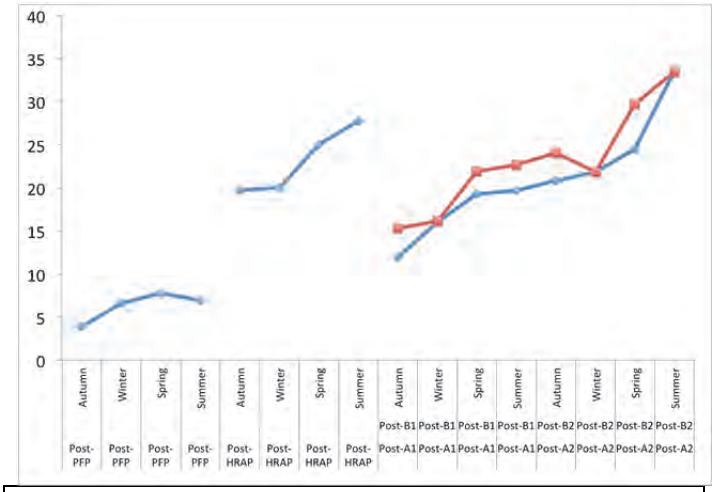


Post-B1



Post-B2

Average pH (morning measurements) at the effluent of the systems. All values were higher than Post-AD values. The red line shows the average values for the B-Train.

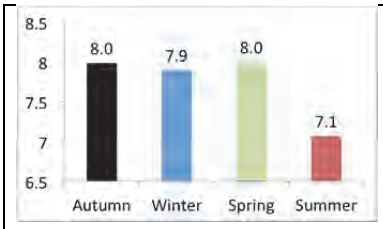


ANOVA table; values are averages; different letters suggest significant differences between treatment means.

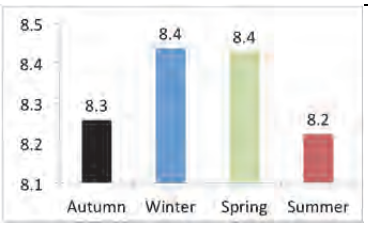
Post-AD	7.96	D	Autumn	9.02	A
Post-PFP	8.45	D	Winter	9.28	A B
Post-HRAP	9.74	A B C	Spring	9.51	A B
Post-A1	9.26	A	Summer	9.29	B
Post-A2	9.93	B C			
Post-B1	9.44	A B			
Post-B2	10.07	C			

System: F = 9.2, p < 0.00001
Season: F = 5.15, p = 0.0027
System x season: F = 0.69, p = 0.80

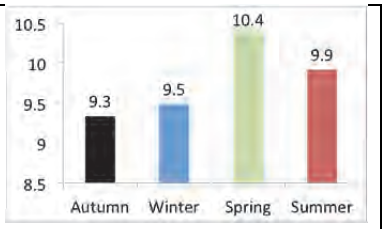
Figure 1.9 Increase of pH measured in the morning in the effluent as percentage of Post-AD levels. Both system design and season influenced pH-values.



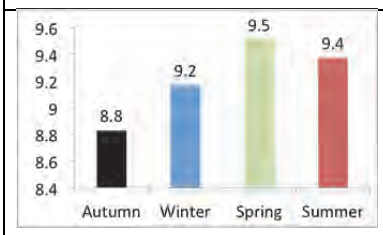
Post-AD



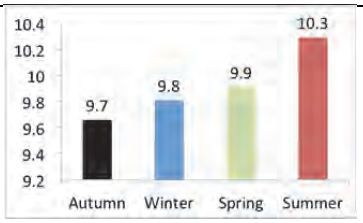
Post-PFP



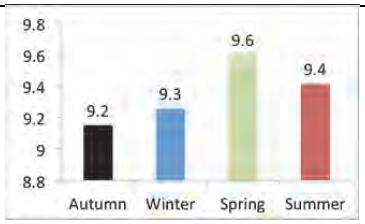
Post-HRAP



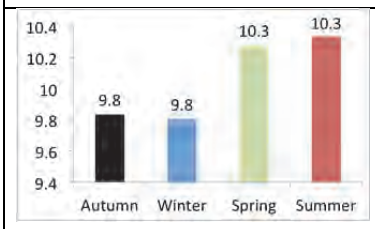
Post-A1



Post-A2



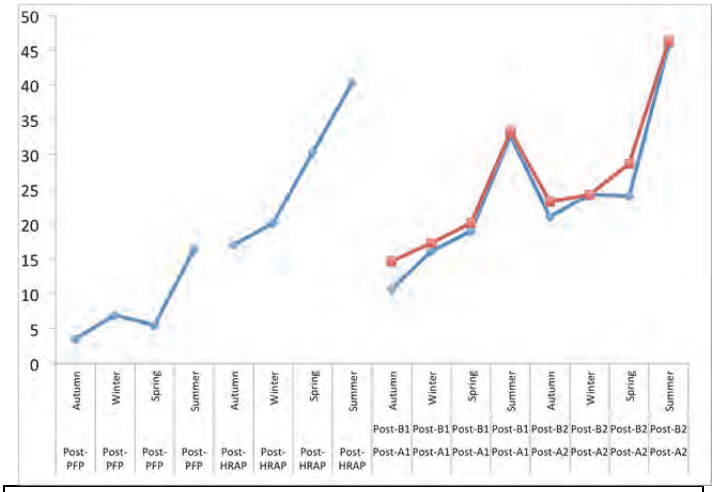
Post-B1



Post-B2

Average pH (evening measurements) at the effluent of the systems. All values were higher than Post-AD values.

The red line shows the average values for the B-Train.

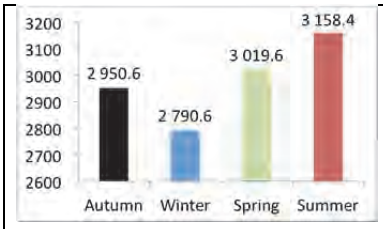


ANOVA table; values are averages; different letters suggest significant differences between treatment means.

Post-Ad	7.70	D	Autumn	8.94	A
Post-PFP	8.30	E	Winter	9.29	B C
Post-HRAP	9.66	A B C	Spring	9.59	C
Post-A1	9.18	A	Summer	9.04	A B
Post-A2	9.85	B C			
Post-B1	9.43	A B			
Post-B2	9.97	C			

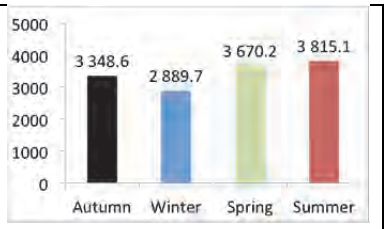
System: F = 60.6, p < 0.00001
Season: F = 7.68, p = 0.0018
System x season: F = 2.12, p = 0.06

Figure 1.10 Increase in pH value measured in the afternoon in the effluent as percentage of Post-AD levels. Both system design and season influenced pH-values.

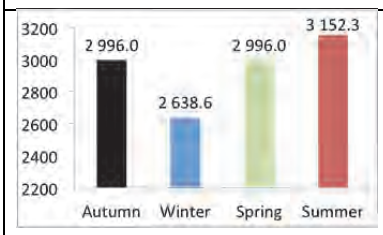


Post-AD

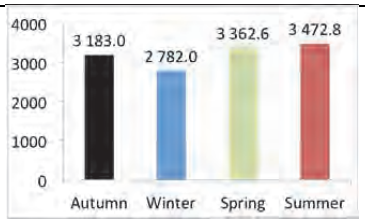
Post-PFP



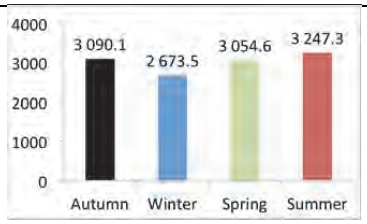
Post-HRAP



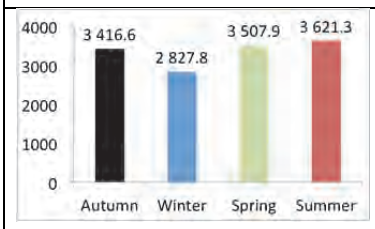
Post-A1



Post-A2



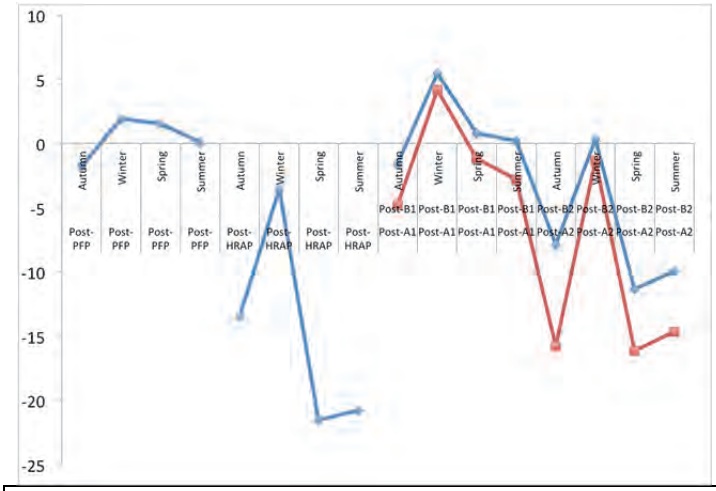
Post-B1



Post-B2

Conductivity (µs/cm) in the effluent as percentage of Post-AD levels.

The red line shows the average values for the B-Train.

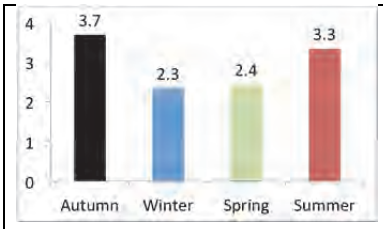


ANOVA table; values are averages; different letters suggest significant differences between treatment means.

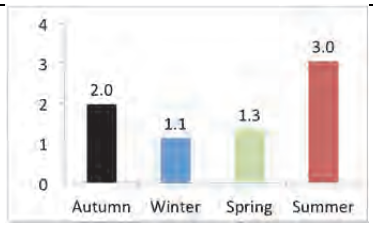
Autumn	3062	A B
Winter	2793	B
Spring	3296	A
Summer	3308	A

System: F = 1.36, p = 0.24
Season: F = 5.97, p = 0.001
System x season: F = 0.14, p = 0.99

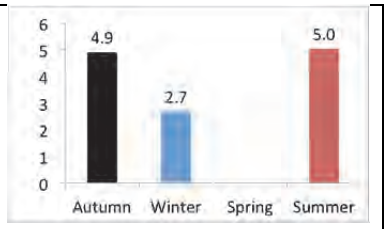
Figure 1.11 Change of conductivity (µs/cm) in the effluent as percentage of Post-AD levels.



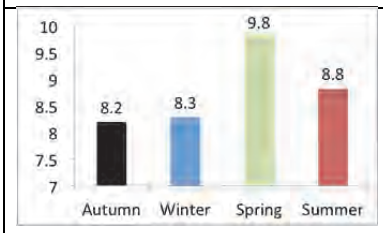
Post-AD



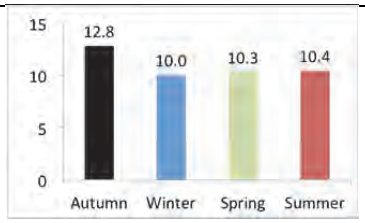
Post-PFP



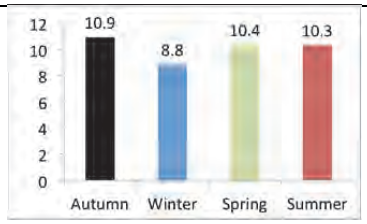
Post-HRAP



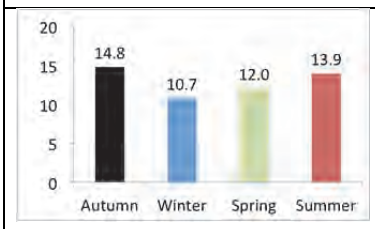
Post-A1



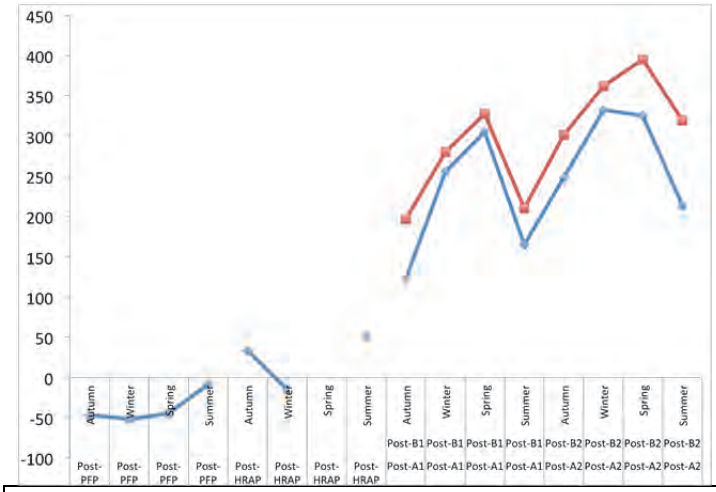
Post-A2



Post-B1



Post-B2



Oxygen concentration (O_2 mg/L) in the effluent as percentage of Post-AD levels. The red line shows the average values for the B-Train. (Some values from the post-HRAP data set were missing.)

ANOVA table; values are averages; different letters suggest significant differences between treatment means.		
Post-AD	2.95	A
Post-PFP	1.97	A
Post-HRAP	4.81	A
Post-A1	8.23	A B C D
Post-A2	11.0	C D
Post-B1	9.62	B C D
Post-B2	13.1	D
System: F = 7.38, p < 0.0006 Season: F = 0.47, p = 0.63 System x season: F = 0.08, p = 0.99		

Figure 1.12 Change of oxygen concentration in the effluent as percentage of Post-AD levels.

2 - Correlation between environmental variables and the concentration of ammonia and chemical oxygen demand at the outflow of high rate algal ponds

For this analysis, ammonia, COD and nitrate values were selected as the dependent variables for several reasons. Ammonia is one the most harmful substances for fish and considering that aquaculture has been chosen for beneficiation of the effluent; knowledge about factors influencing the concentration of ammonia in these unique effluent systems is needed. The pH increased as a result of the effluent treatment, thereby further increasing the toxicity of unionised ammonia to fish. Results from the data analysis in section 1 suggested that a range of factors may have influenced the concentration of ammonia and many other water quality variables. Thus, it was assumed that multiple regression analysis can be used to isolate the most influential variables. COD was chosen as a measure of effluent treatment success. The choice of nitrate as a dependent variable was chosen since nitrate is a nutrient used by plants.

Simple bivariate least-squares regression analysis was used to estimate relationships between variables and select those independent variables that were most likely to contribute to a multiple regression analysis. This was followed by multiple step-wise forward regression tests. During this process some variables can be selected as influential variables, while others do not contribute significantly to a predictive model when used in combination with several independent variables. The distribution of residuals was chosen as a criterion to gauge the suitability of the final model.

In some cases, both the models and the distribution of the residuals could be improved by log-transforming the dependent or independent variables. Thus, some graphs show log-transformed values.

Results

Ammonia

Post-HRAP

Ammonia values were measured regularly during all seasons and years of operations at the outflow of the HRAP system. These values include a range of conditions that allow us to analyse the effects of several variables on ammonia levels.

The concentration of ammonia (mg/L) at the outflow of the HRAP unit was not correlated to the range of conditions tested in this study. Table 2.1 shows the p-values from the regression analysis. Only pH had a small influence on ammonia concentration, but at an r^2-value of 17 % only a small portion of the variance in ammonia levels could be explained by the variability in pH.

Table 2.1 The p-values from regression analysis with ammonia concentration as the dependent variable and various environmental conditions and water quality variables as independent variables. Values were taken at the outflow of the high rate algal pond (HRAP) system. Except for a very low significance of pH on ammonia values, none of the independent variables listed here had a significant influence on the concentration of ammonia.

Independent variable	p-value	Independent variable	p-value
Retention time (days)	0.40	Temperature ^{o}C (a.m.)	0.20
Flow rate (l/h)	0.22	Temperature ^{o}C (p.m.)	0.16
COD (mg/L)	0.86	pH a.m.	0.04
Nitrite (mg/L)	0.85	pH p.m.	0.55
Phosphate (mg/L)	0.36	Conductivity (us/cm)	0.45
Chloride (mg/L)	0.64	Oxygen (O_2, mg/L)	0.45
		Percentage oxygen saturation	0.92

Post-A1, A2 and B1, B2

Values for A1 and A2 as well as B1 and B2, respectively, were pooled for correlation analyses. In contrast to the results of the analysis of the HRAP data, some variables significantly influenced ammonia levels. Figure 2.1 shows these relationships, and the predictive models. Those variables that were not significantly correlated to the concentration of ammonia at the outflow are listed in Table 2.2.

Table 2.2 Coefficient of correlation from regression analysis with ammonia concentration as the dependent variable and various operational conditions and water quality variables as the independent variables. Values were taken at the outflow of the two "train A" and "train B" units and pooled for the analysis. Correlations given as "n. s." did not significantly influence ammonia levels at the outflow of the respective system.

	Coefficient of correlation (r)				
Independent variable	Train A	Train B	Independent variable	Train A	Train B
Time (months)	n. s.	n. s.	Temperature (a.m.)	-0.42	n. s.
Light (minutes/day)	-0.40	n. s.	Temperature (p.m.)	-0.31	n. s.
Retention time (days)	n. s.	n. s.	pH (a.m.)	-0.46	n. s.
COD (mg/L)	n. s.	n. s.	pH (p.m.)	-0.54	n. s.
Nitrite (mg/L)	n. s.	n. s.	Conductivity (us/cm) (a.m.)	n. s.	n. s.
Nitrate (mg/L)	n. s.	n. s.	Conductivity (us/cm) (p.m.)	n. s.	n. s.
Phosphate (mg/L)	n. s.	n. s.	Oxygen (mg/L)	n. s.	n. s.
Chloride (mg/L)	-0.36	-0.31	Oxygen (% saturation)	n. s.	n. s.

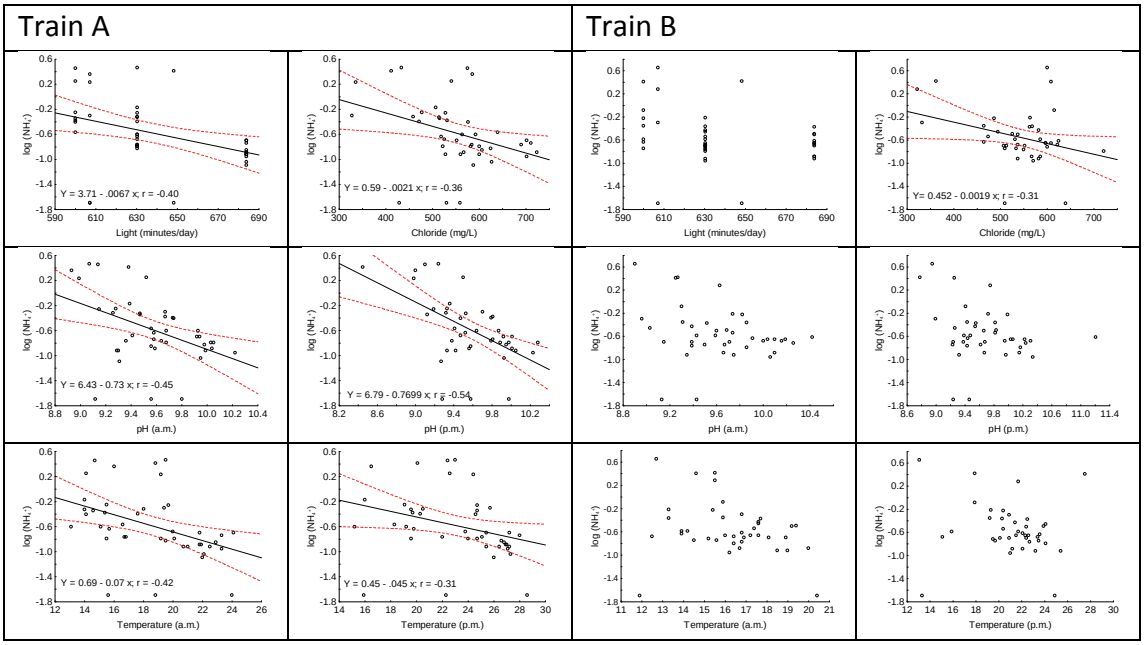


Figure 2.1 Significant relationships and their linear and non-linear estimates of ammonia values at the outflow of train A and train B. Values for A1 and A2 and B1 and B2 were pooled. Except for the correlation between chloride and ammonia, values within train B were not significantly correlated to each other.

The regression models for data from train A show that there were significant correlations between day light length, chloride, pH and temperature (independent) and ammonia levels (Figure 2.1). Data for train B differed in that these independent variables did not significantly influence ammonia values. Thus, with regard to water quality dynamics and relationships between variables the two trains showed different trends. The characteristics of train A may allow for a better manipulation of ammonia values.

Multiple regression analysis with forward step-wise inclusion of variables for those variables that were significantly correlated to the concentration of ammonia at the outflow of train A (Table 2.2) indicated that pH measured in the afternoon was the strongest predictor for the concentration of ammonia. The other independent variables were not included in the model at an error level of 5 %.

Chemical oxygen demand

Post-HRAP

Except for a significant correlation between COD (mg/L) and nitrate (mg/L) there were no significant correlations between operation parameters and water quality with COD (Table 2.3). The positive correlation between COD and nitrate is only based on a small number of valid values (n=4). Thus, more data would be needed to establish reasons for such a correlation.

COD levels were influenced by the number of months the system was in operation and day length for data collected at the effluent of train A. For train B data, these relationships were not significant (Figure 2.2).

Table 2.3 Coefficient of correlation from regression analysis with COD concentration as the dependent variable and various operational conditions and water quality variables as the independent variables. Values were taken at the outflow of the two "train A" and "train B" units. Variables that did not significantly influence ammonia levels at the outflow of the respective system are shown as "n. s."

	Coefficient of correlation (r)				
Independent variable	Train A	Train B	Independent variable	Train A	Train B
Time (months)	-0.32	n. s.	Temperature (a.m.)	n. s.	n. s.
Light (minutes/day)	-0.33	n. s.	Temperature (p.m.)	n. s.	n. s.
Retention time (days)	n. s.	n. s.	pH (a.m.)	n. s.	n. s.
COD (mg/L)	n. s.	n. s.	pH (p.m.)	n. s.	n. s.
Nitrite (mg/L)	n. s.	n. s.	Conductivity (us/cm) (a.m.)	n. s.	n. s.
Nitrate (mg/L)	n. s.	n. s.	Conductivity (us/cm) (p.m.)	n. s.	n. s.
Phosphate (mg/L)	n. s.	n. s.	Oxygen (mg/L)	n. s.	n. s.
Chloride (mg/L)	n. s.	n. s.	Oxygen (% saturation)	n. s.	n. s.

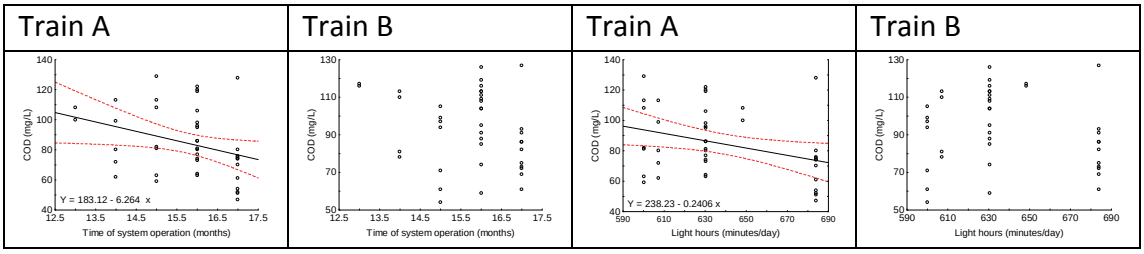


Figure 2.2 Significant correlations and their linear estimates of COD-values at the outflow of train A and train B. Values for A1 and A2 and B1 and B2 were pooled. Correlations for data from train A were significant, while there are no estimates for the non-significant correlations for data from train B.

Nitrate

Similar to the lack of correlations between COD and water quality variables, nitrate levels at the outflow of the HRAP system were not significantly correlated with other variables.

Regression analyses for nitrate values using data collected at the effluent of train A and train B showed no correlation between variables.

3 - Constructed wetlands as effluent treatment systems

The studies conducted on the water quality changes in a constructed wetland system involved testing a range of conditions, such as the measuring of water quality at various distances from the inflow. In addition, since there were uncontrollable changes in environmental conditions, such as ambient temperature or day length, and the inevitable variation in the concentrations of water quality variables at the inflow, an analysis of such a data set should take all factors into account.

The aim of this analysis was therefore to determine which factors influenced water quality, and whether water quality could be predicted as a function of selected independent variables.

It was hoped that the information gained from this exercise would lead to suggestions for future studies aimed at optimising the performance of this wetland system.

The methods used in this study have been described in other documents and reports. Here, we focus on some additional statistical tests.

For this, data collected during the study of the wetlands were used to model selected water quality variables as a function of operating conditions and other water quality variables.

Concentrations of ammonia, COD, PO_4^{3-}, and nitrate were used as the dependent variable in a multiple regression model using all available information as predictors. We modelled water quality using a step-wise forward multiple regression procedure. Variables making a significant contribution to the model had p-values below 5 %. Residual patterns of the selected models were tested for normality and only regression models with normally distributed residuals were accepted and presented. In some cases, models could be further improved by using log-transformed variables.

This method of data analysis allows us to highlight those variables and conditions that most significantly influenced the values of the dependent variables. In many cases, only some variables had a significant effect on water quality. The regression tables show those effects that are valid predictors. However, due to relatively low correlation coefficients, it was not always appropriate to suggest a predictive model.

Meaningful relationships are shown in the figures and the regression models are also given.

Various tests did not show significant relationships. Thus, only those models that allow an interpretation of the relationships between variables are given here.

Scatterplots show considerable variation in the data. Data were collected under a wide range of conditions over a long period during which there were frequent spikes in water quality and changes in operational conditions. Here, all data are shown. The main advantage of the multiple regression analysis is that outliers in such a large data set rarely change the conclusions one can draw from such a study.

Data from one wetland unit

Measurements of water quality were taken at 15-m distances from the inflow to the wetland. While COD values were significantly ($p=0.012$) reduced as a function of distance from the inflow to the wetland, there was a large variability in this data set (Figure 3.1). The model predicts a 26 % reduction from 104.5 mg/L to 71 mg/L at 60 meters from the inflow. However, since only a small percentage of this reduction can be explained by distance alone, it is likely that other factors contributed to the changes in COD concentration.

The pH was significantly reduced as water passed through the wetland. The predicted change of 0.3 units from pH 8.8 to 8.5 could be considered meaningful. At this high pH range even a small reduction in pH can lead to a beneficial reduction of ammonia toxicity if fish are being farmed using the system effluent.

The significant drop of the concentration of ammonia ($p<0.0004$) from an estimated 4.05 mg/L to 0.39 mg/L (87 %) suggests that the wetland system could reduce ammonia levels to below 0.5 mg/L. This, in combination with the reduction of pH favours the addition of wetlands to the treatment plant as it improves the usefulness of the effluent water for aquaculture.

Both the concentrations of nitrite and nitrate could be significantly reduced through the use of wetlands by 74 % and 75 %, respectively.

The wetland system did not significantly reduce conductivity values, total dissolved solids, oxygen and chloride concentration.

There was a significant positive correlation between pH and the concentration of nitrite and nitrate. Thus, as pH decreased by passing the water through the wetland, a reduction of nitrite and nitrate levels can be predicted. In contrast to the negative relationship between ammonia and pH that was found when analysing the effluent of the algal pond systems, such a relationship was not significant for this wetland system.

There were no other significant correlations between water quality variables for this system.

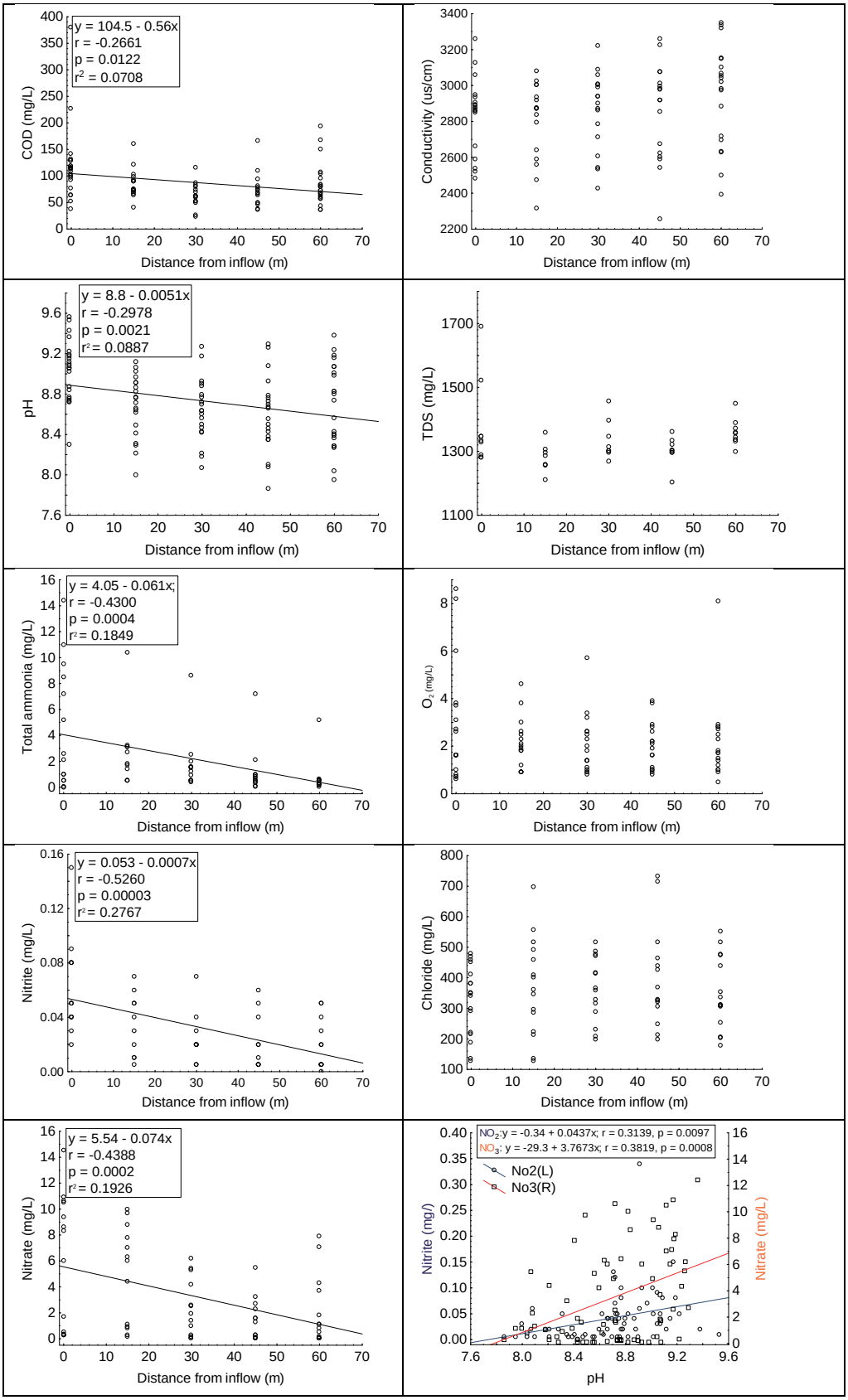


Figure 3.1 Water quality as a function of distance from the inflow to a wetland.

Wetland data – results from a long-term study with variation in conditions

The data set used for the following analysis differs from the previous data set in that this study was conducted over a longer period, under different operating conditions, using four wetlands as replicates. Thus, the data generated in this study covered a wide range of environmental conditions, seasons, and changes in wetland management. In interpreting these results, the circumstances under which the data were collected need to be taken into account.

Similar to the analyses of the data from the other studies, all combinations of correlations and relationships between all data were tested. Here, only the significant correlations will be reported. Several factors significantly influenced the concentrations of ammonia, COD, orthophosphate and nitrate, and the results from the correlation analyses will be reported.

Results

Ammonia

There was an exponential effect of water pH on the concentration of ammonia. The model that describes the ammonia concentration as a function of pH should not be compared to the well-known chemical model, predicting ammonia as a function of water temperature and pH. In this data set, the ammonia concentration is likely a function of both pH and of the biochemical changes in the wetland that are moderated by pH.

The concentration of ammonia can be estimated using the exponential model of Figure 3.2.

Ammonia concentration was significantly negatively correlated to ambient temperature (Figure 3.2) but not to water temperature. This suggests that the effect of ambient temperature on the metabolic activity of the wetland flora may have been more important than the water temperature. In future studies it could be tested whether plants not fully immersed in water will respond more to ambient temperature than water temperature. Should the correlation between ambient temperature and ammonia concentration be confirmed to be stronger than that of water temperature, it may be useful to test wetland performance in tunnels or under shade cloth cover. This suggestion is further supported by the fact that the number of daylight hours was not correlated to ammonia levels.

The exponential model predicting ammonia concentration as a function of the distance from the inflow allows us to predict ammonia levels. Here, the estimated average of 0.69 mg/L NH_4^+ at the inflow to the wetlands was reduced to 0.08 mg/L after 15 metres, an 82 % reduction (Figure 3.2). After 9 m the concentration of ammonia averaged 0.19 mg/L, more than double the concentration predicted for 15 m. Thus, despite the exponential asymptotic drop in ammonia levels, the wetland should not be less than 15 m long, as 9 m still left a concentration in the effluent that is potentially harmful to fish. Similar to the conclusions

drawn from the HRAP study, it is essential to predict ammonia levels at its lower ranges. This data set allows for such a prediction.

Step-wise regression modelling with the log-transformed ammonia concentration as the dependent variable suggests keeping these three variables in the model. The adjusted r^2-value is 33 %, and the estimated coefficients are 0.312 (pH), -0,30 (ambient temperature), and -0.21 (distance from inflow). Residuals were normally distributed.

Chemical oxygen demand (mg/L)

The independent variables that were selected as the best predictors of COD levels were water temperature and ambient temperature, the number of days the system was in operation and the concentration of PO_4^{3-} (Figure 3.3). A forward step-wise regression procedure suggests that all variables should be used as predictors in a multiple regression model. The suggested model has an adjusted r^2-value of 36 % with the logarithm of COD as the dependent variable. The coefficients for water temperature, days in system, ambient temperature and PO_4^{3-} - concentration are 0.307, -0.28, 0.166, and 0.148, respectively, with normally distributed residuals.

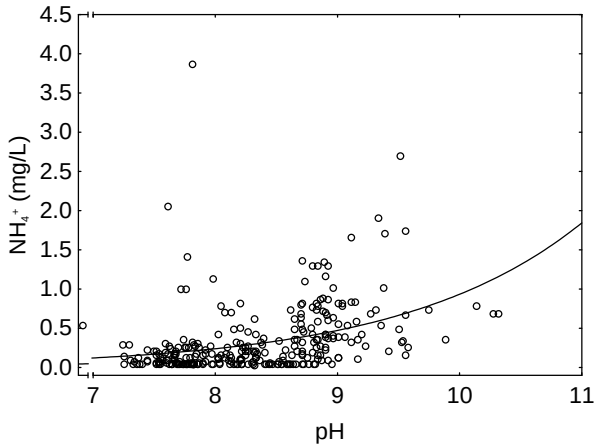
Thus, it is predicted that an increase in temperature and a well-established wetland will be most effective in reducing COD-levels. Future studies should test whether the increase in phosphate levels acted as a nutrient to the wetland flora, thereby leading to improved effluent quality due to a higher nutrient assimilation.

Orthophosphate (mg/L)

Only the number of days in the system and the ambient temperature had a significant influence on orthophosphate levels. Phosphate levels decreased by approximately 0.44 mg/L per day as the wetland matured. An increase in temperature resulted in a slight increase in phosphate concentration (Figure 3.4).

Nitrate (mg/L)

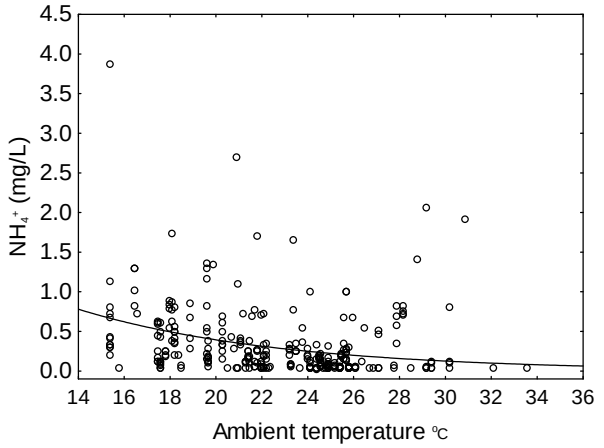
Only water temperature and the duration of wetland system operation influenced the concentration of nitrate in the effluent. The model predicts a drop in nitrate levels of 23 % from 29 mg/L to 21 mg/L (Figure 3.5).



Function:

$$y = 10^{-2.98+2.95\,x}$$

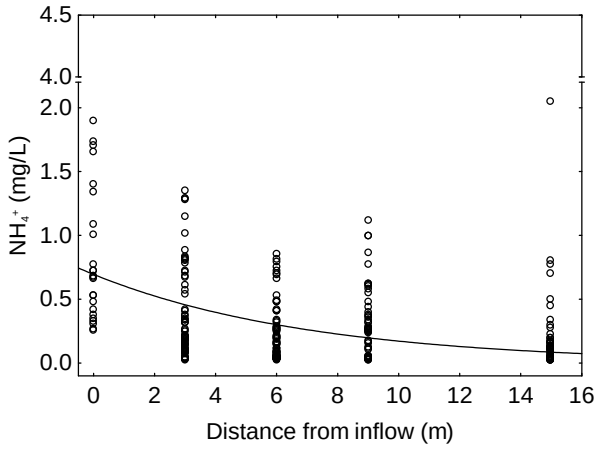
Y = total ammonia (mg/L)
X = pH



Function:

$$y = 10^{0.58-0.05\,x}$$

Y = total ammonia (mg/L)
X = Ambient temperature



Function:

$$y = 10^{-0.16-\,0.061\,x}$$

Y = total ammonia (mg/L)
X = Distance from inflow (m)

Figure 3.2 Variables that significantly influenced the ammonia concentration in an experimental wetland.

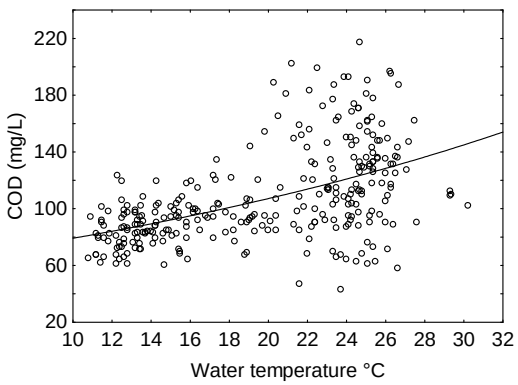
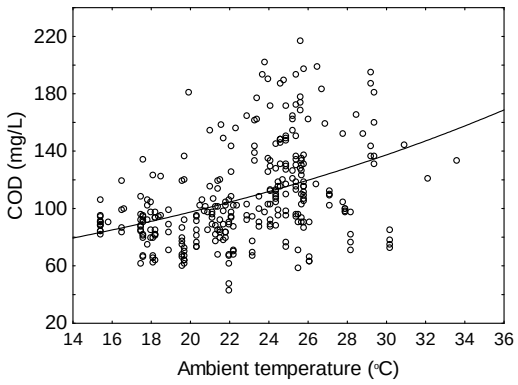
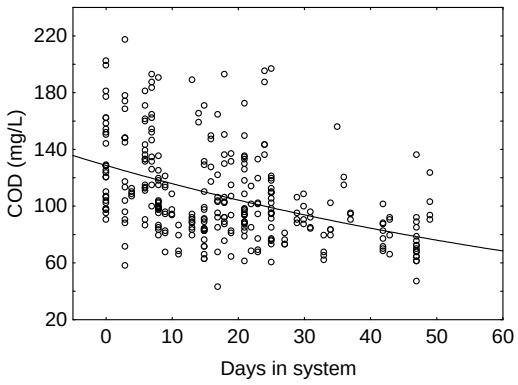
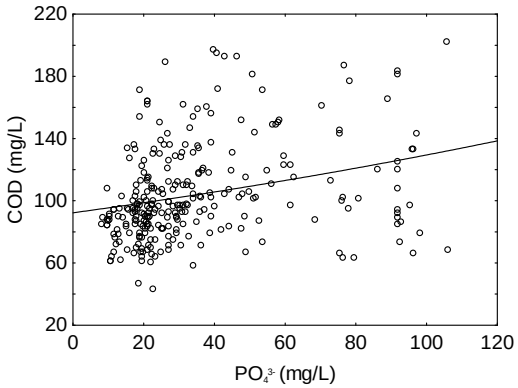
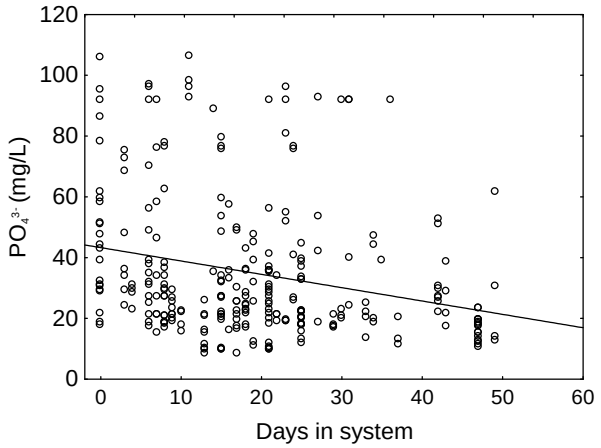
	Function: $y = 10^{1.76 + 0.013\,x}$ Y = COD (mg/L) X = Water temperature
	Function: $y = 10^{1.69 + 0.0149\,x}$ Y = COD (mg/L) X = Ambient temperature
	Function: $y = 10^{2.11 - 0.0046\,x}$ Y = COD (mg/L) X = Number of days of system operation
	Function: $y = 10^{1.965 + 0.00157\,x}$ Y = COD (mg/L) X = Number of days of system operation

Figure 3.3 Variables that significantly influenced the concentration of COD in an experimental wetland.

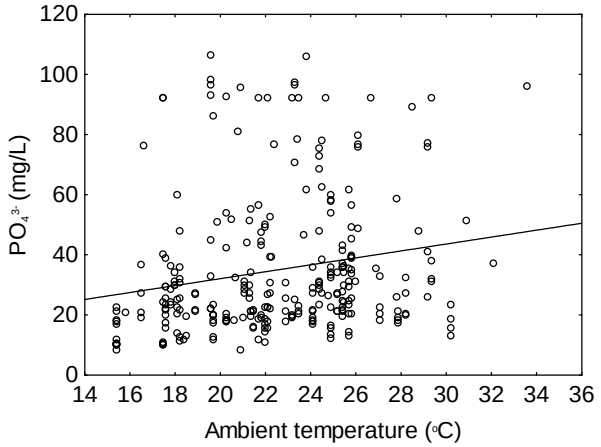


Function:

$$y = 43.287 - 0.439\,x$$

Y = PO_4 (mg/L)

X = Number of days of system operation



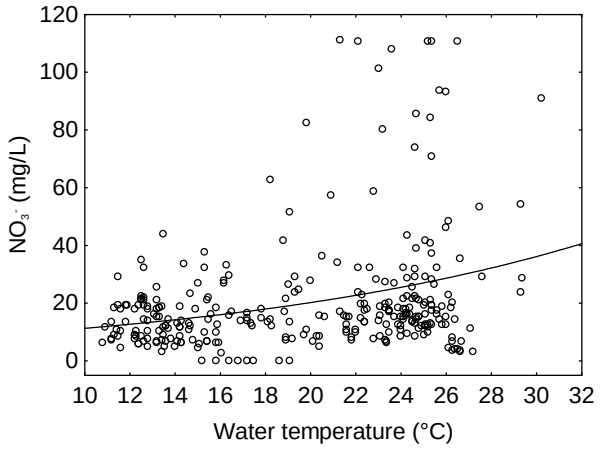
Function:

$$y = 9.02 + 1.152\,x$$

Y = PO_4 (mg/L)

X = Ambient temperature

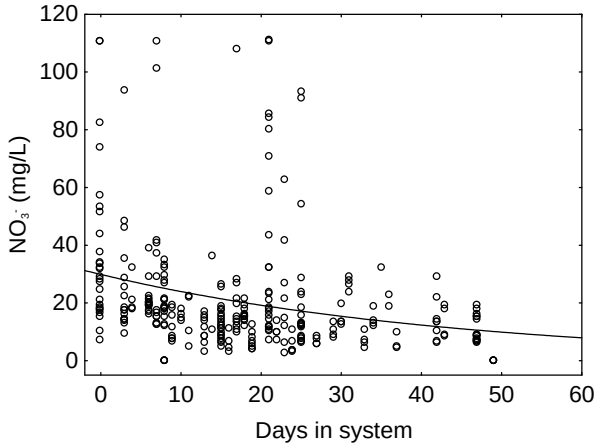
Figure 3.4 Variables that significantly influenced the concentration of phosphate in an experimental wetland.



Function:

$$y = 10^{0.8 + 0.025\,x}$$

Y = NO_3^- (mg/L)
X = Water temperature



Function:

$$y = 10^{1.475 - 0.0096\,x}$$

Y = NO_3^- (mg/L)
X = Number of days of system operation

Figure 3.5 Variables that significantly influenced the concentration of nitrate in an experimental wetland.